Bacopa monnieri (L.) Pennel (Scrophulariaceae)

Sinhala: Lunuwila; Tamil: Pirami; Hindi: Brahmi; English: Thyme-leaved gratiola

Brahmi or Lunuwila possesses numerous medicinal properties. Its uses are documented in ancient Ayurvedic texts and the herb has been widely used to promote the intellect, and treat neurological and mental problems. This plant is commonly distributed in moist and damp areas on the edges of streams and water trenches. It is a prostrate, glabrous and fleshy herb. The leaves are sessile, soft, and succulent up to 2.5 mm long with obscure venation. The stem is 10-30 cm long and 1-2 mm thick, with soft ascending branches. Flowers white or blue with purple veins, axillary and solitary on peduncles usually longer than the leaves. Fruits ovoid, acute capsules include in the persistent calyx.

The herb is mainly used to promote intellect and as a potent nervine, cardiotonic and diuretic. Leaves and whole plant are used in Indian tribal veterinary medicine, especially in the treatment of epilepsy.

Brahmine and Herpestine are major alkaloid present in the aerial parts. Flavonoid such as glucuronyl-7-apigenin and glucuronyl-7-luteolin are present. Bacosides and Bacosaponins are important saponin constituents.

Brahmi has the capacity to improve the higher order cognitive processes and improve learning capability. It also has anxiolytic activity, anti depressant activity, intellect promoting activity antioxidant property, analgesic activity, spasmodic activity, and bronchodilatory activity.

The original paper on page 55 and review paper on page 91 describe the findings of scientific studies of B. monnieri.
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Experimental evaluation of gastroprotective and adaptogenic activity of *Amalakayas Rasayana* and its vehicle (ghee and honey)

S M S Samarakoon¹, S K M K Herapathdeniya², H M Chandola¹, B Ravishankar⁴

**Abstract**

*Amalakayas Rasayana* (AR) was tested for its anti-ulcer activity in forced swimming induced hypothermia and stress induced gastric ulceration. AR was administered in the dose of 270 mg/kg orally for 7 consecutive days prior to the experiment. The adaptogenic activity was assessed by determining and comparing the changes in rectal temperature and ulcer index and compared in AR and vehicle treated group against stress control group. In forced swimming induced gastric ulceration, pretreatment with AR caused significant attenuation of ulcer index when compared with both stress control (p<0.001) and vehicle control (p<0.05) groups. AR exhibits significant reduction in ulcer index in comparison to stress control group (p<0.05) and vehicle control (p<0.001) groups. The results suggest that AR possesses significant adaptogenic and gastro protective activities.

**Introduction**

Ageing is the accumulation of changes in humans refers to a multi-dimensional process of physical, psychological, and social changes [1]. The ageing process is a biological reality which has its own dynamic course that is beyond human control. Ageing is defined as a progressive generalized impairment of function resulting in a loss of adaptive response to stress and in a growing risk of age associated disease [2].

Ayurveda has classified ageing into two viz., Kalaja jara (Physiological ageing) which is natural process of ageing and Akalaja jara (premature ageing) [3]. Ayurveda has described various rejuvenative therapies with the help of special class of medicinal preparations called Rasayana that are believed to rebuild the body, mind, prevent degeneration and postpone ageing [4]. *Amalakayas Rasayana* (AR) is one among many Rasayana formulations mentioned in Ayurvedic classical text Charaka Samhita for the treatment of ageing related disorders [5] and used by Ayurvedic physicians. However, no report on the pharmacological screening on this formulation is available; hence, this study was designed to assess adaptogenic and anti-stress activities of AR to provide pharmacological basis to clinical claims and to justify its use as anti-ageing medicine.

**Materials and Methods**

**Test drug:** The raw materials (Table 1) of the test formulation were collected from Gujarat Ayurveda University pharmacy and were subjected to pharmacognostical studies in order to establish their authenticity. From the raw materials, the test drug AR was prepared following the classical guidelines [6]. The vehicle viz., honey and ghee of standard brands were purchased from the local market.

**Chemicals:** All the chemicals and reagents used in the experimental study were procured from standard and reputed firms and are of analytical grade regularly being used in the laboratory.

**Animals:** Charles Foster strain albino rats of either sex weighing between 200 ± 30g were selected and procured from the animal house attached to the institute (Registration No.548/2002/CPCSEA). They were housed in large spacious polypropylene cages and fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given ad libitum. The animals were acclimatized for at least one week in lab condition before commencement of the experiment in standard laboratory conditions 12 ± 01 hour day and night rhythm, maintained at 25 ± 3°C and 40 to 60% humidity. Before the test, the animals were kept fasting for 12 hours. Institutional Animal Ethics Committee had approved the experimental protocol (Approval number: IAEC 05/09-10/Ph.D.08) and the care of animals was taken as per the CPCSEA Guidelines (Committee for the Purpose of Control and Supervision on Experiments on Animals) [7].

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Effect of drugs on stress-induced ulcer was evaluated by following the method of Parmar and Jagruti [12], which was modified according to the experimental need. The rats after noting their final rectal temperature were again exposed to the swimming stress inside the same container for 16 hours. At the end of 16 hour period blood was obtained from the retro-orbital puncture under light ether anaesthesia using capillary tubes. The body weight was noted and then they were sacrificed. Blood samples were collected for assessing different types of haematological parameters by using automatic haematological analyzer (ACRUS automated haematology auto-analyzer). The vital organs like liver, heart, kidney and adrenals were dissected out, cleaned for extraneous tissues, blotted with tissue paper, weighed and computed per 100 g body weight. The stomach was excised, cleaned and opened along the greater curvature. The inner surface was cleaned gently by washing with cold saline solution and spread on wax board with the mucous surface upwards avoiding corrugation and examined for ulceration with a magnifying lens. Severity of ulcer and total number of ulcers in each rat was recorded for calculating ulcer index. Ulcer index was calculated according to the method described by Kulkarni and Goel [13]. Mean ulcer scores for each experimental group were calculated and expressed as the ulcer index.

**Table 1: Formulation composition of Amalakayas Rasayana**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Botanical Name</th>
<th>Part used</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Amalakai</td>
<td>Emblica officinalis</td>
<td>Fruit</td>
<td>11 Part</td>
</tr>
<tr>
<td>2 Shweta</td>
<td>Alpenia galanga</td>
<td>Rhizome</td>
<td>1 Part</td>
</tr>
<tr>
<td>3 Shatavari</td>
<td>Asparagus racemosus</td>
<td>Root</td>
<td>1 Part</td>
</tr>
<tr>
<td>4 Punarnava</td>
<td>Boerhavia diffusa</td>
<td>Root</td>
<td>1 Part</td>
</tr>
<tr>
<td>5 Manduka parni</td>
<td>Centella asiatica</td>
<td>Whole plant</td>
<td>1 Part</td>
</tr>
<tr>
<td>6 Shalaparni</td>
<td>Desmodium gangeticum</td>
<td>Root</td>
<td>1 Part</td>
</tr>
<tr>
<td>7 Jivanti</td>
<td>Leptadenia reticulate</td>
<td>Root</td>
<td>1 Part</td>
</tr>
<tr>
<td>8 Rasna</td>
<td>Plucheas lanceolata</td>
<td>Root</td>
<td>1 Part</td>
</tr>
<tr>
<td>9 Haritaki</td>
<td>Terminalia chebula</td>
<td>Fruit</td>
<td>1 Part</td>
</tr>
<tr>
<td>10 Guuduchi</td>
<td>Tinospora cordifolia</td>
<td>Stem</td>
<td>1 Part</td>
</tr>
<tr>
<td>11 Lauha Bhasma</td>
<td>Incinerated Iron</td>
<td>–</td>
<td>1.5 Part</td>
</tr>
</tbody>
</table>

**Dose selection and schedule:** The classical dose of AR in human beings is 3 g/day [8]. The dose for experimental animals was calculated by extrapolating the human dose to animals (270 mg/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes (1964) [9]. The drug solution was made by adding unequal quantity of ghee (700 mg/kg) and honey (1350 mg/kg) as per the classical indication [10] and administered to animals orally with the help of gastric catheter sleeved to syringe. The drugs were administered to over night fasted animals.

**Adaptogenic and anti-ulcer activity** [11]: The selected animals were divided in to four groups of six animals in each. Normal control (water control – WC) animals were kept under standard laboratory conditions, left undisturbed in their home cages without stress exposures. Second group received only distilled water and served as stress control (SC) group while the third group received combination of ghee (700 mg/kg) and honey (1350 g/kg) and served as vehicle control (VC). Fourth group (AR) was administered with the AR (270 mg/kg) plus vehicle. For the experimental group, drugs were given for seven consecutive days. On sixth day the rats were kept in individual metabolic cages to prevent coprophagy and fasted for 36 hours with access to water ad libitum. On the seventh day one hour after drug administration, the initial rectal temperature of individual rats was noted. After noting initial rectal temperature rats are kept inside specially arranged containers, which were made up of plexiglass with holed lids. The water level was maintained up to 25 cm height and temperature of water was maintained at 22 ± 2 °C. Rats were placed in the container and exactly after 20 minutes of exposure to stressed condition, the rats were taken out individually and final rectal temperature of each rat was noted. The drop in rectal temperature was noted down.

**Statistical analysis:** The results were presented as mean ± SEM for six rats in each group. Statistical comparisons were performed by unpaired student’s t test and one way Anova with Dunnett’s multiple t test as post-hoc test by using Sigma Stat Software (version 3.1) and the level of significance was set at p<0.05.

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Results

Effect of AR on rectal temperature

Table 2: Effect of AR and vehicle on rectal temperature in rats subjected to forced swimming stress

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>Percentage decrease in rectal temperature (°C)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>QS</td>
<td>28.046 ± 0.835</td>
<td>--</td>
</tr>
<tr>
<td>VC</td>
<td>0.7±1.4</td>
<td>22.608 ± 1.909</td>
<td>19.39↓</td>
</tr>
<tr>
<td>AR</td>
<td>0.27±0.7+1.4</td>
<td>16.470 ± 0.530</td>
<td>41.32↓</td>
</tr>
</tbody>
</table>

*p<0.05 **p<0.001 Vs stress control (unpaired t test); p<0.05 Vs vehicle control (unpaired t test)

Administration of vehicle and test drug significantly decreased rectal temperature in comparison to stress control group. Further, test drug shows statistically significant decrease in rectal temperature in comparison to vehicle control group (Table 2).

Effect of AR and vehicle on ulcer index

Table 3: Effect of AR and vehicle on ulcer index in rats subjected to forced swimming stress

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>Ulcer index</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>QS</td>
<td>47.58 ± 2.44</td>
<td>--</td>
</tr>
<tr>
<td>VC</td>
<td>0.7±1.4</td>
<td>20.70 ± 2.55</td>
<td>56.49↓</td>
</tr>
<tr>
<td>AR</td>
<td>0.27±0.7+1.4</td>
<td>05.90 ± 0.48*</td>
<td>87.60↓</td>
</tr>
</tbody>
</table>

#One way Anova - F value 102.50; p<0.001; p<0.05 for VC and AR Vs stress control.
*p<0.001 Vs vehicle control (Unpaired t test)

Administration of vehicle and test drug significantly attenuated stress induced ulceration in comparison to stress control group. Further, test drug shows statistically highly significant decrease in ulcer index in comparison to vehicle control group (Table 3).

Discussion

Ageing is universal but complex biological process with definite manifestations characterized by impairment of various functions and decreased ability to respond to stress [14]. Rasayana chikitsa is a specialized section of Ayurveda, which mainly deals with the preservation and promotion of health by revitalizing the metabolism and enhancing immunity. Rasayana therapy encompasses procedures of revitalization and rejuvenation to increase the body’s power of resistance to disease and supposed to slow down the advancement of ageing [15].

Swimming stress in small laboratory animals has been widely used for studying the physiological changes and the capacity of the organism to adjust in response to stress [16]. Swimming is not always a simple exercise stress, because emotional factors are difficult to be eliminated. Even short single stress like one day forced swimming stress is as effective as prolonged stressor in bringing about the stress induced alterations in the body [17]. Swimming induced hypothermia is an inevitable outcome of swimming at water temperature lower than the animal’s core temperature. In this study, forced swimming lead to remarkable hypothermia and pre-treatment with both vehicle (p<0.05) and AR (p<0.001) attenuated it in significant manner. The magnitude of attenuation observed in AR treated group is comparatively high in comparison to vehicle (p<0.05). Thus, the observed adaptogenic effect can mainly be due to adaptogenic properties of AR.

Stress ulcers are due to both physiological and psychological factors, which is crucial for gastrointestinal defense and increased accumulation of acid and pepsin leading to auto-digestion of the gastric mucosa [18]. Stress in animals is known to increase gastric motility and acidity which could lead to ulceration manifested by severe mucosal damage and haemorrhage [19]. Importance of impaired mucosal blood flow also appears among the important factors in the pathogenesis of stress-induced ulcers [20].

The other factors that may be involved are platelet-activating factor [21], increase in gastric motility, vagal over activity [22], mast cell degranulation [23] and decreased prostaglandin (PG) synthesis [24]. The reactive oxygen species generated by the metabolism of arachidonic acid, platelets, macrophages, and smooth muscle cells may also contribute to gastric mucosal damage [25]. Results presented in this work showed that oral administration of AR and vehicle before stress induction decreased the incidence and severity of stress induced gastric ulcers in rats (p<0.05). Attenuation of ulcer index in pretreated with AR against vehicle control group is highly significant (p<0.001).

Conclusion

Many of the drugs in AR are reported to have anti-stress and adaptogenic activity. Further the vehicle; combination of ghee and honey is also reported to have adaptogenic activity. Thus, the observed adaptogenic profile of AR may be attributed to one or more bioactive principles present in these drugs. From this study, it can
be concluded that AR is having significant adaptogenic and gastroprotective activity. The observed adaptogenic and anti-stress effect may probably be either through attenuation of stress induced stimulation of hypothalamus-pituitary-axis (HPA), quenching of free radicals, and enhancement of cell proliferation or cellular detoxification mechanisms.

References

10. Acharya JT. Charaka Samhita, Chaukambha Bharati Academy, Varanasi. 2004; 520.
Study of the efficacy of an ayurvedic treatment regimen on Balaka Pakshaghatha with special reference to cerebral palsy

Saroja Weerakoon¹, A P G Amarasinghe¹

Abstract

Balaka Pakshaghatha (BP) is a condition that affects movement, posture and co-ordination, and it is caused by Shiromarmabhighata (damage of the brain), before, during or soon after birth. Because of this problem most of the children have to face many motor activities dysfunction and it becomes a common developmental disability problem. Cerebral palsy (CP) is also a condition, described in modern medical science resulting from damage to the brain before, during or soon after birth. Etiopathogenesis and symptoms of Balaka Pakshaghatha are similar to cerebral palsy. Objectives of this study were to evaluate the efficacy of an Ayurvedic treatment regimen with selected Pancha Karma (bio purification measures) treatments for the management of Balaka Pakshaghatha with special reference to cerebral palsy and to compare the effectiveness of Ayurvedic treatment and Physiotherapy treatment for the management of Balaka Pakshaghatha. Sixty patients in the age group of 1-6 years of CP/BP were taken for the study from the Paediatric Clinic of Ayurveda Teaching Hospital, Colombo and Physiotherapy Unit in Awissawella base Hospital in Sri Lanka. Sixty patients were divided in to two groups, 30 patients in each group. The test group of 30 patients were selected for Ayurvedic treatment. 30 patients were randomly selected for physiotherapy treatments. Ayurvedic treatment regimen was of three rounds of treatment of 45 days in each round with two months gap and six months follow up. The efficacy of each therapy was evaluated by Gross Motor Function Classification System. Both Ayurvedic and Physiotherapy treatments had significant result of 'p' value at < 0.05. In Ayurvedic, treatment 'p' value is 9.44 E-13 and in the Physiotherapy treatment, has been 3.32 E-08. Ayurvedic treatment regimen and Physiotherapy have the capacity to improve the gross motor functions of Balaka Pakshaghatha. However, Ayurvedic treatment regimen is highly effective than Physiotherapy treatment in the management of Balaka Pakshaghatha.

Introduction

Balaka Pakshaghatha (BP) or Cerebral Palsy (CP) is a condition that affects movement, posture and co-ordination, and it is caused by damage to the brain before, during or soon after birth [1]. Most of the children have to face many motor activity dysfunctions due to this problem and it becomes a common developmental disability problem. It has been recognized as one of the Vatha vyadhi in the field of Ayurvedic medicine [2]. Annual incidence of BP/CP is 2-2.5 per 1000 births [3]. Extrapolated statistics for Balaka Pakshaghatha in Sri Lanka is 39810 per 19, 905, 165 [4]. The objectives of this study were to evaluate the efficacy of an Ayurvedic treatment regimen with selected Pancha Karma (Bio Purification measures) treatments available in Paediatrics Ward at Boralla Ayurvedic Teaching Hospital for the management of Balaka Pakshaghatha with special reference to Cerebral Palsy and to compare the effectiveness of Ayurvedic treatment and Physiotherapy treatment for the management of Balaka Pakshaghatha.

Materials and Methods

Sixty patients in the age group of 1-6 years of BP/CP were taken for the study irrespective of their sex, race and religion etc. from the Paediatric Clinic of Ayurveda Teaching Hospital Colombo and Physiotherapy Unit in Awissawella base Hospital.

Patients with history of delayed milestone, spasticity in one or all the limbs, persistence of primitive reflexes were diagnosed by using performa as BP/CP and selected for the study. Children who have history of delayed developmental milestone, body stiffness, communication difficulties, restless behaviour, abnormal reflexes, BP/CP with history of convulsion were included in this present study. Children those who are suffering from convulsion, who are not in progress after two months OPD treatment, children below one year and above 6 years of age, and those who have other systemic disorders like Asthmatic condition, Heart disease etc. were excluded.

The sample of sixty patients was divided in to two groups, including 30 patients of each group. Test Group
(Group A), who had some progress after two months O.P.D. treatments, were selected for Ayurvedic Treatment. Patients in Physiotherapy group (Group B) were selected considering their age range such as 1-2 years, 2-4 years and 4-6 years for Physiotherapy Treatments.

Ayurvedic treatment schedule

Outdoor treatment period was two months. The patients who had some progress were treated in Indoor Patient Department with three rounds of treatments of 45 days, each round with two months gap, and six months follow up.

Methods of administration of drugs

In first two weeks Patients were treated with Etamateduru Decoction [5] and Bhraymi Mandukaparnie Maduyashti Decoction (Bacopa monnieri L., Centella aciatica L., Glyceryrriza glabra L.) 60 ml each twice a day, Vacha Ashvaganda [Acorus calamus L., Wilhania somnifera L. Dunal] powder ¼ tea spoon at night with bee honey, and Mahadalu Anupana [6] with Chandrakalka [7] 250mg twice a day with bee honey. As an external treatment, used Narayana oil application (Taila Abyanga) over upper limbs, lower limbs and head. In third week, Dashamooladi Decoction [8] and Bhraymi Mandukaparnie Maduyashti Decoction 60 ml. each twice a day and Vacha Ashvaganda powder ¼ teaspoon at night with bee honey given internally and externally used Shiro Dara with Narayana oil. During the period of fourth week, used the same internal treatment as third week and externally treated with Pizichil using Narayana oil. During the period of fifth week, internally treated with Danthimoolade Decoction [9] and Bhraymi Mandukaparnie Maduyashti Decoction twice a day, Vacha Ashvaganda Powder at night and Saraswatha Powder [10] ¼-tea spoon with bee honey in the morning. Externally patients were treated with Sashthika shali Pinda sweda.

After the fifth week, patients were treated three days with Darthri Powder [11] ½ tea spoon at night, which has mild purgative action. During the last week of treatment regimen, internally used Mashabalade Decoction and Bhraymi Mandukaparnie Maduyashti Decoction 60 ml each twice a day, Saraswatha Powder ¼ tea spoon in the morning with bee honey and Vacha Ashvaganda Powder ¼ tea spoon with bee honey at night. Administered Vasti treatment (Enema) using one ounce of Narayana oil.

Physiotherapy treatment schedule

Physiotherapy treatment depended on the affected limbs and the body parts. It consists of a number of exercises that include stretching, strengthening, and positioning. Bracing, abduction pillows, knee immobilizers, wheelchair inserts, sitting recommendations and handling techniques were used in physiotherapy according to the affected parts of the body.

Duration for physiotherapy was 45 minutes per day. It continued with three rounds of treatment of 45 days, each round with two months gap and six months follow up.

Assessment criteria

The criteria for assessment of treatment was based on gross motor function classification system (GMFCS) [13]. Before starting the treatment for the children, they were assessed by using the assessment criteria of GMFCS. Then patients were categorized according to the age and levels of their conditions. GMFCS contains of five levels. Level 1 is the best (maximum improvement) and level 5 is the least (minimum improvement). After completion of the treatment, they were assessed by using GMFCS, compare pre treatment, and post treatment levels.

Statistical method

Inter group statistical analysis was performed by using sample t test of pre-treatment and post-treatment. Level of significance was set at p < 0.05 in both groups.

Discussion

The efficacy of each therapy was studied by using GMFCS and results were derived after subjecting to statistical analysis.

In group A, only five patients were found between the age from 1-2 years. One patient was in level 5, three were in level 4 and one was in level 3 at the beginning of Ayurvedic treatments. After completion of this treatment regimen, they were found to be in the levels of 1, 2, and 3 (Table 1) Seventeen patients were between the ages of 2 and 4 years. All of them had a significant progress and came forward (Table 2). Eight patients were found between the ages of 4 and 6 years. Most of them achieved progress after the Ayurvedic treatment (Table 3).

In Group B, 10 patients were found between the age of 1-2 years. They too were in the levels of 3, 4, and 5 at first, after treatments they acquired some improvement. Some of them remained in the same level even after treatment (Table 1). There were only 10 patients between the age group of 2 and 4 years. Some patients remained in the same levels even after the treatments (Table 2). Only 10 patients were found between the age groups of 4 to 6 years. After treatment, they achieved some progress. However, no one succeed to the level 1 and 2 (Table 3).
### Table 1: Effects of treatment of patients between 1 and 2 years of age (Group A and B) (n=15)

<table>
<thead>
<tr>
<th>GMFCS Levels before treatment</th>
<th>No. of patients progressed after treatment (GMFCS Levels)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level 1</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>5</td>
<td>A B</td>
</tr>
<tr>
<td>4</td>
<td>1 5</td>
</tr>
<tr>
<td>3</td>
<td>3 4</td>
</tr>
<tr>
<td>Total</td>
<td>5 10</td>
</tr>
</tbody>
</table>

### Table 2: Effects of treatment of patients between 2 to 4 years of age (Group A and B) (n=27)

<table>
<thead>
<tr>
<th>GMFCS Levels before treatment</th>
<th>No. of patients progressed after treatment (GMFCS Levels)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level 1</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>5</td>
<td>A B</td>
</tr>
<tr>
<td>4</td>
<td>5 3</td>
</tr>
<tr>
<td>3</td>
<td>10 5</td>
</tr>
<tr>
<td>Total</td>
<td>17 10</td>
</tr>
</tbody>
</table>

### Table 3: Effects of treatment of patients between 4 to 6 years of age (Group A and B) (n=18)

<table>
<thead>
<tr>
<th>GMFCS Levels before treatment</th>
<th>No. of patients progressed after treatment (GMFCS Levels)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level 1</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>5</td>
<td>A B</td>
</tr>
<tr>
<td>4</td>
<td>2 5</td>
</tr>
<tr>
<td>3</td>
<td>5 4</td>
</tr>
<tr>
<td>Total</td>
<td>8 10</td>
</tr>
</tbody>
</table>
According to the Table 4, Inter group analysis of pre treatment and post treatment progress levels of both the groups revealed significant 'p' value (p < 0.05). The 'p' value of treated group is 9.44E-13 whereas 'p' value of physiotherapy group is 3.32E-08.

Conclusion

Ayurvedic treatment regimen and Physiotherapy have the capacity to improve the gross motor functions of Balaka Pakshaghatha/Cerebral Palsy. However, ayurvedic treatment regimen is highly effective than that of Physiotherapy treatment in the management of Balaka Pakshaghatha/Cerebral Palsy. Further studies are proposed to evaluate the efficacy of combine therapy, i.e. Ayurvedic treatment regimen and Physiotherapy for the management of Balaka Pakshaghatha/Cerebral Palsy.

Reference

5. Ailapperuma IAS. Vatika Prakaranaya Havest Guli Kalka Pota, Jinalankara Piriwena, Chandra kalka Anupana. 1915; 221: 2424.

Table 4: Intra Group analysis of the overall effect of Ayurvedic Treatment Regimen and Physiotherapy Treatment regimen

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean BT</th>
<th>SD BT</th>
<th>t</th>
<th>P</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayurvedic</td>
<td>4.13333</td>
<td>2.33333</td>
<td>0.62881</td>
<td>11.64127</td>
<td>9.44E-13</td>
</tr>
<tr>
<td>(Group A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significant</td>
</tr>
<tr>
<td>Physio.</td>
<td>4.27586</td>
<td>3.41379</td>
<td>2.06781</td>
<td>7.260223</td>
<td>3.32E-08</td>
</tr>
<tr>
<td>(Group B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Significant</td>
</tr>
</tbody>
</table>

P values are significant at p < 0.05
Clinical efficacy of Dashamoola Taila Matra Vasti on management of primary dysmenorrhoea

Kaumadi Karunagoda¹, Shilpa Donga², Lakshmi Priya Dei³

Abstract

Primary dysmenorrhoea is the most common gynaecological complaint among young women. Prevalence of dysmenorrhoea is estimated as 45% to 85% among reproductive aged women. It is a condition which has no any underline pelvic pathology or anatomical defect. In modern sciences, nonsteroidal anti inflammatory drugs and oral contraceptive pills are used as a symptomatic treatment for this condition but they are having many side effects and they are not curative. Hence study was carried out to find out a reliable and longlasting Ayurvedic management for the condition and to find out the efficacy of Dashamoola Taila Matra Vasti on primary dysmenorrhoea. The dose of Dashamoola Taila Matra Vasti was 60 ml and duration was 7 days for two consecutive cycles. Results were assessed on the basis of specially prepared grading system for pain. The results obtained were highly significant. Total effect of therapy was, 38.89% got complete remission while marked improvement was there in 50%. In the follow up period no patient complaint recurrence of symptoms. The study suggests that Dashamoola Taila Matra Vasti can be established as a reliable longlasting treatment for relieving primary dysmenorrhoea.

Introduction

Dysmenorrhoea is a medical condition characterized by severe uterine pain during menstruation. While many individuals experience minor pain during menstruation, dysmenorrhoea is diagnosed when the pain is so severe as to limit normal activities, or requires medication [1]. It has been defined as painful menstruation of sufficient magnitude so as to incapacitate day to day activity [2]. Primary dysmenorrhoea is the most common gynaecological complaint among young reproductive age women. By 40 high quality studies, prevalence of dysmenorrhoea is estimated as 45% to 85% among reproductive aged women [3]. It is a condition which has no any underline pelvic pathology or anatomical defect [4]. There is several pathophysiology and risk factors have been identified as a etiology of this condition.

Treatment in practice for the condition depends upon analgesics and oral contraceptive drugs which give several unwanted effects as well as short term. Several Ayurvedic oral therapies give significance results on management of primary dysmenorrhoea without adverse effect, but its long lasting effect is debatable. Though Uttara Vast has proven long lasting effect on this condition it cannot be implemented to unmarried girls who are the most common sufferers of Primary dysmenorrhoea [4].

Pain is the main feature of primary dysmenorrhoea, so it has strong relation with Vata Dosha. Matra Vasti was taken as a treatment since Vasti has been mentioned as one of the best therapeutic procedure for alleviation of vitiated Vata [5]. The present study was carried out as a very preliminary step to find out a reliable and longlasting Ayurvedic management for the condition and to find out the efficacy of Dashamoola Taila Matra Vasti on primary dysmenorrhoea.

Materials and Methods

Selection of drug

Kashtartava especially manifesting as primary dysmenorrhoea is a Vata predominant condition and selected drugs are also good Vatashamaka drugs as mentioned in classics. Dashamoola Taila has been mentioned for the treatment of Udavarta Yonivyapad [6], which is one of the main disease conditions coming under primary dysmenorrhoea in Ayurveda.

Contents of Dashamoola [7, 8] are Aegle marmelos Corr., Premna integrifolia L., Oroxylum indicum Vent., Steriospermum suaveolens DC, Gmelina arborea Roxb., Desmodium gangeticum DC., Uraria picta Desv.,

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Ten ingredients of dried Dashamoola are collected from the pharmacy, identified with the help of organoleptic and powder microscopic studies. Equal amount of Dashamoola made in to Yavakuta form is dipped in water for overnight and next day Kwata is prepared. This Kwata along with Kalka of Dashamoola is added in Tila Taila (sesame oil) and oil is prepared as per standard protocol [9].

Selection of patients

Patients attending the O.P.D. and I.P.D. of Department of Striroga and Prasutitantra, Institute of Postgraduate Training and Research in Ayurveda, Gujarat Ayurved University, Jamnagar complaining of pain in menstruation and fulfilling the criteria of inclusion were selected for the present study. An elaborative case taking proforma was specially designed for the purpose of incorporating all aspects of the disease on Ayurvedic and modern parlance. Patients of age group between 15-25 years, coming with chief complaint of painful menses from more than 3 cycles with scanty or average amount of menses. Patients below 15 years and above 25 years, patients with chronic illness, patients with intrauterine contraceptive devices, patients with menorrhagia or any uterine pathology – fibroid, adenomyosis, endometriosis etc. were excluded from the study.

Investigations

Routine haematological and urinary examinations were done before and after treatment. Sonography for uterine and adnexal study was done for exclude pathological cases.

Method of administration

Daily 60 ml of Dashamoola Taila was administered in morning hours through rectal (Matra Vasti) for 07 days for two consecutive menstrual cycles starting from mid cycle. After stopping the administration of the drugs under trial, patients were advised to report weekly for follow up study, which was carried out for 2 months.

Method of administration of Matra Vasti

The patient was advised to take light meal, not more than 3/4th of the usual quantity. Before administration of Vasti, Abhyanga (application of oil) with Tila Taila was done on the region of lower back and lower abdomen. Thereafter, Nadi Sweda was performed.

After this pre preparatory measures (Purvakarma), the patient was advised to take left lateral position with left lower extremity straight and right lower extremity flexed on knee and hip joint. The patient was asked to keep her left hand below the head. 60 ml of lukewarm Taila was taken in enema syringe. Rubber catheter oiled with Taila was attached to enema syringe. After removing the air from enema syringe, rubber catheter was administered into the anus of the patient up to the length of 4 inches. The patient was asked to take deep breath while introducing the catheter and drug.

Criteria of assessment

The effect of the therapy was assessed considering to the overall improvement in signs and symptoms. For this purpose, following categories were maintained.

Severity of pain (multidimensional scoring pattern)

0 Menstruation is not painful and daily activity unaffected
1 Menstruation is painful and daily activity not affected. No analgesic required.
2 Menstruation is painful and daily activity affected. Analgesic drug were needed.
3 Menstruation is painful, she cannot do even her normal routine work and has to absent from class / office during menses. Had to take analgesic but poor effect.

Duration of pain

0 no pain in menstruation
1 pain persist less than 12 hours
2 pain continue for 12 -24 hours
3 pain continue more than 24 hours

Criteria for the assessment of overall effect of the therapies:

- Complete remission: 76%-100% relief in the signs and symptoms were considered as complete remission.
- Marked improvement: 51%-75% relief in the signs and symptoms were considered as markedly improvement.
- Improved: 26%-50% relief in the signs and symptoms.
- Unchanged: Below 25% relief in the signs and symptoms were considered as unchanged.

Investigations: Laboratory investigations – Hb%, WBC/DC, ESR, PCV, were carried out before and after treatment to rule out any other pathological conditions as well as to record any specific change by the treatment.

Results

Total 20 patients were registered for the study among them 18 patients had completed the treatment and 02 left against medical advice. So observations and results drawn from 18 completed patients.
Table 1: Risk factors

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>No. of patients (n=18)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early age (&lt;20 years)</td>
<td>14</td>
<td>77.77%</td>
</tr>
<tr>
<td>Early menarche (12-13 years)</td>
<td>11</td>
<td>61.11%</td>
</tr>
<tr>
<td>Positive family history</td>
<td>12</td>
<td>66.66%</td>
</tr>
<tr>
<td>Lose weight (BMI &lt;20)</td>
<td>6</td>
<td>33.33%</td>
</tr>
<tr>
<td>Null parity</td>
<td>15</td>
<td>83.33%</td>
</tr>
<tr>
<td>Smoking</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 2: Features related to primary dysmenorrhoea

<table>
<thead>
<tr>
<th>Features</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronicity (4-6 years)</td>
<td>9</td>
<td>50%</td>
</tr>
<tr>
<td>Onset of pain (1 day before)</td>
<td>11</td>
<td>54.28%</td>
</tr>
<tr>
<td>Aggravation of pain (1st day)</td>
<td>17</td>
<td>94.28%</td>
</tr>
<tr>
<td>Severity of pain (grade 2)</td>
<td>12</td>
<td>65.71%</td>
</tr>
<tr>
<td>Site of pain (Hypogastrium)</td>
<td>18</td>
<td>100%</td>
</tr>
<tr>
<td>Duration of pain (12-24hrs.)</td>
<td>10</td>
<td>60%</td>
</tr>
</tbody>
</table>

Table 3: Effect of therapy (% of relief)

<table>
<thead>
<tr>
<th>Associated symptoms</th>
<th>% of relief</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity of Pain</td>
<td>75%</td>
</tr>
<tr>
<td>Duration of Pain</td>
<td>68.33%</td>
</tr>
</tbody>
</table>

Table 4: Effect of therapy (paired t test)

<table>
<thead>
<tr>
<th>No of pt.</th>
<th>Mean</th>
<th>%of relief</th>
<th>S.D.</th>
<th>S.E.</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity of pain</td>
<td>18</td>
<td>2.23</td>
<td>0.67</td>
<td>70%</td>
<td>0.922</td>
<td>7.159</td>
</tr>
<tr>
<td>Duration of pain</td>
<td>18</td>
<td>2.00</td>
<td>0.83</td>
<td>63.89%</td>
<td>0.826</td>
<td>6.559</td>
</tr>
</tbody>
</table>

Table 5: Total effect of therapy

<table>
<thead>
<tr>
<th>Effect of therapy</th>
<th>No. of pts.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete remission (76%-100% relief)</td>
<td>07</td>
<td>38.89%</td>
</tr>
<tr>
<td>Marked improvement (51%-75% relief)</td>
<td>02</td>
<td>11.11%</td>
</tr>
<tr>
<td>Improved (26%-50% relief)</td>
<td>09</td>
<td>50%</td>
</tr>
<tr>
<td>Unchanged (25%-0% relief)</td>
<td>00</td>
<td>0%</td>
</tr>
</tbody>
</table>
Discussion

In this study maximum numbers of patients were suffering from dysmenorrhoea since 4-6 years (Table 2) among them grade 2 type of severity was found in 65.71%. The 48.71% of patients were on analgesics/antispasmodics (Table 3). The data are suggestive of the chronicity of the problem and also supports the reality that even people suffering from such common problem visit Ayurvedic clinic quite late and after taking several other trials.

Majority (54.28%) of them had onset of pain one day before to the onset of menstruation (Table 3), pain aggravation on first day on menstruation was found in 94.28% and pain persisted for 12 to 24 hours in 60% (Table 3). These observations show typical characters of primary dysmenorrhoea [3]. It is a known fact that two days prior to onset of menstruation, large quantity of progesterone and estrogen secretes from corpus luteum. This high level of progesterone induce increases the tone in the isthmus and upper part of the cervix [4]. An exaggeration of this could therefore be the basis of the non-coordinating action of the uterus. Again high level of ovarian hormones stop FSH (Folicular Stimulating Hormone) and LH (Lutinising Hormone) secretion causes sudden stoppage in secretion of progesterone and oestrogen which results to menstruation. Withdrawal of progesterone preceding menstruation probably causes break down of lysosomes and the synthesis of various prostaglandins [10]. These prostaglandins are responsible for pain in menstruation by myometrial contraction, vasoconstriction and increase sensitivity of nerve endings for pain [11]. It is the course of strong pain on starting of menstruation and withdraws after 24 hours as prostaglandins are of short lifespan [12].

On the basis of site all the patients showed pain in hypogastrum (Table 3), while in 71.43% complain pain radiated towards inner and front aspect of the thighs. It could be because sympathetic nerves, arising from segments T5 and T6 in the case of motor nerves and from segment T10 to L1 in the case of sensory nerves, pass down from the celiac plexus through the intermesentric plexus, lying retroperitoneally in the front of the abdominal aorta [13].

Discussing the risk factors of disease, it is similar to those mentioned for dysmenorrhoea. Early onset of menarche and early age; below 22 years, which are mentioned as risk factors for dysmenorrhoea, were found in 61.11% and 77.77% (Table 2) patients respectively.

The theory postulated behind this finding is that in this age, pituitary and other endocrine gland do not attain their maturity till the age of 20 [14]. It can lead to hormonal imbalance and thus, dysmenorrhoea.

Concept of 'Bija' given as the Nidana of Yonivyapada in classics was supported by the data obtained, as positive family history was found in 66.66% (Table 2) of patients. It suggested that there is a definite relation of a person’s genetic trait and Prakriti with the condition of dysmenorrhoea. Nulliparity, which is considered as the risk factor for primary dysmenorrhoea was found in 83.33% (Table 2) of patients. It is believed that vaginal delivery removes the stenosis or pin hole of cervical canal and internal os [1] and thus, facilitates the flow of menstrual blood. It reduces that pain during menstruation, because the flow of blood through the cervix becomes easier. Lose weight also a risk factor given as per modern science. Present study it is noticed 33.33% (Table 2) patients are below 20 in B.M.I. (Body Mass Index). It supports the modern findings regarding in this issue [15].

Effect of therapy on primary dysmenorrhoea shows 75% relief on severity of pain and 68.33% relief in duration and (Table 4) according to ‘t’ value highly significant results (P < 0.001) on both the components (Table 5) evidence that treatment is effective on both severity as well as duration of menstrual pain. When considering total effect of therapy 50% of patients got complete or marked remission. Not a single show negative respond to the therapy (Table 6).

In follow up period, most of the patients show prolong and lasting effect on primary dysmenorrhoea. The prolongation in recurrence of symptom can be due to the strong anti-inflammatory and analgesic property of Dashamoola. It is a proven drug, effective on primary neurological disorder and improves nerve conduction velocity [16]. This effect of Dashamoola on nervous system can be the responsible factor behind its lasting effect.

Conclusion

Dashamoola Taila Matra Vasti is effective to relieve primary dysmenorrhoea. Matra Vasti seems to be better than oral analgesics and oral contraceptive pills used in dysmenorrhoea, because it is found efficacious in whole the feature complex related to dysmenorrhoea. Dashamoola Taila Matra Vasti helps to protect from the recurrence of dysmenorrhoea. With some further researches, Dashamoola Taila Matra Vasti can be established as line of treatment for Primary dysmenorrhoea.

Reference


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In vitro evaluation of different aqueous extracts of Senna alata leaves for antibacterial activity

E Christy Jeyaseelan1, S Tharmila1, A C Thavaranjith

Abstract

The present study was to enrich the knowledge of antimicrobial activity of aqueous extracts (cold, hot and fresh juice) of leaf of Senna alata (L.) Roxb. and to confirm their effect through qualitative phytochemical analysis. The preliminary antibacterial assay was performed against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Proteus vulgaris by agar well diffusion method. The Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the cold and hot extracts were determined by macro broth dilution method. Streptomycin and sterile distilled water were used as the standard and control respectively. Qualitative phytochemical analysis was done to identify the chemical compounds present in the extracts. The antibacterial activity of the test extracts differed significantly (P <0.05); the hot and cold extracts were able to inhibit the growth of all tested bacteria, while the fresh juice inhibited only B. subtilis and S. aureus. The cold extract revealed significantly (P <0.05) higher inhibition on all test bacteria except B. subtilis, which was highly inhibited by fresh juice. The MIC of the cold extract ranged from 5 mg/ml to 40 mg/ml. The lowest value was against P. vulgaris. The MBC of the cold and hot extracts ranged from 20 mg/ml to 160 mg/ml. Phytochemical analysis revealed the presence of glycosides, alkaloids, saponins, cardiac glycosides, tannins, phlobatannins, flavonoids, terpenoids and anthraquinones. This study has proved the feasibility of in vitro control of tested bacteria by aqueous extracts of S. alata leaves.

Introduction

Senna alata (L.) Roxb (formerly known as Cassia alata Linn.) (Family-Fabaceae) (Tamil-Vandugolli, Sinhala-Alata) is a tropical plant, widespread in South East Asian countries [1]. It is a large shrub with very thick, finely downy branches; leaves large, sub sessile and pinnate; flowers are irregular, bisexual, golden yellow in spiciform pedunculate racemes; pod is long, ligulate with a broad wing down the middle of each valve [2]. This plant is traditionally used for the treatment of various ailments including several infections caused by bacteria, protozoa, fungi and viruses. The aqueous leaf extracts are used in the treatment of ringworm and parasitic skin diseases [3]. In Belgian Congo, the plant is employed as a remedy for leprosy. In Ghana the leaves are crushed, mixed with black peppers and applied on dhoby's itch, claw - claw and ringworm on the head and skin. In the Pacific Island and Mauritius the leaves are used for skin disease [2].

Owoyale et al. (2005) carried out antibacterial and antifungal screening of different organic solvent extracts of Senna alata, and they reported that the flavonoid glycoside is an active ingredient of the extracts [4]. Adedayo et al. (1999) demonstrated the antifungal activity of methanol crude extract and partially purified fractions of flowers of Senna alata against standard and local fungal isolates [5]. Sule et al. (2010) reported the in vitro control of fungi; Microsporum canis, Trichophyton jirrucosum, Trichophyton mentagrophytes and Epidermophyton jirrucosum using hot ethanolic leaf extract of Senna alata [6]. The present study is an attempt to enrich the knowledge of antibacterial activity of different forms of aqueous extract of leaf of S. alata against some selected bacteria known to be pathogenic in human. The phytochemical components were also investigated as a scientific assessment of the claim of therapeutic potency of the extracts.

Materials and Methods

Preparation of plant extracts

The healthy plant leaves were collected from botanical garden of Department of Botany, University of Jaffna, Sri Lanka. They were dried in shade. Completely dried leaves were ground into fine powder using an electric blender. The powder was used to get cold and hot aqueous extractions as described below.

a). Cold extract

20 g powder was soaked in 60 ml sterile distilled water with intermittent shaking for one hour at ambient temperature. Then the mixture was filtered through doubled layered muslin cloth and the filtrate was further filtered through Whatman no 1 filter paper. The filtrate was completely dried in an oven at 45 °C [7].

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b). Hot extract
20 g powder was soaked in 60 ml sterile distilled water and kept in boiling water bath with intermittent shaking for one hour. Then the mixture was filtered through double layered muslin cloth and the filtrate was further filtered through Whatman no 1 filter paper. The filtrate was completely dried in an oven at 45 °C [8].

c). Fresh extract
20 g fresh healthy leaves were crushed with 20 ml distilled water using motor and pestle. The crushed material was filtered through two layered muslin cloth, and the filtrate was further filtered through Whatman no 1 filter paper. The filtrate was immediately used for the study [9].

Test microorganisms
Test bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were obtained from a bacterial culture collection, Department of Botany, University of Jaffna, Sri Lanka.

Phytochemical analysis
The phytochemical analysis of the fresh leaf juice was carried out to determine the presence of the following biomolecules using the standard qualitative procedures as described by Trease and Evans (1989) [10].

a). Test for tannins
1 ml of distilled water and one to two drops of ferric chloride solution were added to 0.5 ml of extract solution and observed for brownish green or a blue black coloration.

b). Test for terpenoids
5 ml of extract was mixed with 2 ml of CHCl₃ in a test tube. 3 ml of concentrated H₂SO₄ was carefully added along the wall of the test tube to form a layer. An interface with a reddish brown coloration indicated the presence of terpenoids.

c). Test for steroids
0.5 ml of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated H₂SO₄ was added slowly. Bluish green color was observed for steroids.

d). Test for saponins
5 ml of extract was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with three drops of olive oil and observed for the formation of an emulsion, which indicated the presence of saponins.

e). Test for flavonoids
A few drops of 1% NH₃ solution was added to the 2 ml of extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

f). Test for cardiac glycosides
1 ml of concentrated H₂SO₄ was taken in to a test tube. 5 ml of extract was mixed with 2 ml of glacial CH₃CO₂H containing one drop of FeCl₃. The above mixture was carefully added to the 1 ml of concentrated H₂SO₄. Presence of cardiac glycosides was detected by the formation of a brown ring.

g). Test for phlobatannins
10 ml of extract was boiled with 1% HCl in a boiling tube. Deposition of a red precipitate indicated the presence of phlobatannins.

h). Test for Alkaloids
1ml of 1% HCl was added to the 3 ml of extract in a test tube. Then it was treated with a few drops of Meyer’s reagent. A creamy white precipitate indicated the presence of alkaloids.

i). Test for Resins
5 ml of copper solution was added to the 5 ml of extract. The resulting solution was shaken vigorously and allowed to separate. A green precipitate indicated the presence of resin.

j). Test for Glycosides
10  ml of 50% H₂SO₄ was added to the 1 ml of extract in a boiling tube. The mixture was heated in a boiling water bath for 5 min. 10 ml of Fehling’s solution (5 ml of each solution A and B) was added and boiled. A brick red precipitate indicated the presence of glycosides.

k). Test for Anthraquinones
Extract was mixed well with benzene, and then half of its own volume of 10% ammonia solution was added. Presence of a pink, red or violet coloration in the ammonial phase indicated the anthraquinones.

Determination of antibacterial activity
Agar well diffusion method was used to determine the antibacterial activity. 20 ml molten nutrient agar media were mixed with 1 ml of 10⁶ colony forming units/ml each test bacterial inoculum and poured into sterile Petri dishes separately. After complete solidification, 8 mm diameter wells were made using sterile cork borer.

The cold and hot test extracts were dissolved in distilled water. The wells were filled with filter sterilized 100 μl of (50 mg) cold extract, (50 mg) hot extract, fresh juice, (50 μg) streptomycin and sterile distilled water. Streptomycin and sterile distilled water were used as standard and control respectively. The plates were incubated at 37°C for 24 hours and antibacterial activity was determined by measuring the diameter of clear zone around the well using Vernier caliper [11]. Each experiment was repeated three times.
Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration was determined by the macro broth dilution method [12]. The hot and cold extracts were diluted to 320, 160, 80, 40, 20, 10, 5, 2.5, and 1.25 mg/ml and the streptomycin was diluted from 5.12 mg/ml to 0.02 mg/ml as two fold dilution in nutrient broth. The tubes were inoculated with 1.0 ml (0.5 McFarland standards) of test bacteria and incubated at 37°C for 24 hours. The MIC was taken as the lowest concentration of test samples that did not permit any visible growth. For the determination of MBC, two loops full of culture were taken from each of the broth tubes that showed no growth in the MIC tubes and inoculated onto fresh nutrient agar plates. After 24-hour incubation, the plates were observed for the growth of bacteria. The concentrations of the extracts that showed no growth were recorded as the MBC. Each experiment was repeated three times.

Statistical analysis

Results were expressed as mean ± SD of three experiments. Statistical significance was determined using analysis of variance and Tukey test at p = 0.05 using statistical software SPSS Windows version 13.0.

Results

The qualitative tests for the presence of phytochemicals revealed that the fresh juice of S. alata possess glycosides, alkaloids, saponins, cardiac glycosides, tannins, phlobatannins, flavonoids, terpenoids and anthraquinones. But, the tests for steroids and resins did not show positive results (Table 1).

The hot and cold extracts were able to inhibit the growth of all test bacteria, while the fresh juice failed to inhibit the growth of E. coli, P. vulgaris and P. aeruginosa. The cold extract showed significantly highest inhibition on all test bacteria except B. subtilis compared to other two test extracts and the largest zone of inhibition was produced against P. vulgaris (23.1 ± 0.2 mm). There was no significant difference between the inhibitory effects produced by the cold and hot extracts on S. aureus. The B. subtilis was highly inhibited by the fresh juice of S. alata leaf (Table 2).

The antibiotic streptomycin inhibited the growth of all test bacteria except P. aeruginosa. In most of the cases the diameter of clear zone produced by the (50 mg/ml) crude test extracts on test bacteria were found to be larger than that produced by the (50 µg/ 100 µl) streptomycin to the respective bacteria (Table 2).

The minimum inhibitory concentration (MIC) of hot and cold extracts ranged between 5 mg/ml and 80 mg/ml. The lowest MIC value, 5 mg/ml was exerted by cold extract on P. vulgaris. The required MIC value of the cold extract for E. coli, P. vulgaris and P. aeruginosa were found to be lower than the hot extract. However, for B. subtilis and S. aureus, the required MIC values were equal in both hot and cold extracts. The MIC value of streptomycin was found between 0.04 mg/ml and 1.28 mg/ml. The minimum bactericidal concentration (MBC) of test extracts ranged from 20 mg/ml to 160 mg/ml, and the hot and cold extracts revealed the lowest MBC against P. vulgaris (Table 3).

Table 1: Phytochemical constituents of fresh leaf juice of Senna alata

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Presence / Absence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+ present, - absent</td>
</tr>
</tbody>
</table>

Table 2: Antibacterial activity of different form of aqueous extracts of S. alata leaf

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diameter of inhibition zone (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>Fresh extract</td>
<td>20.6±0.6a</td>
</tr>
<tr>
<td>Cold extract</td>
<td>14.6±0.7b</td>
</tr>
<tr>
<td>Hot extract</td>
<td>13.8±0.4b</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>14.5±0.6</td>
</tr>
</tbody>
</table>

- No activity; * Zone of inhibition includes the diameter of well (8 mm); Values are mean ± SD, Values with different superscript on the same column are significantly (P < 0.05) different.
The present study was undertaken to determine the feasibility of in vitro control of bacteria by using three different forms of aqueous extracts of leaf of Senna alata. In indigenous medicine, these three forms of extracts are widely used for the treatment of various diseases. The results revealed that both cold and hot extracts were more effective than fresh juice, and these two extracts were able to inhibit the growth of both Gram negative and Gram positive bacterial species selected for this study.

The activity of the plant extracts against both Gram positive and Gram negative bacteria is an indication of the presence of broad spectrum antibacterial compounds [13]. Generally Gram positive bacteria shows higher sensitivity to plant extracts than Gram negative bacteria [14-17]. This variation is due to the differences in the cell wall structure and composition of Gram positive and Gram negative [18]. In this study, even though the cold extract had broad spectrum of activity, the Gram negative bacteria were highly inhibited than Gram positive bacteria. This suggests that there might be specific substances which inhibit the growth of Gram negative bacteria more.

The lower or absence of bacterial growth inhibition by the fresh juice of the leaf may be due to the lower concentration of active ingredients which are toxic to bacteria. Further study with higher concentration may give better inhibition. The amount of active ingredients in plant extracts depend on the climate conditions where the plants grow. Wandee (2010) reported that the amount of anthraquinone glycosides in the leaves of S. alata varied with season. In winter (November-February) and summer (March-May) plants contain the highest amount of total anthraquinone glycosides (1.24% dry weight). But, the samples collected in rainy season (June-October) contain only 0.16% dry weight [1]. In the present study, the sample was collected from botanical garden where the plant is irrigated well. Therefore, the amount of active ingredients may be lower than that grow in wild.

The inhibitory effect of a plant extract resulted from the activity of phytochemicals was present in the extract. The type of phytochemical present in an extract depends on the type of solvent used for the extraction and the mode of extraction. In this study, the plant material was extracted in three different methods with water. It can be clearly seen the variation in the inhibitory effect with the variation of extraction method (Table 2).

The result of this experiment correlates with a former study, where the E. coli, S. aureus and P. aeruginosa were inhibited by aqueous leaf extract of S. alata in agar well diffusion method. In a previous study done by Okoro et al. (2010) documented that S. aureus was susceptible to polyphenol extracts of S. alata, while E. coli appeared to be resistant to the extracts [19]. But in the present study both bacteria were inhibited by aqueous extracts. In another study, hot (soxhlet) aqueous leaf extract of S. alata failed to inhibit the growth of S. aureus and P. vulgaris, where the antimicrobial screening was performed by agar disk diffusion method [20]. The variation in the results may be due to the variation in the extraction method or method of antibacterial screening or by both. It was already reported that agar well diffusion method is more effective than disc diffusion method for antibacterial screening as filter paper disc composed of cellulose where many free hydroxyl groups present on each glucose residues makes the surface of the disc hydrophilic [21], and therefore if an extract contains cationic active constituents with a good antibacterial activity it will not be expressed in disc diffusion method [22]. In the present study the hot extract showed comparatively lower activity than cold extract. Generally, treatment of plant extracts to high temperature could inactivate volatile compounds, but could also increase the release of active components and free radicals [7].

The result for the qualitative phytochemical analysis also correlates with some previous studies [4,7]. It has been reported that different phytoconstituents have

<table>
<thead>
<tr>
<th>Test bacteria</th>
<th>MIC (mg/ml)</th>
<th>MBC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hot</td>
<td>Cold</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>E. coli</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>P. vulgaris</td>
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<td>5</td>
</tr>
<tr>
<td>S. aureus</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>80</td>
<td>20</td>
</tr>
</tbody>
</table>
different degree of solubility in different type of solvents depending on their polarity [7]. In traditional preparations water is largely used as the solvent.

The inhibition of tested bacteria by S. alata leaf extracts confirmed their antibacterial activity and this is most likely due to the action of different phyto-constituents present in the extract. Owowale et al. (2005) reported that the antimicrobial activity of S. alata is associated with the presence of phytochemicals such as phenols, tannins, saponins, alkaloids, steroids, flavonoids and carbohydrates [4]. Flavonoids act as cytoplasmic poisons and also they have been reported to inhibit the activity of enzymes [23]. Saponins are surface active agents which interfere with or alter the permeability of the cell wall. Therefore, this facilitates the entry of toxic materials or leakages of vital constituents from the cell. Tannins act by coagulating the cell wall proteins [24]. Anthraquinones react irreversibly with amino acids in proteins, often leading to inactivation of the protein and loss of function. The alkaloids have ability to intercalate with DNA [6].

The standard antibiotic streptomycin showed higher activity with lower MIC and MBC values compared to the test extracts. Streptomycin is refined and purified product, whereas the test extracts are a mixture of varies plant constituents. Some of these constituents can interfere within them and this ultimately affects the antibacterial activity of the extract [7]. Therefore, further study with bioassay guided fractionation and isolation of pure compound(s) is necessary to authenticate the effect of these extracts.

Conclusion

The results of the present study have confirmed the long history of the use of aqueous leaf extracts of S. alata in traditional medicine for the treatment of microbial infections. Even though the hot and cold extracts had inhibition on all test bacteria, the cold extract showed comparatively better effect. Therefore, cold aqueous leaf extract of S. alata can be used for antibacterial treatment and antibacterial drug screening.

References


Jeyaseelan et al. Antibacterial activity of Senna alata leaves... SLJIM 2011;01(02): 64-69


Selection of the most suitable pot height and harvesting stage for higher growth, yield and oil quality of Vettiver (Vetiveria zizanioides)

N D N Priyadarshani¹, M K T K Amarasinghe¹, S Subasinghe¹, I R Palihkakara¹, H K M S Kumarasinghe¹

Abstract

Vetiveria zizanioides (L.) Nash is a valuable medicinal and aromatic plant used in both indigenous medicine and perfumery industry. Economically most important part of the Vettiver is root system. Vettiver roots are directly used for the medicinal purposes and indirectly for extraction of essential oils. Low yield and poor quality roots as well as oil are the problems associated with Vettiver production. Yield and quality of Vettiver roots depend on climatic conditions, growing media, agronomic practices, time of harvesting etc. Objective of the present study was to select the most promising pot height and harvesting stage in order to enhance bio-mass production, oil content and quality of Vettiver. A pot experiment was conducted at Medicinal Plant Garden, Faculty of Agriculture, from March 2008 to April 2009. Three pot heights, namely, 35, 40 and 45 cm with four different harvesting intervals such as 3, 6, 9 and 12 months after planting were used for this experiment. Data on number of tillers, number of leaves, dry weight of roots and shoots were recorded at 3, 6, 9 and 12 months of planting as different harvesting stages. Root oil contents, chemical composition of oils such as Khusimol, β-Vetivenene, β-Vetivone, α-Vetivone, Iso-valencinol and fiber content were also analyzed. Results revealed that, Vettiver planted in 45 cm pot height showed higher biomass production. Oil content of Vettiver increased with the increasing harvesting intervals. Higher oil content (2.15%) was recorded 12 months after planting. Subsequently higher oil percentage (2.13%) was recorded in 9 months after planting. However, there were no significant differences between oil content of 9 and 12 months after planting. It was also observed in the present study that the Vettiver harvested at 9 months of planting had significantly (P< 0.05) high Khusimol (14.5%), β-Vetivone (1.4%) and Iso-valencinol (4.9%) contents in root oil. Relatively lower fiber contents (36%) were associated with 9 months after planting compared to 12 months after planting. Therefore, Vettiver planted in 45 cm pot height and roots harvested at 9 months after planting could be used as most promising pot height and harvesting interval in order to enhance bio-mass production, oil content and quality of Vettiver.

Introduction

Vetiveria zizanioides (L.) Nash (Sinhala – Sevendara, Tamil – Vettiver) which belongs to the family Poaceae is one of the most important medicinal and aromatic plants widely used in indigenous medicine and perfumery industry. Vettiver oil is one of the most valuable product of Vettiver roots. Vettiver oil has 442 extensive applications in the soap and cosmetic industries, pharmaceutical companies and as antimicrobial and anti-fungal agent [1]. In Sri Lanka, annual national demand for Vettiver is 41175 Kg (dry basis) and this is valued as 4 million rupees [2]. The root system of Vettiver consists of long fibrous roots and rootlets. These roots grow more than 2 m in depth and about 80% of the roots can be found in the first 30-35 cm [3]. Even after the careful harvesting, 40% of the roots remain in the soil yielding highly damaged roots. One of the main problems in Vettiver production is poorly developed low quality roots. These roots produce lower oil yields as well as low quality oils. Such problems in Vettiver production could be avoided by adopting proper agronomic and crop management practices. Therefore, the present study was carried out to select the most promising pot height and harvesting stage in order to enhance bio-mass production, oil content and quality of Vettiver.

Materials and Methods

A pot experiment was conducted from March 2008 to April 2009 at Medicinal Plant Garden, Faculty of Agriculture, University of Ruhuna. Three pot heights, namely, 35, 40 and 45 cm with four different harvesting intervals such as 3, 6, 9 and 12 months after planting were used for this experiment. Three different heights of black polythene bags were filled using top soil: sand (1:2). Leaves of tillers were cut down by keeping 3 cm from the base. Tillers were planted in pots keeping one tiller per pot. Pots were arranged in a Completely Randomized Design (CRD) with four replicates. Watering was done at

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two day intervals up to four weeks after planting and thereafter plants were subjected to rain fed condition. Hand weeding was practiced at two month intervals.

As non destructive measurements, number of tillers per bush and number of leaves were taken in 3, 6, 9 and 12 months after planting. Roots were harvested manually and roots were air dried in the laboratory for three weeks period to a constant weight. Dry weight of roots and shoots were taken in Vetiver roots harvested at 3, 6, 9 and 12 months after planting as different harvesting stages. Total root oil content, chemical composition of oil (Khusimol, β-Vetivenene, β-Vetivone, α-Vetivone, Iso-valencinol) and fiber content were determined. Vetiver root samples were air dried in the laboratory for three weeks period and roots were cut into 1 cm length of root pieces using a secatier. Then prepared root samples were used for the oil extraction and the residue after the oil extraction was used for the analysis of fiber content of Vetiver roots with the four replicates from each treatment. Samples were subjected to AOAC method for determination of crude fiber [4]. Oil content and chemical compounds in oil were analyzed using Steam Distillation Procedure and Gas Chromatography Internal Normalization method, respectively. Data with percentage values were subjected to angular transformation where necessary and analyzed using ANOVA (analysis of variance) with Statistical Analysis System (SAS version 6.12).

Results

Effect of different pot height and harvesting stages on biomass production of Vetiver

A pot height of 45 cm (T₃) showed higher root (dry) weights 84.5 g, 242 g, 641 g and 777 g respectively at 3, 6, 9 and 12 months after planting. At 9 months after planting it was more than double the root (dry) weight at 6 months after planting (Figure 1).

Higher shoot weights (dry) were recorded in 45 cm pot height (T₃) 192.25 g, 584g, 1572.8 g and 1836.8 g respectively at 3, 6, 9 and 12 months after planting (Figure 2).

Figure 1: Changes in dry root weight of Vetiver as affected by different pot heights (cm) at different harvesting stages (3, 6, 9 and 12 months after planting) (α=0.05). T₁-35 cm, T₂-40 cm T₃-45 cm.

Figure 2: Changes in shoot dry weight of Vetiver as affected by different pot heights (cm) at different harvesting stages (3, 6, 9 and 12 months after planting) (α=0.05). T₁-35 cm, T₂-40 cm T₃-45 cm.
Pot height had not shown significant differences (P>0.05) in number of leaves up to 3 months after planting. However, numbers of leaves were significantly (P<0.05) affected by pot height after 6 months of planting. Significantly higher (P<0.05) number of leaves of 215, 471 and 543 were recorded in 45 cm pot height (T₃) at 6, 9 and 12 months after planting respectively (Figure 3).

Similarly, pot height had not shown significant differences (P>0.05) in number of tillers up to 3 months of planting. However, it was significantly affected by pot height after 6 months of planting. A significantly higher (P<0.05) number of tillers of 36, 67 and 79 was recorded in 45 cm pot height (T₃) at 6, 9 and 12 months after planting respectively (Figure 4).

All the growth and yield parameters (root dry weight, shoot dry weight, number of leaves and number of tillers) of Vetiver were higher in 45 cm pot height (T₃) compared to other treatments (pot height of 35 and 40 cm).

Effect of different harvesting stages on oil content and quality of Vetiver

Oil content of Vetiver increased with the increasing harvesting intervals. Highest oil content (2.15%) was observed 12 months after planting (T₄). Subsequently higher oil percentage (2.13%) was recorded in 9 months after planting (T₃) (Figure 5).

Results of chemical compound analysis revealed that a significantly highest (P<0.05) Khusimol content (14.5%) was recorded in Vetiver harvested 9 months after planting (T₃). It varied as 10.5%, 7.7 % and 13.5 % respectively at the 3, 6 and 12 months after planting. Khusimol content was higher in 3 months old plants (10.5%) than in the 6 months old plants (7.7%). Production of Khusimol showed a twofold increase 9 months after planting (14.5%) compared to the 6 months after planting (7.7%) (Figure 6).
A significantly (P<0.05) higher β-Vetivenene (0.8%) content was recorded 6 months old plants (T₂) compared to 3, 9 and 12 months old plants (Figure 7).

A significantly higher (P<0.05) β-Vetivone content (1.4%) was recorded at the 9 months after planting (T₃) compared to the other harvesting intervals. Production of β-Vetivone increased during the first nine months and after that it decreased to 0.8%, when it reached to 12 months after planting (Figure 8).

There was an increasing trend in α-Vetivone content (%) with increasing intervals of harvesting. Significantly high (P<0.05) α-Vetivone content (5.2%) was recorded 12 months after planting (T₄) (Figure 9).
A significantly higher (P<0.05) iso-valencinol content (4.9%) was recorded at 9 months after planting (T3) compared to other harvesting intervals (Figure 10).

Figure 10: Iso-valencinol content (%) of Vetiver oil at different harvesting periods. Means with the same letter are not significantly different at α=0.05. T1-3 MAP, T2-6 MAP, T3-9 MAP, T4-12 MAP.

Different harvesting intervals showed significant differences (P<0.05) in fiber content of roots. Fiber content of roots increased with the increasing harvesting intervals. A significantly high (P<0.05) root fiber content (44.1%) was recorded in Vetiver harvested 12 months after planting (T4) (Figure 11).

Figure 11: Changes in root fiber content of Vetiver as affected by different harvesting intervals. Means with the same letter are not significantly different at α=0.05. T1-3 MAP, T2-6 MAP, T3-9 MAP, T4-12 MAP.

Discussion

There was a positive correlation between biomass productions of Vetiver and pot height. Increase in pot heights facilitates the downward movement of roots providing more space. This may be the reason for higher growth and yield observed in 45 cm than other treatments. It is not practically feasible to handle pot heights above 45 cm. Yoon (1993) found that, larger bag sizes of 6” × 13”, 7” × 15” and 8” × 12” are considered too large for practical use and there was a decrease in the number of tillers and top dry weights production from the largest bag to the smallest bag, which is in agreement to results in this study [5]. Chomchalow (2001) reported that digging of soil for root harvesting may be environmentally undesirable, an alternative means of growing Vetiver could be in poly-bags and other containers [6]. He further pointed out that, this would not only mitigate soil erosion concerns but also increase cost benefit ratio of Vetiver cultivation for its roots and root oil, as well as optimum utilization of degraded lands as poly-bag platforms.

It was reported in the present study that oil content (1.24%) doubled 6 months after planting when compared to the oil content (0.63%) at 3 months after planting. Similarly, when considering the oil content between 6 and 9 months after planting it was nearly double at 9 months after planting. But there was no such increment between 9 and 12 months after planting. The most promising harvesting time with respect to the root yield was 9 months after planting. Therefore, it is not economically viable to keep extra 3 months in the field as it increases the cost of production.

Maffi (2002) pointed out that the Vetiver roots give a yield of about 0.3 to 2 % essential oil depending upon the biotype, cultural practices, age of roots and mode and duration of distillation [7]. However, in the present study, the oil yields were 2.13% and 2.15% respectively, 9 and 12 months after planting on a dry weight basis and there were no significant differences oil contents between 9 and 12 months. Therefore, harvesting interval of 9 months after planting could be recommended to obtain an economically viable root and oil yields.

It was also observed in the present study that the Vetiver harvested at 9 months of planting (T3) had significantly (P<0.05) high Khusimol, β-Vetivone and Iso-valencinol contents in root oil. However, during the period of 9 to 12 months of planting α-Vetivone content (5.2%) of Vetiver increased while Khusimol, β-Vetivone and Iso-valencinol contents in Vetiver oil decreased.

There were no remarkable changes in temperature, monthly average rainfall and number of rainy days up to nine months of planting. However, there were remarkable reductions in monthly average rainfall and number of rainy days during the period between harvesting intervals of 9 and 12 months. Present study was conducted under the rain fed conditions (watering was done at two day intervals up to four weeks after planting).

Water stress conditions are highly associated with the secondary metabolites production of Vetiver. These may be the reasons for such changes in active ingredients. Maffei (2002) reported that in North India, there is no definite period for harvesting and the roots are harvested both for the manufacture of articles and for oil distillation when plants are 10-12 months old. Chadha (1995) pointed out that tremendous diversity of oil composition exists.
with respect to pattern of growth, orientation and thickness of roots, as well as for occurrence of secondary roots and harvesting time [8]. Aggarwal et al. (1998) demonstrated that the age, quality and stage of root harvest, and processing for distillation are vital components for essential oil distillation [9].

High fiber content reduces the yield and quality of roots as well as oil. Therefore, it is necessary to select best possible harvesting interval with lower fiber content for yield and quality improvement of the Vettiver oil. Anon (1976) reported that Vettiver has a high content of hemicelluloses and its cellulose content is 45.8% (Dry Weight basis) [10]. He also revealed that Vettiver containing short fiber and pulp has to be used in admixture with 30-40% of a long-fibered pulp. Though the high fiber content of Vettiver is important for the paper industry it is not a good feature in oil distillation as it creates practical difficulties in processing, oil extraction as well as loses the essential ingredients in oil.

Conclusion

Pot height of 45 cm and Vettiver harvested at 9 months of planting could be used as most appropriate pot height and harvesting interval in order to enhance biomass production, oil content and quality of Vettiver. Period of harvesting highly depends on the soil and climatic conditions, agronomic practices adopted and purpose of harvesting.

Therefore, further research has to be carried out to select proper harvesting time in relation to the soil types and climatic condition of the different regions to obtain maximum yield in good quality.

Acknowledgement

Department of Ayurveda, Ministry of Indigenous Medicine is greatly acknowledged for the funds provided for this research project.

References

Anti hyperlipidemic effect of Vara Asanadi Kwatha against high fat diet induced hyperlipidemic rats

Anju P Ramachandran¹, M Shyam Prasad¹, Vijay Kumar², B K Ashok³, B Ravishankar⁴, H M Chandola⁵

Abstract

Changing life style and diet patterns along with significant role played by genetics made Hyperlipidemia/Dyslipidemia as one of the most common metabolic aberration of lipids among people all over the world. An extra cavernous research study on classical Ayurvedic formulations which are not in the limelight of routine clinical practice is essential to explore the most effective and target oriented anti hyperlipidemic drugs. Objective of this study was to evaluate anti-hyperlipidemic activity of Vara asanadi kwatha against high fat diet induced hyperlipidemic Wistar strain albino rats. Wistar strain albino rats of either sex weighing 180 ± 25 g six animals were selected and housed with each cage containing 6 animals. Test drug treated animals were managed with Vara Asanadi Kwatha at a dose of 8 ml/kg in which the efficacy of medicine has been assessed on various serum biochemical parameters, histopathological sections and weights of liver, kidney and heart. One group kept as cholesterol control and the remaining as water control. Findings are in favour of mild anti hyperlipidemic and significant hepatoprotective and nephroprotective activities of the test formulation. Vara Asanadi Kwatha is a mild anti-hyperlipidemic and potent hepatoprotective as well as renoprotective drug.

Introduction

Lipid and lipo-protein abnormalities have become enormously common in the general populace. The metabolic aberrations of lipids are linked as risk factor with numerous numbers of serious systemic illnesses including cardio vascular disorders and metabolic syndrome [1]. Obesity and hyperlipidemia often exist together clinically and share much in common from the etio-pathology to the complications [2]. Vara Asanadi Kwatha [VAK] is a classical Ayurvedic formulation in the form of decoction, which claims to be effective in the management of overweight and obesity [3]. An experimental evaluation of the drug on its anti-hyperlipidemic action will certainly give thoughts regarding the efficacy of Vara Asanadi Kwatha in counteracting the ill effects of dyslipidemia. With this judgment the present experimental study was carried out to screen anti-hyperlipidemic potential of VAK in experimental animals.

Materials and Methods

Test formulation:

The ingredient wise composition of Vara Asanadi Kwatha has been provided in Table 1.

Each raw constituent of VAK was subjected to pharmacognostical identification and was certified as genuine and of good quality in the Department of Pharmacognosy, Institute of Post Graduate Teaching and Research in Ayurveda (IPGT and RA), Gujarat Ayurved University, Jamnagar. The test drug was prepared by adding one part of the crushed raw drug to sixteen parts of water, boiled and reduced to half. Thin sheets of Iron was added during the boiling period of kwatha and later removed while filtering. The prepared drug was procured from Ayurveda Pharmacy, Kannur, Kerala.

Animals:

Wistar strain albino rats of either sex weighing 180 ± 25g were obtained from animal house attached to Pharmacology Laboratory of IPGT and RA Gujarat Ayurved University, Jamnagar. Six animals were housed in each cage made up of poly-propylene with stainless steel top grill. The dry wheat (post hulled) waste was used as bedding material and was changed every morning. The animals were exposed to 12 hour light and 12 hour dark
cycle with the relative humidity of 50 to 70% and the ambient temperature during the period of experimentation was 22 ± 03ºC. Animals were fed with Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited. For their drinking purpose tap water ad libitum was used. The experiment was carried out after obtaining the permission from institutional animal ethics committee. (Approval number; IAEC (Institutional Animal Ethics Committee) 06/09-11/PhD/05).

Dose fixation and schedule:

The human dose of *V* *a* *ra Asanadi Kwatha* is 45ml twice a day (90 ml per day) [4]. The suitable dose for rats was calculated by referring to table of Paget and Barnes (1964) [5] and the dose obtained thus was 8 ml/kg rat. The test formulation was administered with the help of oral catheter attached to a disposable syringe.

Anti-hyperlipidemic activity:

The effect of test formulation on diet induced hyperlipidemia was carried out as per previous study [6]. The selected animals were divided into three groups of six animals each. First group was kept as normal control (NC) which received only tap water.

To second group hyperlipidemic diet was administered and served as cholesterol control (CC) group. Third group received hyperlipidemic diet and *V* *a* *ra Asanadi Kwatha* (VAK). Test drug was administered at morning hour and hyperlipidemic diet (to second and third group) was administered at evening hours for 20 consecutive days. The hyperlipidemic diet includes hydrogenated vegetable oil (Vanaspati Ghee - 'Raag' brand, Batch No. BA 76, Adani Wilmar Ltd., Gujarat) and cholesterol extra pure powder (Batch No. 14036 Suvidhnath Laboratories, Baroda) made in to 20% suspension in coconut oil (Parachute coconut oil, Batch No. GSW002, Ponda-Goa.). The suspension was administered at the dose of 0.5 ml/100 g rat. On the 21st day after overnight fasting, the animals were weighed again and blood was collected from retro-orbital plexus under ether anaesthesia. From separated serum; biochemical parameters like glucose [7], serum total cholesterol [8], serum triglyceride [9], and serum high density lipoprotein cholesterol (HDL-C) [10], Serum low density lipoprotein cholesterol+ very low density lipoprotein cholesterol (LDL-C + VLDL-C) were estimated. Serum (LDL+VLDL) was calculated by subtracting HDL cholesterol value from total cholesterol instead using both values separately, as in rats whose serum cholesterol is <100 mg/dl Friedewald formula overestimates LDL levels [11]. Further blood urea [12], serum creatinine [(13], serum glutamic oxaloacetic transaminase (S.GOT), serum glutamic pyruvic transaminase (S.GPT) [14], alkaline phosphatase [15], total bilirubin [16], direct bilirubin [17] and serum uric acid[18] were also estimated as per standard procedure. Further, all the rats were sacrificed by overdose of ether anesthesia and from the sacrificed animals liver, kidney, heart and aorta were excised out. The liver, kidney and heart were weighed and fixed in 10% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin and serial sections were cut and each section was stained with hematoxylin and eosin [19]. The slides were viewed under trinocular research microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

Statistical analysis:

The results were presented as mean ± SEM for six rats in each group. Statistical comparisons were performed by unpaired student’s t test by using Sigma stat software (version 3.1) for all the treated groups with the level of significance set at P<0.05.

Results

Data related to effect of VAK on body weight of albino rats have been provided in Table 2.
In normal control rats a progressive gain in body weight was occurred in comparison to its initial values. In contrast to this, significant increase in body weight was occurred in cholesterol control group in comparison to both initial values. In VAK treated group also significant increase in body weight was occurred in comparison to its initial value. Marginal increase of relative weight of liver and heart was found in cholesterol control group in comparison to normal control group which is found to be statistically non-significant (Table 3).

### Table 2: Effect on body weight

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Actual change in body weight (g)</th>
<th>% change in comparison to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC(n=6)</td>
<td>203.00 ± 6.38</td>
<td>218.00 ± 6.97**</td>
<td>15.00 ± 3.49</td>
<td>–</td>
</tr>
<tr>
<td>CC(n=6)</td>
<td>174.67 ± 8.88</td>
<td>196.00 ± 11.41*</td>
<td>21.33 ± 5.74</td>
<td>42.20↑</td>
</tr>
<tr>
<td>VAK(n=6)</td>
<td>177.00 ± 3.96</td>
<td>217.33 ± 7.04**</td>
<td>40.33 ± 6.56</td>
<td>89.08↑</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM (standard error of mean), ↑- Increase, ∗∗∗∗P<0.05, ∗∗∗P<0.01, (Compared with initial body weight, paired t test)

In normal control rats a progressive gain in body weight was occurred in comparison to its initial values. In contrast to this, significant increase in body weight was occurred in cholesterol control group in comparison to both initial values. In VAK treated group also significant increase in body weight was occurred in comparison to its initial value. Marginal increase of relative weight of liver and heart was found in cholesterol control group in comparison to normal control group which is found to be statistically non-significant (Table 3).

### Table 3: Effect on weight of important organs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight of liver (mg/100g)</th>
<th>Weight of heart (mg/100g)</th>
<th>Weight of kidney (mg/100g)</th>
</tr>
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<tbody>
<tr>
<td>NC (n=6)</td>
<td>3061.83 ± 102.96</td>
<td>335.13 ± 6.26</td>
<td>605.00 ± 08.04</td>
</tr>
<tr>
<td>CC (n=6)</td>
<td>3356.85 ± 185.82</td>
<td>337.39 ± 14.58</td>
<td>642.19 ± 10.32*</td>
</tr>
<tr>
<td>VAK (n=6)</td>
<td>2934.58 ± 43.99</td>
<td>330.69 ± 6.93</td>
<td>591.58 ± 10.42 ααι</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM

∗∗∗ P<0.001 (Compared with normal control group, unpaired t test)

αα P<0.01 (Compared with cholesterol control group, unpaired t test)

Treatment with VAK attenuated weight of these organs in non-significant manner. Further, cholesterol control group significantly increased the kidney weight and treatment with VAK significantly attenuated it. The data related to the effect of VAK on serum biochemical parameters were provided in Table 4.

Feeding of cholesterol diet led to significant increase in serum glucose in comparison to normal control group and treatment with VAK non-significantly attenuated it. Further blood urea, serum creatinine and serum lipid profiles were significantly increased by feeding with hyperlipidemic diet. These parameters were also non-significantly elevated by administration of VAK. S.GOT, Aspartate transaminase (AST) and alkaline phosphatase activities were significantly enhanced by feeding with hyperlipidemic diet in rats. VAK significantly attenuated activity of these enzymes in comparison to cholesterol control group. Further total bilirubin and serum uric acid levels were also elevated by feeding of hyperlipidemic diet and VAK significantly attenuated them.

Histopathological sections from control group shows normal cytoarchitecture of liver, kidney and heart (Fig. 1A, 2A and 3A). In contrast, hyperlipidemic diet produced perivascular cell infiltration and micro fatty changes in liver, cell infiltration and fatty changes in kidney and cell infiltration and fatty changes in majority of sections of heart (Fig. 1B, 2B and 3B). Simultaneous treatment with VAK significantly attenuated cholesterol induced pathological changes in all the three organs (Fig. 1C, 2C and 3C).
<table>
<thead>
<tr>
<th>Parameters</th>
<th>NC</th>
<th>CC</th>
<th>% change in comparison to NC</th>
<th>% change in comparison to CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood sugar (mg/dL)</td>
<td>87.50 ± 4.33</td>
<td>111.67 ± 3.44**</td>
<td>27.62 ↑</td>
<td>101.67 ± 4.08</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>58.00 ± 2.87</td>
<td>87.33 ± 2.40***</td>
<td>50.56 ↑</td>
<td>80.50 ± 4.39</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>81.16 ± 2.54</td>
<td>164.00 ± 16.54***</td>
<td>102.06 ↑</td>
<td>134.16 ± 15.07</td>
</tr>
<tr>
<td>LDL+VLDL (mg/dL)</td>
<td>27.50 ± 2.51</td>
<td>42.80 ± 4.42*</td>
<td>55.63 ↑</td>
<td>42.73 ± 4.38</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>30.50 ± 1.25</td>
<td>39.50 ± 3.08*</td>
<td>29.50 ↑</td>
<td>38.00 ± 3.58</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>19.83 ± 0.30</td>
<td>14.16 ± 0.94***</td>
<td>28.59 ↓</td>
<td>17.16 ± 0.87*</td>
</tr>
<tr>
<td>Blood urea (mg/dL)</td>
<td>75.33 ± 3.48</td>
<td>42.33 ± 1.05***</td>
<td>43.80 ↓</td>
<td>44.80 ± 4.56</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.48 ± 0.03</td>
<td>0.68 ± 0.03***</td>
<td>41.66 ↑</td>
<td>0.60 ± 0.03</td>
</tr>
<tr>
<td>S.GOT (IU/L)</td>
<td>152.50 ± 5.29</td>
<td>236.00 ± 25.73***</td>
<td>54.75 ↑</td>
<td>144.66 ± 11.22**</td>
</tr>
<tr>
<td>S.GPT (IU/L)</td>
<td>46.67 ± 3.31</td>
<td>73.00 ± 4.81**</td>
<td>56.41 ↑</td>
<td>63.66 ± 2.01*</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>211.16 ± 9.81</td>
<td>526.33 ± 22.64***</td>
<td>149.25 ↑</td>
<td>402.50 ± 28.00**</td>
</tr>
<tr>
<td>Bilirubin total (mg/dL)</td>
<td>0.35 ± 0.02</td>
<td>0.55 ± 0.06*</td>
<td>57.14 ↑</td>
<td>0.35 ± 0.05*</td>
</tr>
<tr>
<td>Bilirubin D (mg/dL)</td>
<td>0.15 ± 0.02</td>
<td>0.20 ± 0.02</td>
<td>33.33 ↑</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>0.90 ± 0.10</td>
<td>1.35 ± 0.15*</td>
<td>50.00 ↑</td>
<td>0.80 ± 0.11*</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM, ↑ - Increase, ↓ - Decrease,
P<0.05, **P<0.01, ***P<0.001 (Compared with normal control group, unpaired ‘t’ test)
P<0.05, aP<0.01 (Compared with cholesterol control group, unpaired ‘t’ test)
Figure 1A. NC-liver showing normal cytoarchitecture, Hc-hepatocytes, Kc-Kupffer cell, S-sinusoid.

Figure 1B. CC-liver showing micro and macro (Fc) fatty changes, (CI) cell infiltration.

Figure 1C. VAK liver showing almost normal cytoarchitecture.

Figure 2A. NC-kidney showing normal cytoarchitecture, G-glomerulus, Ct-convoluted tubule.

Figure 2B. CC-kidney showing micro (Fc) fatty changes, and (CI) cell infiltration.

Figure 2C. VAK kidney showing almost normal cytoarchitecture.

Figure 3A. NC-heart showing normal cytoarchitecture, Mc-myocardium.

Figure 3B. CC-heart showing CI-cell infiltration.

Figure 3C. VAK heart showing normal cytoarchitecture.

Discussion

Elevated levels of different types of lipids have been implicated in the production of atherosclerosis. In this stipulation, the blood vessel wall thickens due to cholesterol deposition ensuing to inflammatory reaction. This ultimately leads to loss of elasticity of affected vessel wall and becomes the major pathology involved in the occurrence of a number of serious systemic disorders such as cardiovascular diseases, cerebrovascular accidents, peripheral arterial disease which account as the significant culprit for mortality/disability in both developed and developing countries. Even after the prescription of dietetic, lifestyle and therapeutic interventions the incidence and prevalence of lipid abnormalities and resultant fatal complications are hiking up. Hence there is huge scope for the introduction of effective hypolipidemic and anti-hyperlipidemic drugs in to existing therapeutic armamentarium.

In the current experimental work, in comparison to cholesterol control group, the VAK treated animals exhibited moderate level of decrease in S.cholesterol, S.triglycerides and HDL-C, but the variations were statistically non significant.
Statistically significant changes were attained in the values of SGOT, SGPT, alkaline phosphatase, total bilirubin and uric acid revealing the high hepatoprotective and nephroprotective properties of the test formulation. SGOT determination is of immense value in the assessment of coronary artery diseases and myocardial infarction.

Elevated serum enzyme activity associated with cardiac disorder is assumed to reflect activity of enzyme released from the injured cardiac tissue too [20]. The significant change attained in the value of SGOT may also have noteworthy role in the cardio-protective activity of the trial drug. The observations attained in bio chemical parameters are in line with the histopathological findings of this study. The normal cytoarchitecture and absence of cholesterol induced pathological changes in the histopathological sections of liver, heart and kidney shows the efficacy and capability of VAK in the management of dyslipidemia induced complications.

Vara is well known as triphala (combination of Terminalia chebula, Terminalia bellirica and Emblica officinalis) in Ayurveda is the foremost ingredient of VAK and Ayurvedic science has identified its benefits in obesity, diabetes mellitus and hepatic disorders. There are many reports with regard to pharmacological effects of triphala, including its anti-hypercholesterolemic, anti-oxidant and hepatoprotective properties [21,22]. Emblica officinalis (Amalaki) given in a ration of rabbit at 1g/kg has found to have anti-hypercholesterol activity. In one of the study; T. arjuna, T. bellirica and T. chebula was fed to rabbits on cholesterol rich diet inducing atherosclerosis which showed that T.chebula as the most potent hypolipidemic agent among the three drugs and induced partial inhibition of rabbit atheroma as seen from plasma and tissue lipid content and the lesions of aorta. Haritaki (T. chebula) is also well known for its anti-hepatotoxic activities. Hepatoprotective activity of T.bellirica is also been reported as the alcoholic extract of fruit of T. bellirica in a dose of 30 mg/kg given I/V to dogs showed significant bile stimulant activity and increased solids in bile secretion. Further numerous studies have been conducted on the anti-hyperlipidemic activity of Citraka (Plumbago zeylanica), which is the one of the ingredient of VAK. In the study conducted on hyperlipidemic rabbits – Plumbagin; the active constituent of Plumbago zeylanica reduced serum cholesterol by 53-86% and elevated decreased HDL cholesterol significantly [23].

Curcuma longa (Haridra) is an established hepatoprotective drug and is been used widely in the management of jaundice and hepatic disorders [24]. C. longa prevents the formation of fatty liver by the modulation of expressions of enzymes that are important to fat metabolism [25].

In a usual mutant obesity, Curcuma longa had significantly reduced cholesterol and triglyceride concentration, while increasing HDL cholesterol. Advance evidences indicate that it diminishes the oxidation of LDL, blood glucose and renal lesions. It had been demonstrated to reduce smooth muscle cell proliferation and endothelial dysfunction [26]. As per recent research studies Curcumin has been reported to have the nephroprotective effect to improve creatinine and urea clearance and also can protect the chronic renal allograft nephropathy [27].

Furthermore the hypolipidemic and hepatoprotective activities of Pierocarpus marsupium (Asana) are also well established by several studies [28,29]. Most of the Ayurvedic drugs such as E. officinalis and C. longa are stronger and efficient anti-oxidants; which may be helpful in preventing lipid peroxidation [30].

Thus multiple constituents of VAK are reported to have antihyperlipidemic, anti-hepatotoxic and hepatoprotective activities. The ingredient such as Curcuma longa is having nephroprotective properties also and the same is reflected in present study. The non-significant changes obtained in the most of the values of lipid profile and blood sugar cannot be interpreted negatively, as the results are definitely pointing towards the direction of reduction. The weak action obtained in terms of these parameters may be because of the fact that the drug is administered in the form of decoction. Otherwise most of the individual components of the Vara Asanadi Kwatha are proven anti-hyperlipidemic drugs when used in single or in combinations. The alcoholic extract of the same drugs may show more potent and significant anti-hyperlipidemic activity as reported by the various studies in this regard.

Conclusion

From the present study it can be concluded that Vara Asanadi Kwatha is having mild anti-hyperlipidemic and remarkable hepatoprotective and nephroprotective activities. Exclusive experimental works on hepatoprotective and nephroprotective properties of Vara Asanadi Kwatha may reveal hidden and highly informative facts regarding this wonderful classical formulation.

Acknowledgement

The authors wish to thank Dr Sulakshan Chavan, Miss Hetal Aghera and staff of pharmacology laboratory, IPGT and RA, and Jamnagar for their technical support.

References

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Antibacterial properties of “Accmus” mouth wash

S Tharmila¹, T Thileepan², A C Thavaranjit¹, R Srikaran³

Abstract

Antimicrobial herbs can be used individually or in combination to prepare mouth wash which is healthier and safer than the synthetic ones. In this study a new “Accmus” herbal mouth wash was prepared and its antibacterial properties were evaluated. Alcoholic, boiled alcoholic and aqueous extracts of “Accmus” mouth wash were prepared from the bark of Acacia arabica, Acacia speciosa and root of Calamus rotang in combination by tincture and hot extract methods respectively. Alcohol content and pH were also determined. Antibacterial properties of the above extracts were also studied against Staphylococcus aureus, Bacillus sp of Gram (+)ve and Pseudomonas aeruginosa, Klebsiella sp of Gram (-)ve in vitro by using agar well diffusion method. This study showed that the alcohol content and pH of mouth wash preparations were in acceptable levels. Aqueous extract exhibited better antibacterial activity compared with alcoholic extract and had maximum sensitivity towards Bacillus sp and low towards Klebsiella sp. Staphylococcus aureus was only inhibited by all preparations of mouth wash. So the hot extraction method was efficient than the alcoholic extraction and this could be recommended with antibacterial properties rather than the alcoholic extract of mouth wash. Further study is needed for further purification and characterization of active constituents from various solvent extracts of mouth wash against oral diseases.

Introduction

Mouth wash or mouth rinse is a product used to enhance oral hygiene. Commercial brands of mouth wash contain synthetic and semisynthetic chemical substances such as thymol, methyl salicylate, menthol, chlorhexidine gluconate, methylparaben, hydrogen peroxide etc [1] and also include water and sweetness such as sorbitol, sodium saccharin [2]. Sometimes a significant amount of alcohol is added as the carrier for the flavour. Sodium benzoate is a common preservative in commercial mouth washes [3]. The risk of acquiring cancer rises almost five times for users of alcohol containing mouth wash who neither smoke nor drink [4]. Mouth washes containing cetylpyridinium chloride are also associated with loss of taste sensation and brown discoloration of teeth [4]. To overcome such harmful effect natural mouth washes are available in markets and are produced from plant based healthy ingredients such as organic aloe vera, peppermint, clove bud essential oils, perilla seed extract etc. The present study is to prepare a new “Accmus” mouth wash from the bark of Acacia arabica, bark of Acacia speciosa and root of Calamus rotang. Acacia arabica (Karuvel-“T”) is a tree, becomes under family leguminosae. Its bark has medicinal properties, mainly used in oral diseases. Hence, it has 24-42% of tannin. Acacia speciosa (Kadduvakai – “T”) becomes under family mimosaceae. Its bark decoction is being used in orodental diseases for gargle. Powder of root bark is used for bleeding. Calamus rotang is a climber one and it is classified under family palmae. In traditional medicine the root of Calamus rotang has been used against many oral diseases such as gum bleeding and aphthous ulcer in form of decoction for gargling [5,6]. The objective of this study is to prepare a natural new “Accmus” mouth wash and test its antibacterial activity against Gram (+) ve and Gram (-) ve bacteria.

Materials and Methods

Collection of plant materials

The plant Acacia speciosa was collected by the Unit of Siddha Medicine, University of Jaffna, Sri Lanka and it was identified based on herbarium records in the Department of Botany, University of Jaffna and other relevant materials [7,8]. And healthy bark was obtained, washed under running tap water, dried in sun shade for three weeks. Then ground into fine powder. Bark of Acacia arabica and root of Calamus rotang were also collected from local market and their characters were compared with herbarium records [7,8]. The above parts were washed under running tap water, dried in sun shade for five days and then ground into fine powder, by using electric blender. The powder was stored in air tight dark bottles at room temperature.

Preparation of mouth wash

“Accmus” mouth wash was prepared by two methods.

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Tincture method

25 g of each of the above herbal powder was mixed and mixture was soaked in 93.75 ml of 25% ethanol and 281.25 ml distilled water for two weeks under direct sunlight with occasional shaking. The mixture was filtered through double layered muslin cloth and the filtrate (355 ml) was collected into a clean dried dark bottle.

Half of the above volume of the filtrate was boiled at 85°C for 30 minutes and poured into a clean dried dark bottle as boiled alcoholic extract [9].

Hot extract method

25 g of each of the above herbal powder mixture was mixed with 250 ml distilled water in a sterile beaker. It was heated at 50°C on hot plate for 6 hours continuously till the final volume of extracts reached as 150 ml. Then extracts were filtered through double layered muslin cloth and the filtrate was concentrated by heating. It was kept at 4°C until used for assay [10].

Determination of pH was determined by pH meter.

Determination of alcohol content

Alcohol content of mouth wash was determined by ebuliometer. Durability of mouth wash also noted based on its characters such as color change, (odour) smell formation, turbidity and change in viscosity.

Antibacterial assay

Culture preparation.

The bacterial isolates of Staphylococcus aureus, Bacillus sp from Gram positives and Gram negative Pseudomonas aeruginosa, Klebsiella sp were obtained from bacterial culture collection, Department of Botany, University of Jaffna for this study. Test organisms were stored on nutrient agar slants at 4°C and these were sub cultured before 24 hours of the experiment and incubated at 37°C. After the incubation a loop full of young bacterial inoculum was transferred into the 10 ml of sterile saline water (0.85%) in an aseptic condition. Inoculum concentration was estimated by haemocytometer and the number of cells per ml was adjusted to 10⁶ cells by using tenfold dilution [11].

Determination of antibacterial activity

Nutrient agar medium was autoclaved and cooled to 40°C. The antibacterial assay was performed by agar well diffusion method [12]. 1 ml of test culture (10⁶ CFU/ml) was inoculated into a sterile petridish with 20 ml sterile nutrient agar and mixed well and allowed to solidify. Then wells were made by using sterile cork borer (8 mm in diameter) on the surfaces of agar plates and were filled with 100 µl of each extracts using sterile Pasteur pipette. 100 µl of commercially available “Chlorhexidine digluconate” mouth wash was used as standard and alcohol and water were used as control. Then plates were incubated at 37°C for 24-48 hours. Antibacterial activity was determined by measuring the diameter of the clear zone around the well. The above experiment was repeated five times and the mean diameter of the zone of inhibition was calculated.

Results and Discussion

Table 1: Antibacterial activity of mouth wash extracts on test bacteria

<table>
<thead>
<tr>
<th>Mouthwash extracts</th>
<th>Mean zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus (+)ve</td>
</tr>
<tr>
<td>Alcoholic extract of mouthwash (Tincture)</td>
<td>12</td>
</tr>
<tr>
<td>Alcoholic extract of mouthwash after boiling (Tincture)</td>
<td>10</td>
</tr>
<tr>
<td>Aqueous extract of mouthwash</td>
<td>15</td>
</tr>
<tr>
<td>Chlorhexidine digluconate (Standard)</td>
<td>16</td>
</tr>
</tbody>
</table>

Zone of inhibition includes the diameter of the well (8mm in diameter). (-) No activity.

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Out of five samples of alcoholic mouth wash, turbidity was observed after 8 months in two samples and 11 months in other three samples. Whereas in aqueous mouth wash, cloudiness and colour change were observed after 3 days. This indicated that the durability period of alcoholic mouth wash was higher (8-11 months) than that of aqueous mouth wash (2-3 days) at room temperature. But aqueous mouth wash could be kept safe at 4°C for 6-8 months.

In commercially available mouth wash, alcohol content goes up to 27% and the pH ranges from 5-7 [13]. These two parameters were in acceptable level. In newly prepared mouth wash (Table 2). Results also showed that aqueous extract of mouth wash containing natural ingredients, exhibited better antibacterial activity when compared to alcoholic extract. It had maximum sensitivity towards Bacillus sp, while it had low sensitivity towards the Klebsiella sp. Among the tested bacterial growth, Staphylococcus aureus was only inhibited by both preparations of mouth wash. All tested bacterial growth was inhibited by the aqueous extract of mouth wash and the positive control “Chlorhexidine digluconate”. But alcohol alone (control) didn’t inhibit the growth of any tested bacteria (Table 1). This is due to less alcoholic concentrations and the tolerance of test bacteria. Aqueous natural mouth wash showed greater antibacterial activity than alcoholic extracts of mouth wash. Hot extract method was highly efficient for the extraction of antibacterial compounds rather than tincture method. Long term use of alcoholic mouth wash is not preferable, because of the hazardous effects especially for children and causes dehydration in mouth [14]. Even though the durability period of aqueous mouth wash was low at room temperature, it showed greater range of antibacterial activity against test bacteria and absence of alcohol. So this could be recommended rather than the alcoholic extract of mouth wash.

Further studies should be done clinically and test the effectiveness of this “Accmus” aqueous extract of mouth wash against oral diseases.

**Conclusion**

In both preparations of mouth wash pH and alcohol content were in acceptable level. Staphylococcus aureus growth was only inhibited by both mouth wash preparations. Hot extraction method was efficient than that of alcoholic extraction. Aqueous mouth wash showed greater antibacterial activity against test bacteria and it could be recommended with antibacterial activity rather than the alcoholic extract of mouth wash.

**Table 2: pH and alcohol content of mouth wash extracts**

<table>
<thead>
<tr>
<th>Mouth wash extracts</th>
<th>pH</th>
<th>Alcohol content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic extract of mouth wash</td>
<td>4.5</td>
<td>18</td>
</tr>
<tr>
<td>Alcoholic extract of mouthwash after boiling</td>
<td>5.1</td>
<td>3</td>
</tr>
<tr>
<td>Aqueous extract of mouthwash</td>
<td>5.8</td>
<td>-</td>
</tr>
</tbody>
</table>

References

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease often resulting in increased morbidity, mortality and disability. Cytokines regulate a broad range of inflammatory processes that are implicated in the pathogenesis of rheumatoid arthritis. In rheumatoid joints, it is well known that an imbalance between pro and anti-inflammatory cytokine activities favours the induction of autoimmunity, chronic inflammation and thereby joint damage. During recent decades a better understanding of the pathogenesis of RA has led to the development of new strategies for disease control which have transformed the management of RA. However, none of them are effective in curing rheumatoid arthritis. Furthermore, the potentially greater efficacy of treatment with TNF antagonists comes at a cost that is too high for the majority of the world's population and with more side effects. In this review we discussed about effective compound herbal drug Yi Shen Juan Bi (YJB) derived from traditional medicine as cytokine target in rheumatology. According to our published research findings YJB significantly ameliorate symptoms and prevented severe arthritis development in rats. Our studies showed that YJB significantly reduced the production of IL-1β, IL-6 and TNF-α in vivo and in vitro. These data indicate that YJB may have the potential to regulate the immunomodulatory cytokines. So these herbal compound drugs are more effective in the treatment of RA. It is our hope that this kind of traditional drugs can be developed to become new pharmaceutical agents that can be used in cost and clinically effectively for people suffering from rheumatic diseases.

Introduction

Rheumatoid arthritis (RA) is a common, chronic disease, for which multiple pharmacotherapies are generally applied. RA is a prevalent condition often leading to a high burden of suffering in patients [1]. Conservative treatment is mostly symptomatic and often associated with adverse effects. Therefore, it is understandable that many RA patients seek complementary and alternative medicine (CAM) to manage their illness [1]. In the USA, about 60 to 90% of arthritis patients use CAM [2]. An Indian study reported that around 40% of RA patients use either Ayurvedic or homeopathic medicines or TCM alongside conventional medicines [3]. According to the World Health Organization (WHO), traditional herbal preparations account for 30-50% of the total medicinal consumption in China [4].

The suppression of auto immunity in RA can be observed either as the induction of cell cycle arrest, which slows down inappropriate or uncontrolled cell division, or as the induction of apoptosis in stressed cells. Some anti-inflammatory plant natural products have been found to be very effective regulators of the cell cycle of autoimmunity by targeting specific cell signaling molecules, leading to apoptosis or cellular senescence. Many anti-inflammatory plant natural products have molecular signaling targets that can be potentially employed for treatment of rheumatoid arthritis. A main feature of a number of anti-inflammatory plant natural products is their action on the suppression of autoimmunity by upregulating key signaling molecules like Bax, Bak, and Bid and the subsequent down regulation of expression of various other key signaling molecules such as NF-κB, Bcl-2, and activate the caspases, in the nucleus and/or cytoplasm, which eventually induces apoptosis of target cells. Also most plant natural compounds respond to inflammatory mediators including IL-1, 4, 6, 8, 10, 12, 13, 17, 18, 21, TNF-α, TGF-β, IFN-γ, VIP, INOS, and cyclooxygenase-2, prostaglandin E2. In this review we discussed herbal medicine cytokines targets in rheumatology special regards to Yi Shen Juan Bi (YJB) [5-8] (Table 1).
Table 1: Ingredients of Yi Shen Juan Bi (patent number: ZL200510040550) [5-8].

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Composition (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rehmannia glutinosa</td>
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</tr>
<tr>
<td>Eupolyphaga sinensis</td>
<td>210</td>
</tr>
<tr>
<td>Angelica sinensis</td>
<td>210</td>
</tr>
<tr>
<td>Bombyx batryticatus</td>
<td>210</td>
</tr>
<tr>
<td>Herba epimedii</td>
<td>210</td>
</tr>
<tr>
<td>Herba erodii</td>
<td>210</td>
</tr>
<tr>
<td>Buthus martensi</td>
<td>262.5</td>
</tr>
<tr>
<td>Corydalis yanhusuo</td>
<td>31.3</td>
</tr>
<tr>
<td>Scolopendra subspinipes</td>
<td>210</td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>210</td>
</tr>
<tr>
<td>Polistes mandarinus (stir-baking)</td>
<td>210</td>
</tr>
<tr>
<td>Cynanchum paniculatum</td>
<td>262.5</td>
</tr>
<tr>
<td>Rhizoma drynariae</td>
<td>31.5</td>
</tr>
<tr>
<td>Polygonum cuspidatum</td>
<td>262.5</td>
</tr>
<tr>
<td>Pyrola rotundifolia</td>
<td>31.5</td>
</tr>
<tr>
<td>Millettia reticulata</td>
<td>262.5</td>
</tr>
<tr>
<td>Zaocys Dhumnades (stir-fried with wine)</td>
<td>210</td>
</tr>
<tr>
<td>Humulus scandens</td>
<td>262.5</td>
</tr>
<tr>
<td>Rehmannia glutinosa (dried)</td>
<td>210</td>
</tr>
</tbody>
</table>

The cytokine network in rheumatoid arthritis

Cytokines regulate a broad range of inflammatory processes that are implicated in the pathogenesis of rheumatoid arthritis. In rheumatoid joints, it is well known that an imbalance between pro-and anti-inflammatory cytokine activities favours the induction of autoimmunity, chronic inflammation and thereby joint damage. However, it remains less clear how cytokines are organized within a hierarchical regulatory network, and therefore which cytokines may be the best targets for clinical intervention. Analysis of cytokine mRNA and protein in rheumatoid arthritis tissue revealed that many proinflammatory cytokines such as TNF alpha, IL-1, IL-6, GM-CSF, and chemokines such as IL-8 are abundant in all patients regardless of therapy [9]. This is compensated to some degree by the increased production of anti-inflammatory cytokines such as IL-10 and TGF beta and cytokine inhibitors such as IL-1ra and soluble TNF-R. In rheumatoid joint cell cultures that spontaneously produce IL-1, TNF alpha was the major dominant regulator of IL-1.

Subsequently, other proinflammatory cytokines were also inhibited if TNF alpha was neutralized, leading to the new concept that the proinflammatory cytokines were linked in a network with TNF alpha at its apex. This led to the hypothesis that TNF alpha was of major importance in rheumatoid arthritis and was a therapeutic target. This hypothesis has been successfully tested in animal models of, for example, collagen-induced arthritis, and these studies have provided the rationale for clinical trials of anti-TNF alpha therapy in patients with long-standing rheumatoid arthritis. Several clinical trials using a chimeric anti-TNF alpha antibody have shown marked clinical benefit, verifying the hypothesis that TNF alpha is of major importance in rheumatoid arthritis. Retreatment studies have also shown benefit in repeated relapses, indicating that the disease remains TNF alpha dependent [9].

Immunomodulatory and anti-arthritic potential of traditional medicine

Ayurveda, traditional Chinese medicine (TCM), and other traditional systems are today yielding their theoretical and experiential frameworks to investigation by modern scientific techniques, applied mainly for the purpose of illustrating the effectiveness of remedies that have been developed over the centuries. In this context, the underlying theoretical framework fades away, and the tested substances become the focus of a new international effort at preventive health care and disease treatment. Herbal formulas developed today rely on a combination of traditional and modern indications for the use of the medicinal materials [10].

Arthritis has been a recognized medical condition since ancient times, and the Chinese had developed numerous formulas for its treatment. Chinese herbal formulas were not specifically designed for either of the two major types of arthritis defined today. The basis for Chinese doctors differentiating arthritis into subgroups was not the microscopic details of the pathology. Instead, arthritis was divided into traditional medicine categories: hot and cold types, upper and lower body involvement, deficiency or excess syndrome, pain characteristics (such as variability and severity), and whether the site of the arthritis was fixed or “moving.” Both rheumatoid arthritis and osteoarthritis fall under the heading of bi syndrome, a disorder of qi and blood circulation that leads to symptoms of pain, numbness, swelling, and stiffness [11]. Rheumatoid arthritis fits most closely those syndromes characterized by the Chinese as wind-damp invasion affecting the joints. Osteoarthritis more closely fits the syndrome of liver/kidney deficiency syndrome causing weakness and stiffness in the legs with painful joints. In China, syndromes similar to rheumatoid arthritis were an area of special concern, generating considerable literature on the subject, since the condition could arise suddenly and could rapidly become severely debilitating [11]. Osteoarthritis, on the other hand, tended to be lumped together with other disorders of aging, in which stiffness...
and pain, especially of the legs, was considered just one part of the gradual deterioration of body functions that occurs with old age. As such, it is usually not the subject of much discussion separate from antiaging therapies. The closest traditional Chinese medicine term to rheumatoid arthritis is fengshi bing which literally means wind-damp disease [12-13]. The wind and damp factors can complex with either cold or heat factors to yield arthralgia. Almost all of the traditional approaches apply to the complex involving cold factors rather than heat. Gout, which has some characteristics in common with arthritis, usually fits the cold-dominated category or the cold-damp category of bi syndromes [12].

Chinese researchers have attempted to elucidate how the herbs used in traditional arthritis formulas alleviate the symptoms—from the modern viewpoint—by carrying out numerous studies of the blood constituents of patients [13]. According to studies that have been carried out recently the mechanism of action that may be dominant in the situations with good therapeutic results is a reduction in the levels of pro-inflammatory cytokines, such as interleukin-1 (IL-1), TNF etc [13]. The effect is then to alter the levels of T-cells and the production of activated antibodies and other components. In addition, or as a result, the properties of the blood and its circulation also change, with lowered sedimentation rate and improved circulation to the extremities. The herbs may also act on the prostaglandin synthesis and degradation pathways, yielding a lower level of pro-inflammatory prostaglandins [13].

**YJB as selective cytokine targeted anti rheumatic drug**

The past studies evaluated anti-arthritis potential of YJB in vivo and invitro rat models, which is very close to its human counterpart. In these studies, we used adjuvant arthritis (AA) induced and collagen induced experimental rat models for our experiments. One of the most imperative features of these models is chronic synovitis, including inflammatory cell infiltration, panes formation, and destruction of cartilage and bone erosion. According to our research findings YJB significantly ameliorate symptoms and prevented severe arthritis development in rats [5, 6, 7, 8, 14].

To elucidate the effect of YJB on immunomodulatory cytokines such as TNF-α, IL-1β and IL-6, an ELISA assay was performed. TNF-α and IL-1β are considered key mediators in the joint inflammation and in the destruction of cartilage and bone in patients with RA [14]. TNF-α is a pivotal mediator in inflammatory arthritis including RA [15]. TNF-α is an autocrine stimulator as well as a potent paracrine inducer of other inflammatory cytokines such as IL-1β and IL-6. The blockade and inhibition of TNF-α reduces the production of other inflammatory cytokines in RA patients [16]. IL-6 is a proinflammatory cytokine with a wide range of biological activities in immune regulation, inflammation and oncogenesis [17]. IL-6 is known to be responsible for the increase of serum g-globulin and the emergence of rheumatoid factors [18]. High levels of IL-6 have been observed in both sera and synovial fluids from the affected joints of patients with RA [19]. Our studies showed that YJB significantly reduced the production of IL-1β, IL-6 and TNF-α [5-8]. These data indicate that YJB may have the potential to regulate the immunomodulatory cytokines. Further our studies clearly confirmed that anti-arthritic property of YJB substantiated lower TNF-α, IL-1 production capacity of macrophages in *in vitro* [5].

The contribution made by proinflammatory cytokines in RA, such as tumour necrosis factor TNF-α and IL-1 has been validated in preclinical animal models and in humans [20]. It is also well documented in human RA and in animal models that IL-1 and TNF-α synergistically mediate synovitis and destruction of cartilage and bone [21]. TNF-α is an important regular factor in inflammation and immunity response, which can stimulate the synoviocyte and cartilage cells to synthesize the PGE2 and collagenase causing synovium and cartilage destruction as well as those of IL-1, IL-6, and IL-8 [22]. Therefore it is one of the most important factors in the cytokine network. In RA patients, IL-1β is overexpressed in inflamed synovial tissue, in particular in the lining layer and in sublining cells [23] and it is elevated in draining lymph from affected joints [20]. Furthermore cartilage from arthritis patients’ exhibits upregulation of IL-1β mRNA as compared with normal cartilage [24, 25]. In addition, increased production of IL-1β in fibroblast like synoviocytes of susceptible individuals may lead to a higher risk of developing severe joint damage even in the absence of systemic inflammation. In general, TNF-α causes early joint swells in RA, while IL-1β combining with the immune complex leads to the cartilage erosion [25].

Considering these investigations it can be concluded that TNF-α and IL-1β have a pivotal role in the pathogenesis of RA. Furthermore based on these views, it can be pointed out that blocking of both TNF-α and IL-1β is necessary in the treatment of RA.

The study revealed that TNF-α mRNA and IL-1β mRNA expression in synovial cells of model group was significantly higher than that of normal rats. These are correlated with above research findings in RA. Furthermore pro-inflammatory cytokines, such as tumour necrosis factor α and interleukin-1β, are expressed in the arthritic joints in both AA rats and human rheumatoid arthritis, and blockade of these molecules results in amelioration of disease [26]. Our results confirmed that YJB could significantly decrease the TNF-α mRNA and IL-1β mRNA expression in synovial cells. This may be the one of the underlying mechanism that how YJB ameliorate inflammation in RA. Therefore it is a promising drug for the treatment of cytokine expression *in vivo*. 

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Conclusion

Taken together, our past results suggested that YJB can be effectively applied to inflammatory and immune diseases at the level of proinflammatory cytokines and mediator regulation (see proposed mechanism of YJB’s activation in Figure 1).

Figure 1: The mechanisms of YJB activation proposed here in scheme summarizes that the active components of YJB down regulate TNF-α, IL-1β, IL-6, NO, PGE2, NF-κB and COX-2 expression, which results in enhance anti inflammatory and immunomodulatory action. YJB potently induces the apoptosis of synovium, via ultimate executioner caspase 3. YJB also down-regulates cytochrome-c related Bcl-2 expressing and up-regulates Bax expressing leading to triggered apoptosis cascade, which results in enhance apoptosis in the RA synovium and potentially limiting disease progression. ‘+’ : positive effects ‘−’ : negative effects ‘solid-line arrow’: known actions ‘dotted-line arrow’: our researches discovered actions [5-8].

References


Evidence based Ayurveda for revitalization of mental health

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Abstract

Mental health is an integral part of health. Mentally healthy people are able to function well in home and society and meet the ordinary demands of daily life. They enjoy a positive quality of life and are free of disabling symptoms of psychopathology. On the other hand, any type of mental disorder is associated with a totally disturbed life. According to the World Health Organization, 10% of the world’s population has some form of mental disability and 1% suffers from severe incapacitating mental disorders. In India community-based surveys conducted during the past two decades showed that the total prevalence of psychiatric disorder was around 5.8%. For children, only a few studies have reported a prevalence rate ranging from 8.17-35.6% in India. Although the current used drugs are the first choice medication, however, these agents produce various unacceptable side effects like, loss of appetite, stomach aches/cramps, headache, dizziness, irritability, drowsiness, staring, tics etc., which should be a matter of concern in both adults and children. In this area, Ayurvedic herbs having Medhya property may prove safe and effective. Review of various experimental and clinical studies offer clue to use different Medhya drugs, judiciously for the management of psychiatric and behavioural disorders.

Introduction

Mental health may be defined as a state of emotional and psychological well being in which an individual is able to use his or her cognitive and emotional capabilities, function in society and meet the ordinary demands of everyday life [1]. According to World Health Organization mental health is defined as “a being of well-being in which the individual realizes his or her own abilities, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her community” [2]. The World Health Organization states that 10% of the world’s population has some form of mental disability and 1% suffers from severe incapacitating mental disorders [3]. In India community-based surveys conducted during the past two decades showed that the total prevalence of psychiatric disorder was around 5.8% [4]. Another study reports the prevalence rate for mental disorders in India as 65.4 per 1000 population [5].

Mental health in children is defined by the achievement of expected developmental cognitive, social and emotional milestones and at the same time satisfying social relationships and effective coping skills. Mentally healthy children enjoy a positive quality of life; function well at home, in school, and in their communities; and are free of disabling symptoms of psychopathology [6]. For children, only a few studies have reported a prevalence rate ranging from 8.17-35.6% in India [7-11].

The most commonly occurring mental disorders are anxiety disorders, mood disorders, personality disorders, dementia and that of children as mentioned in DSM-IV, include anxiety disorders, attention deficit and disruptive behaviour disorders, autism and other pervasive disorders, eating disorders, learning and communication disorders, mood disorders, tic disorders etc. These disorders are characterized by abnormal behaviour, thoughts, emotions and relationship with others.

The current medications used in the treatment of mental disorders including children incorporate antipsychotic, antidepressants, anti anxiety drugs, stimulants and mood stabilizing groups. Although these drugs are the first choice medication, however, these agents produce various unacceptable side effects like, loss of appetite, stomach aches/cramps, headache, dizziness, irritability, drowsiness, staring, tics etc., which should be a matter of concern in both adults and children. In this area, Ayurvedic herbs having Medhya property may prove safe and effective. The drugs promoting Medha (intellect) are termed as Medhya drugs.

Review of various experimental and clinical studies offer clue to use different Medhya drugs, judiciously for the management of psychiatric and behavioural disorders. The review is taken from articles cited on Pubmed and in MAPA (Medicinal and Aromatic Plant Abstracts) by using the key words learning and memory, cognition, Bacopa, Centella etc.

Nootropic activity

In a study, daily administration of Ashwagandha root extract (50,100 and 200 mg/kg orally) for 6 days significantly improved memory consolidation in mice receiving chronic electroconvulsive shock (ECS) treatment. Ashwagandha administered on day 7, also attenuated the disruption of memory consolidation, produced by chronic treatment with ECS. On the elevated plus maze Ashwagandha reversed the scopolamine (0.3

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mg/kg) induced delay in transfer latency on day 1. On the basis of these findings it is suggested that Ashwagandha exhibits a nootropic like effect in naive and amnesic mice [12]. A study indicate that treatment during postnatal developmental stage with Centella asiatica extract can influence the neuronal morphology and promote the higher brain functions of juvenile and young adult mice [13].

Regeneration of nerves/Induction of Axon-or Dendritic outgrowth

Sub fractions of Centella asiatica ethanolic extract were tested (100 microg mL–1) for neurite elongation in the presence of nerve growth factor (NGF). Greatest activity was found with a non-polar fraction (GKF 4). Relatively polar fractions (GKF 10 and GKF 13) also showed activity, albeit less than GKF 4. The findings indicate that components in Centella ethanolic extract may be useful for accelerating repair of damaged neurons [14].

In a study, it was found that six of the 18 compounds isolated from the methanol extract enhanced neurite outgrowth in human neuroblastoma SH-SY5Y cells. In Withanolide A – treated cells, the length of NF-H-positive processes was significantly increased compared with vehicle treated cells, whereas, the length of MAP2-positive processes was increased by Withanosides IV and VI. These results suggest that axons are predominantly extended by Withanolide A, and dendrites by Withanosides IV and VI [15].

Treatment with Withanolide A (WL–A) isolated from Ashwagandha, induced significant regeneration of both axons and dendrites, in addition to the reconstruction of pre and post synapses in the neurons. WL–A (10 micro mol Kg -1 day -1, for 13 days, p.o.) recovered A beta (25-35) induced memory deficit in mice. At that time, the decline of axons, dendrites and synapses in the cerebral cortex and hippocampus was almost recovered. WL–A is therefore an important candidate for the therapeutic treatment of neurodegenerative diseases, as it is able to reconstruct neuronal networks [16].

Effect on cognitive function

In a study, Bacopa monnieri significantly improved speed of visual information processing measured by the IT task learning rate and memory consolidation compared to placebo, with maximal effects evident after 12 weeks [17].

In an experimental study, an extract of B. monnieri was given to albino rats to measure its effect on three newly acquired behavioural responses: brightness discriminating, condition avoidance and continuous avoidance. The facilitating effect of the Bacopa was clearly discernible in all three learning responses, augmenting both the rat’s cognitive function and mental retention capacity. The rats learned faster, retained more of what they had learned, and remembered it longer. The chemical constituents responsible for the facilitating effect of Bacopa on learning schedules were identified as a mixture of bacosides A and B. The bacosides also enhanced vital protein activity and produced an increase in protein synthesis in the hippocampus, a part of the brain that is important for long-term memory [18].

In an open 4 week trial of Bacopa in 35 patients with anxiety neurosis 12g/day of dried Bacopa herb was given in the form of syrup. Significant improvement in anxiety (P<0.05), concentration (P<0.05) and immediate memory span (P<0.01) were seen as a result of the treatment. Work related mental fatigue, measured as total work output and errors committed per unit time, also improved significantly with Bacopa treatment (P<0.001). Improvements were also seen in symptoms such as insomnia, headache, palpitation and irritability [19]. To investigate the effect of Bacopa in school children aged 6-8 years, 40 children were given Bacopa syrup equivalent to 1 g dried herb daily for 3 months, in a single-blind design. Immediate memory, perception and reaction/ performance times improved with Bacopa treatment [20].

Neonatal rat pups (7 days old) were given different doses of fresh leaf juice of C. asiatica (CeA) orally for different period of time. These rats were then subjected to spatial learning (T-Maze) and passive avoidance tests along with the age matched normal saline control rats. The result showed improvement in spatial learning performance and enhanced memory retention in neonatal rats treated with higher doses [21].

In a study on the effects of Brahmi (B. monnieri) on human memory, seventy six adults aged between 40 and 65 years took part in a double blind randomized, placebo control study in which various memory functions were tested and levels of anxiety measured. The results showed a significant effect of the B. monnieri on a test for the retention of new information. Follow up tests showed that the rate of learning was unaffected suggesting that B. monnieri decreases the rate of forgetting of newly acquired information [22]. Bacopa has also demonstrated a significant memory promoting effect in animal models of Alzheimer’s disease [23].

In an experimental study, fresh C. asiatica plant extract was given orally to rat pups (n=5), from P7-P49 (6 weeks, 2 ml/kg/day). These and age matched normal control (n=5) rats were subjected to learning tests in T-maze and passive avoidance test. Following this, rats were sacrificed and amygdaloid nucleus was processed for Golgi staining. Results showed a significant increase in the percent correct response (control: 86.44 + 2.33 percent Vs Expt. 93.44 + 3.90 percent) in plant extract treated rats. Passive avoidance retention test revealed a significantly memory retention, dendritic intersection was significantly increased at all concentric circles, except at 100 micron. Dendritic branching points also significantly increased in the inner three zones. These results indicate a correlation between improved learning capacity and increased
dendritic arborization in amygdaloid nucleus. This may be the neural basis for enhanced learning in *Centella asiatica* treated rats [24].

A study undertaken to assess the potential of *Nordostachys jatamansi* as a memory enhancer, elevated plus maze and the passive avoidance paradigm were employed to evaluate learning and memory parameters. Three doses (50, 100, and 200 mg/kg p.o.) of an Ethanolic extract of *N. jatamansi* were administered for 8 successive days to both young and aged mice.

The 200 mg/kg dose of *N. jatamansi* ethanolic extract significantly improved learning and memory in young mice and also reversed the amnesia induced by diazepam (1 mg/kg, i.p.) and scopolamine (0.4 mg/kg, i.p.). Furthermore, it also reversed aging induced amnesia due to natural aging of mice. Hence, *N. jatamansi* might prove to be a useful memory restorative agent in the treatment of dementia seen in elderly persons [25].

A study undertaken with the objective of studying the effect of *Tinospora cordifolia* (Tc) on learning and memory in normal rats and on cyclosporine induced memory deficits, both alcoholic and aqueous extract of *T. cordifolia* enhanced the cognition in normal rats as were seen in behavioural tests – Hebb William maze and the passive avoidance task [26].

To investigate the effects of *Glycyrrhiza glabra*, on learning and memory, the elevated plus-maze and passive avoidance paradigm were employed to evaluate learning and memory parameters. Three doses (75, 150 and 300 mg/kg p/o) of aqueous extract of *G. glabra* were administered for 7 successive days in separate groups of mice. The dose of 150 mg/kg of the aqueous extract of liquorice significantly improved learning and memory of mice. Furthermore, this dose reversed the amnesia induced by diazepam 1 mg/kg i.p.), scopolamine (0.4 mg/kg i.p) and ethanol (1 mg/kg i.p) [27].

The effect of *Withania somnifera* extract prepared by two different methods were assessed on behavioral parameters using open field exploratory behavior, behavior despair and passive avoidance tests and were compared in young and old stressed Wistar rats. *W. somnifera* extracts prepared with 50% methanol and solvent containing water, ghee and honey were administered orally as fine suspension, during the shock period. The results revealed that stress induced depression anxiety and retention deficit in young and old rats. Administration of *W. somnifera* methanolic extract 250 mg/kg during shock period in young and old rats attenuated the stress-induced depression and enhanced memory. *W. somnifera* traditional extract 250mg/kg produced memory enhancement in both control and stressed young and old rats [28].

Isolated constituents of *W. somnifera* (Sitoindosides VII-X and Withaferine – A) increased cortical muscarinic acetylcholine receptor capacity partly explaining the cognition enhancing and memory improving effects traditionally attributed to *Ashwagandha* [29].

In a study, one half of a group of 40 healthy children (ages 6-8) were given *Bacopa* in a syrup base three times a day (a total of 1.05 g/day) over the course of four weeks, while the other half were given a placebo. Those children taking *Bacopa* were superior in matters of speed and accuracy in solving maze problems. Overall, these improvements “vitalized” the children’s efficacy and their propensity to choose exploratory behaviour and to opt for novel experiences in preference to familiar ones [30].

**Effect in ADHD**

In a study 36 children in the 8-10 year age group were selected for a double blind, randomized trial. 19 were given 50 mg of *Bacopa* twice daily, 17 others received placebo. After 12 weeks of treatment, the children were subjected to a battery of specialized tests. The data revealed a significant improvement in the areas of sentence repetition, logical memory and pair associative learning (matching things that go together, e.g. “test” and “grade”) in all 19 ADHD children who took *Bacopa* [31].

A study to test the efficacy of *Bacopa* on children for six weeks, 50 normal school children split into two groups were given either *Bacopa* or placebo. At the conclusion, they were evaluated for attention, concentration, and memory. *Bacopa* was shown to improve mean reaction time (auditory and visual) significantly [32].

**Effect on behaviour**

In an experimental study, rats were individually trained in a simple T-maze until they reached a predetermined level of performance. They were then divided into three groups and given either nothing, diazepam (Valium), or *Bacopa*. At the end of 10 days, they were evaluated by repeating the T-maze trial. Those animals given *Bacopa*, showed remarkable learning and memory enhancement compared with the control and valium groups. Furthermore, the neurochemical content of their brain tissue showed an increase in the level of serotonin. Serotonin has been identified with improved spatial memory as well as anxiolytic benefits [33].

**Antidepressant activity**

In a study, *B. monneiri* extract given in the dose of 20 and 40 mg/kg, orally once daily for 5 days was found to have significant antidepressant activity in forced swim and learned helplessness models of depression and was comparable to that of imipramine [34].

A 15 day treatment with *N. jatamansi* resulted in a significant increase in the levels of NE, DA, 5-HT, 5-HIAA and GABA. These data indicate that the alcoholic extract of the roots of *N. jatamansi* causes an overall increase in the levels of central monoamines and inhibitory amino acids [35].

In an experimental study a bioassay-guided isolation of the ethanol extract from the fruits of *Piper longum* yielded, a known piperidine alkaloid, piperine, which suggests a mechanism for the antidepressant effects observed in the behavioral studies.
showed an inhibitory effect against monoamine oxidase (MAO). The results suggest that piperine possesses potent antidepressant-like properties that are mediated in part through the inhibition of MAO activity and therefore represent a promising pharaco therapeutic candidate as an antidepressant agent [36].

Anxiolytic/antistress activity

The bioactive glycowithanolides (WSG), isolated from W. somnifera roots WSG (20 and 50 mg/kg) was administered in rats orally once daily for 5 days and the results were compared by those elicited using the benzodiazepine lorazepam (0.5mg/kg, i.p.), for the antidepressant investigations. WSG induced an anxiolytic effect, comparable to that produced by lorazepam, in the elevated plus-maze test, social interaction and feeding latency in an unfamiliar environment. WSG also exhibited an antidepressant effect, comparable with that induced by imipramine, in the forced swim induced ‘behavioral despair’, and ‘learned helplessness’, tests [37].

A number of herbal drugs mostly in the form of their bioactive isolated from them, were evaluated for their antistress activity by a number of tests. W. somnifera, Ocimum sanctum, T. cordifolia, C. asiatica, G. glabra were reported with encouraging results [40].

Neuroprotective effect

The effect of an aqueous extract of C. asiatica (100, 200 and 300 mg/kg for 21 days) was evaluated in i.c.v. STZ induced cognitive impairment and oxidative stress in rats. The findings indicated that an aqueous extract of C. asiatica is effective in preventing the cognitive deficits, as well as the oxidative stress, caused by i.c.v. STZ in rats [41].

The protective effect of N. jatamansi (NJ) on neurobehavioural activities was studied in middle cerebral artery (MCA) occlusion model of acute cerebral ischaemia in rats. All the alternations induced by ischemia were significantly attenuated by 15 days pretreatment of NJ (250 mg/kg p.o.) and correlated well with histopathology by decreasing the neuronal cell death following MCA occlusion and reperfusion [42].

CNS Depressant/sedative effect

The methanol extract of the whole plant of Shankhpushpi, Convolvulus microphyllus sieb ex spreng (convolvolaceae), was found to produce alternations in the general behaviour pattern, reduction in spontaneous motor activity, hypothermia, and potentiation of phenobarbitone-sleeping time, reduction in exploratory behavioural pattern and suppression of aggressive behaviour. The extract also showed an inhibitory effect on conditioned avoidance response and antagonism to amphetamine toxicity. The findings explicitly suggested that the whole plant extract of C. microphyllus possesses a potential CNS depressant activity [43].

Effect of chronic administration of ethanolic extract of Acorus calamus (AC) was studied on spontaneous electrical activity and monoamine levels of brain. AC seemed to exert its depressive action by changing electrical activity and by differentially altering brain monoamine levels in different brain regions [44].

A sedative action and potentiation of barbiturate effect (increased sleeping time, reduced body temperature) was observed in small animals (mice, rats, rabbits and cats) following intraperitoneal administration of the aqueous and ethanolic extracts of both European and Asian varities of A. calamus [45].

CNS stimulating action

The effects of a 50% ethanol extract of the root of Plumbago zeylanica were investigated on locomotor behavior and central dopaminergic activity in rats. The results showed that the extract of the root of P. zeylanica specifically enhanced the spontaneous ambulatory activity without inducing stereotypic behaviour. The neuro-chemical estimations revealed elevated levels of DA and its metabolite homovanillic acid (HVA) in striatum compared with the control rats. These behavioural and biochemical results indicated stimulatory properties of the extract of the root of P. zeylanica, which may be mediated by dopaminergic mechanisms in the rat brain [46].

Discussion

Review of the various clinical and experimental studies of different Ayurvedic Medhya drugs reveals that these drugs possess nootropic, cognition enhancing, anxiolytic, antidepressant, anticonvulsant, CNS depressant, sedative and neuroprotective effect.
Ashwagandha and Centella possess nootropic activity. Bacopa and Ashwagandha have the potential for corrective effect in cognitive deficit, while Centella can influence the neuronal morphology and can thereby promote higher brain functions.

The drugs C. asiatica and W. Somnifera (Ashwagandha) are useful for repair of damaged neurons and in neurodegenerative disorders as these drugs have the capacity to reconstruct the neuronal networks.

Regarding the effect on cognition, the drugs Bacopa, Centella, N. jatamansi, T. cordifolia, G. glabra and W. somnifera may be useful. Bacopa has potential for revitalization of intellectual functions and may improve the higher order cognitive functions such as learning and memory. It has also showed effect on positive behavior modification by increasing the level of serotonin. Centella can induce memory retention an improved learning capacity by increasing dendritic arborization in amygdaloid nucleus.

N. jatamansi is useful as memory restorative agent as it facilitates cholinergic transmission in the brain. Both T. cordifolia and G. glabra are helpful in improving the learning capacity and memory restoration. W. somnifera, by increasing cortical muscarinic acetylcholine receptor capacity, can enhance memory.

Studies also indicate that Bacopa can produce significant improvement in different areas of child’s functioning in ADHD children and it can also improve the mean reaction time (Auditory and Visual) significantly. Bacopa can also improve the behaviour by increasing the serotonin level in brain.

The drugs B. monnieri, N. jatamansi and Piper longum possess significant antidepressant activity. N. jatamansi can cause overall increase in the levels of central monoamines and inhibitory amino acids and therefore can be used as an antidepressant agent. P. longum also can be used as a promising antidepressant agent as it acts through the inhibition of MAO activity.

Studies regarding the anxiolytic or antistress activity revealed that Bacopa, Centella, W. somnifera, T. cordifolia and G. glabra have the potential to ameliorate the adverse conditions like stress. W. somnifera can be used as a mood stabilizer in clinical conditions of anxiety and depression. G. glabra posseses significant anxiolytic activity. Bacopa has potential to modulate the activities of HSP 70, P450 and SOD (super oxide dismutase) thereby possibly allowing the brain to be prepared to act under adverse conditions.

C. asiatica by its potentiation of cellular oxidative defense mechanism acts as neuroprotective drug. Effectiveness of N. jatamansi in local ischaemia is most probably by virtue of its antioxidant property.

C. microphyllus shows potential CNS depressant activity by producing alternations in the general behaviour pattern, reduction in spontaneous motor activity, hypothermia, and potentiation of phenobarbitone-sleeping time, reduction in exploratory behavioural pattern and suppression of aggressive behaviour. A. calamus seems to exert its depressive action by changing electrical activity and by differentially altering brain monoamine levels in different brain regions. P. zeylanica possesses CNS stimulant activity.

Conclusion

The studies indicate that Ayurvedic medhya drugs act in different way on the brain and different drug have specific action. Thus these drugs have potential to improve the overall mental system. Studies also reveal that Brahmi (B. monnieri) is the best intellect promoting drug which can prove safe and effective in promoting mental health in children. The review provides a clear view about the specific properties of different medhya drugs thus their judicious use will be very much helpful for the management of mental and behavioural disorders of adults and children.

References


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All authors including the co-authors should have been responsible for a significant part of the manuscript. All authors and co-authors should have taken part in writing the manuscript reviewing it, and revising its intellectual and technical content. Any author whose name appears on a paper assumes responsible for the results.

Length of papers

Original research papers should be written under the following sub headings: Title page, Abstract, Keywords, Introduction, Materials and methods, Results, Discussion, Acknowledgements, References, Tables and Figures. It should not exceed 4000 words.

Review papers should include an abstract, an introduction that outlines the main points, under brief sub headings and references. Review papers may exceptionally be longer up to 5000 words.

Short communications should contain novel preliminary findings. It should be written under the subheadings similar as for a research paper but should include one figure, a table and only few key references. It should not exceed 2000 words.

Title page

The title of the paper must be as brief as possible. The title of the paper should correctly reflect its contents and must not exceed 100 characters in length. A short running title of up to 40 characters should be provided. The name(s) and initials of the author(s) with the highest academic degree and their affiliation(s) should be given. The name, postal address and e-mail address of the corresponding author should be stated.

Abstract

Contributors are requested to submit an abstract of not more than 200 words of the article/paper with the following components: Objectives, Methods and materials, Setting, Interventions, Main outcome measures, Results and Conclusions. The abstract should not contain any reference.

Manuscript layout

Manuscript of articles should be organized as follows: Title page, Abstract, Key words, Introduction, Methods and materials, Results, Discussion, Conclusions, Acknowledgement and Reference.

Introduction

Introduction should supply enough information to allow the reader to understand and evaluate the results and discussion that follows. It should be restricted to reason for undertaking the present study and provide only the most essential background. It should state the problem, consider events and literature up to the present problem, State any assumptions, give an outline of the work.

Materials and Methods

The nomenclature, the source of materials and equipments used with the manufacturers’ details should be clearly mentioned within parenthesis. The procedure adopted should be explicitly stated to enable other workers to reproduce the results.
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Results

The results are concisely and logically presented. Only data essential for the main conclusion emerging from the study should be included. Interpretations of data should be left to the discussion section. Presentation of the same results in the figures and text should be avoided.

Discussion

Long, rambling discussions should be avoided. The discussion should deal with the interpretation of results without repeating information already presented in results. It should logically relate new findings to old ones. Unqualified statements and conclusions not completely supported by data should be avoided. All hypotheses should be clearly identified as such. Recommendations may be included as part of the discussion, only when considered absolutely necessary and relevant.

Acknowledgement

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