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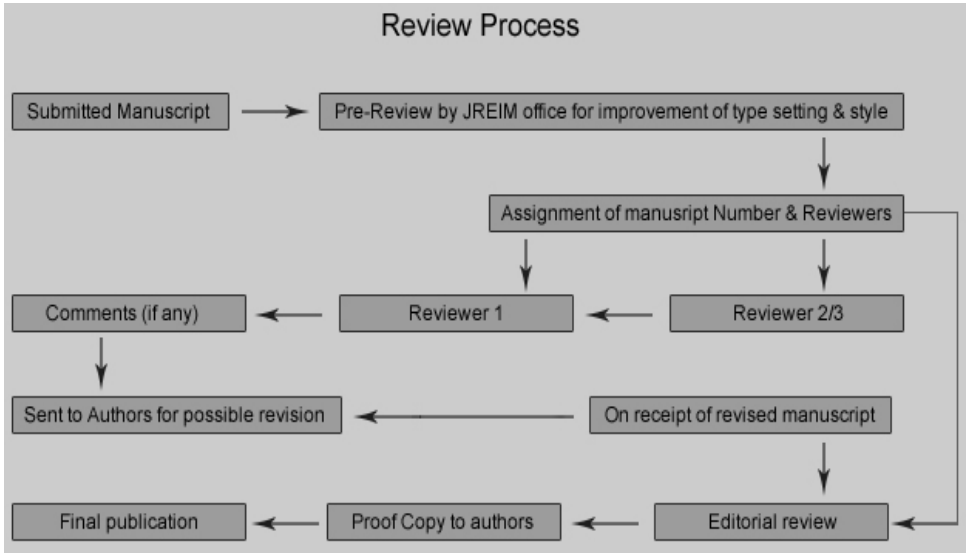

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AN OVERVIEW OF POSTGRADUATE AND DOCTORAL LEVEL RESEARCH CONDUCTED IN AYURVEDA EDUCATION INSTITUTIONS DIABETIC NEUROPATHY :

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Abstract: Diabetes has become one of the major causes of premature illness and death in most countries. Vascular complications; both micro and macro vascular predominate the features of Indian diabetic patients due to delayed diagnosis and late presentation of the syndrome. Therefore many complications like Polyneuropathy are present at diagnosis. It is common, often severe but frequently unreported and inadequately treated. Lot of research on diabetic neuropathy is being done and reported but an considerable portion of it which is conducted by Ayurveda academic institutions go as part of PG & Doctoral thesis go unnoticed and unpublished. Hence an attempt is made to review the available academic clinical researches concerned with the management of diabetic polyneuropathy in the present article. A sudden surge of interest towards the complications of Diabetes can be noted by the number of works done after 2005 (out of 15, 12 were carried out after 2005). The works reviewed are based on obtained transcripts of the thesis or by personal communication or by acquaintances. Seven works/ abstracts (46.67%) were retrieved out of 15.

Keywords: Diabetic neuropathy, Ayurveda, Systematic review, PG and Ph.D.Thesis.

Introduction

At least 171 million people worldwide have diabetes; this figure is likely to be more than double by 2030.[1] At present India have 35 million diabetics (5.5% of population), which is likely to reach 80 million by 2030.

Overall, direct health care costs of diabetes range from 2.5% to 15% of annual health care budget, depending on local diabetes prevalence and the sophistication of the treatment available. In developed countries most people with diabetes are above the age of retirement whereas in developing countries those most frequently affected are in the middle, most productive years between 35 and 64 of age.[2]

Diabetes has become one of the major causes of premature illness and death in most countries. The number of deaths attributed annually to diabetes is around 3.2 million,[3] six deaths every minute. Vascular complications; both micro and macro vascular predominate the features of Indian diabetic due to delayed diagnosis and late presentation of the syndrome. Therefore many complications including polyneuropathy are present at diagnosis. Diabetic foot accounts for one of the largest in patients admissions in India. The neuropathies are among hospital the most common of the long-term complications of diabetes, affecting up to 50% of patients.[4,5] Long-standing peripheral neuropathic pain associated with peripheral neuropathy occurs in one of six diabetic subjects.[6]

These complications of diabetes have been recognized in modern science only since last two centuries. In the late 1800s, a series of papers appeared in which many of the subtypes of diabetic neuropathies were defined **(Althaus 1885, Leyden 1887, Auche 1890, Pryce 1893).** Included in these descriptions are patients not only with diabetic sensorimotor polyneuropathy but also others with proximal diabetic, truncal, median and ulnar neuropathies. Bruns focused further on the entity of proximal diabetic neuropathy (1890). Diabetic polyneuropathy was recognized as having various manifestations;

1. Asst. Professor 2. Director

Leyden identified 3 subtypes: painful, ataxic and paralytic. Autopsy studies on several patients showed peripheral nerve degeneration **(Leyden 1887, Auche 1890).**[7]

Major risk factors of this condition are the level and duration of elevated blood glucose. Neuropathy can lead to sensory loss and damage to the limbs. It is also a major cause of impotence in diabetic men. Diabetic foot disease, due to changes in blood vessels and nerves, often leads to ulceration and subsequent limb amputation. Diabetes is the most common cause of non-traumatic amputation of the lower limb. Simple measures like good glycaemic control and neuroadjuvants, visual inspection of feet and foot-care can save and salvage feet at risk.

With longstanding diabetes mellitus progressive damage to nerves is seen and the symptoms are most profound at the extremities of the limbs; this condition is known as Diabetic Polyneuropathy; the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes.[8]

The most common form of neuropathy is distal symmetrical sensory motor polyneuropathy. Early distal sensory motor neuropathy is usually asymptomatic, but sensory abnormalities may be detectable by neuro-physiological testing. Symptomatic distal sensory-motor neuropathy is manifested by sensory loss, and may be accompanied by paraesthesiae and/or pain. Peripheral neuropathy may be asymptomatic. When symptoms are present, they may be negative or positive. Negative symptoms include loss of sensation and loss of strength, while positive symptoms include pricking or pain.[9] One of the most distressing symptoms that people can suffer from is neuropathic pain and paraesthesia.[10] Chronic painful diabetic peripheral neuropathy can cause symptoms that last for years and severely impair quality of life.[11, 12] Severe distal sensory-motor neuropathy is manifested by motor involvement, and may be accompanied by disabling symptoms and the potential for ulceration, which can lead to infection, necrosis, gangrene, and loss of the limb.[13]

The recent study by **Daousi** *et al.*[14] investigated the prevalence, severity, and current treatment of chronic painful neuropathy was concluded that chronic painful peripheral neuropathy was "common, often severe but frequently unreported and inadequately treated."

Hyperglycemia associated with diabetes is thought to be central to the effect on nerve structure through a number of possible mechanisms, including increased activity in the polyol pathway, altered myo-inositol metabolism and non-enzymatic glycation. Other mechanisms may also be involved, e.g. alterations in nerve growth factor activity, blood viscosity, circulating platelets and the rate of synthesis and transport of intra-axonal protein. There may also be interactions between these pathways.

The loss of sensation in feet plays an important cause for the development of pressure sores which does not heal and ultimately terminating into diabetic gangrene. Hence in diabetics, prevention and management of Polyneuropathy is of utmost importance.

Diabetic polyneuropathy is a sequel to *Madhumeha* which occurs due to further vitiation of the *Doshas* or due to *Vyadhi karshana*. The disease diabetic polyneuropathy is not directly mentioned in *Ayurvedic* texts. But the *lakshanas* of diabetic polyneuropathy i.e. burning sensation, tingling sensation, numbness etc. are explained under *purvaroopa* and *upadrava* of *Prameha*. When *Prameha* is neglected or ill-treated it will lead to *Madhumeha* by *dhatukshayajanya Vata prakopa* as a *Paratantra Vyadhi*.

Madhumeha is of two types according to *samprapti*; Due to *Shuddha Vata* & due to *Avarana*. In *Shuddha Vatajanya Madhumeha, Nidana* causes primary *Vataprakopa*. And in *Avaranjanya Madhumeha* aggravated *Kapha* and *Pitta* obstructs normal pathway of *Vata* and it leads to secondary *Vataprakopa*. The *prakopa* of *Vata* due to *marganirodha* leads in to diverse clinical scenarios of *Vataja nanatmaja vikara, karma kshaya* of *Vata* and increase of function of opposite factors i.e., *Pitta* and/or *Kapha.*

In both the types, this *prakupita Vata* along with *Kapha* and *Pitta* circulates all over the body and produces symptoms like *padadaha, pada supti, padaharsha*. In *Ayurveda, lakshanas* like *Pada supti, Padaharsha* are dealt under *Vataja nanatmaja vikaras*. These are correlated to diabetic polyneuropathy as told in modern system of medicine.

Management

The main objectives of therapy for diabetes mellitus are to reduce or eliminate microvascular and macrovascular complications of diabetes mellitus along with near normal glycaemic control, to treat associated disorders and to allow the patient to achieve as normal a lifestyle as possible.[15]

Management and prognosis of diabetic polyneuropathy depend largely on the underlying condition. The mechanisms of diabetic neuropathy are poorly understood since from 1864 Marchal de Calvi recognized the condition. At present, treatment alleviates pain and can control some associated symptoms, but the process is generally progressive. Treating the diabetes may halt progression and improve symptoms of neuropathy but recovery is slow. The painful symptoms of diabetic polyneuropathy may become severe enough to cause depression in some patients.

Despite advances in the understanding of the metabolic causes of neuropathy, treatments aimed at interrupting these pathological processes have been limited by side effects and lack of efficacy.[4] Thus, treatments are symptomatic and do not address the underlying problems.

The first line of treatment in diabetic polyneuropathy is the management of diabetes itself. *Vata dosha* is invariably involved as *Madhumeha* as it is of *Vataja* variety and the degeneration of nerves occurs due to *Vata kopa*. The morbid increase in the *Dhatus* prior to *Medas* and the resultant lack of *poshana* of *uttara dhatus* i.e, *Asthi*, *Majja* and *Shukra* causes various complications in *Madhumeha*. The present condition is nothing but a manifestation of *majja dushti* causing demyelination of nerve fibers due to microvasculopathy and hyperglycemic insult to nerves. The concept of *Avarana Vata* helps in understanding the condition better. The *roopavriddhi*, *roopahani* and *roopantara* of *Vata* are clearly observable in this condition. The *Avarana* may be due to primary *vriddha dosha* or *dushya* leading to *gatihanana* of *Vata* or primary *Vata prakopa* leading to the *dushti* of *ashrayee Dhatus*. Ultimately *Avarana* of *Vata* leads to *Vataprakopa*.

Objectives

To review the available academic research works (PG and PhD thesis) on the management of diabetic neuropathy through Ayurveda.

Methodology

The authors have consulted only Postgraduate and Doctoral level dissertations submitted in Ayurveda educational institutions for this study. Other academic researches that might have been submitted as dissertations in medical colleges, pharmacy colleges etc were not consulted. The published papers available on Google scholar or Pubmed were also not included in the study. The academic research works done were searched with key words on Diabetic Neuropathy and Diabetic Polyneuropathy yielded the following results;

1. **Dwivedi KN:** Effect of *Dashamoola* on Diabetic Polyneuropathy, Banaras Hindu University, Varanasi, **1986**.
2. **Dwivedi KN:** Role of *Jeevaneeya* and *Balya* drugs in Diabetic neuropathy. PhD thesis, Banaras Hindu University, Varanasi, **1996**.
3. **Vijaya K:** To evaluate the effect of *Takradhara* in the management of *Madhumeha upadravas* with special reference to Diabetic Peripheral Neuropathy- An observational study. Govt. Ayurvedic Medical College, Mysore, RGUHS, Bangalore, **2003**.
4. **Pravith N.K:** Clinical study on the efficacy of an Ayurvedic package in the management of Diabetic neuropathy with special reference to *Nadi Kwatha*, Govt. Ayurveda College, Trivandrum, **2005**

5. Nandeshwar Manisha: Clinical study of *Amritadi ghritam* as *anuvasana basti* in *Madhumehajanya Upadrava* w.s.r. ro Diabetic Neuropathy. KG Mittal Punarvasu Ayurveda College, Mumbai, **2006**.

6. Tiwari Priyaranjan: Clinical evaluation of *Dashamuladi Ghana Vati (Kalpita Yoga)* in the management of Diabetic Neuropathy, National Institute of Ayurveda, Jaipur, **2007**.

7. Nisha K: Comparative clinical trial to evaluate the efficacy of an Ayurvedic compound in Diabetic Neuropathy, Govt. Ayurveda College, Trivandrum, **2007**.

8. Karishma: Evaluation of the Efficacy of *Sapta-Avartita Guduchi Taila* in *Twak - Gata-Vata* (Diabetic Peripheral Neuritis) - A Comparative Clinical Study. Govt. Ayurveda Medical College, Bangalore, RGUHS, Bangalore, **2008**.

9. Kokane Deepti: Ayurvedic Management of Diabetic Polyneuropathy, Ayurveda Mahavidyalaya, Hubli, RGUHS, Bangalore, **2008**.

10. Sawant Manish: Clinical study of *Ardhamatrika basti* in *Madhumehajanya Upadrava* w.s.r. to *Vatanadi pratana shosha* (Diabetic Neuropathy), KG Mittal Punarvasu Ayurveda College, Mumbai, **2008**

11. Kumar Sanjay: A clinical study on *Naimittika Rasayana* effect of *Shilajatu* and *Mamajjaka* in patients of Diabetes mellitus with special reference to Diabetic Neuropathy, Banaras Hindu University, Varanasi, **2009**.

12. Jaideep: A clinical study to evaluate the effect of Ayurvedic formulation in patients of Diabetic Neuropathy, RGGPG Ayurveda College, Paprola, **2009**.

13. Manish Jain: A comparative clinical management of Diabetic neuropathy with *Nishoshiradi tailam* as external application, BNMR Ayurvedic Medical College, Bijapur, RGUHS, Bangalore, **2010**.

14. Vyasaraja Tantri A: A Comparative Study on the efficacy of *Shamanoushadhis* in the management of Peripheral & Proximal Diabetic Neuropathy, Govt. Ayurveda Medical College, Mysore, RGUHS, Bangalore, **2011**

15. Niranjan Y: A clinical study on the management of Diabetic Polyneuropathy with Dashamooladi Rasayana compound, PhD thesis, IPGT&RA, Gujarat Ayurveda University, Jamnagar, **2011**

Discussion

Research in medical sciences should be a process that converts data into information, information into knowledge and knowledge into wisdom of physician for usefull clinical application of the gained wisdom. It should be: Balanced and comprehensive with equal emphasis on literary, field, experimental and clinical research, able to impact the fields of education, pharmacy and practice in a profound way. Present day Ayurvedic Researches are failing in this respect as they are unable to disseminate the knowledge gained from the researches as research work becomes valid and accepted when it is published in peer reviewed journals. Documentation and publication of research findings is the main issue faced by Ayurveda in the global arena as the publications are scarce.

Lacunae in Ayurvedic Research:

⇒ Majority of the studies belongs to evidence levels 2nd to 4th. A few studies fall under level 1a to 1b.

⇒ Small sample size making them vulnerable for methodological error.

⇒ Rationale for selected study designs is not always properly described.

⇒ Understanding of Ayurvedic classical terminologies and their grading for objectivity and universalization

⇒ Dependency of researches on symptomatology alone.

⇒ Missing values complicate the calculation of probability and power.

⇒ There are no networks of competence or centers for excellence.

⇒ No publication on health services research (HSR) and health technology assessment (HTA)

⇒ The evidence of Ayurveda is difficult to survey. As there are no comprehensive electronic databases for Ayurvedic studies.

⇒ Many publications are only retrievable via hand - search of references and interviews of experts.

⇒ In common western databases and CAM databases, only a small number of Ayurvedic studies are listed.

⇒ Various studies are published in regional languages, many of them only as abstracts. A large number is not available at all.

Hence to make this overview more precise, the work is restricted to academic researches at various Ayurvedic institutes across the nation. The authors have restricted their study to Postgraduate and Doctoral level dissertations submitted in Ayurveda educational institutions due to the scarcity in obtaining reports on this regard. The authors of certain studies were reluctant to provide their findings for review.

A sudden surge of interest towards the complications of Diabetes can be noted by the number of works done after 2005 (out of 15, 12 were carried out after 2005). The works reviewed are based on obtained transcripts of the thesis by personal communication or by acquaintances. Seven works/ abstracts were retrieved out of 15 (46.67%).

The work of **Dwivedi KN**[16] **(1986)** is the first of its kind on Diabetic Neuropathy. Role of *Jeevaneeya* and *Balya* drugs in Diabetic neuropathy was also worked out by the same scholar in 1996. The abstract of the work was published[17] in Sachitra Ayurved entitled "Sushruta's *dashamula* and its application in diabetic neuropathy" in 2003. The efforts to retrieve the transcripts of the thesis/ article went unproductive.

In the work done by **Tiwari Priyaranjan**[18] *et al.* **(Table 1)** the trial drug was a combination of *Dashamula, Madhyama Panchamula (Bala, Punarnava, Eranda, Mudgaparni and Mashaparni)* and *Vanga Bhasma*. 25 subjects are included for this study among them 18 completed the schedule. The study had three groups; Methylcobalamin (500 mcg BD) as Control group, Trial group and Mixed therapy. Three groups of 6 patients are statistically tested after 2 months of trial. Among the three groups studied, mixed therapy yielded maximum results and Trial and control groups fared moderate and mild results. The authors concluded that *Dashamuladi Ghana vati* might have the property of myelin sheath repair which has resulted in improvement in nerve conduction studies.

Nisha K *et al.*[19] **(Table 2)** opined that Diabetic neuropathy can be considered as *upadrava* of *madhumeha*. A definite clinical syndrome which simulates neuropathy is not seen in Ayurveda. The *prakupita kapha, pitta medas* and other *dhatus* cause the *avarana* to *vata,* thereby manifesting symptoms of neuropathy. *Dhatukshaya* and *ojokshaya* causes neuropathy with predominant motor symptoms. A comparative clinical trial was conducted to assess efficacy of *Bhoonimbadi choornam* (one *karsha* with *madhu* and *ghrita* for six months) comparing with conventional medicine i.e. gabapentin, methylcobalamin, alpha lipoic acid & gamma linoleic acid. Twenty patients participated in study, 10 each in study wherein clinical, electrophysiological and biochemical parameters were assessed and analyzed statistically. *Bhoonimbadi choornam* was found to be effective in reducing signs and symptoms of diabetic neuropathy especially in subjective sensory symptoms. The general status of peripheral nerves is improved as revealed by electrophysiological studies. NCV increase to normal or near normal velocity, so that it can be concluded that the trial drug promotes remyelination. The FBS, PPBS, S. Cholesterol was found to be decreased significantly.

Karishma *et al.*[20] **(Table 3)** considered the condition as *Madhumehajanya Twak gata Vata* and conducted an open clinical trial using *Sapta-avartita Guduchi taila* (10-20 drops B.D. with warm milk) with and without *Padabhyanga* for one month in 30 cases. The results were based on the improvement in Neuropathy Total Symptom Score, Quantitative Sensory Tests (Monofilament, Biothesiometer, HCP Sensitometer) and Quality of Life using Nottingham health profile (NHP). Significant

improvement was observed in the symptoms, no change was noticed in the findings of neurological assessment (Quantitative Sensory Testing). Both the groups showed statistically significant improvements in the Quality of life.

Kokane Deepti[21] **(Table 4)** tried to explore the unexplored field of *Swarna Bhasma* in the management, comparator agent was *Dashamoola Kwatha*. Conceptual framework was based on Diabetic Polyneuropathy as *Madhumeha Upadrava*. Emphasis was given on the role of *Vata* causing symptoms due to *dhatukshaya* or *avarana*. It was an open study on 24 subjects receiving either *Dashamoola Kwatha* 40 ml. BD or Tablets of *Vasantkusumakar Rasa* 125 mg BD for 3 weeks. The oral hypoglycemic agents were continued as per diabetologist's recommendation. Burning sensation, Tingling sensation, Pain and Paraesthesia were graded and assessed. No objective parameters were used. Both the trial drug and comparator showed statistically highly significant results on parameters, but, *Dashamoola Kwatha* yielded better result in terms of overall effects.

Jaideep[22] **(Table 5)** studied 23 patients of diabetic neuropathy with 500 mg *Dashmool* extract + 50 mg *Pushkarmool* extract and 25 mg Hingu orally twice in a day and local *abhyanga* with *Masha taila* for 6 weeks. The oral drugs were referred from *Jhinjhinivata chikitsa* of Bhaishajya Ratnavali[23]. Vibration perception, Pinprick and deep tendon reflexes were assessed along with subjective parameters. It was an observational study without any control and the type of diabetes studied is not described. The response of the trial drug was found highly significant ($p<0.001$) in criteria but there was no much improvement in feeling of gloves and stockings.

Tantri V. *et al.*[24] **(Table 6)** studied on 40 subjects of NIDDM with signs and symptoms of peripheral & proximal neuropathy with *Gokshuradi Guggulu* (2 Tablets of 500mg TID with hot water after food) and *Twak Choorna Lepa* in group A and *Sahacharadi Kashaya* (15 ml TID after food) with *Moorchita Tila Taila* (5 ml) and *Ela Choorna Lepa* in group B and patients of both groups were given with *kataka khadiradi kashaya* of 15 ml TID with hot water before food without disturbing hypoglycemic agents. Assessment was done based on clinical graded signs and symptoms, FBS and PPBS after one month. The Group B has shown a better result than that of the *Gokshuradi guggulu* and *twak lepa*.

Niranjan *et al.*[25] **(Table 7)** studied Diabetic Polyneuropathy as a complex multifactorial disorder with varied clinical features due to *Avarana* of *Vata*. The underlying pathological phenomenons are postulated as a result of intricate *Vata Dushti* including *uttara dhatu kshaya*, *margavarana*, *Rakta dushti*, *indriya pradosha* and *ojakshaya*. The work is described in a unique way explained as per **Charaka Samhita**.[26] The trial drug was a combination of *Gokshura*, *Guduchi*, *Amalaki* and *Ashwagandha* processed with *Dashamoola Kashaya*. It stands as the biggest study based on the sample size, on 69 subjects. It is also registered in Clinical trial registry of India vide CTRI/2011/07/001885. The assessment was done based on changes in Neuropathy Symptom Score (NSS) and Michigan Neuropathy Screening Instrument (MNSI). Quality of life was assessed in detail using both traditional and WHO parameters.

Among 15 academic works on Diabetic Polyneuropathy carried out across the nation, all were PG thesis except two; **Dwivedi KN (1996)** and **Niranjan Y (2011).** No concrete conceptual decision was made in any of the works regarding *Ayurvedic* term equivalent for the condition. However there was an effort to understand the *Samprapti* in the lines of Ayurveda. Majority of the works revolve around DPN as *Madhumeha Upadrava*. The role of Vata in the manifestation of the condition is recognized by almost all the scholars. *Avarana* and *dhatukshaya* are considered as major pathological process terminating in neuropathy.

Regarding the interventions, four studies (Maximum) use *dashamoola* as sole or part of treatment strategy. New approach in the selection

of drug can be seen in the works of **Jaideep**[22] wherein the formulation was derived from *Jhinjhinivata chikitsa* of Bhaishajya Ratnavali. **Karishma (Bangalore, 2008)**[20] proposed a new understanding of the condition under *Twakgata Vata* and treated with *Guduchi*. **Sawant M. (2008)** considered DPN as *Vatanadi pratana shotha*. Jaideep, Tantri V and Jain Manish included local treatment (*Abhyanga*) along with systemic interventions. Nisha and Tiwari P. used modern drugs as control; methylcobalamin was the common drug in both the works. The criteria of inclusion were not quite rigid as the autonomic and proximal neuropathies are also included in certain trials.

Regarding the methodology, the works of Karishma and Nisha stands apart due to detailed account of methodical precision. The outcome measures were clinical in majority of works relying on graded symptoms and their assessment. Quality of Life (QoL) parameters were assessed in only one (Karishma) trial. Nerve Conduction Studies (NCS) were used to give objectivity in two trials. Majority of them had sample size < 40. All were randomized trials, but none mentioned method used for the same. No blinding/ masking were reported in any of the trials conducted so far.

Emerging trends

There has been an improvement in the quality of research over the years in terms of methodology. Majority of the works carried out are in the department of Kayachikitsa. The common method followed to avoid bias and to bring objectivity in such studies were randomization of sampling; however majority of them fail to report the methods followed for the same. The quality of theoretical discussions is constantly being upgraded and many hypotheses regarding the understanding of the condition are put forth looking the research question at different angles.

We could identify certain trends in the development of research methodology in the field of diabetic neuropathy research in Ayurveda.

· Inclusion of QoL parameters in assessing the response.

· An increasing effort to quantify and objectivism the parameters of assessment.

· Better application of fundamental doctrines of Ayurveda in understanding Diabetic neuropathy

· Better methodological rigors

· Adaptation of more validated and reproducible assessment scales instead of symptom scoring in assessing the efficacy.

Randomized controlled trials are considered gold standard when appropriately designed, conducted and reported. To assess a trial accurately readers require complete, clear and transparent information on its methodology and findings. One can notice a sustained improvemet in the quality of research being carried out in this regard. Application of CONSORT model[27] in describing the methodology will make the report widely acceptable and transparent.

Future Prospects

A long term prospective study to evaluate the prophylactic efficacy of *Dashamoola* may be taken up with a larger sample. More sensitive and precise quantification using Biothesiometer, Nerve Conduction Velocity, and Nerve biopsy may be used for better assessment. Pharmacological models to understand pathophysiological, pathobiochemical and structural abnormalities of diabetic polyneuropathy and the role of *Dashamoola* in preventing the development of these alterations, to halt their progression, or to induce their regression, despite concomitant hyperglycemia may be taken up.

Table 1. Tewari Priyaranjan

Study ID: Tiwari P *et al.*
Title: Clinical evaluation of *Dashamooladi Ghana Vati* (*Kalpita yoga*) in the management of Diabetic neuropathy
Authors: Tiwari P et.al.
Settings: Dept. of Kayachikitsa, National Institute of Ayurveda, Jaipur, India
Year: 2007 **Study Design:** Clinical study
Methods
Objectives: To assess the efficacy of compound drug- *Dashamooladi Ghana Vati* (*Kalpita yoga*) in the management of Diabetic neuropathy

Sample Size: Male 13; Female 12; Total 25

Eligibility Criteria: Symptomatic Diabetic polyneuropathy above the age of 12 years with evidence of diminished/ absent muscle jerks, pain, touch, vibration sense, orthostatic hypotension

Randomization: Done, method not specified

Adverse Effects: No adverse effects reported

Intervention

Sample size and Drug

Group I. 8 Patients : Tab. Methylcobalamin 500 mcg twice daily with lukewarm water after meals for two months.

Group II. 9 Patients : Tab. *Dashamooladi Ghana Vati* 500mg twice daily with lukewarm water after meals for two months.

Group III. 8 Patients : Tab. Methylcobalamin 500 mcg twice daily with lukewarm water after meals for two months along with Tab. *Dashamooladi Ghana Vati* 500 mg twice daily with lukewarm water after meals for two months.

Drop Outs: 7

Statistical Test Used: Subjective symptom rating scale developed by Prof. AK Sharma, BP, weight, pulse rate, respiratory rate, urine routine, GHb, FBS, PPBS, Lipid profile, Nerve conduction studies were assessed using students t test.

Overall Result: The clinical studies carried out on subjective and objective parameters revealed an overall mild improvement in I (Allopathic) group, moderate improvement in II (Ayurvedic) group and maximum improvement in third group. Group II was better than group I. Group II showed significant improvement in most of the symptoms and in nerve conduction studies in shorter duration of time. Mixed therapy proved to be most effective.

Conclusion and Remarks: *Dashamooladi Ghana Vati* has shown highly significant clinical and electrophysiological recovery in shorter duration of time without any complications. Physiological and haematological recovery noticed after the therapy was statistically insignificant.

Mixed therapy has shown highly significant results by producing marked symptomatic improvement and electrophysiological improvement in a very short span of time, without any complications, with early normalization of neurological abnormalities.

Dashamooladi Ghana Vati might be having the property of myelin sheath repair which ultimately increases the nerve conduction velocity. It may stimulate the property to induce the nerve growth factor naturally found in the body.

Trial dug was potent in the management of diabetic neuropathy

Table 2. Nisha K

Study ID: Nisha K *et al.*

Title: A comparative clinical trial to evaluate the efficacy of an Ayurvedic compound in Diabetic Neuropathy

Authors: Nisha K, Radhakrishnan VN, Lila AS, Chandra SR

Settings: Dept. of Kayachikitsa and Panchakarma, Govt. Ayurveda Medical College. Thiruvananthapuram, Kerala, India

Year: 2007 **Study Design:** Comparative clinical trial

Methods

Objectives: To evaluate the efficacy of *Bhoonimbadi choornam* in reducing signs and symptoms of diabetic neuropathy and to evaluate the changes in blood biochemical parameters

Sample Size 20 (Group A-10, Group B-10)

Eligibility Criteria Patients with clinical and laboratory evidence of diabetic neuropathy

History of diabetes mellitus of more than 5 years

Age group: 40-70 years of both sex

Randomization: Not mentioned

Adverse Effects: The drug administered with hot water produced gastric irritation

The drug was not patient friendly in terms of disagreeable taste.

Intervention

Sample size and Drug

Group A. 10 patients were given *Bhoonimbadi choornam* (*A.Hr. Kushta Chikitsa*) 6g with *madhu* and *sarpi* before food twice daily as *leha* for 6 months with oral hypoglycaemic drugs.

Group B. 10 patients were given Gabapentin 300mg, Methylcobalamin 500mcg, Alphalipoic acid 50m, Gammalinolenic acid 50mcg with oral hypoglycaemic drugs.

Drop Outs: Not reported

Statistical Test Used: Quantitative sensory testing, vibration sense test, pin prick, monofilament sensory test, temperature discrimination, motor system examination and autonomic system examination were done.

Nerve conduction study (NCV) was done to assess the condition of nerves and to differentiate axonal degeneration or demyelination type of neuropathy. Motor nerve conduction velocity was done in median, ulnar, common peroneal and posterior tibial nerve. Sensory NCV was done in median, ulnar and sural nerves. Amplitude of impulse was also assessed to know the effect on axonal degeneration.

Overall Result: Statistical significance at $P < 0.05$ level were noted within study group in SNCV velocity of median nerve and symptoms like tingling sensation, burning sensation, numbness, feeling of walking on cotton / wool, pain which worsens at night inducing sleeplessness, muscle weakness ,stumble while walking, indigestion, constipation, dizziness, wasting and signs like impaired pain and touch sensation. The biochemical parameters like FBS, PPBS and Serum cholesterol also have statistical significance at $P < .05$ level. Comparing both groups the study group had improvement in the clinical responses within group than that of control group. In other criteria, it can be concluded that, the intervention in both groups are equally effective.

Conclusion and Remarks: A definite clinical syndrome which simulates neuropathy is not seen in Ayurveda.

Diabetic neuropathy can be considered as *upadrava* of *madhumeha*. The *prakupita kapha*, *pitta*, *medas* and other *dhatus* cause the *Avarana* to *Vata*, thereby manifesting symptoms. *Dhatukshaya* and *ojokshaya* causes neuropathy with predominant motor symptoms.

The treatment with *Bhoonimbadi choornam* was found to be effective in reducing signs and symptoms.

The general status of peripheral nerves was improved as revealed by electrophysiological studies. NCV increase to normal or near normal velocity, so that it can be concluded that the trial drug promotes remyelination.

The FBS, PPBS, S. Cholesterol was found to be decreased significantly. So drug is effective in improving the blood biochemical parameters i.e., on blood sugar and lipid levels. The drug was found more effective in subjective sensory symptoms.

Table 3. Karishma

Study ID: Karishma *et al.*

Title: Evaluation of the efficacy of *sapta-avartita Guduchi taila* in *twakgata vata* (Diabetic peripheral neuritis)- A comparative clinical study

Authors: Karishma *et al.*

Settings: Dept. of PG studies in Dravyaguna, Govt. ayurveda Medical College, Bangalore and SJIM Hospital, Bangalore

Year: 2008

Study Design: Comparative clinical trial

Methods

Objectives: To compare and evaluate the efficacy of *Sapta avartita Guduchi taila* in the form of oral administration and *Padabhyanga* in *madhumehajanya twakgata Vata* (Diabetic peripheral neuritis)

Sample Size: Male 15; Female 15; Total 30

Eligibility Criteria: Diabetic patients (NIDDM) type, presenting with classical features of peripheral neuropathy within the age group of 35-55 years of either sex, who are on a standard anti diabetic medicine.

Randomization: Open clinical trial

Adverse Effects: None mentioned

Intervention

Sample size and Drug

Group A. 15 Patients : *Sapta avartita Guduchi taila* orally 10-20 drops twice daily with warm milk for one month

Group B. 15 Patients : *Sapta avartita Guduchi taila* orally 10-20 drops twice daily with warm milk for one month and *Padabhyanga* with the same at night for one month

Drop Outs: Nil

Statistical Test Used: Neuropathy total symptom score scale, Quantitative sensory tests including Monofilament, Biothesiometer, HCP sensitometer, Quality of life (Nottingham health profile) were used as parameters of assessment of response.

Symptoms were analysed by non-parametric Friedman test, Wilcoxon signed rank test (Paired t test), and between the groups assessment was done using non-parametric Mann-Whitney test.

Overall Result: Statistically significant results were observed in Hyperalgesia, *daha*, shooting pain.

Statistically non-significant results were seen in *Twak bheda* (aching pain), numbness, tingling.

No change observed in neurological assessment even though it was statistically significant. Statistically significant changes were brought in Quality of life parameters, however on comparison it was non-significant.

Conclusion Remarks: Patients receiving *Sapta avartita Guduchi taila* both orally and in the form of external application exhibited a significant improvement in the symptoms of diabetic peripheral neuropathy when compared to the other group.

Though significant improvement was observed in the symptoms, no changes were noticed in neurological assessment (Quantitative sensory testing).

Table 4. Kokane Deepti

Study ID: Kokane D *et al.*

Title: Ayurvedic management of Diabetic Polyneuropathy

Authors: Kokane DG, Subbanagowda PG, Joshi H

Settings: Dept. of Kayachikitsa, Ayurveda Mahavidyalaya, Hubli, Karnataka India

Year: 2008

Study Design: Clinical Trial

Methods

Objectives: To assess the efficacy of Ayurvedic formulations in the management of Diabetic Polyneuropathy

Sample Size: Male 14; Female 10; Total 24

Eligibility Criteria: Type II Diabetes with Diabetic Polyneuropathy

History of Diabetes type II less than 10 years

Good blood sugar maintenance

Preserved tendon reflexes in lower limbs

Randomization: Done, method not described

Adverse Effects: None described

Intervention

Sample size and Drug

Group A. 12 Patients : *Dashamoola Kwatha* 40 ml twice daily with Oral Hypoglycemic agents, *Sukhoshna jala anupana* for 21 days

Group B. 12 Patients : *Vasantakusumakara Rasa* 125 mg twice daily with Oral Hypoglycemic agents, *Godugdha anupana* for 21 days

Drop Outs: Nil

Statistical Test Used: Symmetrical burning sensation, tingling, pricking pain (pins & needles), pain and parasthesiae in lower limbs were graded and assessed using Students t test

Overall Result: 100% of group A showed good improvements. 58.3% , 33.3%, 8.33% in group B showed good, marked and moderate improvements Group A showed better results than group B

Conclusion Remarks: *Madhumeha* resembles and coincides with the disease Diabetes mellitus

Most of the symptoms mentioned under *Poorvarupa* and *Upadrava* of *Prameha* such as *Pada daha, pada suptata* and *shoola* are similar to diabetic polyneuropathy symptoms.

Diabetic polyneuropathy can be studied under *Madhumeha Upadrava*

Dashamoola Kwatha is very effective for diabetic polyneuropathy, it can also be managed with *Vasantakusumakara Rasa*

Good sugar control plays important role in diabetic polyneuropathy Diabetic polyneuropathy can be managed with Ayurvedic preparations

Table 5. Jaideep

Study ID: Jaideep *et al.*

Title: A clinical study to evaluate the effect of Ayurvedic formulation in patients of Diabetic Neuropathy

Authors: Jaideep, Mishra A, Mehra BL

Settings: Dept. of Kayachikitsa, RGG PG Ayurveda College, Paprola, Himachal Pradesh, India

Year: 2009 **Study Design:** Observational clinical study

Methods Objectives: To evaluate the efficacy of trial drug in the management of Diabetic Neuropathy.

To study its adverse effect (if any).

Sample size: Male 08; Female 15; Total 23

Eligibility Criteria: Patients who are already diagnosed as Diabetics.

Patients whose blood sugar level >120mg/dl for more than 3 weeks. Patients with symptoms of peripheral neuropathy such as Tingling sensation, Burning Sensation, Numbness, Pain.

Randomization: Not applicable

Adverse Effects: No side effects were seen

Intervention

Sample size and Drug

23 Patients : Each capsule (wt-575mg) containing *Dashmool* extract 500mg, *Pushkarmool* extract 50mg, *Hingu* 25mg (*Chakradatta, Vatavyadhi Chikitsa*).

Dose- One capsule twice a day for 6 weeks

Abhyanga with *Masha taila* for 15-20 min once daily

Drop Outs: 01

Statistical Test Used: Neuropathy signs were assessed with the help of Neuropathy kit- containing Buck reflex hammer to elicit deep tendon jerks, sterilised needle to check for pin sensitivity, tip therm to check for perception of cold sensation, test tube for adding hot water to check for perception of hot sensation and a vibration tuning fork of 128Hz to assess the vibration perception of the patients.

Assessment was done on graded symptoms and signs- *karapada daha*, tingling, numbness, *shoola*, gloves and stockings, thermal perception, vibration perception, pin sensitivity, deep tendon reflexes.

The results were analyzed using Students t test.

Overall Result: Highly significant ($p<0.001$) improvements in maximum assessment criteria except gloves and stockings, statistically highly significant results on blood sugar.

Conclusion Remarks: The response of the trial drug was found highly significant ($p<0.001$) in maximum of assessment criteria but there was no much improvement in feeling of gloves and stockings.

As the patients were taking Oral hypoglycaemic drugs so the effect on fasting blood sugar was found highly significant. The other routine baseline haematological and biochemical investigations were normal before the therapy and remained, normal after the treatment also, so they were not altered by therapy on these profiles.

Table 6. Vyasaraja Tantri A

Study ID: Tantri VA *et al.*

Title: A comparative study on the efficacy of *shamanaoushadhis* in the management of Peripheral and Proximal Diabetic Neuropathy

Authors: Tantri VA *et al.*

Settings: Dept. of Kayachikitsa, Govt. Ayurveda Medical College, Mysore, Karnataka, India

Year: 2011 **Study Design:** Pre & post test clinical study

Methods

Objectives: To evaluate the efficacy of *Kataka Khadiradi Kashaya* and *Gokshuradi Guggulu* internally & *Twak Lepa* externally in group A.To evaluate the efficacy of *Kataka Khadiradi Kashaya* and *Sahacharadi Kashaya* internally with *Moorchita Tila Taila* & *Ela Lepa* externally in group B

To compare and assess the efficacy of combined treatment in group A & B.

Sample Size: Male 31; Female 09; Total 40

Eligibility Criteria: NIDDM patients with signs and symptoms of peripheral & proximal neuropathy

Patients of either sex of age group : 25 – 75 years

Randomization: Done, method not described

Adverse Effects: Palatability of *Kataka Khadiradi Kashaya* was a reason for dropouts

Intervention

Sample size and Drug

Group A: 20 : Patients of both groups were given *kataka khadiradi kashaya* of 15 ml t.i.d. with hot water before food without disturbing hypoglycemic agents.

Two tablets of *gokshuradi guggulu* t.i.d. (each tab. of 500mg) with hot water internally after food and *Twak lepa* externally to the affected area

Duration- One month

Group B: 20 : Patients of both groups were given *kataka khadiradi kashaya* of 15 ml t.i.d. with hot water before food without disturbing hypoglycemic agents.

15 ml *sahacharadi* kashaya t.i.d with 5ml *moorchita tila taila* internally after food and *Ela lepa* externally to the affected area

Duration: One month **Drop Outs:** 13

Statistical Test Used: Graded signs and symptoms like pain and burning sensation, altered sensation, muscle weakness, sensory loss, FBS, PPBS. Physical examinations like muscle strength, reflexes, touch, vibration, temperature, position and pain sensation were assessed pre and post test. t test, Chi square test, repeated measure ANOVA were used for statistical analysis.

Overall Result: Group B was better than group A. Burning sensation is the only parameter which was reduced to highly significant level in group A

Conclusion Remarks: The specific references for the detailed concept of the Diabetic Peripheral and Proximal Neuropathy are not found in the Ayurvedic treatises.

The *Karapada Daha* and *Suptata* even though found in the *poorvaroopa* of *Madhumeha*, explanation of manifestation of *Vatavyadhi* in the improper treatment of *Vataja Prameha* by Acharya Vagbhata holds good to explain Diabetic neuropathy as a complication.

Diabetic Peripheral & proximal Neuropathy can be explained as *Madhumeha Vyadhi Karshana Janya Vatavyadhi*

The study was conducted to compare the effect of *Prameha chikitsa* and the *Prameha chikitsa* with *Vatavyadhi chikitsa*.

Both the groups have shown the highly significant result in the reduction of FBS and PPBS. But there was a comparatively more decrease in Group B.

The neuropathic symptoms were better cured by *Prameha Chikitsa* Added with the *Vatavyadhi chikitsa* (*Sahacharadi Kashaya* with *Moorchita tila taila*) than only *Prameha chikitsa*.

Table 7. Niranjan Y

Study ID: Niranjan Y *et al.*

Title: A clinical study on the management of Diabetic Polyneuropathy with *Dashamooladi Rasayana* Compound

Authors: Niranjan Y, Santwani MA, Baghel MS

Settings: Dept. of Kayachikitsa, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar

Year: 2011

Study Design: Open ended randomized controlled clinical study

Methods

Objectives: To review the etiopathogenesis of Diabetic Polyneuropathy in light of available classical literatures of Ayurveda

To assess the efficacy of Ayurvedic formulation-*Dashamooladi Rasayana* Compound in Diabetic Polyneuropathy.

Sample size: Male 36; Female 35; Total 69

Eligibility Criteria: Metabolically stable diabetic patients with symptomatic diabetic sensorimotor polyneuropathy

Patients of either sex between the age group of 35-70 years.

Patients with Diabetic Polyneuropathy in stage N3 of Dyck's staging[1]

Randomization: Computer generated randomization

Adverse Effects: No Adverse effects reported

Intervention

Sample size and Drug

Group A. 38 Patients : Tab. *Dashamooladi Rasayana* Compound- 3 tablets of 500mg twice daily after food with *Sukhoshna Jala*

Group B. 33 Patients : Cap. Pregabalin75mg + Methylcobalamin 750 mcg One capsule OD

Duration-8 weeks **Drop Outs:** 6

Statistical Test Used: Primary outcome measures: Changes in Neuropathy Symptom Score (NSS) and Michigan Neuropathy Screening Instrument (MNSI).

Secondary outcome measures: Changes in *Agni Bala*, *Deha Bala*, *Chetas Bala* , WHO Quality of Life (WHO QoL) BREF

The data generated in the clinical study was analyzed by applying student't' test using Statistical software-Sigmastat 3.5.

Overall Result: In Group A 33 (94.29%) subjects responded with Moderate positive response and 2 (5.71%) showed mild positive response. In Group B, 27 (90%) responded with moderate positive results and 3 (10%) showed mild positive response to the allocated treatment. Out of sample size of 65, 60 (92.31%) showed moderate positive response and 5 (7.69%) showed mild positive response.

Conclusion Remarks: Diabetic polyneuropathy is a complex multifactorial disorder with varied clinical features due to *Avarana* of *Vata*. It cannot be directly correlated to any predefined condition in Ayurveda as the nature of *Avarana* of *Vata* decides clinical presentation.

The alternate hypothesis is accepted as *Dashamooladi Rasayana* Compound radically improved both primary and secondary outcome measures.

Based on the results on diabetes profile; *Dashamooladi Rasayana* compound cannot be a standalone drug in diabetes with neuropathy, but an excellent adjuvant with anti diabetic drugs in the management of Diabetic Polyneuropathy. However it has some positive role on Diabetes as evident by the results on biochemical parameters. The hematological and biochemical investigations generate evidence that the trial drug is well tolerated and safe for long term use.

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EVALUATION OF THE EFFICACY AND SAFETY OF AYURVEDIC DRUG (*VACHA BRAHMI GHAN)* IN THE MANAGEMENT OF *MANODWEGA* (ANXIETY NEUROSIS)

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Abstract: An open randomized clinical trial was conducted to evaluate the efficacy of Ayurvedic drug (*Vacha Brahmi Ghan* tablets) in clinically diagnosed and confirmed 110 patients belonging to the age group of 20-60 yrs with the symptoms of *Manodwega* (Anxiety neurosis) like fear (*bhaya*), excessive sweating (*swedabahulayta*), indecisive (*asthairya*), palpitation (*hritkampa*), fatigue (*anavasthitachittata*), breathlessness (*swaskrichchhata*), trembling (*vepathu*), sleeplessness (*anidra*), unexplained stomach aches (*udarsula*), frequent headaches (*sirahsula*), nausea (*utklesha),* dryness in mouth (*mukhasushkata*), chest pain (*urahsula*) and giddiness (*bhrama*). The trial drug was administered in the dose of 500 mg thrice times in a day with lukewarm water for six weeks. Overall response at the end of the study was rated to be good in 9.18%, fair 70.40% and poor 16.32% of the patients. No adverse effects were reported. Statistically the result was determined significant ($p<0.001$).

Keywords: Ayurveda, Traditional medicine, *Acorus calamus*, *Bacopa monnieri*, Anxiety neurosis, *Manodwega,* Mental Health, *Manas roga.*

Introduction

Anxiety disorders are most common of all psychiatric disorders throughout the world.[1-3] Recent surveys have found that 18% of Americans may be affected by them.[4] The anxiety is of a greater degree than just every day worries and patients do mention that they are not able to control the worries. They are frequently accompanied with physical symptoms as well. These symptoms have to present for most days at least for several weeks at a time. The term of anxiety is used to describe the feelings of uncertainty, uneasiness and apprehension or tension that a person experiences in response to internal or external stimuli and can result in physical, emotional, cognitive and behavioural symptom.[5] The anxiety is an entirely psychological and physiological disorder and is characterized by somatic, cognitive, emotional and behavioural components to generate regular patterns of fear, worry, uneasiness and nervousness.

Anxiety neurosis, also entitled as generalised anxiety disorder is a neurotic disorder characterised by persistent excessive anxiety, which is not caused by organic brain disease or any other psychiatric disorder. The symptoms range from mild feelings of fatigue, apprehension and tension to more intense states of restlessness and irritability that may lead to aggressive acts. In extreme cases, the uncontrollable emotional discomfort is accompanied by physical reactions including tremors, sustained muscle tension, dyspnoea, hypertension and profuse perspiration. Other physical sign and symptoms include nausea, diarrhoea, frequency of micturation, insomnia, changes in appetite, all occurring without any underlying organic causes.[6]

The phenomenon of anxiety is not new; the description of anxiety disorders in classical literature of Ayurvedic medicine is available, in separate expressions as *Manodwega*, *Chittodwega* and *Atatwabhinivesha* under *Manas roga.*[7] As

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per Ancient scholars of Ayurveda, entity of this disease is due to predominance of *Rajoguna* and *Tamoguna* which is present in *Manovaha srotas* of the body.

Conventional treatments for anxiety in allopathic system of medicine indicated as fluoxetine, sertraline as first line agents. Benzodiazepines, such as alprazolam, clonazepam and diazepam are also sometimes indicated for short-term use. Antidepressants, such as monoamine oxidase inhibitors (MAOIs) like phenelzine and tranylcypromine are also considered for therapeutic management and are especially useful in resistant cases. As most of the allopathic drugs used for the management of anxiety disorders have one or more side effects like sedations and impaired psychomotor performance.[8] Hence need of alternatives and safe herbal Ayurvedic drugs is the need of time.

In Ayurvedic system of medicine, several drugs have been reported in *Manasroga* (psychiatric disorders). The Ayurvedic drug Vacha (*Acorus calamus* L.) Brahmi (*Bacopa monnieri* (L.) Pennal), Jatamansi (*Nardostachys jatamansi* DC.) etc. are used since long for *Manasroga* in day to day practices.[9]

Keeping in view the high prevalence ratio of *Manodwega* (anxiety neurosis) and the adverse effects of conventional allopathic treatment, it was intended to discover safer and effective treatment of *Manodwega* (anxiety neurosis). Thus this study was designed to evaluate the clinical efficacy and safety of *Vacha Brahmi Ghan* tablets in *Manodwega* (anxiety neurosis).

Material and Methods

Study design

An open**,** non comparative randomized clinical study was conducted at RA Poddar Central Research Institute for Ayurveda, Mumbai, India. The study protocol, case record forms (CRFs), regulatory clearance documents, product related information and informed consent form (in Marathi and English) were submitted to the Institutional ethics committee (IEC) and were approved by the same.

Inclusion criteria

The patients within age group of 12 to 60 yrs with symptoms of *Manodwega* (anxiety neurosis) like fear (*bhaya*), excessive sweating (*swedabahulayta*), indecisive (*asthairya*), palpitation (*hritkampa*), fatigueness (*anavasthitachittata*), breathlessness (*swaskrichchhata*), trembling (*vepathu*), sleeplessness (*anidra*), unexplained stomach aches (*udarsula*), frequent headaches (*sirahsula*), nausea (*utklesha)*, dryness in mouth (*mukhasushkata*), chest pain (*urahsula*) and giddiness (*bhrama*) were included in this study.

Exclusion criteria

Patients suffering from severe illness, which necessitated the use of other medications were excluded from the study. Subjects with other anxiety disorders (e.g. panic attacks, obsessive compulsive disorder, post traumatic anxiety and depression) were excluded. Patients of cardiac or respiratory disease, hypothyroidism and hyperthyroidism were also excluded. No lactating or pregnant women were included in the study.

Study procedures

All patients/parents/guardians were informed about the drug, its effects and duration of the study, patients' responsibilities and rights, importance of compliance, ethical aspects, and overall plan of the study. Informed written consent document was obtained from all the subjects and their parents/ guardians.

During the initial visit, a detailed medical history was obtained by interviewing the subjects, which was followed by a thorough clinical examination. Details of the clinical examination were recorded in the structured CRF. All the subjects were advised to take two tablets (each 250 mg) thrice daily with lukewarm water for 6 weeks.

Follow up and monitoring

All the subjects were followed up every two weeks for a period of 6 weeks. The efficacy was assessed on the basis of scoring in

improvement of clinical symptoms like fear (*bhaya*), excessive sweating (*swedabahulayta*), indecisive (*asthairya*), palpitation (*hritkampa*), fatigueness (*anavasthitachittata*), breathlessness (*swaskrichchhata*), trembling (*vepathu*), sleeplessness (*anidra*), unexplained stomach aches (*udarsula*), frequent headaches (*sirahsula*), nausea (*utklesha)*, dryness in mouth (*mukhasushkata*), chest pain (*urahsula*), and giddiness (*bhrama*) and other associated symptoms during each visit with the help of VAS score 0-10. At every visit the return tablet count was made to ascertain the compliance to the medication. The interpretation of results were done on the basis of response above 75%, 50% and 25% and less than 25% as Good, Fair, Poor and No Response respectively.

Adverse events

All adverse events, either reported or observed by subjects/parents/guardians, were recorded in the CRF with information about severity, onset, duration and action regarding the study drug.

The subjects were allowed to voluntarily withdraw from the study if they experienced serious discomfort during the study or sustained serious clinical problem requiring specific treatment.

Statistical analysis

The result were analyzed statistically using Student 't' test wherever applicable. The level of significance for all determination was calculated as per standard table of probability.[10]

Observation and Results

110 subjects were enrolled in the study amongst which 98 subjects completed the trial. The age group of the 98 subjects who had completed the trial was 12 to 60 years (Male 32.65% and Female 67.34%). The duration of the disease was 02 to 60 months.

Results of the study showed a significant decrease in the mean score for fear (*bhaya*) 55.72%, excessive sweating (*swedabahulayta*) 46.31%, indecisive (*asthairya*) 55.85%, palpitation (*hritkampa*) 56.03%, fatigueness (*anavasthitachittata*) 57.73%, breathlessness (*swaskrichchhata*) 48.43%, trembling (*vepathu*) 58.18%, sleeplessness (*anidra*) 44.53%, unexplained stomach aches (*udarsula*) 52.83% and frequent headaches (*sirahsula*) 55.81% at the end of 6 weeks, when compared to their respective baseline values **(Table 1, Figure 1).**

The result also showed a highly significant reduction in the nausea (*utklesha)* 80%, dryness in mouth (*mukhasushkata*) 65.51%, chest pain (*urahsula*) 69.76%, giddiness (*bhrama*) 64.36% at the end of 6 weeks when compared to their respective baseline values **(Table 1, Figure 1).**

No clinically significant adverse reactions were reported or observed during the entire study period.

Overall efficacy and tolerability

In this study a good response was observed in nine subjects (9.18%), fair response in sixty nine subjects (70.40%), poor response in sixteen subjects (16.32%) while four patients reported no response **(Table 2, Figure 2).** All the ninety eight patients who completed the trial reported a very good tolerability to the treatment and not reported any side effects.

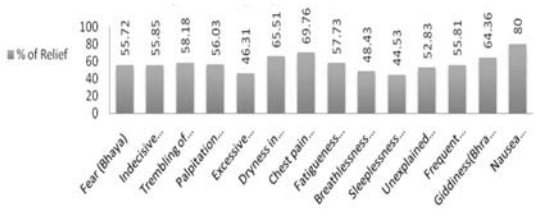
Discussion

The aim of the present study was to re-evaluate and promote an effective and safe drug (*Vacha brahmi ghan* tablet) for *Manodwega* (anxiety neurosis) and to ascertain its anxiolytic effect. In this open, non comparative, randomized clinical study, the effect of the drug was monitored on the 98 subjects of *Manodwega* (anxiety neurosis). All the subjects completed the study. The trial drug had significant ($p<0.001$) symptomatic results. It is well tolerated; no significant adverse effect was reported during and after the treatment.

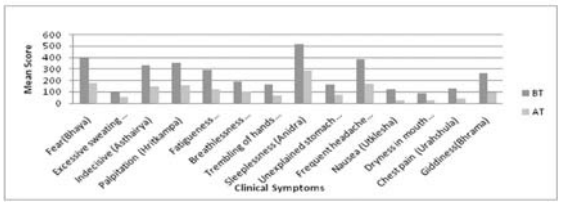
Conventional treatments for anxiety in allopathic system of medicine indicated as fluoxetine, sertraline as first line agents.

Table 1. Effect of the trial drug on clinical sign and symptoms (n=98)

Clinical symptoms	Mean Score			Percentage of Relief (%)
	B.T.	A.T.	Difference	
Fear (*Bhaya*)	393	174	219	55.72
Excessive sweating (*Swedabahulya*)	95	51	44	46.31
Indecisive (*Asthairya*)	333	147	186	55.85
Palpitation (*Hritkampa*)	348	153	195	56.03
Fatigueness (*Anavasthita chittata*)	291	123	168	57.73
Breathlessness (*Swasakrichchhata*)	192	99	93	48.43
Trembling of hands (*Vepathu*)	165	69	96	58.18
Sleeplessness (*Anidra*)	512	284	228	44.53
Unexplained stomach aches (*Udara sula*)	159	75	84	52.83
Frequent headache (*Sirahsula*)	387	171	216	55.81
Nausea (*Utklesha*)	120	24	96	80.00
Dryness in mouth (*Mukhasushkata*)	87	30	57	65.51
Chest pain (*Urahshula*)	129	39	90	69.76
Giddiness(*Bhrama*)	261	93	168	64.36



Percentage of relief of the trial drug on Clinical sign and symptoms



Effect of trial drug on mean score of Clinical symptoms

Figure 1.

Table 2. Overall response of the drug on *Manodwega* (Anxiety neurosis) subjects.

Result	No. of patients (n=98)	Percentage (%)
Good response	09	09.18
Fair response	69	70.40
Poor response	16	16.32
No response	04	04.08

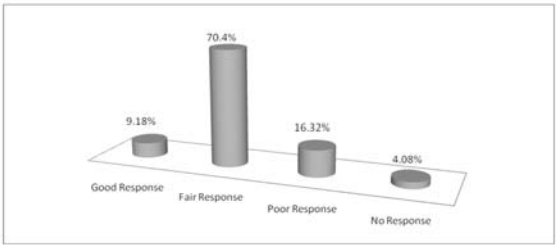


Figure 2. Results of the treatment

Table 3. Statistically overall clinical improvements on the treated patients (n=98).

Mean BT	Mean AT	Difference	% of relief	S.D.	S.E.	't' value	'p' value	Result
35.42	15.63	19.79	55.87	0.886	0.23	0.025	<0.001	S

BT= Before treatment; AT= After treatment; S.D.= Standard deviation; S.E.= Standard error; t= test of significance; p= probability; S= Significant

Benzodiazepines, such as alprazolam, clonazepam and diazepam are also sometimes indicated for short-term use. Antidepressants, such as monoamine oxidase inhibitors (MAOIs) like phenelzine and tranylcypromine are also considered as effective drugs and are especially useful in drugs resistant cases.[11] All the categories of allopathic drugs are associated with certain adverse effects like sedation, impaired psychomotor performance and thus there is a strong demand for safe herbal drugs.

Studies had also indicated that anxiety disorders are more likely among those with family history of anxiety disorders, especially certain types.[12]

The incidences of *Manodwega* (anxiety neurosis) were more in females than the males and *pittakaphaj prakriti* persons were mostly affected in our study. The percentage of relief in clinical sign and symptoms like nausea *(utklesha)* 80%, giddiness (*bhrama*) 64.36%, palpitation *(hritkampa)* 56.03%, fear (*bhaya*) 55.72%, indecisive (*asthairya*) 55.85% and sleeplessness (*anidra*) 44.53% etc. were reduced after completing the study **(Table 1, Figure 1).** The physiological changes were also noticed in all the treated patients in terms of their systolic and diastolic blood pressure, pulse rate and body weight. The over all response of the drug was good (9.18%), fair (70.40%) **(Table 2, Figure 2).** Statistically the result was significant **(Table 3).**

The ingredients of the trial drug Vacha (*Acorus calamus* L.) and Brahmi (*Bacopa monnieri* (L.)Pennal.), are *medhya* (intellectual enhancer) drugs. In properties, both the drugs are *tikta* (bitter), *katu* (pungent) in *rasa* (taste); *laghu* (light), *ruksha* (dryness), *teekshna* (sharpness) in *guna* (properties); *ushna* (hot) in *veerya* (potency); *katu* (pungent) in *vipaka* (post digestion metabolic state) and *medhya* (intellectual promoter) as *prabhava* (a specific action distinct to that particular substance). These are *kaphavatshamaka* in *doshaghnata*.[13-15] Earlier pharmacological studies reported that the Brahmi (*Bacopa monnieri* (L.) Pennel) and Vacha (*Acorus calamus* L.) as anti anxiety agents having adaptogenic effects.[16]

Earlier studies on *Acorus calamus* also shows that active alkaloids asarone and β-asarone give effects on central nervous system of the body.[17] **Singh HK (1997)** also reflecting the effects of *brahmi* as neuropsycopharmacological action.[18] **Sharma** *et al.,* also evaluated the effect of *vacadi churna* in the management of depressive illness.[19]

On the basis of Ayurvedic pharmacology both the drugs Vacha and Brahmi[20-21] are pacifying vitiated *kapha* and *vata dosha* and bring it back to normalised state.[22] These drugs are breakdown the *avarana* of *rajoguna* and *tamoguna* which are present in *manovaha srotansi* due to their *ushna* and *teekshna guna* and these drugs also increase the level of *satva guna*. At the same time these are nourishing the *sadhaka pitta*. Simultaneously these drugs correct the mental weakness (*manodaurbalyaavstha*) and nourish the *Mana* (brain) by their *medhya* effect.[23] Thus the patient attain's the feeling of happiness. Concurrently these drugs clean the micro channels of the body (*srotasa*) that thus the level of *Agni* (digestive capacity) is increased which activates the *dhatuposhana karma* and normal physiological state of the body and mind.

Conclusion

It is concluded that trial drug *Vacha Brahmi Ghan* tablets holds potent anxiolytic activity and is safe and valuable drug for the treatment of *Manodwega* (Anxiety neurosis). However, further research work is required to make out the exact mechanism of action.

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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF URSOLIC ACID IN *Ocimum sanctum* LINN IN HUMAN PLASMA

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Abstract: *Ocimum sanctum* (Holy basil), belonging to Lamiaceae family, is a herb native to India. It is regarded as one of the most important plant used in Ayurvedic medicine which constitute ursolic acid (3-â-hydroxyurs-12-en-oic acid), a pentacyclic triterpene acid that is used in cosmetics and also capable of inhibiting various types of cancer cells. The current study describes the development and validation of a simple and rapid reverse phase HPLC assay method for estimation of Ursolic acid in *Ocimum sanctum* herb powders and extract in human plasma. A HPLC system comprising of RP-C_{18} column (250 x 4.6 mm I.D., 5 micrometer (im) particle size) with solvent system consisting of acetonitrile and water (90:10, v/v) in isocratic mode using diethyl ether-hexane (8:2, v/v) as extracting solvent system and detection at 210 nanometer (nm) using PDA detector. The proposed method was validated for its LOD, LOQ, linearity, accuracy, precision, sensitivity, system suitability and stability. The proposed method was found to be linear in the range of 2 – 100 microgram (ig)/ml with correlation coefficient of 0.9999. The LOD was 1ig/ml and LOQ was 5 ig/ml. The developed HPLC method was found to be precise, sensitive, accurate and reproducible and may be used for qualitative as well as quantitative estimation of ursolic acid in *Ocimum sanctum* herb powders and extracts in human plasma.

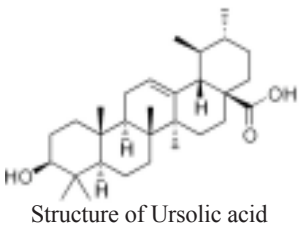
Keywords: Medicinal Plants, *Ocimum sanctum*, Ursolic acid, Human plasma, RP-HPLC.

Introduction

Ocimum sanctum Linn. (Family Lamiaceae) commonly known as Holy basil, is widely used in Indian system of medicine. The medical plants are widely used by the traditional medical practitioners for curing various diseases in their day to day by practice[1]. Traditionally *Ocimum sanctum* is used in malarial fevers, gastric disorders and in hepatic infections. The leaves of the plant have been used as an expectorant, diaphoretic, antioxidant, anti-ulcer, anthelminthic, antiseptic, analgesic and tonic rejuvenator[2].

Ocimum sanctum leaves are also used in bronchial asthma ringworm and other cutaneous diseases and earache[3]. The Indian holy basil also possesses Radio protective, anticarcinogenic and antioxidant properties[4]. *Ocimum sanctum* L. fixed oil has hypotensive, anti-inflammatory, anticoagulant and immune-modulatory activities[5]. Essential oil of Holy basil leaves has been shown to possess antifungal and antibacterial properties[6].

Ursolic acid (also known as urson, prunol and malol) is a pentacyclic triterpenoid compound found in large no. of vegetarian fruits and medicinal herbs, presenting several important biological activities. These include anti-inflammatory,



Structure of Ursolic acid

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antitussive, antitumoral and antistress property[7-10]. *In vitro* study of Ursolic acid has shown that it is about twice as anti-inflammatory compared to Indomethacine. Topical application of 1 or 2 mmol of Ursolic acid along with 5 mmol of TPA for 20 weeks inhibited 45 or 61 % of skin tumors respectively[9]. Other relevant activities such as anti-diabetic[11], antioxidant[12] and anticancer[13, 14] properties are attributed to the presence of Ursolic acid in many plants.[13]

The variation in Ursolic acid in eight species of the genus Ocimum: O.americanum, O.basilicum, O.basilicum var. purpurascens, O.basilicum var. minimum, O. grattissimum, and O.micranthum, O.selloi and O. tenuiflorum grown in Northeast of Brazil was studied by HPLC[15].

HPTLC method has been used for the quantification of the ursolic acid in black (*Krishna Tulasi*) and green (*Sri Tulasi*) varieties of *Ocimum sanctum* Linn.[16] Also LC MS method has been studied for the analysis of Ursolic acid in rat plasma in *Sambucus chinesis*[17]. Also HPLC method has been studied for the analysis of bioactive triterpenes in *Perilla frutescens.*[18]

No HPLC method was developed for determination of ursolic acid in *Ocimum sanctum* herb powders and extracts in human plasma. Thus a rapid and validated method based on HPLC has been developed for quantitative determination of the compound ursolic acid in the leaf extract of *Ocimum sanctum* Linn in human plasma.

Materials and Methods

The herbal extract was prepared by Pharmanza Herbal Pvt. Ltd.; standard was from Chromadex, USA. Blood plasma was from K.E.M. Hospital, Mumbai, India. Methanol and acetonitrile were from Rankem, New Delhi, India. All the other reagents were of analytical grade. Distilled water, prepared from demineralised water, was used throughout the study. All dilutions were performed in standard volumetric flasks.

Chromatographic separation was performed on Shimadzu High Performance Liquid Chromatography equipped with IC-20AT liquid chromatographic pump equipped with HPLC binary gradient with Auto sampler and photodiode detector.

Preparation of mobile phase and stock solution

The mobile phase comprising a mixture of acetonitrile and water (90:10, v/v) was prepared.

The standard solution of Ursolic acid (5000 µg/ml) was prepared by dissolving 50 mg Ursolic acid and diluting to 10 ml with methanol in a standard volumetric flask. Further serial dilutions were given to the above stock solution to prepare the concentrations of 2500ìg/ml, 1000ìg/ml, 500ìg/ml, 100ìg/ml and 50ìg/ml in methanol as follows.

Chromatographic conditions

A Phenomenex C_{18} column (250 ´ 4.6 mm, 5 µm) equilibrated with mobile phase acetonitrile and water (90:10, v/v) was delivered at a flow rate of 1.0 ml/min and efûuents were monitored at 210 nm. The sample was injected using a 20 µl ûxed loop, and the total run time was 10 min. A typical chromatogram for determination of Ursolic acid is shown in **Figure 1.**

Calibration curve for URSOLIC ACID in plasma

Appropriate aliquots of stock solution were spiked in different 10 ml volumetric flasks containing 1 ml of drug free human plasma to obtain final concentrations of 5, 10, 20, 40, 60, 100 µg/ml. Three Quality Control samples were prepared by spiking from above stock solution in 3 different 10 ml volumetric flasks containing 1 ml of drug free human plasma to prepare 15, 50 and 70 µg/ml labeled as LQC, MQC and HQC respectively.

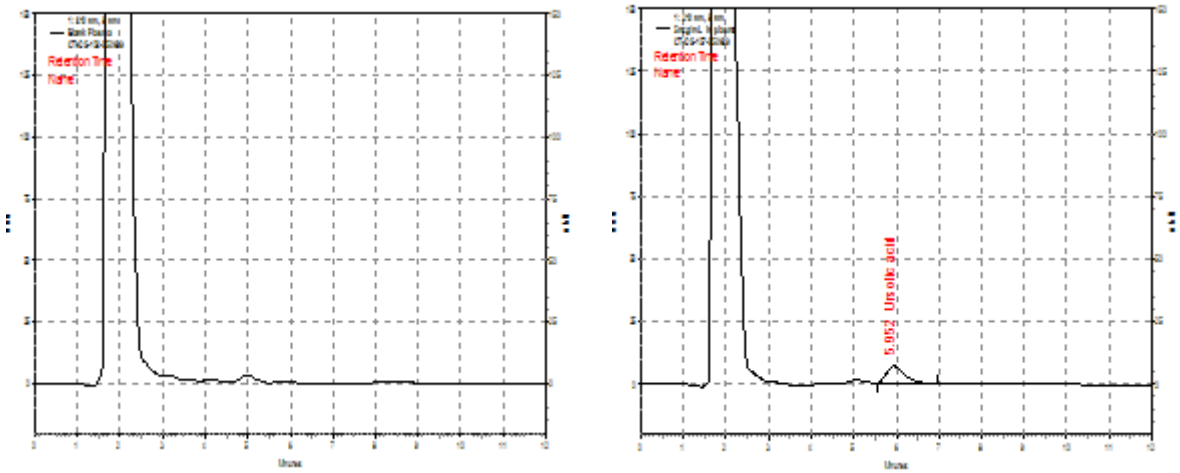

Figure. 1. (A) Chromatogram of blank solution (B) Chromatogram of spiked sample solution

Preparation of plant extract

Ocimum Sanctum raw material was collected and cleaned properly. Then it was extracted with sufficient amount of specific solvent (Ethanol) for 2 hrs under reflux on heating mental by adjusting temperature 40oC from the beginning.

After 2 hrs allowed it to cool and filtered through filter cloth (cycle 1).

The remaining residue was again extracted with the same solvent by repeating the same procedure for second and third cycle. The residue of third cycle was again extracted with water instead of solvent at same conditions.

All the filtrates were combined and concentrated on rotary vaccum evaporator by adjusting the temperature 80-90°c. Mixed all extracts of cycle 1, 2 and 3 and 10% Malto dextrin as an Excipient was added to it.

Vaccum dried it to get it in powder form. Spray dried the water extract - cycle 4 separately to get powder.

Pressure: 600mmHg and temperature: 60°c.

Sample Preparation

Weigh accurately about 250 mg of alcoholic extract of *Ocimum sanctum* (2.5% *Ursolic acid* extract) in a 50-mL volumetric flask, dissolved in methanol and filtered through 0.25 ì filter paper and 50 ìL was spiked in 1 ml drug free human plasma to prepare 6.125 ìg/ml solution. **(Figure 3).**

Procedure for sample and standard cleanup

Liquid-liquid extraction method was used. One ml of drug free human plasma was taken in tubes. Then 2ml of methanol was added to it. This mixture was vortexing for 1min. Then it was extracted with mixture of 10 ml of diethyl ether-hexane (8:2, v/v).The resulting mixture was shaken mechanically for 3min.

After centrifugation for 10 min, the supernatant was transferred to a clean test tube and evaporated to dryness in a water bath at 40°c under a stream of nitrogen. The residue was reconstituted in 0.5ml methanol with vortexing for 60 seconds. This solution was injected into the separation system.

All the calibration and Quality control samples were cleaned by above method and then injected into the separation system.

Calibration curve was constructed by plotting average peak area versus concentrations and regression equation was computed for Ursolic acid. A representative calibration curve of Ursolic acid from human plasma is depicted in **figure 2.**

Results and Discussion

Method Validation

Specificity

There was no peak found in the blank plasma solution at the retention time of standard Ursolic acid solution in plasma.

System suitability

Standard Ursolic acid of 20 µg/ml conc. was injected 5 times on the same day one by one. Mean ± SD., %CV for the area was found to be 793016 ± 4389.73, 0.55; for retention time it was found to be 6.0202 ± 0.0590356, 0.98.

Linearity

Linearity was evaluated by determining seven different concentrations of the standard solutions of Ursolic acid. The peak area ratio and concentration of standard was subjected to regression analysis to calculate the calibration equation and correlation coefficients.

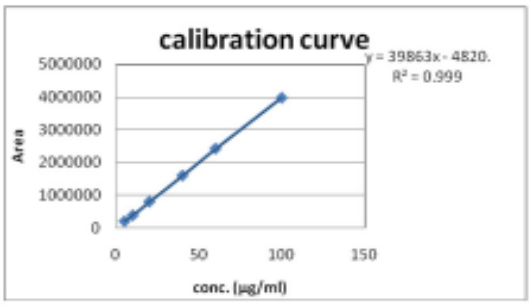

Figure 2. Calibration curve for ursolic acid from blank plasma

The regression equations obtained for the standard solution of Ursolic acid was Y= 39798X-389.48 (R^2 = 0.9999). The linearity range was individually from 5 - 100 µg/ml. The results show that within the concentration range indicated, there was an excellent correlation between peak area ratio and concentration. Results of linearity are given in **Table 1. (Fig. 2)**

Limit of Detection and Limit of Quantitation ??

LOD and LOQ were calculated from the RSD and slope (S) of the calibration curves using equations, LOD = 3.3 × (RSD/S) and LOQ = 10 × (RSD/S) [18]. The LOD and LOQ of Ursolic acid were found to be 1 µg/ml and 5 µg/ml respectively.

Accuracy and Precision

The accuracy was expressed as the percentage of analytes recovered by the assay (% Nominal) and Precision was expressed as percent variation (%CV). Mean recoveries for Ursolic acid are shown in **Table 1.** The within series results and between series result at 15, 50, 70 (LQC, MQC and HQC samples respectively), 5 and 100 µg/ml indicate good accuracy and precision of the method for the determination of the Ursolic acid by mean recovery data. Results are tabulated in **Table 1**.

Table 1. Summary of the validation parameter

Parameters	Observations
Specificity	No interference was found
Linearity Regression equation Correlation coefficient (r^2)	5 to 100 µg/ml Y= 39863x - 4820 0.9999
Accuracy (% Recovery)	98.87 to 100.15 %
Precision (n=3) (% CV) Within series Between series	 0.43 to 1.55 % 0.36 to 1.30 %
LOD	1 µg/ml
LOQ	5 µg/ml
Extraction efficiency	73.61 to 74.45%.

Percentage extraction yield

The percent extraction efficiency was performed for Quality control samples. For one sample 2 test tubes were taken. In one test tube required volume of standard solution was spiked to 1ml of blank plasma and sample was extracted as per the developed extraction procedure. This sample was labeled as extracted. While in another test tube 1ml plasma was processed as per the extraction procedure and required volume of standard solution was added at reconstitution

stage. This sample was labeled as unextracted. Both samples were injected in to chromatographic system. A result of extraction yield was also tabulated in **table 1.**

Stability Experiments

Stock Solution Stability

The stock solution stability was evaluated by comparing the peak area values of stability samples versus peak area values of fresh samples (100µg/ml). The fresh sample was immediately injected and stability sample was kept at room temperature for period of 6 hrs for short term stock solution stability and kept at 4°c for period of 7 and 14 days for long term solution stability and then injected under same chromatographic conditions.

The stability of stock solution was determined by monitoring the peak area ratio of fresh solutions of the standard Ursolic acid and stability sample. Samples were considered to be stable if retention times and peak area ratios of Ursolic acid remain almost unchanged and no significant degradation is observed within the

Table 2. Short term stock solution stability for ursolic acid

Observation Number	Std. 100µg/ml	
	Fresh 0 hrs	Stability 6 hrs
1	5341026	5348726
2	5349072	5349152
3	5351641	5350100
Mean	5347246.33	5349326
S.D.	5537.99	703.33
%CV	0.10	0.01
%Difference	0.04	

Table 3. Long Term Stock Solution Stability for Ursolic acid

Observation Number	Standard. 100µg/ml			
	Fresh	Stability 7 days	Fresh	Stability 14 days
1	5348026	5349453	5348200	5349444
2	5349072	5350984	5349587	5350896
3	5351641	5351698	5349756	5350521
Mean	5349580	5350711.7	5349181	5350287
S.D.	1860.20	1147.01	853.76	753.75
%CV	0.03	0.02	0.02	0.014
% Difference	0.021		0.02	

Table 4. Bench Top Stability for Ursolic acid

Observation Number	L.Q.C. (15µg/ml)		M.Q.C. (50µg/ml)		H.Q.C. (70µg/ml)	
	Initial	Stability	Initial	Stability	Initial	Stability
	0.0 hrs	**6.00 hrs.**	**0.0 hrs**	**6.00 hrs.**	**0.0 hrs**	**6.00 hrs.**
1	14.99	15.33	50.06	49.85	69.17	70.29
2	15.39	15.12	49.98	49.34	70.41	70.46
3	14.98	15.16	49.77	49.81	70.34	70.93
Mean	15.12	15.20	49.94	49.67	69.97	70.56
S.D.	0.23	0.11	0.150	0.28	0.70	0.33
%CV	1.55	0.73	0.30	0.57	1.00	0.47
% Nominal	100.8	101.33	99.88	99.34	99.96	100.8
%Difference	0.53		-0.54		0.84	

Table 5. Freeze Thaw Cycle for Ursolic acid

Observation Number	L.Q.C. (15µg/ml)		M.Q.C. (50µg/ml)		H.Q.C. (70µg/ml)	
	Initial	Stability	Initial	Stability	Initial	Stability
	0.0 hrs	**72.0 hrs**	**0.0 hrs**	**72.0 hrs**	**0.0 hrs**	**72.0 hrs**
1	15.39	14.95	51.26	50.27	70.91	69.36
2	15.34	15.36	51.07	49.86	70.15	70.3
3	15.03	14.96	51.31	50.08	70.52	69.24
Mean	15.25	15.09	51.21	50.07	70.53	69.63
S.D.	0.20	0.23	0.12	0.21	0.38	0.58
%CV	1.28	1.55	0.24	0.41	0.54	0.83
%Nominal	101.66	100.6	102.42	100.14	100.75	99.47
%Difference	-1.04		-0.99		-1.27	

Table 6. Post Extraction Stability (Auto sampler) for Ursolic acid

Observation Number	L.Q.C. (15µg/ml)			M.Q.C. (50µg/ml)			H.Q.C. (70µg/ml)		
	Initial	Stability		Initial	Stability		Initial	Stability	
	0.0 hrs	**6 hrs**	**18 hrs**	**0.0 hrs**	**6hrs**	**18 hrs**	**0.0 hrs**	**6 hrs**	**18 hrs**
1	15.02	14.97	15.15	49.47	49.68	49.48	69.94	69.09	70.01
2	15.09	14.87	14.69	50.47	50.73	50.19	68.76	68.36	69.59
3	15.06	15.12	15.18	50.33	50.5	50.22	69.42	68.11	68.14
Mean	15.06	14.99	15.01	50.09	50.3	49.96	69.37	68.52	69.25
S.D.	0.04	0.13	0.27	0.54	0.55	0.42	0.6	0.51	0.98
%CV	0.23	0.84	1.83	1.08	1.09	0.84	0.86	0.74	1.42
%Nominal	100.4	99.93	100.06	100.18	100.6	99.92	99.1	97.88	98.92
%Difference	-0.46		-0.33	0.41		-0.25	-1.22		-0.17

given period. Stock solution stability results are tabulated in **Table 2, 3** and **4**.

Freeze thaw cycle stability

Spiked samples of three concentrations (15, 50, and 70 µg/mL) were assessed after storage at room temperature for 6 hrs (the fresh samples were immediately injected whereas the stability samples were kept at room temperature for 6 hrs and then extracted and injected – bench top stability) and after three freeze-thaw cycles [storage at -20°c ± 5°c for 3 days (samples were thawed at room temperature and analyzed at every 24 hrs)]. Results are tabulated in **table 5**.

Table 7. Summary of results of stability experiments.

Stability experiments	% Difference
Stock solution stability	
Short term stability	0.04 (at 6 hrs)
Long term stability	0.021 (for 7 days) and (0.02 for 14 days)
Bench top stability	
LQC (15 µg/mL)	0.53
MQC (50 µg/mL)	0.54
HQC (70 µg/mL)	0.845
Freeze thaw cycle stability	
LQC (15 µg/mL)	-1.04
MQC (50 µg/mL)	-0.99
HQC (70 µg/mL)	-1.27
Post extraction stability	
LQC (15 µg/mL)	-0.46 at 6hrs and .33 at 18 hrs
MQC (50 µg/mL)	0.41 at 6hrs and -0.25 at 18 hrs
HQC (70 µg/mL)	-1.22 at 6hrs and -0.17 at 18 hrs

Stability of standard under Auto sampler (Post Extraction Stability)

Post Extraction Stability (Auto sampler maintained at 4°c) for Ursolic acid in plasma was evaluated by comparing triplicate injections of stability versus fresh samples of LQC, MQC and HQC (15, 50, and 70 µg/mL). Post extraction fresh samples were immediately injected and the stability samples were the samples re-injected after 6 and 18 hrs at the temperature mentioned above. The QC concentrations of the fresh and stability samples were determined from the

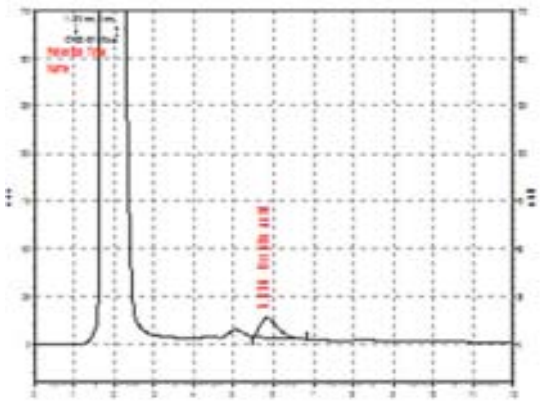

Figure 3. Chromatogram of *Ocimum sanctum* extract in Human plasma.

linearity that was assayed on the same day along with QC samples. Stability conditions were within the acceptance criterion recommended by the Food and Drug Administration (U.S. FDA). Results for the fresh sample and stability samples were given in **Table 6.**

Sample Preparation

100 ìl of Sample of *Ocimum sanctum* extract (2.5% *Ursolic acid* extract) in blood plasma prepared above was injected in Liquid Chromatographic system.**(Figure 3).**

Conclusion

This method as standardized and validated is simple, reproducible, sensitive and specific for the determination of Ursolic acid from *Ocimum sanctum* extract in human plasma. Hence, this method can be applied for the estimation of the Ursolic acid from *Ocimum sanctum* extract in human plasma.

Summary

This manuscript will describe the method development for estimation of Ursolic acid in *Ocimum sanctum* extract in human plasma.

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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF URSOLIC ACID IN *Ocimum sanctum* LINN IN HUMAN PLASMA

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Abstract: *Ocimum sanctum* (Holy basil), belonging to Lamiaceae family, is a herb native to India. It is regarded as one of the most important plant used in Ayurvedic medicine which constitute ursolic acid (3-â-hydroxyurs-12-en-oic acid), a pentacyclic triterpene acid that is used in cosmetics and also capable of inhibiting various types of cancer cells. The current study describes the development and validation of a simple and rapid reverse phase HPLC assay method for estimation of Ursolic acid in *Ocimum sanctum* herb powders and extract in human plasma. A HPLC system comprising of RP-C_{18} column (250 x 4.6 mm I.D., 5 micrometer (im) particle size) with solvent system consisting of acetonitrile and water (90:10, v/v) in isocratic mode using diethyl ether-hexane (8:2, v/v) as extracting solvent system and detection at 210 nanometer (nm) using PDA detector. The proposed method was validated for its LOD, LOQ, linearity, accuracy, precision, sensitivity, system suitability and stability. The proposed method was found to be linear in the range of 2 – 100 microgram (ig)/ml with correlation coefficient of 0.9999. The LOD was 1ig/ml and LOQ was 5 ig/ml. The developed HPLC method was found to be precise, sensitive, accurate and reproducible and may be used for qualitative as well as quantitative estimation of ursolic acid in *Ocimum sanctum* herb powders and extracts in human plasma.

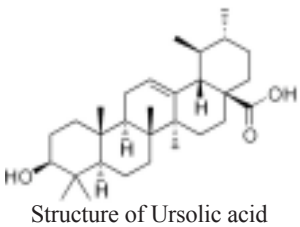
Keywords: Medicinal Plants, *Ocimum sanctum*, Ursolic acid, Human plasma, RP-HPLC.

Introduction

Ocimum sanctum Linn. (Family Lamiaceae) commonly known as Holy basil, is widely used in Indian system of medicine. The medical plants are widely used by the traditional medical practitioners for curing various diseases in their day to day by practice[1]. Traditionally *Ocimum sanctum* is used in malarial fevers, gastric disorders and in hepatic infections. The leaves of the plant have been used as an expectorant, diaphoretic, antioxidant, anti-ulcer, anthelminthic, antiseptic, analgesic and tonic rejuvenator[2].

Ocimum sanctum leaves are also used in bronchial asthma ringworm and other cutaneous diseases and earache[3]. The Indian holy basil also possesses Radio protective, anticarcinogenic and antioxidant properties[4]. *Ocimum sanctum* L. fixed oil has hypotensive, anti-inflammatory, anticoagulant and immune-modulatory activities[5]. Essential oil of Holy basil leaves has been shown to possess antifungal and antibacterial properties[6].

Ursolic acid (also known as urson, prunol and malol) is a pentacyclic triterpenoid compound found in large no. of vegetarian fruits and medicinal herbs, presenting several important biological activities. These include anti-inflammatory,



Structure of Ursolic acid

1. Asst. Professor 2. Asst. Professor 3. Asst. Professor 4. QC Officer 5. Asst. Professor

antitussive, antitumoral and antistress property[7-10]. *In vitro* study of Ursolic acid has shown that it is about twice as anti-inflammatory compared to Indomethacine. Topical application of 1 or 2 mmol of Ursolic acid along with 5 mmol of TPA for 20 weeks inhibited 45 or 61 % of skin tumors respectively[9]. Other relevant activities such as anti-diabetic[11], antioxidant[12] and anticancer[13, 14] properties are attributed to the presence of Ursolic acid in many plants.[13]

The variation in Ursolic acid in eight species of the genus Ocimum: O.americanum, O.basilicum, O.basilicum var. purpurascens, O.basilicum var. minimum, O. grattissimum, and O.micranthum, O.selloi and O. tenuiflorum grown in Northeast of Brazil was studied by HPLC[15].

HPTLC method has been used for the quantification of the ursolic acid in black (*Krishna Tulasi*) and green (*Sri Tulasi*) varieties of *Ocimum sanctum* Linn.[16] Also LC MS method has been studied for the analysis of Ursolic acid in rat plasma in *Sambucus chinesis*[17]. Also HPLC method has been studied for the analysis of bioactive triterpenes in *Perilla frutescens.*[18]

No HPLC method was developed for determination of ursolic acid in *Ocimum sanctum* herb powders and extracts in human plasma. Thus a rapid and validated method based on HPLC has been developed for quantitative determination of the compound ursolic acid in the leaf extract of *Ocimum sanctum* Linn in human plasma.

Materials and Methods

The herbal extract was prepared by Pharmanza Herbal Pvt. Ltd.; standard was from Chromadex, USA. Blood plasma was from K.E.M. Hospital, Mumbai, India. Methanol and acetonitrile were from Rankem, New Delhi, India. All the other reagents were of analytical grade. Distilled water, prepared from demineralised water, was used throughout the study. All dilutions were performed in standard volumetric flasks.

Chromatographic separation was performed on Shimadzu High Performance Liquid Chromatography equipped with IC-20AT liquid chromatographic pump equipped with HPLC binary gradient with Auto sampler and photodiode detector.

Preparation of mobile phase and stock solution

The mobile phase comprising a mixture of acetonitrile and water (90:10, v/v) was prepared.

The standard solution of Ursolic acid (5000 µg/ml) was prepared by dissolving 50 mg Ursolic acid and diluting to 10 ml with methanol in a standard volumetric flask. Further serial dilutions were given to the above stock solution to prepare the concentrations of 2500ìg/ml, 1000ìg/ml, 500ìg/ml, 100ìg/ml and 50ìg/ml in methanol as follows.

Chromatographic conditions

A Phenomenex C_{18} column (250 ´ 4.6 mm, 5 µm) equilibrated with mobile phase acetonitrile and water (90:10, v/v) was delivered at a flow rate of 1.0 ml/min and efûuents were monitored at 210 nm. The sample was injected using a 20 µl ûxed loop, and the total run time was 10 min. A typical chromatogram for determination of Ursolic acid is shown in **Figure 1.**

Calibration curve for URSOLIC ACID in plasma

Appropriate aliquots of stock solution were spiked in different 10 ml volumetric flasks containing 1 ml of drug free human plasma to obtain final concentrations of 5, 10, 20, 40, 60, 100 µg/ml. Three Quality Control samples were prepared by spiking from above stock solution in 3 different 10 ml volumetric flasks containing 1 ml of drug free human plasma to prepare 15, 50 and 70 µg/ml labeled as LQC, MQC and HQC respectively.

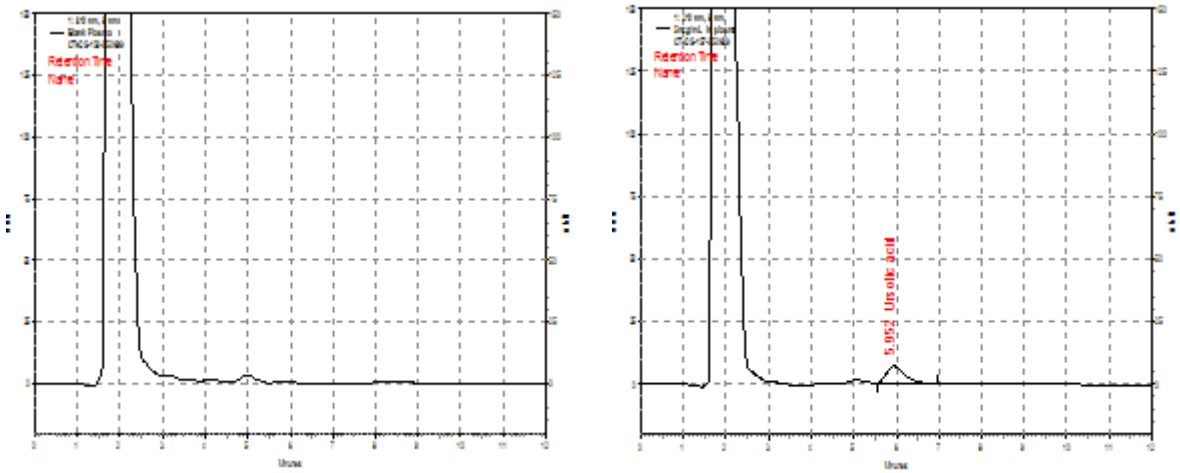


Figure. 1. (A) Chromatogram of blank solution (B) Chromatogram of spiked sample solution

Preparation of plant extract

Ocimum Sanctum raw material was collected and cleaned properly. Then it was extracted with sufficient amount of specific solvent (Ethanol) for 2 hrs under reflux on heating mental by adjusting temperature 40oC from the beginning.

After 2 hrs allowed it to cool and filtered through filter cloth (cycle 1).

The remaining residue was again extracted with the same solvent by repeating the same procedure for second and third cycle. The residue of third cycle was again extracted with water instead of solvent at same conditions.

All the filtrates were combined and concentrated on rotary vaccum evaporator by adjusting the temperature 80-90°c. Mixed all extracts of cycle 1, 2 and 3 and 10% Malto dextrin as an Excipient was added to it.

Vaccum dried it to get it in powder form. Spray dried the water extract - cycle 4 separately to get powder.

Pressure: 600mmHg and temperature: 60°c.

Sample Preparation

Weigh accurately about 250 mg of alcoholic extract of *Ocimum sanctum* (2.5% *Ursolic acid* extract) in a 50-mL volumetric flask, dissolved in methanol and filtered through 0.25 ì filter paper and 50 ìL was spiked in 1 ml drug free human plasma to prepare 6.125 ìg/ml solution. **(Figure 3).**

Procedure for sample and standard cleanup

Liquid-liquid extraction method was used. One ml of drug free human plasma was taken in tubes. Then 2ml of methanol was added to it. This mixture was vortexing for 1min. Then it was extracted with mixture of 10 ml of diethyl ether-hexane (8:2, v/v).The resulting mixture was shaken mechanically for 3min.

After centrifugation for 10 min, the supernatant was transferred to a clean test tube and evaporated to dryness in a water bath at 40°c under a stream of nitrogen. The residue was reconstituted in 0.5ml methanol with vortexing for 60 seconds. This solution was injected into the separation system.

All the calibration and Quality control samples were cleaned by above method and then injected into the separation system.

Calibration curve was constructed by plotting average peak area versus concentrations and regression equation was computed for Ursolic acid. A representative calibration curve of Ursolic acid from human plasma is depicted in **figure 2.**

Results and Discussion

Method Validation

Specificity

There was no peak found in the blank plasma solution at the retention time of standard Ursolic acid solution in plasma.

System suitability

Standard Ursolic acid of 20 µg/ml conc. was injected 5 times on the same day one by one. Mean ± SD., %CV for the area was found to be 793016 ± 4389.73, 0.55; for retention time it was found to be 6.0202 ± 0.0590356, 0.98.

Linearity

Linearity was evaluated by determining seven different concentrations of the standard solutions of Ursolic acid. The peak area ratio and concentration of standard was subjected to regression analysis to calculate the calibration equation and correlation coefficients.

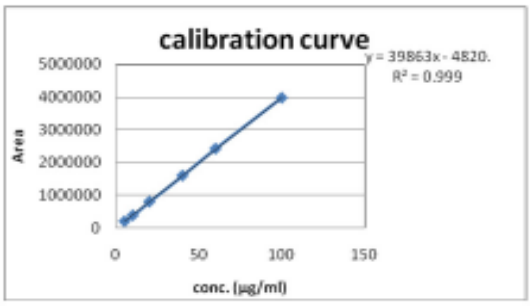


Figure 2. Calibration curve for ursolic acid from blank plasma

The regression equations obtained for the standard solution of Ursolic acid was Y= 39798X-389.48 (R^2 = 0.9999). The linearity range was individually from 5 - 100 µg/ml. The results show that within the concentration range indicated, there was an excellent correlation between peak area ratio and concentration. Results of linearity are given in **Table 1. (Fig. 2)**

Limit of Detection and Limit of Quantitation ??

LOD and LOQ were calculated from the RSD and slope (S) of the calibration curves using equations, LOD = 3.3 × (RSD/S) and LOQ = 10 × (RSD/S) [18]. The LOD and LOQ of Ursolic acid were found to be 1 µg/ml and 5 µg/ml respectively.

Accuracy and Precision

The accuracy was expressed as the percentage of analytes recovered by the assay (% Nominal) and Precision was expressed as percent variation (%CV). Mean recoveries for Ursolic acid are shown in **Table 1.** The within series results and between series result at 15, 50, 70 (LQC, MQC and HQC samples respectively), 5 and 100 µg/ml indicate good accuracy and precision of the method for the determination of the Ursolic acid by mean recovery data. Results are tabulated in **Table 1**.

Table 1. Summary of the validation parameter

Parameters	Observations
Specificity	No interference was found
Linearity Regression equation Correlation coefficient (r^2)	5 to 100 µg/ml Y= 39863x - 4820 0.9999
Accuracy (% Recovery)	98.87 to 100.15 %
Precision (n=3) (% CV) Within series Between series	 0.43 to 1.55 % 0.36 to 1.30 %
LOD	1 µg/ml
LOQ	5 µg/ml
Extraction efficiency	73.61 to 74.45%.

Percentage extraction yield

The percent extraction efficiency was performed for Quality control samples. For one sample 2 test tubes were taken. In one test tube required volume of standard solution was spiked to 1ml of blank plasma and sample was extracted as per the developed extraction procedure. This sample was labeled as extracted. While in another test tube 1ml plasma was processed as per the extraction procedure and required volume of standard solution was added at reconstitution

stage. This sample was labeled as unextracted. Both samples were injected in to chromatographic system. A result of extraction yield was also tabulated in **table 1.**

Stability Experiments

Stock Solution Stability

The stock solution stability was evaluated by comparing the peak area values of stability samples versus peak area values of fresh samples (100µg/ml). The fresh sample was immediately injected and stability sample was kept at room temperature for period of 6 hrs for short term stock solution stability and kept at 4°c for period of 7 and 14 days for long term solution stability and then injected under same chromatographic conditions.

The stability of stock solution was determined by monitoring the peak area ratio of fresh solutions of the standard Ursolic acid and stability sample. Samples were considered to be stable if retention times and peak area ratios of Ursolic acid remain almost unchanged and no significant degradation is observed within the

Table 2. Short term stock solution stability for ursolic acid

Observation Number	Std. 100µg/ml	
	Fresh 0 hrs	Stability 6 hrs
1	5341026	5348726
2	5349072	5349152
3	5351641	5350100
Mean	5347246.33	5349326
S.D.	5537.99	703.33
%CV	0.10	0.01
%Difference	0.04	

Table 3. Long Term Stock Solution Stability for Ursolic acid

Observation Number	Standard. 100µg/ml			
	Fresh	Stability 7 days	Fresh	Stability 14 days
1	5348026	5349453	5348200	5349444
2	5349072	5350984	5349587	5350896
3	5351641	5351698	5349756	5350521
Mean	5349580	5350711.7	5349181	5350287
S.D.	1860.20	1147.01	853.76	753.75
%CV	0.03	0.02	0.02	0.014
% Difference	0.021		0.02	

Table 4. Bench Top Stability for Ursolic acid

Observation Number	L.Q.C. (15µg/ml)		M.Q.C. (50µg/ml)		H.Q.C. (70µg/ml)	
	Initial	Stability	Initial	Stability	Initial	Stability
	0.0 hrs	**6.00 hrs.**	**0.0 hrs**	**6.00 hrs.**	**0.0 hrs**	**6.00 hrs.**
1	14.99	15.33	50.06	49.85	69.17	70.29
2	15.39	15.12	49.98	49.34	70.41	70.46
3	14.98	15.16	49.77	49.81	70.34	70.93
Mean	15.12	15.20	49.94	49.67	69.97	70.56
S.D.	0.23	0.11	0.150	0.28	0.70	0.33
%CV	1.55	0.73	0.30	0.57	1.00	0.47
% Nominal	100.8	101.33	99.88	99.34	99.96	100.8
%Difference	0.53		-0.54		0.84	

Table 5. Freeze Thaw Cycle for Ursolic acid

Observation Number	L.Q.C. (15µg/ml)		M.Q.C. (50µg/ml)		H.Q.C. (70µg/ml)	
	Initial	Stability	Initial	Stability	Initial	Stability
	0.0 hrs	**72.0 hrs**	**0.0 hrs**	**72.0 hrs**	**0.0 hrs**	**72.0 hrs**
1	15.39	14.95	51.26	50.27	70.91	69.36
2	15.34	15.36	51.07	49.86	70.15	70.3
3	15.03	14.96	51.31	50.08	70.52	69.24
Mean	15.25	15.09	51.21	50.07	70.53	69.63
S.D.	0.20	0.23	0.12	0.21	0.38	0.58
%CV	1.28	1.55	0.24	0.41	0.54	0.83
%Nominal	101.66	100.6	102.42	100.14	100.75	99.47
%Difference	-1.04		-0.99		-1.27	

Table 6. Post Extraction Stability (Auto sampler) for Ursolic acid

Observation Number	L.Q.C. (15µg/ml)			M.Q.C. (50µg/ml)			H.Q.C. (70µg/ml)		
	Initial	Stability		Initial	Stability		Initial	Stability	
	0.0 hrs	**6 hrs**	**18 hrs**	**0.0 hrs**	**6hrs**	**18 hrs**	**0.0 hrs**	**6 hrs**	**18 hrs**
1	15.02	14.97	15.15	49.47	49.68	49.48	69.94	69.09	70.01
2	15.09	14.87	14.69	50.47	50.73	50.19	68.76	68.36	69.59
3	15.06	15.12	15.18	50.33	50.5	50.22	69.42	68.11	68.14
Mean	15.06	14.99	15.01	50.09	50.3	49.96	69.37	68.52	69.25
S.D.	0.04	0.13	0.27	0.54	0.55	0.42	0.6	0.51	0.98
%CV	0.23	0.84	1.83	1.08	1.09	0.84	0.86	0.74	1.42
%Nominal	100.4	99.93	100.06	100.18	100.6	99.92	99.1	97.88	98.92
%Difference	-0.46		-0.33	0.41		-0.25	-1.22		-0.17

given period. Stock solution stability results are tabulated in **Table 2, 3** and **4**.

Freeze thaw cycle stability

Spiked samples of three concentrations (15, 50, and 70 µg/mL) were assessed after storage at room temperature for 6 hrs (the fresh samples were immediately injected whereas the stability samples were kept at room temperature for 6 hrs and then extracted and injected – bench top stability) and after three freeze-thaw cycles [storage at -20°c ± 5°c for 3 days (samples were thawed at room temperature and analyzed at every 24 hrs)]. Results are tabulated in **table 5**.

Table 7. Summary of results of stability experiments.

Stability experiments	% Difference
Stock solution stability	
Short term stability	0.04 (at 6 hrs)
Long term stability	0.021 (for 7 days) and (0.02 for 14 days)
Bench top stability	
LQC (15 µg/mL)	0.53
MQC (50 µg/mL)	0.54
HQC (70 µg/mL)	0.845
Freeze thaw cycle stability	
LQC (15 µg/mL)	-1.04
MQC (50 µg/mL)	-0.99
HQC (70 µg/mL)	-1.27
Post extraction stability	
LQC (15 µg/mL)	-0.46 at 6hrs and .33 at 18 hrs
MQC (50 µg/mL)	0.41 at 6hrs and -0.25 at 18 hrs
HQC (70 µg/mL)	-1.22 at 6hrs and -0.17 at 18 hrs

Stability of standard under Auto sampler (Post Extraction Stability)

Post Extraction Stability (Auto sampler maintained at 4°c) for Ursolic acid in plasma was evaluated by comparing triplicate injections of stability versus fresh samples of LQC, MQC and HQC (15, 50, and 70 µg/mL). Post extraction fresh samples were immediately injected and the stability samples were the samples re-injected after 6 and 18 hrs at the temperature mentioned above. The QC concentrations of the fresh and stability samples were determined from the

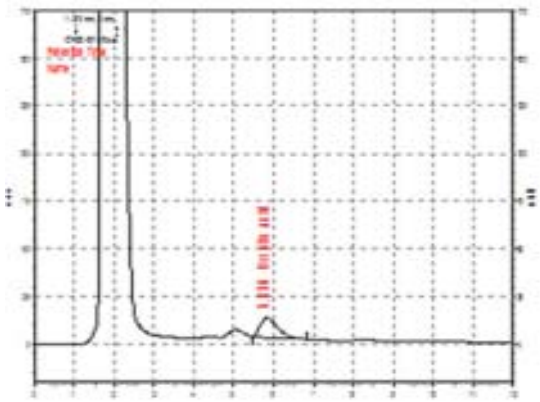


Figure 3. Chromatogram of *Ocimum sanctum* extract in Human plasma.

linearity that was assayed on the same day along with QC samples. Stability conditions were within the acceptance criterion recommended by the Food and Drug Administration (U.S. FDA). Results for the fresh sample and stability samples were given in **Table 6.**

Sample Preparation

100 ìl of Sample of *Ocimum sanctum* extract (2.5% *Ursolic acid* extract) in blood plasma prepared above was injected in Liquid Chromatographic system.**(Figure 3).**

Conclusion

This method as standardized and validated is simple, reproducible, sensitive and specific for the determination of Ursolic acid from *Ocimum sanctum* extract in human plasma. Hence, this method can be applied for the estimation of the Ursolic acid from *Ocimum sanctum* extract in human plasma.

Summary

This manuscript will describe the method development for estimation of Ursolic acid in *Ocimum sanctum* extract in human plasma.

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PHYTOCHEMICAL STUDIES ON *Piper hapnium* BUCH.-HAM

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Abstract: The fruiting spike of *Piper hapnium* which resembles that of long pepper and used as an adulterant, is studied for it's phytochemical and pharmacognostic characters for the first time. The spikes yielded 6.716% of a brown aromatic volatile oil consisting of apiole as the principal component (63.9%) and the rest being sesquiterpenes such as β-bisabolene (6.3), (E) β-farnesene (2.6), β-caryophyllene oxide (2.6), nerolidol (2.0) and (Z) β-farnesene (1.5) etc. Piperine, acacetin and phenolics acids also were located in these fruits. Large polygonal starch grains, rectangular oil cells, uniseriate filaments and sclereids are the important pharmacognostic characters. The presence of apiole as the major component of oil and the above- mentioned pharmacognostic characters and absence of piperlongumine distinguish fruiting spike of *P. hapnium* from that of *P. longum.*

Keywords: *Piper hapnium, P. longum,* Volatile oil, Pharmacognosy, Apiole, Acacetin.

Introduction

Piper hapnium Buch-Ham is a species of *Piper*, endangered and endemic of the Western Ghats. It is a dioecious, glabrous, green, slender climbing warty shrub, with leafy folded stipules, chartaceous 7 nerved, elliptic-ovate auricled leaves, leaf basebeing very unequal auricled on one side and 3-5 cm long erect slender cylindrical male spikes. Female spikes thick, cylindrical 3.5 cm long with peltate-orbicular bracts, each covering partly four adjacent flowers. Fruit obovoid, ca. 0.2 cm across, pungent and black **(Hooker, 1999).**

The fruiting spikes are collected by tribals from forests of Kerala and sold as "long pepper" in markets of Wayanad, Kerala. These spikes, being slightly larger than that of *Piper longum* (long pepper), are thus often used as a substitute of the latter in medicines. The comments of **Mehra and Puri, (1970)** that in Indian market, fruiting spikes of *P. longum* are usually not available and instead, under the name of 'chotipeepal', spikes of *P. peepuloides* and under the name of 'Baripeepal', spikes of *P. chaba* are supplied, are highly significant in this context. Therefore the pepper markets in Kerala are replete with fruits looking like long pepper **(Mathew, 1997).** *Piper hapnium* fruiting spike, as it is, is not recognized in the market, because this plant is never available in good amounts for taxonomic or ethnobotanical studies. Only recently **Saji and co-workers (2001)** have located this plant in a few natural habitats. Absolutely nothing is known on the chemistry or pharmacognosy of this material. Therefore, in the present work, the fruiting spike of *Piper hapnium* were subjected to a phytochemical analysis of its volatile oil, alkaloids and phenolics, as well as pharmacognostic studies to find out the constituents and biomarkers of this product.

Materials and Methods

The fruits of *P. hapnium* were procured from the markets of Wyanad, Kerala. The identity of this drug was confirmed by comparing the same with the authentic material preserved in Indian Institute of Spices Research Centre, Calicut. The Volatile oils were extracted by

1. Lecturer 2. Lecturer 3* Professor

hydrodistillation in a Clevenger apparatus for 4-5 hrs. The volatile oil that condensed in the graduated arm of the apparatus was measured and collected. The percentage yield of the oil was calculated. The analysis of the oil was done using Shimadzu GCMS-5000 with CBPI capillary column and non-polar polydimethyl siloxane phase at Southern Petrochemical and Industrial Corporation (SPIC), Science Foundation, Chennai. The identification of the terpenoid constituents of the oil was done by matching the spectral data with three libraries, WILEY 139.LIB, NIST 62.LIB and NIST 12.LIB, present in the same instrument.

The alkaloids were extracted from 50 gm fruits by keeping the powder in 5% ammoniacal ethanol for 48 hours in the dark. This extract was concentrated and alkaloids were re-extracted in 0.1N H_2SO_4. The soluble acidic extract was made basic with NaOH and fractionated with chloroform. The chloroform fraction was evapourated and the residue was analysed by LCMS at Regional Sophisticated Instrumentation Center (RSIC), Central Drug Research Institute (CDRI), Lucknow with the standards of piperlongumine and piperine, procured from Sigma – Aldrich.

The electrospray mass spectra were recorded on a MICROMASS QUATTRO II triple quadrupole Mass spectrometer. The samples dissolved in acetonitrile : water (1:1) were introduced into the ESI source through a syringe pump at the rate of 5ml per min. The ESI capillary was set at 3.5 KV and the cone voltage was 40V. The spectra were collected in 6s scans and the print outs are averaged spectra of 6-8 scans.The LC traces given are total ion chromatograms (TIC), Mass chromatograms and UV (analog) chromatograms. The accompanying mass spectra give mainly the [M + H] + ions of the components.On the chromatograms the entry at the right hand top corner gives the type; eg. 280 NM An 1 is the UV chromatogram. Scan ES + 360 is the mass chromatogram of M/Z 360. The value 5.60 e7 is the signal strength. Scan ES + TIC is the total ion current chromatogram. MS are recorded throughout the LC run. Mass

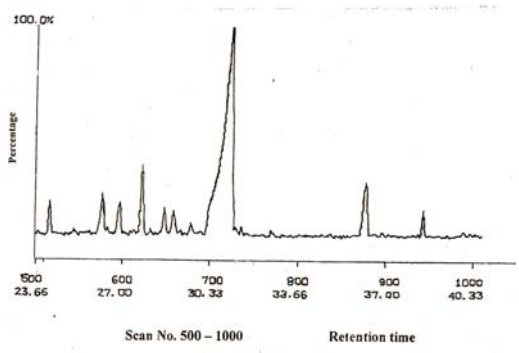

Figure 1. Gas chromatogram of Volatile oil from the fruits of *Piper hapnium*

Spectrum of the component can be picked up whenever the LC peak appears.

Other chemical components of the fruiting spikes were analysed by standard procedures. Flavonoids were analysed following **Harborne (1984)** and **Mabry and co-workers (1970).** For identification of phenolics acids the method recommended by **Ibrahim and Towers (1960)** is followed. The identification of all the compounds was done by co-chromatography with authentic markers. For pharmacognosy studies, the dry fruiting spikes were softened by keeping them in water and heating for about 30 seconds in a microwave oven.. Sections were stained with safranin**(Wallis, 1953),** phloroglucinol-HCl **(Youngken, 1951)**, iodine solution **(Johasen, 1940)** and Sudan Red IV **(Krishnamurthy, 1988)**. The measurements on the cells and other ingredients were done using stage and ocular micrometers and Camera-lucida diagrams were prepared.

Results

The fruits yielded 6.716% of a brown aromatic volatile oil. The odour was pleasant and penetrating the taste was pungent with a cooling sensation. The other physical properties were d^{20} =0.9978 and α_D^{20} = -13°. The gas Chromatogram of the oil is presented in **Fig. 1** and the data in **Table 1.** The major constituents of the volatile oil were monoterpenes. The total number of components was 13 of which the components which present in more than 1% were 11. The

Table 1. GC data of the volatile oil from the fruits of *Piper hapnium.*

Sl. No	Retention time	% Total	Retention area	Name of the Component
1	17.26	0.9	1.4	unidentified
2	23.16	1.5	2.4	(Z) β–farnesene
3	24.20	2.6	4.1	(E) β–farnesene
4	26.20	4.3	6.8	unidentified
5	26.86	3.8	6.0	unidentified
6	27.73	6.3	9.8	β–bisabolene
7	28.60	2.6	4.1	β–caryophylle-neoxide
8	28.93	3.0	4.7	unidentified
9	29.56	1.5	2.3	unidentified
10	31.16	63.9	100.0	apoile
11	32.63	0.8	1.2	unidentified
12	36.23	6.7	10.5	unidentified
13	38.43	2.0	3.1	nerolidol

number of components could be identified with the help of GC-MS libraries were only 6 which amounted to 78.9%. Apiole was the principal component amounting to 63.9%. The minor components identified were β-bisabolene (6.3), (E)β-farnesene (2.6), β-caryophyllene oxide (2.6), nerolidol (2.0) and (Z) β-farnesene (1.5).

The alkaloid fraction gave six spots in TLC (Silica gel) which gave a positive reaction to Dragendorff's reagent. The LC-MS gave twelve peaks of which only piperine could be identified.

Peaks in UV chromatogram (280 nm):

Twelve at 2.13, 2.62, 3.15, 3.50, 5.48, 7.32, 9.12, 10.25, 11.27, 13.80, 16.0,and 24.66

Peaks in Total Ion Chromatogram (ES + TIC):

1.49, 2.23, 3.29, 5.60, 6.66, 8.09, 10.33, 11.43, 13.34, 16.60, 17.85, 21.00, 24.92 and 27.49

Based on Mass Chromatogram:

Molecular weight of alkaloids:

167, 222, 224, 232, 236, 238, 272,276,300,302,314, 326

Total number of alkaloids : 12

Alkaloid identified :

Piperine

Other chemical constituents

Phenolic acids, saponins and steroids were present in the fruit. studied. A flavone acacetin and phenolic acids such as syringic, gentisic and ferulic acids were located. Proanthocyanidins, tannins and iridoids were found to be absent.

Pharmacognosy

The fruiting spike of *P. hapnium* is a brownish black long cylindrical structure in which individual fruits are arranged in spirals. These spikes have an average length of 4 cm and diameter of 5mm. The fruit tastes slightly pungent with a cooling aftertaste. Each fruit is oval in shape and about 2.5mm in length and 1mm in breadth and terminated by a raised portion of the remnant of style **(Fig. 2).** There were small reticulations on the surface. In the T.S. the spikes are found to contain about 8 fruits. Total number of fruits in a spike is about 300. The bracts are peltate with a round head. Bracteoles terminate in a tuft of hairs.

In the T.S of the spike **(Fig. 3)** the cross sections of fruits, bracts and the axis were seen. The stylar region appeared as a crest. The epidermis consisted of barrel shaped cells covered by a thick cuticle. The hypodermis contained polygonal collenchyma. The mesocarp consisted of parenchyma cells with a large number of oval/ spherical oil cells (35 to 42µ in diameter). The innermost region of mesocarp adjoining the endocarp is a continuous layer of vertically placed rectangular oil cells (50 µ x 30 µ). At some regions near the stylar region there were two layers of oil cells. The endocarp was a continuous lignified band with no cell differentiation and was 35-40 µ broad. The embryo consisted of polygonal parenchyma cells near the stylar end. The perisperm contained angular parenchyma containing starch. Towards the outer side, 2-3 layers of cells contained compactly packed clusters of starch grains of

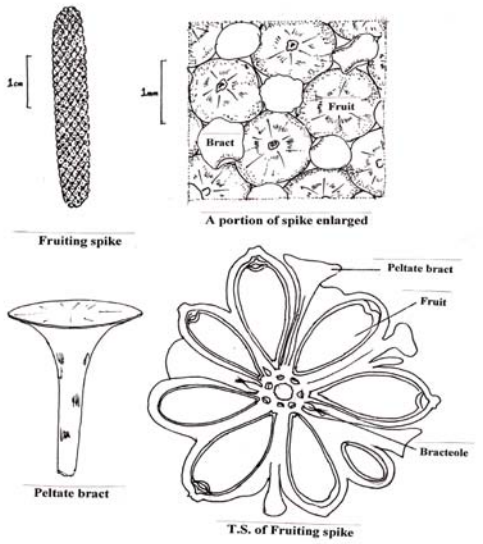

Figure 2. Fruiting spike of *Piper hapnium*

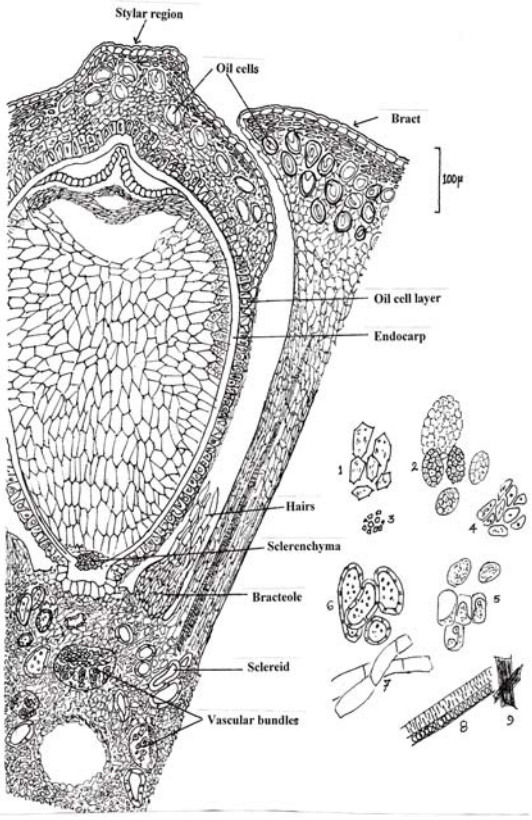

Figure 3. Fruiting spike of *Piper hapnium* T.S. and Powder study: 1. Single large starch crystal, 2. Compound crystal of starch, 3. Starch grains, 4. Collenchyma, 5. Oil cells

about 15 µ in diameter appearing as single crystals. Towards the centre all the starch grains of a cell were found fused together to form a single large polygonal transparent crystal (40 µ x 35-45 µ). Basal end of the seed contained a rectangular patch of sclerenchyma. In between the fruits there were a few reduced bracts terminating in a tuft of uniseriate hairs. Each hair contained 4-6 thin walled rectangular cells (60 µ x 18-20 µ). In the centre of the axis of spike there was a central cavity surrounded 6-8 vascular bundles and a number of oval/elongated sclereids.

Powder study

The powder of the fruiting spike of *P. hapnium* is distinguished by large polygonal starch grains, compound starch grains and small grains. Rectangular as well as oval oil cells, uniseriate filaments, sclereids, tracheids and broken fragments of endocarp are other characteristic features. Polygonal parenchyma, portions of epidermis, and a few fibres were also observed in the powder **(Fig. 3).**

Discussion

The present study unearths the chemistry of the volatile oil, alkaloids and phenolics of the fruits of *P. hapnium* as well as the Pharmacognostic features of the same for the first time. The volatile oil is rich in monoterpenes such as apiole, which forms the principal component amounting to 63.9% and the remaining being sesquiterpenes like bisabolene, â-farnesene, â-caryophyllene oxide etc. The presence of apiole is highly significant in that this monoterpene is endowed with a number of pharmacological activities like antipyretic, antispasmodic **(Weiss, 1988)** CNS-stimulant, diuretic, emmenagogue **(de Walle and Renne, 2001),** uterotonic and vasodilator properties. The presence of piperine (and a number of other alkaloids) add to the pharmacological properties of this plant. Acacetin, the flavone detected, is anti-aflatoxin, antimalarial, hepatoprotective, anticancer and antihistaminic **(Mota *et al.*, 2009).**

Table 2. Table showing the Phytochemical and Pharmacognostic differences between the fruiting spikes of Piper *longum* and *P. hapnium*.

Sr. No.	*Piper hapnium*	*Piper longum*
1.	Monoterpenes 66%	Monoterpenes in traces
2.	Sesquiterpenes 20%	Sesquiterpenes 40%
3.	Aliphatic hydrocarbons absent	Aliphatic hydrocarbons 33%
4.	Apiole (63.9%)	----------
5.	β-Farnesene	----------
6.	β-Caryophyllene oxide	----------
7.	Bisabolene (6.3%)	Bisabolene (11%)
8.	----------	Caryophyllene
9.	----------	Pentadecane
10.	Starch grains large polygonal (40 µ x 35-45 µ)	Starch grains small (2-5 µ)
11.	Oil cells rectangular	Oil cells round
12	Sclereids	Stone cells
13	Uniseriate hairs	----------

It possesses anti-peroxidative, anti-inflammatory and anti-HIV properties **(Wu *et al.*, 2011)** also. A number of studies have shown that acacetin induces apoptosis in human lung, human prostrate and human breast cancer cells **(Pan *et al.*, 2005).** Among the phenolics acids, syringic, gentisic and ferulic acids also possess distinct pharmacological properties **(Duke, 2012).** Ferulic acid is analgesic, antiallergic, anti-inflammatory, hepatoprotective and antihepatotoxic. It is antiviral, immunostimulant, antiallergic and acts as an arteriodilator. It is also found to acts against cancer in the colon, forestomach, liver and skin. Gentisic acid is analgesic, antibacterial, antiimflammatory, antioxidant, antirheumatic and antiviral. It inhibits oxidation of low density lipoproteins. Syringic acid is known to be allelopathic, antioxidant, anti-peroxidant and anti-radicular **(Daniel, 2006).** Therefore the fruits of *P. hapnium*, on its own, can be treated a medicine of immense value.

The essential oil of this plant is much different from that of *Piper longum* for which it is an adulterant. The volatile oil of *P. longum* is rich in sesquiterpenes such as caryophyllene and pentadecane (both about 17.8%) and bisaboline (11%) poor in fragrant monoterpenes **(Zaveri *et al.*, 2011)**. Absence of piperlongumine is another character differentiating *P. hapnium* from *P. longum*. The data on pharmacognostic characters give a large number of biomarkers of *P. hapnium* with the help of which this powder can be identified from that of *P. longum* for which it is an adulterant **(Table 1).** The large polygonal starch grains, rectangular oil cells, uniseriate filaments and sclereids are absent in *P. longum* fruit powder **(Gupta *et al.*, 2007)** and therefore these two plants can be easily distinguished from each other. The Phytochemical and Pharmacognostic differences between the fruiting spikes of *Piper longum* and *P. hapnium* are summarized in **Table 2.**

Abreviations used:

1. WILEY 139. LIB.= Wiley Registry TM of Mass Spectral data 139 Library,

2. NIST 62. LIB= National Institute of Standards and Technology 62 Library.

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AN AYURVEDIC PERSPECTIVE TOWARDS CEREBRAL PALSY

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Abstract: Cerebral palsy is an all encompassing a group of non-progressive, non-contagious motor conditions that cause physical impairment in brain development, chiefly in the various areas of body movement. Its incidence is about 2 to 3 per 1,000 live births. Its prevalence is increased among low birth weight infants, particularly those weighing less than 1000 g at birth. While in certain cases there is no identifiable cause, typical causes include problems involving intrauterine development, birth asphyxia, and birth trauma during labour and certain complications in the perinatal period or during childhood. Cerebral palsy is also more common in multiple births. An exact Ayurvedic correlation to cerebral palsy is not particularly evident in its classical literature. Different aspects of this condition can be found scattered in various contexts of antenatal, natal and postnatal stages. However, considering the disease classification and respective clinical features, Cerebral palsy seems similar to *vatavyadhi* or *vatavikar* particularly afflicting the *shiromarma* or shiromarmabhighata vatavikar which may manifest in form of *pakshaghat*, *ekangaroga*, *pangu* etc.

Keywords: Cerebral palsy (CP), *Vatavyadhi*, Herbal treatment, Medicinal plants.

Introduction

Cerebral palsy (CP) is one of the most common causes of disability in childhood, leading to functional limitations. It is characterized by the inability to normally control motor functions, which affect the child's ability to explore, speak, learn, and become independent. Effective management can improve the quality of life for the child and family. *Cerebral* refers to the cerebrum, which is the affected area of the brain; *palsy* refers to disorder of movement. Cerebral palsy was first described by William John Little, an orthopaedic surgeon in 1862 and initially was called Little's disease who observed that children with tone and developmental abnormalities often have had prolonged labour, prematurity or breech delivery. Later, in 1897, Sigmund Freud suggested that Cerebral palsy might be rooted in the brain's development in the womb and related aberrant development of factors influencing the developing foetus. According to **Michael V. Johnston (2008)**[1] "Cerebral palsy is a diagnostic term used to describe a group of motor syndromes resulting from disorders of early brain development. Although it has been considered a static encephalopathy, but term is not entirely accurate because of the recognition that the neurologic features of cerebral palsy often change or progress over time". **Clayton L. Thomas (2005)**[2] describes cerebral palsy as **"**An umbrella term for a group of nonprogressive, but often changing, motor impairment syndromes secondary to lesions or anomalies of the brain arising in the early stages of its development. Cerebral palsy is a symptom complex rather than a specific disease". **OP Ghai (2009)**[3] state that Cerebral palsy is a non-progressive neuromotor disorder of cerebral origin.

Incidence

It is about 2 to 3 per thousand live births[1,4,5,6,7] Prevalence of cerebral palsy is increased among low birth weight infants, particularly those weighing less than 1000 gm at birth, primarily because of intracerebral haemorrhage and periventricular leukomalacia.[1]

1. Lecturer in Balroga 2. Lecturer in Basic Principle of Ayurveda

Ayurvedic concept related to Cerebral palsy

An exact correlation to cerebral palsy is not directly available in the Ayurvedic texts. As in etiopathogenesis of Cerebral palsy, various factors may operate prenatally, during delivery or in the postnatal period. Likewise in Ayurvedic texts, various factors which may result in condition like cerebral palsy are also discussed regarding imperfections as in *garbhadhan* (impregnation), *garbhini paricharya* (antenatal care), *akalaprasava* (untimely labour) and *prasvoottera* (postpartum) stages etc. However, considering the Ayurvedic disease classification and their respective features, Cerebral palsy can be compared with *vatavyadhi* or *vatavikar* (diseases of the nervous system) which specifically afflict the *shiro-marma* which may noticeable in various clinical forms as *pakshaghat, ekangaroga, pangu, sarvangroga, aakshepka* etc. It is also worth mentioning that just like cerebral palsy, *vatavyadhi* too may emerge at any age (before birth, during birth and after birth till old age) i.e. since conception up to old age. Here, the main causative factor is said to be the vitiation of *vata dosha* {*vata* is one of the *tridosha* /three humors- *vata, pitta* and *kapha,* which are said to be responsible for maintenance of homeostasis in the body. *Vata dosha* keeps *pitta, kapha,* all the *saptdhatus* (seven bodily tissue) and the *malas* (excretory products or wastes) in motion} or *shiromarmabhighata* (injury of brain) etc.

Nidana (Etiological Factors)

Cerebral palsy may result from one or more aetiologies, with the actual cause difficult to determine in all cases. Risk factors for cerebral palsy are consanguinity, history of spontaneous abortion/stillbirth, family history of cerebral palsy, malpresentation and low socio-economic status. Cerebral palsy is caused by damage to the motor control centers of the developing brain and can occur during pregnancy, during childbirth or after birth up to about age three. Prematurity and low birth weight is most commonly linked to cerebral palsy, due to under development of brain. Birth asphyxia is most prominent cause of cerebral palsy in infants as per research findings.[8] Preterm birth entails a high risk for CP, others are several obstetric factors and low Apgar scores are associated with CP.[9,10] Most of cerebral palsy cases are acquired prenatally and from largely unknown causes.[11] Cases of postnatally acquired cerebral palsy are approximately 10%.

1. Prenatal causes of cerebral palsy includes congenital brain malformations and infections, vascular events (e.g. middle cerebral artery occlusion), maternal infections during the first and second trimesters of pregnancy (TORCH e.g. rubella, cytomegalovirus, toxoplasmosis). Less common causes of cerebral palsy includes metabolic disorders (sever hypoglycemia etc.), maternal ingestion of toxins and rare genetic syndromes, chromosomal abnormalities, rhesus incompatibility, maternal diseases during pregnancy.
2. Perinatal causes (or problems during labour and delivery) causes include asphyxia, prematurity and various obstetric emergencies (e.g. antepartum haemorrhage, obstructed labour or cord prolapse which may compromise the foetus causing hypoxia) etc. Severe hypoglycaemia, untreated jaundice and severe neonatal infection (CNS) may be responsible for cerebral palsy.
3. Postnatal causes mainly are brain infections (e.g. bacterial meningitis, viral encephalitis), septicaemia, traumatic brain injury (head injuries caused by motor accidents or fall), toxins (drugs) etc.

A) Garbhakaleen nidan (prenatal factors)

1. Marriage as in *sagotriya* (consanguineous) (***Charaka Samhita Sharirsthana 2/3,[12] Astanga Sangreha Sharirsthana 1/2[13]***). Some studies also noticed that consanguineous families showed an apparent genetic form of spastic CP with microcephaly and mental retardation.[14] other studies also showed genetic aetiological role in cases of cerebral palsy in Asian families.[15] Ataxic cerebral palsy accounts for 5–10% of all forms of cerebral palsy and it is estimated that

approximately fifty percent of ataxic cerebral palsy is inherited as an autosomal recessive trait.[16]

2. Conception in early (first three days) or late days of *ritukala* (proliferative phase including ovulation) can give birth to child with gross defects **(*Kashyapa Samhita Sharirsthana 5/5)*[17].**

3. Acharyas also described various results of *garbhadan* (impregnation) in *atibala* (very young or below sixteen years age) or *ativridha* (old woman or above sixty years age by Acharyas *Charaka, Vagbhata,* and above 70 years age by Acharyas *Sushruta*, *Kashyapa* and *Bhel*) as *alpaayu* (short life), ill health, deformed body parts etc. (***Charaka Samhita Sharirsthana 8/6,[12] Sushruta Samhita Sharirsthana 10/59-60,[18] Astanga Sangreha Sharirsthana 1/3[13]*** *and* ***Astanga Hridayam Sharirsthana 1/9[19]***). Teenage mothers between 15–19 years were more likely to have anaemia, preterm delivery, and low birth weight than mothers between 20–24 years old.[20,21] It is noticed that development disabilities and behavioural issues are increased in children born to teen mothers.[22] During pregnancy, the women over 35 years are at increased risk of gestational diabetes, placenta praevia, pre-eclampsia, miscarriage and pregnancy induced hypertension as well as caesarean sections.[23,24] Induction of labour, augmentation with primiparae and assisted deliveries are also associated with women of advanced maternal age.[25] Perinatal mortality, perinatal and neonatal death, and intra-uterine fetal death also increase with increasing age.[26] The paternal age effect was also detected in athetoid/dystonic cerebral palsy and congenital hemiplegic patients.[27]

4. *Vata* vitiation during the time of fertilization resulting in *Yugma garbha* (twin pregnancy) (***Charaka Samhita Sharirsthana 2/14***).[12] Twin pregnancy is associated with more pregnancy complications and poorer pregnancy outcome than singleton pregnancy[28] as well as perinatal mortality and foetal growth retardation.[29,30] Perinatal morbidity is at least six to seven times among twins as compared to singletons.[31] Twin pregnancy is considered also more prone to get asphyxia especially during the birth of second baby. It is also noticed that babies of multiple birth are at increased risk of cerebral palsy.[32] Within twin pregnancy, there is also an increased risk of cerebral palsy if the co-twin has died in utero.[33,34]

5. Couple having grossly detectable vitiation of *sukra* (sperms) or *artava* (ovum) result in abnormality in newborn (***Charaka Samhita Sutrasthana 28/18-19,[12] Charaka Samhita Chikitshasthana 28/34,[12] and Astanga Hridayam Nidansthana 15/13[19]***). According to Bhavaprakasa, sperm vitiated with *vata* (*vatadushit sukra*) is prone to cause a *vikrita* (deformed), *kubja* (hump backed), *kuni* (deformed armed), *pangu* (lame), *muka* (dumb) or *minmina* (speech defects) child (***Bhavprakash Poorvakhanda 3/61***).[35]

6. Lady with *dosha* vitiated *artava* (ovum), who do not follow *garbhini paricharya* (monthwise specified dietetic protocol) and with *aashya dusti* or *dusti of garbhashya* (abnormalities of uterus) may lead to deformity in child (***Charaka Samhita Sharirsthana 2/29-30,[12] Astanga Sangreha Sharirsthana 2/37[13]*).** Maternal infection is a risk factor for CP in both term and preterm infants, posing a two-fold increased risk.[36] Maternal genitourinary infection occurring in the first two trimesters was associated with increased risk of CP in preterm or low-birthweight children.[37] Marked increase in risk of cerebral palsy in infants noticed when intrauterine exposure of foetus to maternal infection.[38] Chorioamnionitis during pregnancy is a risk factor for both cerebral palsy and cystic periventricular leukomalacia.[39] On other side, study confirms that maternal infection, acidosis at birth and meconium-stained amniotic fluid increase the risk of periventricular leukomalacia in preterm infants.[40]

7. *Dauhridavimanana* (Neglecting the desires of pregnant lady) in the first trimester of pregnancy is said to deliver a child with gross anomalies (***Charaka Samhita Sharirsthana 4/15-19,[12]***

Astanga Sangreha Sharirsthana 2/12,[13] Sushruta Samhita Sharirsthana 3/18-19[18]). In this context Acharya Sushruta comments that suppression of desires related to a specific *indriya* (means specialized sense organ) can produce abnormally of the corresponding *indriya* of the fetus (***Sushruta Samhita Sharirsthana 3/21-22***).[18]

8. Lady consuming food capable of vitiating *vata dosha* (***Sushruta Samhita Sharirsthana* 10/3**)[18] will give birth to an inactive child with deformed limbs and neurological deficits (***Astanga Sangreha Sharirsthana 2/34,[13] Astanga Hridayam Sharirsthana 1/48,[19]*** *and* ***Charaka Samhita Sharirsthana 2/29-30[12]***).

9. Effect of *nija* (internal) and *aagantuja* (external factors e.g. traumatic injuries and accidents) disorders of mother on baby e.g. various maternal diseases like TORCH infection may affect foetus and result to CNS manifestations**.**[31] The relative risk of cerebral palsy increased approximately fourfold with a neonatal history of sepsis.[41]

10. Acharyas described various physical strains which causes harm to mother and foetus such as excessive exercise, coitus, sleeping during daytime, travelling by vehicles or animals, abnormal body postures like squatting, suppression of natural urges causing excessive jerks, trauma etc (***Astanga Sangreha Sharirsthana 2/37,[13] Astanga Hridaya Sharirsthana 1/44-48[19]***).

11. Following dietetics and mode of life contraindicated for pregnant woman (*garbhopaghatkara bhava*): According to *Charaka*, use of excessive *guru* (heavy), *ushan* (hot) and *tikshan* (pungent) substances, use of *madkaraka dravyas* (intoxicated substances) and *madya* (wine), running on uneven path etc (***Charaka Samhita Sharirsthana 4/18***).[12] According to Acharya *Sushruta*, pregnant woman should not eat *sushak* (dried up), *parushit* (stale), *kuthita kleen anna* (putrefied or wet or moistened food) because these things likely to harm the foetus (or affects the foetal nutrition) (***Sushruta Samhita Sharirsthana 10/2***).[18] Similar description was also given by *Vagbhata* (***Astanga Sangreha Sharirsthana 2/37[13]*** *and* ***Astanga Hridayam Sharirsthana 1/44-47[19]***). Various complications of alcohol abuse are known during pregnancy as stillbirths or spontaneous abortions, poorer pregnancy outcome or neonatal deaths etc.[42]

B) Prasvakaleen Nidan (related to intrapartum period)

1. According to Acharya *Sushruta*, birth injuries occurring as a result of *moodhagarbha* (malpositioning or abnormal presentation of the foetus) (***Sushruta Samhita Nidansthana 8/11***).[18]

2. According to *Sushruta*, *akalpravahan* (or bearing down efforts made in absence of labour pains) may results in newborn with abnormalities as deafness (*badhir*), dumbness (*muka*), *hanuvyasta* (dislocation of mandible), *murdhaabhighata* (injury to head), *kasa* (cough), *shavas* (dyspnoea), emaciation and *vikat* (abnormal location of body parts) (***Sushruta Samhita Sharirsthana 10/11).[18]***

3. According to Acharya Charaka, *akalpravahan* (or bearing down efforts made in absence of labour pains) or *vilambhit pravahn* (delayed bearing down efforts) may results in newborn with abnormalities (because of hypoxic injury due to obstructed labour) (***Charaka Samhita Sharirsthana 8/40***).[12] There is high risk of cerebral palsy, mental retardation and seizures in the survivor which is depend on the duration and severity of perinatal hypoxia**.**[31]

4. Acharya *Charaka* and *Vagbhata* also gives more stress for immediate *Pranapratyagamana* (neonatal resuscitation) (***Charaka Samhita Sharirsthana 8/42,[12] Astanga Sangreha Uttertantra 1/3-4[43])***. It is well known that

delayed/improper neonatal resuscitation may result hypoxic conditions which may result in brain damage etc.

5. Acharya Sushruta has advocated against the use of surgical instruments in cases of *jivitmoodhagarbha* (obstructed live foetus) (***Sushruta Samhita Chikitshasthana 15/11***).[18] During difficult or obstructed delivery, there are chances of birth trauma due to application of vacuum extraction and forceps which results in damage to the tissues and organs of an infant caused by mechanical forces during childbirth accompanied by impaired blood circulation and organ functioning. The most frequent and significant birth injuries may include injuries to the skull, brain and spinal cord, extremities etc. Head trauma during delivery may results in number of conditions which includes haemorrhage (subgaleal, subarachnoid, intraventricular etc.), cephalohematoma etc.

(*C*) *Prasvotterkaleen nidan* (related to postpartum)

1. Improper care of the umbilical cord (*nabhinala*) causes umbilical sepsis or septicemia.

2. Breast milk vitiated by *vata* when taken by newborn results in emaciation and various *vataj* disorders in the infant (***Charaka Samhita Sharirsthana 8/55***).[12] According to Acharyas Madhava and Bhavprakasha, milk vitiated by *kapha dosha* (one of the three doshas i.e. humor) result in disorders of *kapha* and infant become *nidralu* (sluggish) and *jada* (numb or idiot) etc. (***Madhava Nidana 68/3***[44] *and* ***Bhavprakasha Madhyamkhanda 71/120***[45]). Acharya *Kashyapa* described that milk vitiated with *tridoshas* when consumed by child results in *panguta* (diplegia), *jadata* (numb or dullness), *mukata* (aphasia) and *charmdala* or allergic dermatitis (***Kashyapa Samhita chikitsasthana 17***).[17]

3. Avoiding of prescribed *rakshakarma* (protective measures) to safeguard the child and mother from infections (***Charaka Samhita Sharirsthana 8/47,***[12] ***Sushruta Samhita Sharirsthana 10/23***[18] ***and Astanga Sangreh Uttarasthana 1/35-41***).[43]

4. Effect of *nija* (internal) and *aagantuja* (external) disorders e.g. *vyadhija phakka* (or marasmic protein energy malnutrition) (***Kashyapa Samhita Chikitshasthana, Phakkachikitsa Chapter***),[17] *graha rogas (graha word means* seizing, holding. So graha rogas are various demons or diseases which influences or attacking the foetus and neonates or childhood age), traumatic head injury in infancy (*siromarmabhighata*). Unconjugated hyperbilirubinemia during neonatal period may lead to kernicterus which further have various sequelae as athetoid cerebral palsy, choreoathetosis, deafness and various grades of intellectual retardation and learning disabilities etc. if not treated early.[31] Neonatal sepsis may also contribute to the development of cerebral palsy and delayed development.[46]

Samaprapti (Pathogenesis)

The various causative factors like *prasvaporva* (antenatal), *prasvakaleen* (durng labour) and *prasvaotter* (postpartum) results in pathogenesis of cerebral palsy by different steps, but at last all they cause derangements of *shiromarma* (brain). Likewise, dietetics and mode of life contraindicated for pregnant woman (or *garbhopaghatkara bhavas)* works by various routes as:

1. Improper nourishment and growth of the foetus or
2. Precipitating the maternal general diseases or
3. Favouring the attack of infectious diseases in mother or foetus or
4. By increasing the chances of pregnancy complications or
5. Harming the foetus directly.

Various maternal diseases like TORCH infection may affect foetus and result to CNS manifestations. Both *akalpravahan* and delayed extraction of obstructed foetus by surgeon results in traumatic head injury and hypoxic insults

which may damage the *mastulunga* (brain) by way of *shiromarmabhighata*. Prematurity should be considered as a complication of improper care of pregnant woman or improper *garbhini paricharya*. Delayed *prana-pratyagum* also result to hypoxic ischemic enancephalopathy. After birth, various disorders squeals may manifest into cerebral palsy such as cases of kernicterus, meningitis, intrauterine or acquired infections, maldeveloped brain etc.

Classification

Cerebral palsy is classified on the basis of topographic distribution, neurologic findings and etiology. But the major classification comprises variants as : Spastic cerebral palsy (which further includes spastic quadriparesis, diplegia, hemiplegia respectively), Hypotonic (Atonic) cerebral palsy, Extrapyramidal (athetoid/dyskinetic) cerebral palsy, Cerebellar (ataxic) and Mixed type cerebral palsy.[3]

Clinical manifestations

Cerebral palsy may invoke muscle stiffness (spasticity), poor muscle tone, and problems with speech, swallowing, balance, coordination, posture, walking, and many other functions. Skeletal deformities, seizures, breathing problems, bowel and bladder control problems, dentition problems, eating difficulties, digestive problems, hearing and vision problems, learning disabilities, mental retardation and are often linked to cerebral palsy. The severity of these problems varies widely, from mild and subtle to very profound. Depending upon the severity of *Dosha-Dusya Sammurcchana* (complex of abnormal body humour and body component), clinical presentations may vary in *vatic* disorders, but clinical presentation present in cerebral palsy can be equated with certain disorders of *vatic vyadhi* as:- *Pakshaghata* : hemiplegia, *Pangulya*: diplegia, *Ekangaroga* : monoplegia, *Sarvangroga* : quadriparesis, *Kubjatva* : kyphosis, *Aaksepaka* : convulsion / involuntary shaking movements, *Ardita* : facial palsy. Common associations are as *Mukatva*: aphasia, *Vaksanga*: dysarthria, lalling speech, *Badhirya*: sensory neural hearing loss (deafness), *Anavasthika chitta*: behavioural disorders, *Apasmara*: epilepsy/seizures and *Khanjatva*: lameness.

Management

The clinical picture in CP ranges from very mild to severe depending on the extent of the CNS lesion. There is no cure for this lifelong condition, but education, therapy and technology can maximize the affected child's potential by improving his functional abilities and quality of life. Treatment for cerebral palsy is a lifelong multi-dimensional course of action. All treatment efforts are directed towards gaining independence in activities of daily living, attending school and having a decent social life. The treatment strategy is based on a realistic evaluation of the child's present functional status and possible future improvements. But ambulation potential often is dependent on the type and severity of cerebral palsy. Rehabilitation is actually team management which includes Pediatric Neurologist, Physiotherapist, Occupational therapist, Orthopedic & Neuro Surgeon, Speech therapist, Social Workers and Nutritionist. Impairments related to hearing, vision, speech and learning defects require prompt assessment and appropriate management. Physical therapy in patients of cerebral palsy aims to promote self help in simple daily tasks as feeding and dressing. Medical management is required in associated epilepsy, behavioural problems, sleeping difficulties, drooling and feeding difficulties etc. This includes medications as anticonvulsants, antispastic drugs, transquilizers etc., for example, Botulinum toxin is found quite effective in the treatment of children with spastic cerebral palsy[47,48,49] and its uses has got some good results. There is also growing interest in the use of hyperbaric oxygen therapy for children with cerebral palsy are found to be promising in some cases[50] and yet no benefit in certain other similar cases[51]. Surgical intervention is found beneficial for correcting anatomical abnormalities or to release tightened muscles in cases where

spasticity non- responsive to drugs as in cases of contractures and deformities as talipes equines, severe scoliosis. Most importantly, Physiotherapy is of prime importance in cases of spastic cerebral palsy as it aims to maintain maximum range of joints movement thus helping in attaining capability in performing essential daily activities as self feeding, combing hair, brushing teeth, bathing, toilet, dressing etc. The concerned affected family also requires social and emotional support to help coping to live with the child's handicap.

Role of Ayurvedic treatment

Though cerebral palsy may not be fully cured, Ayurvedic treatment can definitely help to reduce disability and improve the functioning of the affected individual to a great extent. According to Acharya Sushruta, *nidanparivarjan* (prevention from etiological factors) and *vatadipratighata* (specific measures against particular diseases) are two important measures for treatment of diseases (***Sushruta Samhita Uttertantra 1/25***).[52]

A. Preventive measures

1. Before conception: avoid *sagotriya vivah* (consanguineous marriages) so that congenital anomalies can be minimized in future.
2. During pregnancy, following the principles of *Garbhini-Paricharya* and avoiding of *Garbhopaghatakara Bhavas*. Strict restrictions of pregnant lady for smoking, use of alcohol and drugs which have potency to harm fetus.
3. During labour: educating pregnant woman regarding bearing down effort, by avoiding any *moordhabhighata* (cranial injury) during labour and by preventing infection during management of delivery.
4. During neonatal period: *pranapratyagamana* (resuscitation), *jatakarma* (ceremony performed after birth), *rakshakarma* (protective measures) and *ahara-vidhan* (feeding) are to be done and advised properly. Well-timed and skilled *pranapratyagamana vidhi* of the newborn (resuscitation) should be followed(***Charaka Samhita Sharirsthana 8/42***)[12]
5. Proper care of *nabhinala* (***Charaka Samhita Sharirsthana 8/44-45***).[12]
6. Application of *Taila Pichu* (cotton soaked with medicated oil over bregma) and *balataila abhyanga* (massage the newborn's body with *Bala taila*) (***Sushruta Samhita Sharirsthana 10/13[18] and Astanga Hridaya Uttarasthana 1/1-2[19]***).
7. Prevention of various maternal infections, fetal or perinatal injuries, good maternal care and freedom from any postnatal damage reduces the prevalence of cerebral palsy.
8. Early diagnosis, prompt and adequate management plan can reduce the residual neurological, psychological and emotional handicaps for the child and his family.

B. Specific measures

1. The treatment plan should be that of *vatashamana*, *vata* being dominant *dosha* involved. Management of *vata* disorders includes *snehana* (oleation), *swedana* (sudation) and *vasti (medicated enema)*. According to Vagbhata, *snehana* is more important while treating *vatika* disorders (***Astanga Hridayam Sutrasthana*** 16/5).[19] *Vasti* is said to be the best treatment for *vata roga* (***Sushruta Samhita Chikitshasthana 4/20***).[18] *Vasti chikitsa* is restricted till the child has attained a crawling age (***Kasyapa Samhita Khilsthana 1/11-13***).[17] But *anuvasana vasti* is promoted from early infancy. As far as possible *shodhana* therapies (cleansing and purifying therapy) should be avoided (***Kashyapa Samhita Sidhisthana 3***).[17]
2. As the condition is of *Dhatuksaya* mainly of *mastulunga*, treatment should be *brimhianiya chikitsa* (growth promoting treatment). *Dipana* (digestive stimulant), *Pachana* (digestive) and *Srotosodhaka* (cleansing the micro channels) medicines should be started before going into *brihiangna* therapy to correct any deranged state of *jatharagni* (digestive fire) or *dhatvaagni* (digestive fire at tissue levels) or the invariable *srotorodha* (obstructed microchannels).
3. Taking into account the age and the immature level of *dosha*, *dushya* and *agni* of the

patient, drugs employed should be of *snigdha* (unctuous), *mridu* (soft) and *laghu* (light) properties (***Kashyapa Samhita Sutrasthana 27/66***).[17]

4. Abhyanga, shalishashtika pinda sweda, upanaha etc. of Kerala's traditional Ayurvedic culture has progressed much in this field. Massage of the entire body with medicated oils like *Bala oil* (***Charaka Samhita chikitsasthana 28/148-156***),[12] *Narayan oil* (***Sharangdhar Samhita Madhyamkhanda 9/101-111***),[53] *Prasarni oil* (***Sharangdhar Samhita Madhyamkhanda 9/119-123***),[53] and *Mashadi oil* (***Sharangdhar Samhita Madhyamkhanda 9/124-132***)[53] are very useful. Massage involving concomitant stretching manoeuvres is very beneficial in patients with spastic diplegia resulting from cerebral palsy.[54]

5.Shiroabhyanga (application of oil on scalp), *shiroseka* (pouring oil over the scalp), *shiropichu* (putting a cloth soaked with oil over the head) and *shirovasti* (retaining oil on head for a specific period using a cap) are mentioned with increasing potency respectively in shiroroga (***Astanga Hridayam Sutrasthana 22/23-34***).[19]

6.Nasya (medicated nasal drops) has a definite role in treatment because *nasya* is said to be beneficial in various *shiro roga* as said by Acharya *Charaka* "*dvaram hi shirsho nasa tena tadavyapya hanta tana*" (***Charaka Samhita Sidhisthana 9/88***).[12] So, *pratimarsa nasya* may have beneficial results in patients of Cerebral palsy because according to *Charaka*, it gives strength to *shira* (head) and *shirogata indriyas* (sense organ in head) (***Charaka Samhita Sidhisthana 9/117***).[12]

7.Use of ras-aushadhi described by Acharyas has good results in treatment of various vatavikaras. Some of these are - *Yogendra rasa* (***Bhaisjyaratnavali Vatavyadhirogaadhikara, 531***),[55] *Vatagajankush rasa* (***Bhaisjyaratnavali Vatavyadhirogaadhikara,528***),[55] *Brihat vatchintamani rasa* (***BR Vatavyadhi-rogaadhikara, 530)***[55] etc. Amongst herbs, acharyas have advocated use of *rasayana* dravyas having *medhya prabhava* (or intellect promoting properties) which help improve the mental and physical growth of the child. Here, herbs as Ashwagandha (*Withania somnifera*), Brahmi (*Bacopa monnieri*), Guduchi (*Tinospora cordifolia*), Haritaki (*Terminalia chebula*), Jyotishmati (*Celastrus panniculatus*), Mandukparni (*Centella asiatica*), Shankhpushpi (*Convolvulus pluricaulis*), Vacha (*Acorus calamus*), Yashtimadhu (*Glycerrhiza glabra*), Shatavari (*Asparagus racemosus*), etc have been mentioned time and again in various contexts. Presently, there are a number of evidence based studies (whether experimental/animal) which may further enhance our knowledge base of herbs capable of managing cerebral palsy. Some such worthy studies are as documented as follows :

1. *Acorus calamus:* Ethyl acetate and methanolic extract of *Acorus calamus* showed protective effect against noise stress exposed rat.[56]
2. *Asparagus racemosus:* Root extract of *Asparagus racemosus* noticed neuroprotective effects in mice.[57]
3. *Bacopa monnieri:* Extract of *Bacopa monniera* showed improvement in learning and memory in rats.[58]
4. *Bramhi Ghrita:* Bramhi Ghrita (a polyherbal formulation contains *Bacopa monneri, Evolvulus alsinoids, Acorus calamus, Saussurea lappa* and cow's ghee) has effect on learning and memory in experimental animals (rats).[59]
5. *Brahmi rasayana:* Brahmi rasayana (comprises of B. monnieri, *Eugenia caryophyllus, Elettaria cardamomum, Cinnamomum zeylanicum, Piper longum* and *Piper nigrum*) improves learning and memory in mice.[60]
6. *Celastrus paniculatus:* The Jyothismati oil from seeds of *Celastrus paniculatus* showed cognitive enhancing properties in adult rats.[61]
7. *Centella asiatica:* Fresh leaf extract of Centella asiatica enhanced dendritic arborization in rats,[62] neuroprotective effect in rats.[63,64]
8. *Convolvulus pluricaulis:* The aqueous extract of *Convolvulus pluricaulis* reported neuroprotective property in wistar rats[65] and enhance learning and memory in mice.[66]

9. *Emblica officinalis*: Hydroalcoholic extract of *Emblica officinalis* showed antiseizure effect and cognitive enhancing property in rats.[67]

10. *Glycyrrhiza glabra:* Its ethanolic extract is evaluated in albino rats and mice and showed anticonvulsant activity,[68] and possessed cerebroprotective effect in hypoxic rats.[69]

11. *Sesamum indicum:* The sesaminol glycosides (one of the most abundant lignin glycosides in sesame seed) of Sesamum indicum reported cognitive property when evaluated on cognitive deficits and oxidative stress induced by intracerebroventricular injection of B-amyloid protein in mice.[70]

12. *Tinospora cordifolia:* The aqueous extract of *T. cordifolia* enhances verbal learning and memory in healthy volunteers[71] and enhances cognition in normal rats.[72]

13. *Vitex negundo: Vitex-negundo* leaf extract possessed anticonvulsant activity in maximal electroshock seizures in albino rats and pentylenetetarazole induced seizures in albino mice,[73] and protective action on the brain against ethanol-induced cerebral oxidative stress in rats.[74]

14. *Zingiber officinale:* Its extract provide protection against focal cerebral ischemia against oxidative stress-related brain damage in Male adult Wistar rats[75] and improves cognitive function in middle-aged, healthy women.[76]

Conclusion

Cerebral palsy is the common chronic disability of childhood. It occurs all around the world. The effects of this disorder include either motor or sensory and maybe combination difficulties. Much impairment of cognition, epilepsy, behavioural problems, communication, drooling and feeding difficulties go together with the motor disorder seen in cerebral palsy and requires medical intervention. Here, Ayurvedic treatment can be successfully integrated with mainstream treatment modalities in the interest of meaningful rehabilitation of the cerebral palsy cases.

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EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *COCCULUS HIRSUTUS* LEAVES

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Abstract: Inflammation and pain are the most common health problems treated with traditional remedies which mainly comprise medicinal plants. A number of natural products are used in the traditional medical systems in many countries. An alternative medicine for the treatment of various diseases is getting more popular. Many medicinal plants provide relief of symptoms comparable to that of obtained from allopathic medicines. Therefore agents of natural origin with very little side effects are required as substitute chemicals therapeutics. The methanolic leaf extract of *Cocculus hirsutus* (100 and 200 mg/kg) Linn (Menispermaceae) was investigated for its analgesic and anti-inflammatory effects in animals. The analgesic activity of the methanolic leaf extract of *Cocculus hirsutus* was investigated by eddy's hot plate model and acetic acid induced writhing in mice. Anti-inflammatory activity of *Cocculus hirsutus* was studied by both *in vitro* and *in vivo* models. Human red blood cells membrane stabilization method was adopted for the *in vitro* anti-inflammatory activity and for *in vivo*, carrageenan induced paw edema and cotton pellet induced granuloma in rats was employed. In eddy's hot plate analgesic study, both the doses of *Cocculus hirsutus* showed significant ($p<0.05$ and $p<0.01$ respectively) analgesic activity. In acetic acid induced writhing model, the onset of writhing was delayed and duration of writhing was shortened by the methanolic extract of *Cocculus hirsutus*. *In vitro* anti-inflammatory activity of the methanolic leaf extract of *Cocculus hirsutus* showed significant anti-inflammatory activity in a concentration dependent manner. *Cocculus hirsutus* showed significant anti-inflammatory activity on both carrageenan as well as cotton pellet induced granuloma models in rats. From the results, it was concluded that the methanolic leaf extract of *Cocculus hirsutus* possess analgesic and anti-inflammatory.

Keywords: *Cocculus hirsutus*, Analgesic, Anti-inflammatory, HRBC.

Introduction

Inflammation or phlogosis is a pathophysiological response of mammalian tissues to a variety of hostile agents including infectious organisms, toxic chemical substances, physical injury or tumor growth leading to local accumulation of plasmic fluid and blood cells (**Sobota** *et al.***, 2000**). Although inflammation is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain and aggravate many disorders. Hence, the employment of anti-inflammatory agents may be helpful in the therapeutic treatment of those pathologies associated with inflammatory reactions (**Sosa** *et al.***, 2002**). The clinical treatment of inflammatory diseases is dependent on drugs which belong either to the non-steroidal or steroidal chemical therapeutics. The non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin and ibuprofen inhibit early steps in the biosynthesis pathway of prostaglandins by inhibition of COX enzymes and are the main drugs used to reduce the untoward consequences of inflammation (**Albert** *et al.***, 2002**). However, the side effects of the currently available anti-inflammatory drugs pose a major problem in their clinical use. For instance, NSAIDs cause several serious adverse effects like gastric injury and ulceration, renal damage, and bronchospasm due to their non-selective inhibition of both isoforms of the COX enzyme (**Tapiero** *et al.***, 2002**). The use of

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steroidal drugs as anti-inflammatory agents is also becoming highly controversial due to their multiple side effects (**Van den Worm *et al.*, 2001**). Therefore, a need arises for the development of newer anti-inflammatory agents from natural sources with more powerful activity and with lesser side effects as substitutes for chemical therapeutics.

Cocculus hirsutus Linn (Family: Menispermaceae) is a widely growing plant found in the plains of India in dry localities. Indian tribes use various plant parts of this plant for a wide range of ailments, including constipation, kidney problems. A decoction of the leaves is taken in eczema, dysentery and urinary problem. Leaves and stem are used for treating eye diseases. Roots and leaves are given for Sarsaparilla, as diuretic and in gout (**Nadkarni, 1982**).

Ethanolic extract of whole plant showed the presence of isoquinoline alkaloid d-trilobine and dl-coclaurine, cohirsinine (**Viquaruddin and Tahir, 1991**), Jamtinine (**Viquaruddin and Iqbal, 1993**) and Cohirsutine (**Viquaruddin and Iqbal, 1992**). Aerial parts of the plant reported to be used as a diuretic and laxative (**Ganapathy *et al.*, 2002**).

Leaf juice of this plant is used in the treatment of eczema. Hence there is a search for new anti-inflammatory and analgesic agent that retain therapeutic efficacy and yet are devoid of these adverse effects. Since not much study had been done to evaluate the biological activity of the plant, the present study is focused to evaluate the anti-inflammatory and analgesic activity of aerial parts of *Cocculus hirsutus*.

Materials and Methods

Chemicals and Reagents

The chemicals used in the present study were Carrageenan (S.D. Fine Chemicals Limited, Bombay), Indomethacin (IPCA, Bombay), Naproxen (Ranbaxy, Gurgaon), Pentazo cine (Neon labs, Mumbai), Diclofenac (Biochem Pharma, Mumbai).

Plant Material

The fresh leaves of *Cocculus hirsutus (*L.*)* Diels was collected during the month of December 2008 from Vaikalmedu, Erode (Dist.), Tamilnadu (India). The plant was identified and authentified by the Botanical survey of India, Coimbatore, Tamilnadu (No. BSI/SC/5/23/08-09/Tech.1754). The leaves were shade dried, pulverized by a mechanical grinder and stored in a well-closed container for further extraction.

Preparation of Extract

The dried powdered plant material was extracted with methanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained.

Animals

Male Swiss albino mice weighing 20-25 gm and male Wistar rats weighing 150-200 gm were used for this study. The animals were obtained from animal house, IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2ºC and relative humidity of 30-70%. A12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted chaw (M/s. Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by (IAEC) Institutional Animal Ethics Committee (688/2/C-CPCSEA) of Nandha College of Pharmacy and were in accordance with the guidelines of the IAEC.

Analgesic Activity

Eddy's hot plate method in mice

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally mediated analgesic effects of methanolic extract of *Cocculus hirsutus*. The central analgesic drug, Pentazocine, was used

for positive control group. In this experiment, four groups (n=6) of Swiss albino mice (20–25 g) were placed on a hot plate maintained at room temperature for 15 min. Food was withdrawn on the preceding night of the experiment. Group 1 normal control (0.5% CMC p.o.), and group 2 Pentazocine (30 mg/kg, i.p.), whereas groups 3 and 4 animals received methanolic extract of *Cocculus hirsutus* (100 and 200 mg/kg, p. o respectively). Each animal was then individually placed gently on Eddy's hot plate at 55ºC. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate, were determined 15, 30, 45, 60 and 90 min after administration of the test drug or vehicle (**Jeane Silva** *et al*.**, 2003**).

Acetic acid induced writhing response in mice

This method was used to preferentially evaluate possible peripheral effects of methanolic extract of *Cocculus hirsutus* as analgesic substance. Groups of four Swiss albino male mice (n=6) were fasted overnight prior to the start of the experiment, with free access to water. The peripheral analgesic drug, Indomethacin (5 mg/kg), was used as a positive control. Group 1 normal control (0.5% CMC p.o), and group 2 Indomethacin (5 mg/kg, p.o.), whereas groups 3 and 4 animals received methanolic extract of *Cocculus hirsutus* at doses of 100 and 200 mg/kg was administered orally (p.o), to mice. 30 min after treatment, the mice were injected intra peritoneally with 0.1 ml of 1% acetic acid solution to induce the characteristic writhings. 5 min after acetic acid administration, the mice were then placed in an observation box, and the number of writhings was counted in a 5min period. The response of the extract and Indomethacin treated groups were compared with those of animals in the control group (**Mate** *et al*.**, 2008; Jeane Silva** *et al*.**, 2003**).

Anti-inflammatory Activity

In vitro Anti-inflammatory Activity

The human red blood cell membrane stabilization method was used for this study. The blood was collected from healthy human volunteer who was not taken any NSAID's for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% Dextrose, 0.8% Sodium citrate, 0.5% Citric acid and 0.42% Nacl) and centrifuged at 3,000 rpm for 20 min. The Packed cells were washed with Isosaline and a 10% suspension was made. Various concentrations of methanolic extract of *Cocculus hirsutus* were prepared (100, 200 mcg/ml) using with CMC and to each concentration 1 ml of phosphate buffer, 2 ml of hypo saline and 0.5 ml of HRBC suspension were added. It is incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min. The hemoglobin content of the supernatant solution was estimated spectrometrically at 560 nm. Diclofenac (mcg/ml) was used as reference standard and a control was prepared omitting the extracts (**Rajendran and Lakshmi** *et al*.**, 2008**).

In vivo Anti-inflammatory Activity

Cotton pellet induced granuloma method in rats

Cotton pellets, weighing 5 mg each were sterilized. Under ether anesthesia, the pellets were introduced subcutaneously through a skin incision on the back of the animals. Starting from 30 min after the implantation of cotton pellet for all the rats, 0.5% CMC to the normal control group, 25 mg/kg of Naproxen to the positive control group and 100, 200 mg/kg of the methanol extract of *Cocculus hirsutus* to the test groups was administered daily for 5 days (a daily p.o. administration). On the fifth day, the animals were sacrificed with chloroform, the granuloma was removed and the weights were determined (**Perez** *et al*.**, 2005**).

Carrageenan induced paw edema in rats

For this experiment, the male rats (120-150 g) were divided into four groups (n = 6). The first group received 0.5% CMC (10 ml/kg p.o.), while the second group received Indomethacin (8 mg/kg p.o). The third and the fourth groups were treated with the methanol

Table 1. Analgesic effect of methanolic extract of *Cocculus hirsutus* (100 & 200mg/kg),leaves on heat stimulating response in the hot plate test in Swiss albino male mice.

Treatment groups	Dose mg/kg	Reaction Time (Mins)				
		15	30	45	60	90
Control	10 (ml/kg)	2.0 ±0.3	2.6 ± 0.4	2.3 ± 0.2	1.7 ± 0.2	1.3 ± 0.2
Pentazocine	30	5.6 ± 0.5[b]	6.0 ± 0.3[b]	6.3 ± 0.7[b]	8.6 ± 0.7[b]	10.6 ± 0.2[b]
MCH-I	100	3.3 ± 0.4[(ns)]	3.3 ± 0.4[(ns)]	3.3 ± 0.4[(ns)]	5.3 ± 0.8[c]	6.6 ± 0.4[b]
MCH-II	200	4.3 ± 0.4[c]	5.0 ± 0.3[b]	5.0 ± 0.3[b]	6.0 ± 0.3[c]	8.6 ± 1.7[b]

The data represent the Mean ± SEM (n=6); $p<0.05^{(c)}$, $p<0.01^{(b)}$, $p<0.001^{(a)}$ compared to corresponding control; MCH-methanolic extract of *Cocculus hirsutus* (p.o).

extract of *Cocculus hirsutus* (100 and 200 mg/kg p.o.) respectively. Acute inflammation was produced by the sub plantar administration of 0.1 ml of 1% Carrageenan (in 1% CMC w/v) in the right hind paw of the rats. The paw thickness was measured at 0 min, 30 min, 60 min, 120 min and 240 min after Carrageenan injection by using verniar calipers (**Mani Vasudevan *et al.*, 2006**). The animals were pretreated with the drug 1 hour before the administration of Carrageenan (**Perez *et al.*, 2005**).

Statistical Analysis

All the results were expressed as mean ± standard error mean (S.E.M.). Data were analyzed using one-way ANOVA followed by Dunnett's *t*-test. The analysis was carried out using Graph pad software of version 4. $p<0.05$ was considered as statistically significant.

Results

Analgesic Activity

Hot plate method in mice

The analgesic activity of methanolic extract of *Cocculus hirsutus* assessed using hot plate method in Swiss albino mice was illustrated in **Table 1** Methanolic extract of *Cocculus hirsutus* showed significant analgesic activity at 100 and 200 mg/kg, p.o dose. Analgesic activity was comparable with the standard drug Pentazocine. Among the two doses 200 mg/kg showed maximum analgesic activity at reaction time 90 min (8.6 ± 1.7) is slightly lower than the standard drug Pentazocine (10.6±0.2).

Table 2. Analgesic effect of methanolic extract of *Cocculus hirsutus*(100 & 200 mg/kg) and Indomethacin (5 mg/kg) on Acetic acid induced writhing test in mice.

Treatment groups	Dose (mg/kg)	Number of writhes	% Inhibition
Control	10 (ml/kg)	66.67 ± 0.5	-
Indomethacin	5	19.67 ± 0.5[b]	70.50
MCH-I	100	36.33 ± 0.5[b]	45.51
MCH-II	200	23.67 ± 0.7[b]	64.50

The data represent the Mean ± SEM (n=6); $p<0.05^{(c)}$, $p<0.01^{(b)}$, $p<0.001^{(a)}$ compared to corresponding control.
MCH-methanolic extract of *Cocculus hirsutus* (p.o).

Acetic acid-induced writhing response in mice

The analgesic effect of methanolic extract of *Cocculus hirsutus* leaves on acetic acid induced writhing was shown on **Table 2.** Injection of acetic acid into control mice produced 66.67 ± 0.5 writhes. Pretreatment with methanolic extract of *Cocculus hirsutus* at doses of 100 and 200 mg/kg reduced the number of writhes 36.33 ± 0.5 (45.51% protection) and 23.67 ± 0.7 (64.50% protection) respectively. Among the two doses 200 mg/kg showed the slightly lower analgesic activity than standard drug Indomethacin 19.67 ± 0.5 (70.50 % protection). It was observed that the onset of writhing was delayed and duration of writhing was shortened.

Table 3. *In vitro* anti-inflammatory activity of methanolic extract of *Cocculus hirsutus* by HRBC Membrane stabilization method.

Treatment groups	Concentration (mcg/ml)	Absorbance (540nm)	% Inhibition
Control	-	0.461 ± 0.001	-
Diclofenac	50	0.164 ± 0.001 [b]	65.2
MCH-I	100	0.283 ± 0.001 [b]	38.5
MCH-II	200	0.216 ± 0.001 [b]	53.7

The data represent the Mean ± SEM (n=6); $p<0.05^{(c)}$, $p<0.01^{(b)}$, $p<0.001^{(a)}$ compared to corresponding control.
MCH-methanolic extract of *Cocculus hirsutus* (p.o).

Anti-inflammatory Activity

In vitro Anti-inflammatory Activity

The methanolic extracts of the leaves of *Cocculus hirsutus* were studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. The methanolic extract of *Cocculus hirsutus* leaves showed significant anti-inflammatory activity in a concentration dependent manner. Methanolic extract at a concentration of 200 mcg/ml showed 53.7% protection of HRBC in hypotonic solution. All the results were compared with standard Diclofenac, which showed 65.2% protection.

In vivo Anti-inflammatory Activity

Cotton pellet-induced granuloma method in rats

The anti-inflammatory effect of the methanolic extract of *Cocculus hirsutus* assessed using cotton pellet induced granuloma method in Wister rats was illustrated in **Table 4**. The methanolic extract of *Cocculus hirsutus* showed significant anti-inflammatory activity at 100 and 200 mg/kg (p.o.) dose. After 6 days, the mean dry weight of granulomatous tissue surrounding the threads was significantly lower for the group treated with *Cocculus hirsutus* extract as compared to the control group. Among the two doses 200 mg/kg showed maximum decreased formation of granuloma tissue.

Table 4. Anti-inflammatory effect of *Cocculus hirsutus* methanol extract (100 mg/kg & 200 mg/kg) and Naproxen (25 mg/kg) on Cotton pellet-induced granuloma in rats.

Treatment groups	Dose (mg/kg)	Weight of cotton pellet (mg)	% Inhibition
Control	10 (ml/kg)	130.7 ± 2.6	-
Naproxen	25	31.8 ± 0.2 [b]	76.0
MCH-I	100	55.2 ± 0.5 [b]	57.4
MCH-II	200	41.4 ± 0.5 [b] ± 0.5 [b]	68.3

The data represent the Mean ± SEM (n=6);
$p<0.05^{(c)}$, $p<0.01^{(b)}$, $p<0.001^{(a)}$ compared to corresponding control.
MCH-methanolic extract of *Cocculus hirsutus* (p.o).

The results indicate that *Cocculus hirsutus* at the dose levels of 100 mg/kg and 200 mg/kg produced a significant decrease the weight of the granuloma 55.2 ± 0.5 (57.4% inhibition) and 41.4 ± 0.5 (68.3% inhibition) respectively.

Among the two doses 200 mg/kg showed the slightly lower reduced weight of granuloma than standard drug Naproxen 31.8 ± 0.2 (76.0 % inhibition).

Table 5. Anti-inflammatory activity of methanolic extract of *Cocculus hirsutus* (100 & 200 g/kg) and Indomethacin (8 mg/kg) on Carrageenan induced paw edema method in Wistar rats.

Treatment Group	Dose (mg/kg)	Paw thickness (mm)					% inhibition
		0 min	30 min	60 min	120 min	240 min	
Control	10 ml/kg	4.79 ± 0.06	5.09 ±0.03	6.11±0.02	7.23±0.05	8.63 ±0.01	-
Indomethacin	8	4.21 ± 0.06 [b]	4.01 ± 0.01 [b]	4.0 ± 0.05 [b]	3.65 ± 0.04 [b]	3.53 ± 0.02 [b]	59
MCH-I	100	4.68± 0.12 [ns]	4.54 ± 0.09 [b]	4.38 ± 0.04 [b]	4.31 ± 0.01 [b]	4.25± 0.18 [b]	50.3
MCH-II	200	4.6 ± 0.03 [ns]	4.38 ± 0.04 [b]	4.22 ± 0.06 [b]	3.98 ± 0.1 [b]	3.95 ± 0.1 [b]	54

The data represent the Mean ± SEM (n=6); $p<0.05^{(c)}$, $p<0.01^{(b)}$, $p<0.001^{(a)}$ compared to corresponding control; MCH-methanolic extract of *Cocculus hirsutus* (p.o).

Carrageenan-induced paw edema in rats

The anti-inflammatory effect of the methanolic extract of *Cocculus hirsutus* and Indomethacin on the carrageenan induced hind paw edema as shown in **Table 5** the methanolic extract of *Cocculus hirsutus* at doses 100 mg/kg and 200 mg/kg, produced a significant effect against carrageenan induced inflammation after 4.0 h of the administration. The dose of 200 mg/kg exhibited a significant inhibition of 44.95 % after 2.0 h, the effect increased at 4.0 h (54 %). Anti-inflammatory activity of methanolic extract of *Cocculus hirsutus* was significant and similar to that of Indomethacin (8 mg/kg).

Discussion

The inflammation is a complex process, which is frequently associated with pain and involves several events, such as the increase of muscular permeability, increase of granulocytes and mono nuclear cells migration, as well as the granulomatous tissue proliferation. Pain is subjective experience, which is difficult to define exactly even though we all experience it. Pain distinguished as two types, peripheral or neurogenic pain may involve the following pathological states: peripheral nociceptive afferent neurons which are activated by noxious stimuli and central mechanism which is activated by afferent inputs pain sensation (**Mate *et al.*, 2008**).

The hot plate test was selected to investigate central antinociceptive activity because it had several advantages particularly the sensitivity to strong antinociceptive and limited tissue damage. Prostaglandins and bradykinins were suggested to play an important role in pain. Phenolic compounds are reported to inhibit prostaglandin synthesis. A number of Phenolic compounds have been reported to produce analgesic activity (**Hosseinzadeh *et al.*, 2002**). As phytochemical tests showed presence of Phenolic compounds in methanolic extract of *Cocculus hirsutus*, they might suppress the formation of prostaglandin and bradykinins (**Kou *et al.*, 2005; Hosseinzadeh *et al.*, 2002**).

Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response (**Bartolini *et al.*, 1987**). The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and thereby reduction in number of writhes in the animals. The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins (**Berkenkopf and Weichmann, 1988**). The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting anti nociceptives. This response is thought to involve local peritoneal receptors (**Chakraborty *et al.*, 2004**). This result indicates that the analgesic effect of methanolic extract of *Cocculus hirsutus* might be mediated by inhibiting the synthesis or action of prostaglandins.

The centrally acting analgesic activity of the extract was also corroborated in our study by the tail immersion test results. The fact that in thermal stimuli (hot plate and tail immersion tests); the antinociceptive effect should be shown by acting centrally on opioid receptors. Since the drug had shown the analgesic activity in tail immersion test, it seems that the methanolic extract can act centrally. Taking this into consideration the methanolic extract of *Cocculus hirsutus* possess peripheral and central analgesic properties.

The extracts exhibited membrane stabilization effect by inhibiting hypotonicity-induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane (**Chou, 1997**) and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (**Murugasan, 1981**). Some of the NSAIDs are known to possess membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact

mechanism of the membrane stabilization by the extract is not known yet, hypotonicity induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components (**Rajendran and Lakshmi, 2008**).

It is known that the inflammatory granuloma is a typical response of a chronic inflammatory process and it has been established that the dry weight of the pellets is well correlated with the granulomatous tissue.

The chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or in granuloma form. The *Cocculus hirsutus* extract showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions. It reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide during granuloma tissue formation (**Olajide** *et al*., **2000**).

Obtained results showed that methanolic extract of *Cocculus hirsutus* has anti-inflammatory activity on an acute inflammatory process like in carrageenan induced paw edema in rat paws. It is well known that leukocytes migration to the injured tissues is an important aspect of the inflammatory process. Histamine and serotonin are responsible for the immediate inflammation response, whereas kinins and prostaglandins mediate prolonged response.

The anti- inflammatory effect of methanolic extract of *Cocculus hirsutus* in rats with carrageenan induced paw was significant (**Perez** *et al*., **2005**).

Conclusion

The results of the study shows that the methanolic extract of *Cocculus hirsutus* leaf posses peripheral and central analgesic activity in animal model. The *Cocculus hirsutus* leaf extract shows *in vitro* anti-inflammatory activity on HRBC and *in vivo* anti-inflammatory activity on acute and chronic anti-inflammatory activity models in rats.

Further detailed study on *Cocculus hirsutus* plant using different flogestic agents in this area will enable us to understand the mechanism of action underline the above mention activity.

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AYURVEDA IN PEDIATRIC DENTISTRY

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Abstract: Tooth decay is caused by specific types of acid-producing bacteria that cause damage in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. The mineral content of teeth is sensitive to increases in acidity from the production of lactic acid. Specifically, a tooth (which is primarily mineral in content) is in a constant state of back-and-forth demineralization and remineralization between the tooth and surrounding saliva. Pediatric dentistry faces thriving challenges in preventive regimes of child and adolescents and an introduction of ayurvedic medicine in this context holds a lot of over comings and over rulings. The use of alternative herbal medicine has achieved a name in hall of fame over the decades, however, its use in dentistry is restriced. This article aims to review the same.

Keywords: Dentistry, Ayurvedic Dentistry, Pediatric Dentistry.

Introduction

Danta Swasthya, a Sanskrit term for 'Oral Health' is an individualistic term and varies with each person's constitution *(prakriti)* and climatic changes resulting from solar, lunar and planetary influences *(kala-parinama)* according to the appendix of Ayurvedic Medicine.

Dental Caries, known to be the most complex, multifactorial condition and a predominant cause of tooth decay has been raising alarms for preventive stratergies since decades. Two groups of bacteria are responsible for initiating caries: Streptococcus mutans and Lactobacillus.

Tooth decay is caused by specific types of acid-producing bacteria that cause damage in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. The mineral content of teeth is sensitive to increases in acidity from the production of lactic acid. Specifically, a tooth (which is primarily mineral in content) is in a constant state of back-and-forth demineralization and remineralization between the tooth and surrounding saliva.

Pediatric dentistry faces thriving challenges in preventive regimes of child and adolescents and an introduction of ayurvedic medicine in this context holds a lot of over comings and over rulings. Research and development of traditional drugs, as tools in health care, need a special approach, preferably in a multidisciplinary setting.[1] An ethnopharmacognostic approach needs to be practised for the precise use of ayurvedic medication.

Studies in the last decade aim at use of green tea, haritaki and turmeric powder in dentistry. This article aims to review the prospects of use of ayurveda in dentistry as a whole and compatibility of the above products for use in pediatric dentistry.

Alternative health care

The public's interest in alternative health care has grown dramatically in the past few years. In a 1993 article, titled "Unconventional Medicine in the United States," Dr. David Eisenberg and colleagues reported the results of a 1990 telephone survey of U.S. adults.1 Of the 1,031 individuals who completed the survey, 34 percent said they had used at least one unconventional therapy in the previous year, and one-third of those (11.2 percent) had seen

1. Professor & HOD 2. Postgraduate student

providers of unconventional therapy. The latter patients made an average of 19 visits in the course of a year to such providers, mostly for the treatment of chronic illness. Dr. Eisenberg extrapolated the survey results to the U.S. population and concluded that "Americans made an estimated 425 million visits to providers of unconventional therapy vs. only 388 million visits to all primary care physicians. Thus, Dr. Eisenberg concluded that "both the frequency and use of unconventional therapy in the United States is far higher then previously reported." Countries of the south asia pacific region, especially India and China on the other hand have always restricted to use of herbal remedies for treatment of chronic ailments over the centuries. This alternative therapy which is as considered over the western subcontinent, is the primary therapy implied among maximum population in eastern subcontinent. Examples of alternative health care practitioners typically include acupuncturists, chiropractors, massage therapists, yoga instructors, nutritionists, dietitians and naturopathic physicians, aromatherapists, guided imagery caregivers, and crystal therapy healers, to name a few.

Knowledge about alternative medicine is slow getting into medical school curricula in the western region. However, this has been awarded as a Bachelor's qualification of BAMS (Ayurveda) in India for quite sometime. interactions with various dental colleagues and their knowledge of dental school curricula reveal that alternative dental products and treatment modalities are rarely included in dental education. In part, this may be due to the fact that the trend in health care toward alternative therapeutic measures is occurring to a lesser degree in dentistry. Also, many alternative-care protagonists are squarely aligned against orthodox dentistry by being antifluoride or antimercury. A vocal minority of practitioners preach the toxic dangers of root canal fillings and the folly of periodontal surgery in salvaging teeth. Most holistic dental practitioners simply recommend alternative and natural dental products that are available through their practice, in health food stores or by mail order.[2] Unfortunately, most of these products have little or no direct scientific basis for the specific oral health claims.

The scope of Ayurveda

The most important class of alternative health products are herbal products. These products feature herbal sources as the main active ingredient. Echinacea (coneflower), utilized as a tincture or in powder form in the United States is often added to toothpastes and mouthrinses as a remedy for gum problems. Myrrh and echinacea are promoted for their antimicrobial action in toothpaste formulations by the noted therapeutic herbalist David Hoffmann in his book, *The Complete Illustrated Holistic Herbal Element Book*.[3] However, no modern studies have documented the efficacy of these products to treat any dental disease above and beyond the value of effective tooth brushing. Most common herbal ingredients in oral health care products range from cariostatic agents to analgesics to antimicrobials to bleaching/scouring agents including aloe vera; aniseed bayberry; blue flag; burdock root; calendula; cayenne; chamomile; clevers; cloves; fennel; ginger; goldenseal; gotu kola; horsetail; licorice; marshmallow; myrrh; neem; peppermint; poke root; prickly ash; propolis; red sage; rosemary; strawberry and witch hazel; essential oils such as cinnamon bark, clove oil, eucalyptus, red thyme, and true lavender; and, for fetor ex ore (halitosis), fresh parsley, pulverized nettle leaves or watercress, however, most of these do not have much support to their cause in literatures.

***Camellia sinesis* (Green tea)**

Studies during the past 15 years have focused on polyphenols in green tea for their antibacterial and antiviral properties. In particular, Horiba in 1991,[4] and Otake and colleagues in 1991,[5] have demonstrated that polyphenolic compounds may protect teeth from caries by killing causative bacteria. Additionally, Makimura

and colleagues, 1993,[6] proposed that those compounds inhibit bacterial collagenase activity. And Yu and colleagues, 1995,[7] reported that the polyphenols in green tea increase the acid resistance of human enamel. Though brewed green tea is considered to have these attributes, green tea is seldom added to oral health products. Consumption of green tea on daily basis does prove to have a beneficial effect on maintainence of oral health, and hence seems advisable especially in case of children and adolescents.

***Terminalia chebula* (*Haritaki*)**

If all the herbal formulations in Ayurveda's classical texts are reviewed, haritaki will be found to be one of the most frequently used ayurvedic herbs. In most of the compounds it is used as minor adjunct. In many others it is used as the foundation base of the entire formula - like in most of the electuaries or jams. It is the one of the prominent herb in formulations for asthma, cough, tonics, skin diseases, abdominal disorders. Apart of the usual self, an aqueous extract of Terminalia chebula as an oral rinse has already proved its efficacy as an anticaries agent.[8] This acts by increasing the pH and salivary buffering capacity of oral mucosa thereby creating an eco friendly environment and inhibiting initiation of acidic content of the saliva. Only if a suitable base to the oral rinse is provided, it shall be palatable for consumption by children and adolescents at ease.

***Curcuma longa* (*Haldi*-Turmeric powder)**

Turmeric has been used for thousands of years as a dye, a flavoring, and a medicinal herb. In India, it has been used traditionally as a remedy for stomach and liver ailments, as well as topically to heal sores. Ancient Indian medicine has touted turmeric as an herb with the ability to provide glow and luster to the skin as well as vigor and vitality to the entire body. Since turmeric has antimicrobial, antioxidant, astringent, and other useful properties, it is quite useful in Dentistry also. Apart from being a potent analgesic and also known for its efficient action in maintaining gingival and periodontal health[9] , it has also been found that tinted pit and fissure sealant is useful for applying to tooth surfaces for the prevention or reduction of dental caries.[10]

The dental-plaque detection system includes a dental-plaque staining agent, which contains at least one selected from the yellow pigment of beni-koji, turmeric extracts, and curcumin; and a light-emitting apparatus, which outputs light having a wavelength within a range of 250 to 500 nm to an object in the oral cavity where the dental-plaque staining agent is attached. A yellow pigment of beni-koji and turmeric are known as staining agents also used for other purposes.[11] Hence, the use of turmeric powder in Pediatric Dentistry seems promising in diagnostic, preventive and treatment point of view.

***Melaleuca alternifolia* (Tea tree oil)**

Another large group of oral products promoted for their antimicrobial properties contain malleleuca (tea tree) oil. These products are available in mouthwashes, toothpastes, toothpicks and lip balms. The pure oil is also available and can be applied with a toothbrush; however, it has a very pungent taste and strong aroma.

Tea tree oil does have antimicrobial properties on bacterial cultures, but no studies document the efficacy of tea-tree-oil-containing dental products on oral disease. Some herbal products, such as the new Dental Herb Co. products called Tooth & Gum Tonic and Under the Gum Concentrate are promoted as anticaries and antiperiodontitis agents. There are no studies howsoever to support these claims.

Conclusion

The possibilities for advancement of traditional medicine should be outlined as a programmed development aiming at standardization of the quality of ingredients and manufacturing processes and schemes of quality control. However, simultaneous clinical research along with experimentally based standardized production is of vital importance. Within the

framework of such developmental endeavours serious scientific attention should be given to conceptual aspects of the traditional system and the context in which their drugs are used. Pediatric Dentists should be knowledgeable about these traditional and emerging, preventative and therapeutic products because a large number of patients use them or intend to do so. These patients may rely on dental professionals for sound advice in this area.

Numerous natural dental products are available with no research supporting their efficacy. The decision regarding their use must be made by patients and/or their dental health providers and should be based on their oral health needs and the availability of scientific documentation as to their safety, at least, as well as their efficacy, especially for use for children.

There is growing interest in alternative medicine and the use of alternative medical products. However, scientific evidence supporting research and further research proving efficacy of herbs need to be worked on before introducing the same in this speciality of dentistry.

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PHARMACOGNOSTICAL AND PHARMACEUTICAL STUDIES ON *KASAHARA DASHEMANI VATI*

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Abstract: *Background:* In the present era of geometrical rise of demand for indigenous medicines, maintaining quality standards is the need of hour. Absence of reference standards for compound formulations is a hindrance on the way towards standardisation. *Objectives:* The present study is aimed at setting a preliminary pharmacognostical and pharmaceutical profile of *Kasahara* Dashemani Vati (KD tablet). *Methods:* Study included preparation of KD tablets using pre authenticated raw drugs following all SOPs. Later KD tablet was subjected to pharmacognostical, physicochemical and phytochemical analysis as per standard protocols. The final observations were systematically recorded. *Results:* Pharmacognostical findings matched with that of individual raw drugs negating any major change in the microscopic structure of the raw drugs during the pharmaceutical processes of preparation of tablet. Tested physicochemical parameters were within the optimum reference range for a tablet. Phytochemical components such as phytosterols, glycosides, flavonoids and tannins were tested positive. HPTLC gave a preliminary fingerprint of the formulation with 6, 5 and 5 spots on short UV, long UV and visible spectrum of light respectively. *Conclusions:* KD tablet can be screened pharmacognostically for the structures of individual raw drugs to authenticate the ingredients. It is inferred that the formulation meets the required qualitative standards at a preliminary level. Identified phytochemicals support intended actions of the formulation in respiratory system. Thus the quality of KD tablet can be ascertained by pharmacognostical, physicochemical and phytochemical screening for the findings in accordance with the observations in present study. The results of this study may be used as the reference in further research undertakings of its kind.

Keywords: *Kasahara Dashemani Vati*, Pharmacognosy, Chromatography, Standardization.

Introduction

Since ancient times humanity has depended on the diversity of plant resources for food, clothing, shelter, and traditional medicine to cure myriads of ailments. Ayurveda is an Indian system of medicine affluent with vast number of herbal, poly herbal and herbo-mineral formulations for various disease entities. During the past decade, there has been increasing acceptance and public interest in natural therapies in both developing and developed countries. To meet the market demands there is every chance of compromise in the quality of herbal products when there is scarcity of raw materials. Thus quality control for efficacy and safety of herbal products is of paramount importance.[1, 2] Quality can be defined as the status of a drug that is determined by identity and purity of contents; physical, chemical and biological properties and by the manufacturing processes.[3] Maintaining the quality standards of formulations is a challenge encountered by the science with thousands of years' of experience. The development of this traditional system of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in the healthcare.[3 4]

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Scarcity of reference standards for compound formulations is one of the hindrances in the way towards standardisation. First step in quality standardisation of compound formulations is to establish the presence of each ingredient in the finished product,[5] followed by physico chemical and phytochemical analysis. *Kasahara Dashemani* (KD) is a group of ten herbs **(Table 1)** mentioned in *Charaka Samhita* (A fore line treatise of Ayurveda) which is meant for curing the ailments presenting with cough as principal symptom.[6] Tablet form preserves the originality of drug and is also easier for prescription of appropriate dose. In present study KD tablets were subjected to Standard Pharmacognostical (powder microscopy) and Analytical (physicochemical and phytochemical tests along with HPTLC) evaluation in order to prepare a preliminary assay of the formulation.

Materials and Methods

(a) Collection and authentification of raw drugs

All the drugs except *Vrishchira (Trianthema portulacastrum* Linn.*)* were obtained from Pharmacy of GAU, Jamnagar. *Vrishchira (Trianthema portulacastrum* Linn.*)* was collected from the Jamnagar locality during the month of September. The ingredients with botanical source and parts used are given in **Table 1.** Pharmacognostical authentication of all the raw drugs was done based on the morphological features, organoleptic characters and powder microscopy of individual drugs. The API standards were used for authentication.[7]

(b) Method of Preparation of KD tablets

All the pre authenticated drugs (enlisted 1 to 10 in Table 01) taken in equal proportions were properly dried and pulverized in to fine powder of mesh number 100. Later 3.5 % w/w of gum acacia was taken as binding agent and aqueous solution was prepared by dissolving it in sufficient quantity of distilled water and mixed with the fine powder of raw drugs to make a homogenous blend.[8] This blend was then sieved through mesh number 12 to get granules of uniform size and dried in oven at uniform temperature of 50 degree Celsius. On the next day the granules were punched in to tablets of 500 mg each and uncoated tablets were packed in air tight packing. The whole process of tablet preparation was done at the Pharmacy under sterile environment.

(c) Pharmacognostical analysis

Pharmacognostical analysis of KD tablets based on Organoleptic characters i.e. color, odor, taste and texture were recorded. Microscopic

Table 1. The constituents of *Kasahara Dashemani* group along with their part used and their ratio used in preparation of KD tablet

Sl. No.	Sanskrit Name	Botanical Name	Parts Used	Proportion
1	Draksha	Vitis vinnifera Linn.	Fruit	1 part
2	Abhaya	Terminalia chebula Retz.	Fruit	1 part
3	Amalaki	Emblica officinalsGaertn.	Fruit	1 part
4	Pippali	Piper longum Linn.	Fruit	1 part
5	Duralabha	Fagonia cretica Linn.	Whole Plant	1 part
6	Shringi	Pistacia integerrima Stewart ex Brandis	Gal	1 part
7	Kantakarika	Solanum surattenseBurm.	Whole Plant	1 part
8	Vrishchira	Trianthema portulacastrum Linn	Whole Plant	1 part
9	Punarnava	Boerhavia diffusa Linn.	Whole Plant	1 part
10	Bhumyamalaki	Phyllanthus urinaria Linn.	Whole Plant	1 part

studies i.e. dissolving KD tablets in small quantity of distilled water, filtering through filter paper and the precipitate treated with and without stain to find out the lignified materials along with other cellular constituents and later compared with the findings of individual ingredients of the KD tablet. The micro photographs were taken under Carl Zeiss Binocular microscope attached with camera.[9,10, 11]

(d) Physicochemical analysis

KD tablet was analyzed with appropriate protocols for standard physicochemical parameters such as aqueous extractive, alcohol extractive, hardness, uniformity of weight, total ash, acid insoluble ash, disintegration time and loss on drying as per CCRAS recommendations at the Pharmaceutical chemistry lab of the institution.[12-14]

(e) Phytochemical analysis

Qualitative tests: The methanol extract of the sample was analyzed for different functional groups.[15]

HPTLC: Methanol extract of KD tablet was spotted on pre coated silica gel GF 60_{254} aluminum plate by means of Camang Linomate V sample applicator fitted with a 100 µL Hamilton syringe. The mobile phase consisted of Toluene, Ethyl acetate and Acetic acid in a ratio of 6:3:1 v/v. After development densitometric scan was performed with a Camag T. L. C. scanner III in reflectance absorbance mode at 254 and 366nm under control of Win CATS Software (V 1.2.1. Camag). Then the plate was sprayed with Anisaldehyde sulphuric acid followed by heating and then visualized in day light.[16]

Observation and Results

Pharmacognostical

Organoleptic Characters

The sample (powdered KD tablet) was greenish brown solid powder with predominant *Kashaya* (astringent) taste followed by *Katu* (pungent) and *Amla* (sour) and characteristic smell.

Microscopic Characters

Powder microscopy of KD showed the striking characters of all individual ten drugs of KD group. Such as prismatic crystals and cluster crystals of *Draksha*, fibers, sclerides and tannins of *Abhaya*, stone cells and pitted vascular fibres of *Amalaki*, beaker shaped stone cells and starch grains of *Pippali*, pitted vessels and schlerides of *Duralabha*, tannins of *shringi*, Unicellular Multi serrated trichomes of *Kantakari*, prismatic crystals of *Vrishchira*, trachieds of *Punarnava*, cluster crystals of *Bhumyamalaki* **(Fig. 3-5).**

Physicochemical

The observations of physicochemical parameters such as aqueous extractive, alcohol extractive, uniformity of weight, total ash, acid insoluble ash, disintegration time and loss on drying were shown in **Table 2.**

Table 2. Observations of various physicochemical parameters of KD tablet

Sl. No.	Test	Result
1.	Aqueous Extractive	37.5 %w/w
2.	Alcohol Extractive	24.9 %w/w
3.	Hardness	4.0 kg/m^2
4.	Uniformity of weight	< 5% Variation
5.	Total Ash	11.39 % w/w
6.	Acid insoluble ash	7.09 % w/w
7.	Disintegration time	23 min
8.	Loss on drying	5.40 % w/w

Phytochemical Analysis & HPTLC

Qualitative tests:

Preliminary phytochemical analysis through suitable tests for the presence of various functional groups indicates the presence of steroids, glycosides, flavonoid and tannins. On performing HPTLC, the chromatogram showed 6 peaks with R_f values 0.17, 0.24, 0.49, 0.58, 0.73 and 0.84 at 254nm. While at 366nm the chromatogram showed 5 spots with R_f values 0.16, 0.25, 0.49, 0.58, and 0.73 **(Fig. 1-2).**

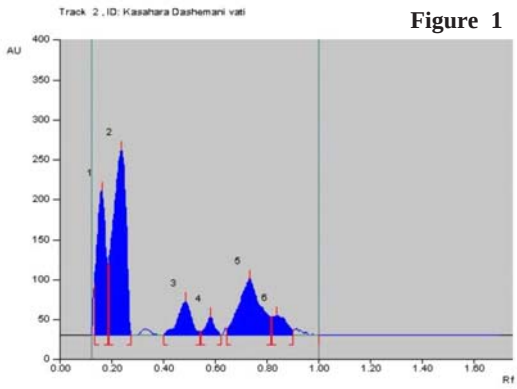

Figure 1

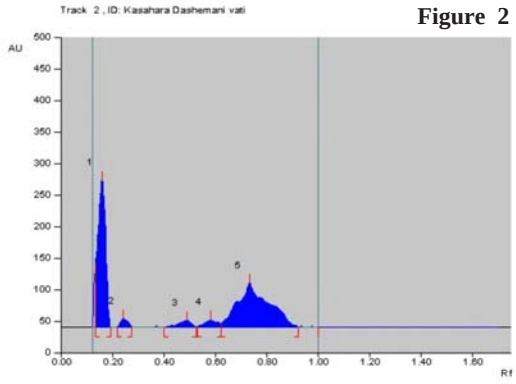

Figure 2

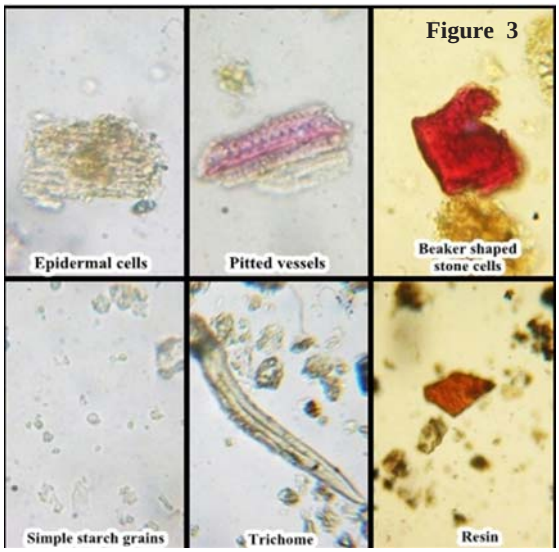

Figure 3

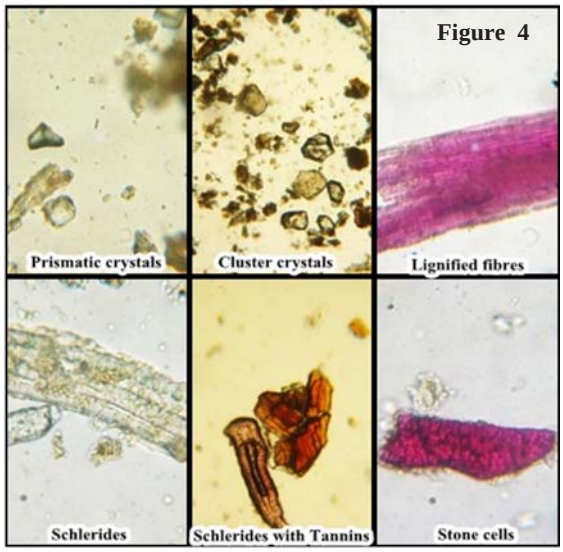

Figure 4

When viewed under day light after post chromatographic derivatisation with Anisaldehyde Sulphuric Acid it showed peaks with Rf values 0.33, 0.38, 0.66, 0.74 and 0.85.

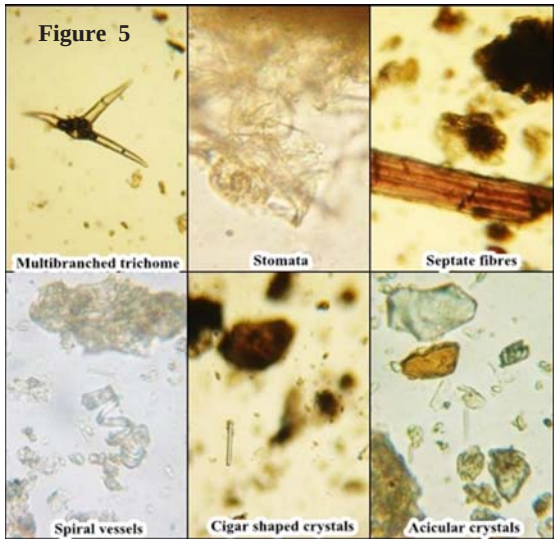

Figure 5

Discussions

Taste of the final product was *Kashaya* (astringent) followed by *Katu* (pungent) and *Amla* (sour) as majority of the ingredients had *Kashaya* taste. Study on the KD tablet is a step towards pharmacognostical and physico-chemical standardization of polyherbal drugs in tablet form. Powder microscopy of KD tablet showed the striking characters of all individual ten drugs of KD group. Few microscopic findings are

overlapping as those are basic structures in plants body, to overcome these in present study only the findings highly specific for the particular herb are considered as its marker, such as cluster crystals of *Draksha*, schlerides of *Abhaya*, pitted vascular fibres of *Amalaki*, beaker shaped stone cells of *Pippali*, unicellular multi branched trichomes of *Kantakari*, trachieds of *Punarnava*, prismatic crystals and paracytic stomata of *Vrishchira*. This confirms there is no major change in the microscopic structure of the raw drugs during the pharmaceutical processes of preparation of tablet excluding minor changes in the larger structures such as stomata, multi-branched trichomes etc.

All the pharmaceutical parameters analyzed showed acceptable values. Variation in weight was less than 5%, hardness of 4 kg/cm^{2}and rate of disintegration was 23 minutes which are well within the permissible limit.[17, 18, 19] Higher percentage of water soluble extractive may be indicating the abundance of water soluble tannins, glycosides, sugars and also the presence of binding agent which is water soluble.

Phytochemicals such as steroids, flavonoids and tannins are proven for their action on various structural and functional components of respiratory system.[20,21] Tannins and flavonoids also have anti-infective action.[22,23] HPTLC study of the drug has yielded standard finger prints of the formulations with 6, 5 and 5 peaks on short UV, long UV and when viewed in day light after post chromatographic derivatisation with Anisaldehyde Sulphuric Acid. Among which few chromophores are susceptive to both short and long UV. This polysusceptivity is frequently expected when there are clusters of larger phytochemical constituents such as tannins.

Conclusions

Pharmacognostical findings confirm the similarities in the microscopic characters of individual ingredients and the finished product with no major changes in the microscopic structures during the pharmaceutical processes of tablet preparation. Physicochemical parameters of the tablet were within the permissible limits. Identified phytochemical components support the intended action of the formulation in respiratory system. It is inferred that the formulation meets the required qualitative standards at a preliminary level. Results of this study may be used as the reference standard for testing the samples of KD tablet or in further research undertakings of its kind.

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