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EFFECTS OF THE FRESH LEAVES OF *SPONDIAS MOMBIN* L. ON MILK PRODUCTION OF WEST AFRICAN DWARF (WAD) EWES AND THEIR LAMB’S GROWTH PERFORMANCE

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ABSTRACT

The high pre weaning mortality recorded in West African Dwarf lambs is caused by insufficient breast milk. To reduce this pre weaning mortality, an experiment was conducted to evaluate the effect of *Spondias mombin* leaves on ewe’s milk production and growth performance of theirs lambs in southern Benin. The assay was conducted on 18 lactating ewes divided into three homogeneous groups of 6 animals each who received supplementation of leaves of this plant. The resulted outcomes indicate that the leaves of *Spondias mombin* have a significant effect on milk production of ewes and lambs weight gain. The average value of 94.00; 94.67 and 68.67 g/day were found in group 1, group 2 and control group, respectively. However, both types of treatment had similar effects on milk production of ewes and growth of lamb. The increase in milk production was on an average 36.40% and 37.2% in group 1 and group 2 respectively. Treatments by leaves of *Spondias mombin* had no significant effect on live weight change of ewes during lactation (p > 0.05). Also, the leaves of *Spondias mombin* had no significant effect on pH and ash content, protein and milk fat. This difference was significant for dry matter content of milk.

KEY WORDS: WAD sheep, *Spondias mombin*, milk production, lamb growth, Benin.

Cite this article:
INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Nostro et al., 2000; Tanaka, 2002). In Republic of Benin, 80% of the population being unable uses the traditional medicines to sustain their primary health care needs Deleke Koko et al., (2011). Lactogenic plants are most of the medicinal plants that were used by traditional healers. The women with milk production deficiencies traditionally use some of these lactogenic plants to induce milk production or to increase milk yield. Breastfeeding in the first six months of life stimulates babies’ immune systems and protects them from diarrhea and acute respiratory infections (UNICEF, 2006).

Milk is a food that is of great biological significance for lamb from the moment they are born and at weaning. The West African Dwarf (WAD) ewe is a poor milker, kept exclusively for meat, as the ewe’s milk yield barely suffices to feed the lambs. Hence, lamb mortality is high in this breed, 20% according to Gbangboché et al., (2005).

Nowadays, different plants found in nature are widely used in different fields including medicine, pharmaceutics, food and health industries, but these plants are not widely used in veterinary. According to Mirzaei F and Hari Venkatesh K R (2012), ethno-veterinary alternatives are an option for small-scale livestock farmers who cannot use allopathic drugs or for those larger conventional farmers whose economic circumstances prevent the use of veterinary services for minor health problems of livestock.

*Spondias mombin* (family: Anacardiaceae) is a plant that is used for leaf, bark, roots and seeds. A juice of crushed leaves and powder of dried leaves of *S. mombin* are used to treat wounds, inflammations and abortifacients (Ayoka, 2008). *Spondias mombin* has been reported to be anti-helminthic (Ademola et al., 2005) and anti-malarial (Caraballo et al., 2004). The traditional healers in Nigeria recommend *Spondias mombin* to have lactogenic activity (Oguike, 2008). Considering the extensive utilization of *S. mombin* in traditional medicine, the study is designed to evaluate the effect of fresh leaves of *Spondias mombin* on the WAD ewes to improve their milk production to reduce lambs mortality.

MATERIALS AND METHODS

Study environment

The Sheep Research Center of Faculty of Agronomy Sciences is located in the University of Abomey Calavi in the town of Abomey Calavi near Cotonou. The climate is of guinean type with two dry seasons (from November to March, July to September) and two rainy seasons (from March to July, September to November). The average rainfall is 1200 mm per year and the annual temperature ranges from 23°C to 30°C.

Collection of Plant material

*Spondias mombin* (Anacardiaceae), aerial parts (leaves) were collected from Abomey-Calavi, in June 2010 and were identified in Laboratory of Applied Ecology (Faculty of Agronomic Sciences, University of Abomey-Calavi, Benin). The dose of leaves of the plant (100 g/animal) administered was based on the quantity of leaves (100 g of fresh leaves) used by traditional healers in the treatment of milk production deficiencies in the women.

Management of animals

Eighteen (18) lactating WAD ewes, in the 2nd lactating season and weighing an average 14.2 ± 1.52 Kg were used in this experiment. Animals were housed in pens and lambs were kept with their dams and remained with them until their weaning at three months of age. They were treated against ecto and endoparasites and equally vaccinated against pests. Ewes were divided into three groups (six animals each) and were assigned at random to receive one of the treatments using complete randomized block design. The treatments included:

- **Control group:** received no treatment.
- **Group1**: Oral administration of *Spondias mombin* leaves - 100 g/animal/day for 3 days at the beginning of lactation.  
- **Group2**: Oral administration of *Spondias mombin* leaves - 100 g/animal/day for the whole lactating period.

Animals grazed from 11 AM to 5 PM (for 6 h) on improved pastures consisting of *Panicum maximum C1*. The ewes received extra cotton oil cake protein of 200 g per day. They also received mineral supplements in the form of licks and water *ad libitum* throughout the trial period.

**Data collection**

For three months, milk production was recorded once in every two weeks, which began a week after lambing and hand milking was done once a day (6:30 AM). On the day of collection, lambs were isolated from their mothers at 6 PM. The next morning, ewes were hand milked before sent to the pasture. During the experimental period, the fresh milk samples were collected at a month’s interval in each animal. The pH was determined immediately after collecting the samples using a pH meter INOLAB 730. The chemical composition of the milk was determined in the laboratory of chemistry, Agricultural Research Center of Agonkanmè (INRAB). Total solids, protein, ash total and fat were determined in the laboratory using the procedures of AOAC (1990).

The body weight of lambs was measured and recorded. Every two weeks, the lambs were weighed before feeding in the morning. Average daily gain (ADG) of lambs was calculated to compare the growth of lambs between groups. The body weight of ewes was also measured once a month.

**Statistical analysis**

The means and standard errors of the means of milk production, milk chemical composition, body weight of ewe as well as those of ADG were determined. Statistical analysis of the differences between mean values obtained for treatments was performed using Minitab. Data were subjected to one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. In all cases, p values ≤ 0.05 were regarded as statistical significance.

**RESULTS**

**Milk production**

From the first week up to the ninth week of lactation, the daily milk production in groups 1 and 2 was higher than control groups. From the ninth week to the end of lactation, the daily milk production was similar in three groups (Figure 1). This is evident on the lactation Table 1. The daily milk production was significantly higher (p < 0.05) in groups 1 and 2 than Control group (p < 0.05) from the beginning to 7th week of lactation. The daily milk production at 7th week was 83.80; 85.56 g/day and 66.97 g/day in Group 1, Group 2 and control group, respectively (Table 1). From the 9th week to the end of lactation, the daily milk production is similar in all groups (p > 0.05).

**Body weight of lambs and ewes**

The body weight of lambs increased gradually and similarly in three groups during the first 2 weeks (Figure 2). After the first two weeks, this increase was much more pronounced in the lambs of groups 1 and 2 (weaning body weight >8.5 Kg) than control group (weaning body weight <7.5 Kg).

Analysis of variance (Table 3) shows that the average daily gain (ADG) in 0–15 days and 15–30 days was significantly higher in the groups 1 and 2 than Control group (ADG in 0–15 days: 94 g/day; 94.67 g/day for Group 1 and 2 respectively, against 68.67 g/day for control group) (p < 0.05) (Table 3). This difference is not significant in 30–45 days, 45–60 days, 60–75 days and 75–90 days (p > 0.05) in all the three groups. The ADG of lamb was comparable in Group 1 and Group 2.
Figure 1: Curves of lactation of ewes according to week of lactation and treatments

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>Group1</th>
<th>Group2</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82.00 ± 6.49 b</td>
<td>128.93 ± 19.44 a</td>
<td>130.60 ± 7.44 a</td>
<td>*</td>
</tr>
<tr>
<td>3</td>
<td>75.18 ± 5.79 b</td>
<td>107.63 ± 13.77 a</td>
<td>114.00 ± 11.16 a</td>
<td>*</td>
</tr>
<tr>
<td>5</td>
<td>68.33 ± 5.78 b</td>
<td>88.49 ± 13.22 a</td>
<td>95.60 ± 7.33 a</td>
<td>*</td>
</tr>
<tr>
<td>7</td>
<td>66.97 ± 4.23 b</td>
<td>83.80 ± 15.06 a</td>
<td>85.56 ± 10.73 a</td>
<td>*</td>
</tr>
<tr>
<td>9</td>
<td>58.31 ± 5.24 a</td>
<td>69.00 ± 4.18 a</td>
<td>70.60 ± 3.21 a</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>45.11 ± 6.83 a</td>
<td>52.60 ± 7.86 a</td>
<td>57.00 ± 8.60 a</td>
<td></td>
</tr>
</tbody>
</table>

* = p < 0.05

Table 2. Milk physico-chemical composition (Mean ± Standard deviation)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Group1</th>
<th>Group2</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph</td>
<td>6.94 ± 0.01a</td>
<td>6.94 ± 0.01a</td>
<td>6.96 ± 0.01a</td>
<td></td>
</tr>
<tr>
<td>Total solid TS (%)</td>
<td>15.06 ± 0.22a</td>
<td>14.15 ± 0.04b</td>
<td>14.13 ± 0.05b</td>
<td>*</td>
</tr>
<tr>
<td>Ash content (% TS)</td>
<td>2.04 ± 0.09a</td>
<td>2.09 ± 0.11a</td>
<td>2.13 ± 0.15a</td>
<td></td>
</tr>
<tr>
<td>Protein (% TS)</td>
<td>5.22 ± 0.12a</td>
<td>5.06 ± 0.01a</td>
<td>5.02 ± 0.03a</td>
<td></td>
</tr>
<tr>
<td>Fat (%TS)</td>
<td>6.53 ± 0.32a</td>
<td>6.46 ± 0.28a</td>
<td>6.31 ± 0.21a</td>
<td></td>
</tr>
</tbody>
</table>

* = p < 0.05
The sex of the lamb had significant effect on average daily gain (ADG) of lamb in 0–15 days (85.24 g/day for female against 95.00 g/day for male) (p < 0.05) (Table 3). However, the sex of lamb had no effect on average daily gain (ADG) of lamb at 15–30 days, 30–45days, 60–5days and 75–90days (p > 0.05). Also the treatments and sex of lamb had no effect on initial and final body weight of ewe (p > 0.05) (Table 3).

Milk physico-chemical composition

The pH of milk found no difference in the three groups making it statistically insignificant (p > 0.05) (Table 2). Regarding the chemical composition, it was noticed that the milk of ewes in control group contained significantly less water compared to groups 1 and 2 (p < 0.05). The average value of 15.06; 14.15 and 14.13 were found for total solids in control group, Group 1 and Group 2 respectively (Table 2). The ash content, protein and fat were not significantly different between the groups (p > 0.05) (Table 2). The protein was 5.22; 5.06 and 5.02 in control group, Group 1 and Group 2 respectively (Table 2).

<table>
<thead>
<tr>
<th>Table 3: Body weight, Average Daily body weight Gain (ADG) (Mean ± Standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>ADG of lamb (g/day)</td>
</tr>
<tr>
<td>0–15days</td>
</tr>
<tr>
<td>15–30days</td>
</tr>
<tr>
<td>30–45days</td>
</tr>
<tr>
<td>45–60days</td>
</tr>
<tr>
<td>60–75days</td>
</tr>
<tr>
<td>75–90days</td>
</tr>
<tr>
<td>Ewes Body weight (kg)</td>
</tr>
<tr>
<td>Day of birth</td>
</tr>
<tr>
<td>30 days</td>
</tr>
<tr>
<td>60 days</td>
</tr>
<tr>
<td>90 days</td>
</tr>
</tbody>
</table>

a, b, c = Means with different superscript letters on the same row differ significantly (p < 0.05).
DISCUSSION

The floristic and ethnomedicinal aspects of lactogenic plants have been studied extensively (Bailey and Day, 2004; Wynn and Fougere, 2007); however, little is known about their biological activities. In this study, Spondias mombin was investigated for its activity on milk production. Our results on milk production indicate that the leaves of Spondias mombin significantly stimulated milk production in treated ewes compared to control group. This activity of the leaves of S. mombin is due to the ability of leaves to stimulate the hormones that initiate milk biosynthesis (Houdebine, 2007) and causing development of breast tissue (Lompo-Ouedraogo et al., 2004). The presence of steroidal, saponins, sapogenins and tannins constituents in leaves of Spondias mombin (Njoku and Akumefula, 2007; Igwe, 2010) contributes in its lactogenic effect (Goyal et al., 2003; Mirzaei and Hari Venkatesh KR, 2012). The increase in milk production was on an average 36.40% and 37.2% in group 1 and group 2 respectively. Our results were comparable to those of Mishra (2006) and Oguike (2008). Mishra (2006) and Oguike (2008) show that Galega officianalis herb and Spondias mombin plant can increase milk supply up to 50% and 32.59%, respectively.

The pH of the milk in the three groups is comparable. Regarding the chemical composition, it is noticed that the milk in the control group contains less water. This difference can be explained by several factors, including genetic factors (individual) and the treatments (feed) Atti and Rouissi (2003). Other chemical constituents are identical in the three groups and are statistically insignificant. These results are similar to those reported by Rouissi et al., (2007) in an assay where the soybeans were replaced by horse bean in the feed of ewes. The protein of milk obtained in this study is similar to the one of Adewumi and Olorunisomo (2009): 5.52% in WAD ewes. Slightly higher values (7.08% from fat and 6.12% from protein) were found in WAD ewes by Ekeocha (2012). This difference could be explained by feeding regimes, ration components and forage, grain ratios that affected milk composition.

The positive effect of the treatment (S. mombin) on growth performance of lamb is due to milk production performance of their mothers. The milk consumed by the lambs in treated groups is higher than the milk consumed by the lambs in control group. Aside
from overwhelming importance of milk to humans, ewe’s milk production is the foundation for good lamb growth performance (Ogunwole, 2004). Increased milk intake is significantly associated with increased body weight (Korman, 2001; Niznikowski et al., 2006).

As shown in Table 3, effect of sex on growth performance of lamb observed in 0–15 days was significant. As for birth weight, Idris et al., (2010) and Gbangboche (2006) also found significant effect of sex on growth performance. They indicated that in the same managerial conditions, males were significantly heavier than females at birth. In this study, the effect of sex on lamb growth disappears after 15 days. These results disagree with Kumar et al., (2007). This author reported that, the body weight of male lamb was higher than the female lambs at all ages. The results obtained by this author could be explained by the difference in the managerial conditions (managerial conditions of males differ to managerial conditions of females).

CONCLUSION

This study on the effect of leaves of Spondias mombin on dairy ewes showed positive results of this plant on milk production and growth performance of the lambs. It was noticed that the two treatments (3 days and continual) had similar effect on the performances of the sheep. It has been concluded that Spondias mombin is believed to assist initiation maternal milk production and not maintain lactation; hence this plant can be considered as an alternative for lactogenic hormones for inducing lactation in WAD ewes.

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REFERENCES


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Conflict of Interest: None Declared
BROAD SPECTRUM ANTIMICROBIAL ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF \textit{ALANGIUM SALVIIFOLIUM} FLOWER EXTRACT

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ABSTRACT

The present investigation was aimed to evaluate the broad spectrum antimicrobial and phytochemical analysis of the alcoholic and aqueous extracts derived from the flowers of \textit{Alangium salviifolium}. Aqueous and ethanolic extracts were prepared and tested on multiple drug resistant Gram-positive (\textit{Staphylococcus aureus, Enterococci and Staphylococcus epidermidis}) and Gram-negative- (\textit{Citrobacter, Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, Klebsiella species, Enterobacter, E. coli and Seratia marcescens}) organism. Agar disc diffusion method was used to determine the sensitivity of the test samples and standard antibiotics (Gentamycin, Chloramphenicol, Norfloxacin and Amikacin) were used as reference antibiotics. The results of antimicrobial assays showed that aqueous extract was active against all tested microbial species in inhibiting 10 out of 11 test microorganisms with 08–17 mm zone of inhibition. Citrobacter species which were most resistant to the reference antibiotics was inhibited by the aqueous extract. These results indicated that most of the active constituents (responsible for exerting antibacterial action) in these plants are expected to be soluble in polar solvent. It authenticates that the entire tested microorganism is susceptible to alcohol extracts in inhibiting 04 out of 11 test microorganisms with 04–14 mm zone of inhibition. Qualitative phytochemical analysis of the extract confirms the presence of various phytochemicals like tannins, saponins, flavonoids, alkaloids, quinines, cardiac glycosides, coumarins, phenols, anthraquinones, steroids, catechin, reducing disaccharide and proteins. The overall results of this study indicate that the extract from \textit{Alangium salviifolium} flower have medicinally important bioactive compounds and interesting broad spectrum antimicrobial activity against multiple drug resistant bacteria.

KEY WORDS: \textit{Alangium salviifolium}, Broad spectrum antimicrobial activity, phytochemical analysis, aqueous extract, ethanolic extracts, multiple drug resistance

Cite this article:
INTRODUCTION

Medicinal herbs are the local heritage with global importance. The expanding bacterial resistance to antibiotics has become a growing concern worldwide (Gardam, M.A. 2000). Multiple drug resistance in both human and plant pathogens has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. Intensive care physicians consider antibiotic-resistant bacteria a significant or major problem in the treatment of patients (Lepape, A. 2011). The limited life span of antibiotics has rendered a necessity to search for new antimicrobial substances from various sources such as medicinal plants. The majority of rural people has limited access to formal and adequate health services and thus heavily resources to traditional healers. Many indigenous plants have been evaluated and used as a source of many effective and potent drugs against various infectious diseases. Herbal medicines has been in practice since long time as one of the basic treatment for cure of various diseases and show minimum or no side effects and are considered to be safe.

A wide range of phyto-chemicals present in plants is known to inhibit bacterial pathogens (Cowan, 1999; Medina et al., 2005; Romero et al., 2005). Phytochemicals are secondary metabolites in one or more parts of the medicinal plants. Phytochemicals may protect human from various diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Phytochemicals are basically divided into two groups that are primary and secondary metabolites according to their functions in plant metabolism. Primary metabolites comprise common sugars, amino acids, proteins and chlorophyll while secondary metabolites consist of alkaloids, flavonoids, tannins and so on (D.Kubmarawa, G.A.Ajoku., 2007; Edeoga HA, Okwu DE.,2005). Medicinal plants represent a valuable source for this kind of compounds (Gopinath, S.M., et al., 2011).

Alangium salviifolium (L.F.) Wangerin is a deciduous, rambling shrub or a tree belonging to the family Alangiaceae (Jubie S, Jawahar N., 2008), popularly in Karnataka region it is called as Ankola. Alangiaceae is a monogenic family of trees and shrubs found in the tropical and subtropical region. A small, thorny deciduous tree/shrub which grows up to a height of 5–10 meters long, bark is yellowish rough and faintly fissured, the leaves are alternate, elliptical oblong, the flowers are yellowish white in axillary fascicles. The flowering season is February to June. The plant is distributed in dry regions, plains and lower hills in India, Africa, Sri Lanka, Indochina and China. In the Indian subcontinent’s ancient Ayurveda system of medicine, the Alangium salviifolium has extensively been used as a source of traditional remedies for thousands of years. The plant has been reported for its anti-tubercular, antispasmodic and anti-cholinesterase activity (Warrier P K, Nambiar, Samakutty., 2005). The Anti-Fertility activity of the stem Bark of Alangium salviifolium in Wistar female rats has also been reported (Murugan V, Shareef H, Ramanathan M., 2000).

In this present investigation efforts were made to explore new bioactive compounds and the study of phyto-chemical and broad spectrum antimicrobial activity of aqueous and ethanolic extract of Alangium salviifolium flower against hospital isolates which was identified to be Gram-positive (Staphylococcus aureus, Enterococci and Staphylococcus epidermidis) and Gram-negative-(Citrobacter, Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, Klebsiella species, Enterobacter, E. coli and Seratia marcescens). Though the availability of the flower is seasonal found only during February-June it has vibrant phyto-chemicals like tannins, saponins, flavonoids, alkaloids, quinines, cardiac glycosides, coumarins, phenols, anthraquinones, steroids, catechin and broad spectrum antimicrobial treasure in it.

MATERIALS & METHODS

Experimental Plant Material

The proposed material of Alangium salviifolium (AS) flower was procured from
Turuvekere Taluk, Tumkur district, Karnataka Region, India during March, 2011 with the help of local tribes and was washed thoroughly and shade dried.

**Preparation of Extracts**

Preparation of the extract of AS powdered flower is done using ethanol and distilled water. The shade dried coarse powder of the flowers (500 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with 90% ethanol until the completion of the extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. Similarly, aqueous extract was prepared and stored at 4°C for further study.

**Phyto-chemical screening**

Qualitative phyto-chemical analysis of the aqueous and ethanolic extract of AS flower was carried out using standard procedures to identify the constituent carbohydrate moiety (Molisch test, Fehlings test, Barfoeds test, Bials test, Cobalt chloride test, Iodine test), Proteins (Biuret test, millons test), steroids and terpenoids (Liberman-Burchard and Salkowski tests), alkaloids (Mayer’s test), cardiac glycosides (Keller-Kilani test), saponins (foam test), anthraquinone (modified Borntragers test), flavonoids (Shinoda test), tannins and phenols (Ferric chloride test), organic acids (Calcium chloride test), catechin (Ehrlich test), coumarin (alcoholic KOH test) as described by (Sofowara.,1993; Trease and Evans.,1989 and Harborne.,1993).

**Antimicrobial Activity:**

**Microbial Strains**

The microorganisms used in the antimicrobial tests are the clinical samples taken from the registered hospitals of Bangalore region and were identified to be Gram-positive (*Staphylococcus aureus, Enterococci and Staphylococcus epidermidis*) and Gram-negative (*Citrobacter, Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, Klebsiella species, Enterobacter, E.coli and Seratia marcescens*). Loop full of the bacterial strain was inoculated in 25 ml of nutrient broth (HiMedia) in a conical flask and incubated at 37°C on a rotary shaker for 24 h to activate the test bacteria. The inoculum size was maintained to be $1 \times 10^8$ cells/ml, and the bacterial strains were maintained on nutrient agar slants for future use at 4°C.

**Chemicals**

Gentamycin, Chloramphenicol, Norfloxicin, Amikecin (Alkem Laboratories, India) was used as reference antibiotic (RA) against bacteria and dimethylsulphoxide (DMSO) (HiMedia, India) was used as solvent for tested samples. Other chemicals were used of analytical grade of S.D. Fine Chemicals, Mumbai, India.

**Culture media**

Mueller Hinton Agar (HiMedia) was used for the antibacterial susceptibility study. The ingredients are weighed and added in 1000 ml distilled water and boiled to dissolve it completely. The pH of media was adjusted to 7.4 ± 0.2 (at 25°C) and sterilized it by autoclaving at 15 lbs pressure (121°C) for 15 min. The solution of the test extracts was prepared at the concentration of 5 mg/ml by dissolving in dimethylsulphoxide (DMSO) in the stopper specific gravity bottle and stored in refrigerator. The solution was removed from the refrigerator one hour prior to use and was allowed to warm up to room temperature.

**Screening for antibacterial properties**

Antibacterial properties of *Alangium salviifolium* flower extracts were tested by Agar disc diffusion method (Harborne JB., 1973; Jamine.R.Daisy, 2007; Gopinath S M. et al., 2012). The culture plates were prepared by pouring 20 ml of the Mueller Hinton agar medium into sterile petriplates. 200 µl of inoculum suspension was spread uniformly over the agar medium using sterile L shaped spreader to get a uniform distribution of bacteria. The test extracts (20 µl) (from stock solution of 5 mg/ml) was impregnated into
sterile discs (7 mm) (Hi-Media) and then allowed to dry. The disc was then introduced into the medium which was initially inoculated with the test organism. And as reference antibiotics Gentamycin, Chloramphenicol, Norfloxacin, and Amikacin 50 mcg/ml was used to check multiple drug resistance of the strain. Then the plates were incubated for 24 h at 37°C. The experiment was performed under strict aseptic conditions. The effectiveness of these extracts was recorded by measuring the diameter of inhibition zone. Triplicate was performed and the experiment was repeated thrice and the results were recorded.

Table 1. Qualitative phyto-chemical analysis of *Alangium Salvifolium* flower extract

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>Aqueous extract</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catechin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Monosaccharides sugar</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pentose sugar</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hexose sugar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2: Qualitative analysis-Broad Spectrum Antibacterial activity of *Alangium Salvifolium* flower extracts against multiple drug resistant organism using reference antibiotics Gentamycin, Chloramphenicol, Norfloxacin and Amikacin determined by the agar disc diffusion method

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Gentamycin</th>
<th>Chloramphenicol</th>
<th>Norfloxacin</th>
<th>Amikacin</th>
<th>Aqueous Extract</th>
<th>Ethanolic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Staphylococcus epidermis</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Enterococci</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Klebsiella species</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>Citrobacter</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Seratia marcescens</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

(−): Not active, (+): Active
Table 3: Quantitative analysis-Broad Spectrum Antibacterial activity of Alangium Salvifolium flower extracts against multiple drug resistant organism using reference antibiotics Gentamycin, Chloramphenicol, Norfloxacin and Amikacin determined by the agar disc diffusion method

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Zone of inhibition (mm)</th>
<th>Gentamycin</th>
<th>Chloramphenicol</th>
<th>Norfloxacin</th>
<th>Amikacin</th>
<th>Aqueous Extract</th>
<th>Ethanolic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td></td>
<td>28</td>
<td>24</td>
<td>0</td>
<td>22</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td></td>
<td>18</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Enterococi</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td>18</td>
<td>16</td>
<td>14</td>
<td>15</td>
<td>08</td>
<td>0</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td></td>
<td>0</td>
<td>18</td>
<td>16</td>
<td>0</td>
<td>08</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td>25</td>
<td>26</td>
<td>0</td>
<td>23</td>
<td>09</td>
<td>0</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td></td>
<td>23</td>
<td>22</td>
<td>16</td>
<td>20</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td>Klebsiella species</td>
<td></td>
<td>23</td>
<td>21</td>
<td>18</td>
<td>18</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Citrobacter</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Seratia marcescens</td>
<td></td>
<td>21</td>
<td>21</td>
<td>22</td>
<td>21</td>
<td>14</td>
<td>04</td>
</tr>
<tr>
<td>Enterobacter</td>
<td></td>
<td>24</td>
<td>24</td>
<td>23</td>
<td>21</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

(0): No Zone of Inhibition

RESULTS AND DISCUSSION

The results of qualitative and quantitative antibacterial screening of aqueous and ethanolic extracts of Alangium salvifolium flower using four different antibiotics (Gentamycin, Chloramphenicol, Norfloxacin and Amikacin) as a reference is presented in Table 2 and 3. Antimicrobial screening assay against bacteria showed that aqueous extract had the most distinct effect on most of the tested organism in inhibiting 10 out of 11 test microorganisms with 08–17 mm zone of inhibition. ‘Citrobacter species which were most resistant to the reference antibiotics was inhibited by the aqueous extract’. These results indicated that most of the active constituents (responsible for exerting antibacterial action) in these plants are expected to be soluble in polar solvent. It authenticates that the entire tested microorganism is susceptible to alcohol extracts in inhibiting 04 out of 11 test microorganisms with 04–14 mm zone of inhibition. Aqueous extract is effective against all tested microorganisms except Enterococci having comparable results with reference antibiotics used. The highest antibacterial activity of 17 mm in Staphylococcus aureus and least consistent activity is recorded by Pseudomonas aeruginosa, Proteus vulgaris and Proteus mirabilis. Alcohol extract was susceptible for the test organism except Staphylococcus aureus, Staphylococcus epidermis, Proteus mirabilis, and Seratia marcescens.

The phyto-chemical screening and qualitative estimation of aqueous and ethanolic extracts of Alangium salvifolium flower are presented in Table 1. The Aqueous extract showed that they are rich in tannins, saponins, flavonoids, alkaloids, quinines, cardiac glycosides, coumarins, phenols, anthraquinones, steroids, catechin, reducing disaccharide and proteins. Whereas ethanolic extract shows the presence of tannins, saponins, flavonoids, alkaloids, quinines, cardiac glycosides, coumarins, phenols, steroids, were reducing disaccharide and proteins with the absence of anthraquinones and catechin.
CONCLUSION

The results of present study conclude that the ethanolic and aqueous extract of *Alangium salviifolium* flowers have a high significance for its valuable secondary metabolites. Plant extracts have antibacterial potential against the clinical samples which had taken from hospital. Water as solvent is better for extraction of bioactive compounds. Plant extracts that inhibit the growth of pathogenic microorganisms may have potential application as therapeutic agents. Exploitation of these pharmacological properties involves further investigation of these active ingredients of an implementation technique of extraction, purification, separation crystallization and identification and can further use as potential drug.

REFERENCES


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Conflict of Interest: None Declared
INDUCTION OF APOPTOSIS IN MYELOGENOUS LEUKEMIC K562 CELLS BY ETHANOLIC LEAF EXTRACT OF ANNONA MURICATA L.

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ABSTRACT

Prevalence of cancer, especially in Nigeria, is silently growing at exponential rates due to its nature and numerous risk factors associated with its development. Most chemotherapeutic drugs used today are expensive and toxic to normal cells, hence the need for alternative treatment options. In this present study, the efficacy of ethanolic extracts of Annona muricata leaves for its cytotoxicity potential and induction of apoptosis in K562 cancer cells, was investigated. Phytochemical screening verified presence of alkaloids, tannins, flavonoids, saponins, anthraquinones and cardiac glycosides. Using Neutral red uptake assay, the ethanolic extract showed peak cytotoxicity levels \(P<0.001\) at 2.5 mg/ml which decreased with increased concentrations. Caspase-3 activity was significantly enhanced \(P<0.001\) during apoptosis induced by the extract at low quantities, with the peak activity shown at 50 \(\mu\)g/ml. Apoptosis was confirmed by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay. Caspase-3 activity and TUNEL results suggested that the ethanolic extract of Annona muricata induced apoptosis in the myelogenous leukemic K562 cell line. This supports the therapeutic application of Annona muricata to be considered as a natural product source for the development of pro-apoptotic drugs.

KEYWORDS: Apoptosis, K562 cells and Annona muricata

Cite this article:
INTRODUCTION

Cancer remains one of the most dreaded diseases causing an astonishingly high death rate, second only to cardiac arrest (Shafi, et al., 2009). Although overall cancer incidence rates in the developing world are half those seen in the developed world in both sexes, the overall cancer mortality rates are generally similar (Jemal, et al., 2011). Cancer survival tends to be poorer in developing countries, most likely because of a combination of a late stage at diagnosis and limited access to timely and standard treatment (Jemal, et al., 2011). In 2000, approximately 256,000 children and adults around the world developed some form of leukaemia, and 209,000 died from it (Mathers, et al., 2001). The myeloid leukaemia are a heterogeneous group of diseases characterized by infiltration of the blood, bone marrow and other tissues by neoplastic cells of the hematopoietic system (Kasper, et al., 2005).

Plant medicines are considered safer and better than synthetic drugs, since the ingredients in plants such as carbohydrates, fats, proteins, vitamins and minerals are also of body composition (Kilham, 1999). The extensive repertoire of traditional medicinal knowledge systems from various parts of the world are being re-investigated for their healing properties. The fact that conventional and newly emerging treatment procedures like chemotherapy, catalytic therapy, photodynamic therapy and radiotherapy have not succeeded in reverting the outcome of the disease to any drastic extent, has made researchers investigate alternative treatment options (Shafi, et al., 2009).

An alternative treatment approach is the induction of apoptosis in tumour cells using herbs. Apoptosis is characterised by distinct morphologic changes, including cell shrinkage, membrane blebbing, chromatin condensation, DNA fragmentation, and the formation of apoptotic bodies (Wyllie, 1997). Upstream initiator caspasas including caspase-9 activate downstream effector caspasas such as caspase-3, playing a pivotal role in the induction of apoptosis by various stimuli (Wyllie, 1997). The hallmark of apoptosis is DNA fragmentation into approximately 200 bp by the action of Caspase activated Dnase (CAD) (Pirnia, et al., 2002). This is usually assayed for ISEL, TUNEL.

Annona muricata L. is the most tropical semi deciduous tree with the largest fruits of the Annona genus. It is also native to Sub-Saharan Africa countries that lie within the tropics (Zafra-Polo, et al., 1998; Alali, et al., 1999). Traditional ethno-botanical uses in the Peruvian Amazon include its actions as a hypotensive, cardiodepressant (Carabajal, et al., 1991), antispasmodic, anticonvulsant (N’Gouemo, et al., 1997) and sedative (Vasques, 1990) agent, besides other numerous documented properties as an emetic, febrifuge, vermifuge, nervine, decongestant, Galactagogue and poison antidote (de Feo, 1992).

Phytochemically, A. muricata is rich in miscellaneous lactones and isouquinoline alkaloids (Alali, et al., 1999). It contains many active compounds and chemicals; these are the natural phytochemicals known as annonaceous acetogenins (Alali, et al., 1999; Kojima & Tanaka, 2009). Studies have demonstrated their antihyperglycemic (Adeyemi et al., 2009a), antihyperlipidemic (Adeyemi et al., 2009b), antimalarial (Antoun, et al., 1993; Gbeassor, et al., 1990), antiparasitic (de Feo, 1992; Jaramilo, et al., 2000; Bories, et al., 1991), antibacterial (Khan, 1998), insecticidal (Alali, et al., 1998; Guadano, et al., 2000), molluscidal (Santos & Sant’Ana, 2001), antiviral (Antoun, et al., 1999) and most importantly, their anticancer properties (Zafra-Polo, et al., 1998; Alali, et al., 1999; Yang, et al., 2009).

Specific acetogenins in extracts of A. muricata have been reported to be selectively toxic in vitro to certain types of tumour cells including: lung carcinoma cell lines; human breast solid tumour lines; prostate adenocarcinoma; pancreatic carcinoma cell lines; colon adenocarcinoma cell lines; mammary adenocarcinoma cell lines; liver...
cancer cell lines; human lymphoma cell lines; and multi-drug resistant human breast adenocarcinoma (Alali, et al., 1999; Yang, et al., 2009; Liaw, 2002).

It is generally accepted that the mode of action of acetogenins is the inhibition of NADH-ubiquinone oxidoreductase (complex I) in mitochondria. Inhibition suppresses ATP production, especially for cancer cells with high metabolic levels, leading to apoptosis (Morré, et al., 1995; Zeng & McLaughlin, 1996; Oberlies, et al., 1997).

Considering the significance of this cancer sub-type, the objective of this study was to investigate, using K562 cells, the in vitro apoptotic activity of the ethanolic extracts of Annona muricata leaves. K562 cells were the first human immortalised myelogenous leukaemia line to be established (Lozzio, et al., 1981). To determine the plant’s antiproliferative activity, we examined the effects of the varying concentrations of ethanolic extracts of Annona muricata on cell viability using the neutral red uptake assay. Apoptosis was assessed by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay and Caspase-3 activity assay.

MATERIALS AND METHODS

Plant material

Fresh leaves of Annona muricata leaves were collected in May 2010, from Ibeme, Isiala-Mbano in Imo State of Nigeria. They were identified and authenticated by Mr. T. I. Adeleke of Pharmacognosy Department, College of Medicine, University of Lagos, Nigeria.

Preparation of organic extract

A. muricata leaves were oven-dried for five days. The dried leaves were powdered in a warring blender and extracted with 85% ethanol using the Soxlet apparatus. The organic phase was later evaporated under reduced pressure to obtain a dried extract.

Phytochemical analysis

The crude ethanolic extract of A. muricata was subjected to qualitative phytochemical screening for identification of basic classes of active chemical constituents. The phytochemical analysis was carried out using reported standard methods (Trease & Evans, 1989).

Maintenance of K562 cell line

K562 cells were procured from Dr. Wolfgang Fisher of Ludwig-Maximilians University, Munchen, Germany. Cell lines were maintained and propagated in RPMI medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were cultured and maintained at 37°C in a humidified atmosphere of 5% CO₂.

Cell viability assay

Cell viability was assessed using neutral red uptake assay (Borenfreund & Puerner, 1984). Neutral red, a weak cationic dye that penetrates cell membranes by non-ionic diffusion, accumulates at the anionic sites in the lysosomal matrix. Briefly, cells were seeded in 96-well tissue culture plates and tested for 6 h with varying concentrations of 0.625 mg/ml, 1.25 mg/ml, 2.5 mg/ml and 5.0 mg/ml of the extract in triplicates. The plates were then incubated for 2 h at with serum-free medium containing neutral red. The cells were subsequently washed, the neutral red dye was extracted from each plate and optical densities were determined at 540 nm on a multi-well spectrophotometer.

Caspase 3 assay

Apoptosis was induced for 12 h and a negative control set at the same time. The cells were collected, washed twice with PBS (phosphate buffer saline) and to a required number of collected cells; a fresh cold prepared lysis buffer was added. The cells were incubated on ice for 20–60 minutes, and vortexed 3–4 times for 10 seconds, each time following centrifugation at 10,000 rpm at 4°C.
for one minute. A small quantity of supernatant was obtained to assay the protein concentration by the Bradford method. To assess Caspase-3 activity, the Caspase-3 Colorimetric Assay Kit (Genscript, USA) was used following the instructions of the manufacturer. The Caspase-3 Colorimetric Assay Kit is based on the spectrophotometric detection of the chromophore p-nitroanilide (pNA) after cleavage from the labelled substrate DEVD-pNA. The pNA was quantified at 405 nm.

**TUNEL assay**

To evaluate apoptosis in the cells, the TUNEL Apoptosis Detection Kit (Genscript, USA) was used following the instructions of the manufacturer. Cells were exposed to the organic extract on chamber slides for 12 h and then fixed with a fixation solution (4% paraformaldehyde in PBS, pH 7.4, freshly prepared) for 1 h at 15–25°C. The slides were rinsed thrice with PBS, and incubated subsequently on ice following procedure with blocking solution (3% H2O2 in methanol) and permeabilization solution (0.1% Triton X-100 and 0.1% sodium citrate in water, freshly prepared). Before beginning the labelling procedures, the fixed and permeabilized cells were incubated with 100 μl DNase I Solution (500 U/ml DNase 1 [grade 1] in 1X DNase buffer) for 10 min at 15–25°C to induce DNA strand degradation. During the labelling procedure, biotinylated nucleotide is labelled at the DNA 3'-OH ends using the recombinant Terminal Deoxynucleotidyl Transferase (rTDT); then horseradish peroxidase-labelled Streptavidin (Streptavidin-HRP) is bound to these biotinylated nucleotides, which are detected using the peroxidase substrate, hydrogen peroxide and 3,3'-Diaminobenzidine (DAB), a stable chromogen. Using this procedure, apoptotic nuclei are stained dark brown.

**Statistical analysis**

Graphpad Prism 5.0 was used for data analysis. The results of each series of experiments (performed in triplicates) are expressed as the mean ± standard error of mean (SEM). The significance of difference in the means of all parameters reported was determined using ANOVA and Bonferroni Post Hoc test.

**RESULTS**

**Phytochemical analysis**

The phytochemical screening of the ethanolic leaf extract of *A. muricata* revealed strong presence of alkaloids, flavonoids, tannins – hydrolysable and condensing tannins – anthraquinone glycosides and saponins; and weak traces of cyanogenic glycosides and cardiac glycosides.

**Neutral red uptake analysis**

Cytotoxicity test was carried out on the ethanolic extract of *A. muricata* leaves via neutral red uptake method in a dose-dependent manner, shown in Fig.1. Maximum cytotoxicity of the extract on K562 cells were observed at 2.5 mg/ml, which revealed a significant increase (P<0.001) compared to the positive control (H2O2). At higher concentrations of the control, a significant decrease (P<0.01) in cytotoxicity was detected of the ethanolic extract.

**Caspase-3 assay**

The results, shown in Fig.2, showed a steady increase in Caspase-3 activity, attaining its peak at 50µg/ml which was highly significant (P<0.001) in comparison to the positive control. A decline in caspase-3 activity was evident with increased concentrations but still showed significant (P<0.05) values.

**TUNEL assay**

The TUNEL assay (TdT- mediated dUTP Nick End Labelling) was developed as a method to identify individual cells that are undergoing apoptosis by labelling the ends of the degrading DNA with the polymerase terminal deoxynucleotidyl transferase (TdT) (Heatwole, 1999). After the induction with 0.625 mg/ml, 1.25 mg/ml, 2.5 mg/ml and
5.0 mg/ml ethanolic extracts of *Annona muricata*, K562 cells were stained dark brown under the light microscope. This observation, as shown in Fig.3, was assessed in comparison with results obtained from the positive control (DNAse 1 solution). The results confirm that apoptosis had taken place in K562 cells when induced with the leaf ethanolic extract of *Annona muricata*.

**Fig.1. Neutral red uptake cytotoxicity assay of ethanolic leaf extract of A.muricata**

![Fig.1](image-url)

**Extract concentrations**

**Fig.2. Plot of absorbance against concentration of Caspase-3 activity during apoptosis of K562 cells induced by leaf ethanolic extract of Annona muricata.**

![Fig.2](image-url)

**Protein concentration**
Fig.3. TUNEL assay results after the induction of apoptosis in K562 cell line (×100). Arrows indicate artefacts. 

Keys: (A) Negative Control; (B) Hydrogen Peroxide (Positive inducer); (C) DNAse I (Positive control); (D) 1.25 mg/ml ethanolic extract of A. muricata

DISCUSSION

The phytochemical investigation on ethanolic leaf extract of A. muricata confirmed its diverse traditional ethnobotanical uses derived from its long recorded indigenous use and recent documented medicinal properties (de Feo, 1992).

Cytotoxic drugs that exert their maximum cytotoxicity on cancer cells during the S-phase of the cycle prevent cells from progressing through the cell cycle to the S-phase; this is accomplished by sublethal inhibition of RNA and protein synthesis (Craig & Stitzel, 2003). Studies have shown that cancer cells at the S phase of their cell cycle are more vulnerable to the compounds that have been, and are still
being, isolated from *A. muricata* called *Annonaceous acetogenins* (Yuan, 2003).

In this present study, it is clearly shown that *A. muricata* has cytotoxic effects on the myelogenous leukemic K562 cell line. Maximum cytotoxicity of the extract on K562 cells were observed at 2.5 mg/ml, which revealed a significant difference (*P*<0.001) to the corresponding molarity of the positive control. This cytotoxic property decreased significantly (*P*<0.01) at higher concentrations of the control. This observation concurs with some clinical findings which have suggested that at very low dosages, *annonaceous acetogenins* of *A. muricata* exhibited highly toxic effects particularly to ovarian, breast, cervical, bladder and skin cancer cell lines (Alali, et al., 1999). Another study observed that some of the derivatives within the different structural types and some positional isomers showed remarkable selectivities among certain cell lines (Yang, et al., 2009). Lack of selective toxicity is the major limiting factor in the chemotherapy of cancer (Craig & Stitzel, 2003).

The TUNEL assay results clearly show that apoptosis had been induced by the ethanolic leaf extract of *A. muricata* on the myelogenous leukemic K562 cell line. This outcome was compared with results obtained from the positive control (DNAse 1 solution), as apoptotic nuclei was stained dark brown.

Furthermore, the results of the Caspase-3 activity further substantiated that apoptosis had taken place. The activation of Caspase-3 is an important downstream event in apoptosis (Crow, et al., 2004). Increased caspase-3 activity was noted to be directly proportional to increased concentration of the ethanolic extract of *A. muricata* reaching a peak at 50 µg/ml. The least (25 mM) and the highest (200 mM) concentrations of protein obtained during the apoptosis induced by the extract, showed no significant difference (*P*>0.05) to the control.

Great progress has been made in the understanding of the basic mechanisms of apoptosis and the gene products involved (Wyllie, 1997). The regulation of apoptosis in normal and malignant cells has become an area of intensive study in cancer research (Johnstone, et al., 2000). Agents that suppress the proliferation of malignant cells by inducing apoptosis may represent a useful mechanistic approach to both cancer chemoprevention and chemotherapy (Khan & Mlugwana, 1999).

CONCLUSION

It is evident in this present study that the significant increase in Caspase-3 activity and TUNEL assay results suggest that the ethanolic leaf extract of *Annona muricata* had induced apoptosis in myelogenous leukemic K562 cell line.

REFERENCES


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Zeng L., and McLaughlin J.L., (1996) "Recent advances in Annonaceous acetogenins"  

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PHARMACOLOGICAL STUDIES OF YASHTIMADHU (GLYCYRRHIZA GLABRA L.) IN VARIOUS ANIMAL MODELS - A REVIEW

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ABSTRACT

Plants are one of the most important sources of medicines. Yashtimadhu [Glycyrrhiza glabra] is one such plant which symbolizes all that is wondrous in nature because, the whole plant has been used as traditional medicine for household remedy against various human ailments from antiquity. The objective of this paper is to review the literature regarding various pharmacological actions. The canvas of the pharmacological activities of Yashtimadhu is very vast. When these activities were complied Yashtimadhu stands out strongly as a drug of choice in various disorders. This paper reviews the available data on use of Yashtimadhu [Glycyrrhiza glabra] in various disorders as evidenced in these topics.

KEYWORDS: Glycyrrhiza glabra, glycyrrhizin, isoliquiritigenin, Licorice, flavonoids.

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INTRODUCTION-

The increasing use of medicinal herbs among the general public has increased the need for scientific-based research to determine the mechanism of action of herbs. In India, the licorice root carries the ancient Sanskrit name of 'Yasthimadhu' (sweet stalk) and it has been a mainstay of Ayurvedic and other traditional medicines. In ancient Ayurvedic System, more than 1250 preparations are described containing Yashtimadhu as one of its Constituents. In traditional Ayurvedic medicine, herbs were used as special foods, serving to eliminate the excesses as well as strengthen the deficiencies, restore and rejuvenate. (Korhalkar A. et al., 2012). Research on the herbs is a developing area in modern biomedical sciences. Scientists who are trying to develop newer drugs from natural resources are looking towards the Ayurveda, the Indian traditional system of medicine. Several drugs of plant, mineral, and animal origin are described in the Ayurveda for their healing properties. Most of these drugs are derived from plant origin. Some of these plants have been screened scientifically for the evaluation of their pharmacological activity in different models, but the potential of most remains unexplored. The available data on some of the major points on the pharmacological activity of Yashtimadhu [Glycyrrhiza glabra L.] in the various disorders is described below.

1] Regulation of gastrointestinal motility

This study explained the gastrointestinal effects of isoliquiritigenin, a flavonoid isolated from the roots of Glycyrrhiza glabra in vivo & in vitro. The results indicated that isoliquiritigenin plays a dual role in regulating gastrointestinal motility, both spasmogenic and spasmylytic (Chen G, et al., 2009).

2] Anti-obesity action–

Licorice flavonoid oil (LFO), was investigated for anti-obesity action in diet-induced obese rats. The addition of 2% LFO in a high-fat diet significantly decreased the weight of abdominal adipose tissue and the levels of hepatic and plasma triglycerides. The enzymatic activities of acetyl-CoA carboxylase and fatty acid synthase, the rate-limiting enzymes in the fatty acid synthetic pathway, were significantly decreased by LFO, whereas the enzymatic activity of acyl-CoA dehydrogenase, the rate-limiting enzyme in the fatty acid oxidative pathway, was significantly increased (Kamisoyama et al., 2008).

The effects of hydrophobic flavonoids from Glycyrrhiza glabra on abdominal fat accumulation and blood glucose level in obese diabetic KK-A(y) mice, were investigated. The results indicated that licorice hydrophobic flavonoids have abdominal fat-lowering and hypoglycemic effects, possibly mediated via activation of peroxisome proliferator-activated receptor-gamma (PPAR-gamma) (Nakagawa K, et al., 2004).

3] Antioxidant action–

The following is a summary of the scientific evidence in support of the capacity of Glycyrrhiza glabra as antioxidant activity.

Spices such as cloves (Syzygium aromaticum), licorice (Glycyrrhiza glabra), mace (aril of Myristica fragans), and greater cardamom (Amomum subulatum) were tested for their antioxidant properties in vitro. The metal chelating activity, bleomycin dependent DNA oxidation, diphenyl-p-picryl hydrazyl (DPPH) radical scavenging activity and the ferric reducing /antioxidant power (FRAP) were measured in rat liver homogenate in presence of spices. The results showed that the spices tested were strong antioxidants and may have beneficial effects on human health (Yadav AS., et al., 2007).

As cancer chemopreventive agents, a new chalcone derivative a novel group of neolignan lipid esters, and seven known phenolic compounds (formononetin, glabridin, hemileiocarpin, hispaglabridin B, isoliquiritigenin, 4'-O-methylglabridin, and paratocarpin B) were isolated from the roots and stolons of licorice (Glycyrrhiza glabra). All isolates were tested in an authentic...
peroxynitrite anti-oxidant assay. Of these compounds, hispaglabridin B, isoliquiritigenin, and paratocarpin B were found to be the most potent anti-oxidant agents. Furthermore, isoliquiritigenin was demonstrated to prevent the incidence of 1, 2-dimethylhydrazine-induced colon and lung tumors in mice when administered at a dose of 300 mg/kg (Chin YW, et al., 2007).

DHC-1, a herbal formulation derived from Bacopa monniera, Emblica officinalis, Glycyrrhiza glabra, Mangifera indica and Syzygium aromaticum was studied for its antioxidative activity. The protective effect of DHC-1 in isoproterenol-induced myocardial infarction and cisplatin-induced renal damage were studied. The results suggested that DHC-1 possesses a protective effect against both damaged heart and kidneys in rats. This beneficial effect may be attributed, at least in part, to its antioxidative activity (Bafna PA, et al., 2005a).

Pepticare, a herbomineral formulation of the Ayurveda medicine consisting of the herbal drugs: Glycyrrhiza glabra, Emblica officinalis and Tinospora cordifolia, was tested for its anti-ulcer and anti-oxidant activity in rats. Effects of various doses of Pepticare were studied on gastric secretion and gastric ulcers in pylorus-ligation and on ethanol-induced gastric mucosal injury in rats. It was concluded that Pepticare possesses anti-ulcer activity, which can be attributed to its anti-oxidant mechanism of action (Bafna PA, et al., 2005b).

Rabbits were treated (orally) with a preparation of Glycyrrhiza glabra L. for 30 days and in parallel were exposed to vibration stress (30 days). The licorice preparation reduced catalase activity in the peripheral blood and increased animal resistance to vibration stress (Oganesyan KR., 2002). The hypocholesterolemic and antioxidative effects of Glycyrrhiza glabra root powder were examined in hypercholesterolemic male albino rats. Administration of Glycyrrhiza glabra root powder (5 and 10 gm% in diet) to hypercholesterolemic rats resulted in significant reduction in plasma, hepatic total lipids, cholesterol, triglycerides and plasma low-density lipoprotein and VLDL-cholesterol accompanied by significant increases in HDL-cholesterol levels. The root powder administration to hypercholesterolemic rats also decreased hepatic lipid peroxidation with a concomitant increase in superoxide dismutase (SOD) and catalase activities and total ascorbic acid content. The normo-cholesterolemic animals when fed with Glycyrrhiza glabra root powder at 10 gm% level, registered a significant decline in plasma lipid profiles and an increase in HDL-cholesterol content. The antioxidant status of these animals also was improved upon treatment (Visavadiya NP, et al., 2006).

4) Protective activity –

It was concluded that Glycyrrhiza glabra is a potential antioxidant and attenuates the hepatotoxic effect of CCl4 by acting as an in vivo antioxidant and thereby inhibiting the initiation and promotion of lipid peroxidation or by an accelerated scavenging of free radicals and their products by conjugation with GSH aided by GST (MG Rajesh, et al., 2004).

Another study evaluated the potential beneficial effect of glycyrrhizin in a mouse model of carbon tetrachloride (CCl4 (4)-induced liver injury. Glycyrrhizin diminished these alterations. These results suggested that glycyrrhizin alleviated CCl4 (4)-induced liver injury, and this protection was likely due to the induction of heme oxygenase-1 and the downregulation of proinflammatory mediators (Lee CH, et al., 2007).

In the study by Zhan, the protective effects of Isoliquiritigenin (ISL) were investigated in transient middle cerebral artery occlusion (MCAO)-induced focal cerebral ischemia-reperfusion injury in rats. Pretreatment with ISL significantly reduced the cerebral infarct volume and edema and produced significant reduction in neurological deficits. These findings indicated that ISL had the protective potential against cerebral ischemia injury and its protective effects were may be due to the
amelioration of cerebral energy metabolism and its antioxidant property (Zhan C, et al., 2006).

The research done by Liu, compared rectal and oral treatments with glycyrrhizic acid for trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats. There were significant pathological changes in colon in TNBS-treated groups, and rectal glycyrrhizic acid significantly attenuated colitis. These results suggested that rectally administered glycyrrhizic acid had significant protective effects against TNBS-induced colitis in rats, and the rectal route could be a complementary treatment for inflammatory bowel disease (Liu Y, et al., 2011).

The effect of *Glycyrrhiza glabra* extract as a natural antioxidant and melatonin (MEL) on ochratoxin A (OTA)-induced histopathological damages on the testes and oxidative stress was evaluated in male rats. The serum total antioxidant power (TAOP) and total thiol molecules (TTM) production were assessed. Both the biochemical and histopathological examinations showed that MEL and *Glycyrrhiza glabra* extract exerted a protective effect on OTA-induced damages. This data suggested that OTA contamination in animal feeds and human foods could cause reproductive abnormalities (Malekinejad H, et al., 2011).

The study was carried out to evaluate the cerebroprotective effect of the aqueous extract of the roots of *Glycyrrhiza glabra* Linn in hypoxic rats. Extract at the tested doses promoted the locomotor activity and spatial behavior significantly, which was impaired in hypoxic rats. The extract administration restored the decreased levels of brain enzymes such as glutamate and dopamine and decreased acetyl cholinesterase (AChE) activity significantly. Levels of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase were restored to near normalcy by administration of ethanol extract of *Glycyrrhiza glabra*. The study suggested that ethanol extract of *Glycyrrhiza glabra* possessed a cerebroprotective effect in hypoxic rats, which may be mediated by its antioxidant effects (P. Muralidharan, et al., 2009).

Another study indicated that the DNA was protected from the deleterious effects of gamma radiation by glycyrrhizic acid in vitro, ex vivo, and in vivo conditions of radiation exposure. The results showed that the glycyrrhizic acid protected bone marrow cells from the radiation-induced damages. It was suggested that glycyrrhizic acid could be a potential drug for the protection of the hemopoietic system from radiation-induced lesions. These results indicated that glycyrrhizic acid offered radioprotection by the scavenging of free radicals (Nitin Gandhi, et al., 2004).

Yu XQ, et al study was designed to evaluate whether glabridin modulated the cerebral injuries induced by middle cerebral artery occlusion (MCAO) in rats and staurosporine-induced damage in cultured rat cortical neurons and the possible mechanisms involved. Findings indicated that glabridin had a neuroprotective effect via modulation of multiple pathways associated with apoptosis (Yu XQ, et al., 2008).

5] Anti-inflammatory activity-

Glycyrrhizin was evaluated on an animal model of spinal cord injury (SCI) induced by the application of vascular clips. The results demonstrated that treatment with glycyrrhizin extract reduced the development of inflammation and tissue injury events associated with spinal cord trauma (Genovese T, et al., 2009).

This study compared the antiarthritic activities and underlying mechanism of LE and rLE (Licorice and Roasted Licorice Extracts) in the CIA mouse model of human RA. The data suggested that supplementation with LE and rLE might be beneficial in preventing and treating both acute and chronic inflammatory conditions (Ki RimKim, et al., 2010).

The anti-inflammatory activities of glycyrrhizin given at 10 mg/kg i.p. 5 min prior to carrageenan in mice model were evaluated in
this study. Glycyrrhizin exerted potent anti-inflammatory effects in this model. The results indicated that prevention of the activation of NF-kappaB and STAT-3 by glycyrrhizin reduced the development of acute inflammation (Menegazzi M, et al., 2008).

6] Immunostimulating –

The uses of ISCOMs (Immunostimulating complexes) formulated with saponins from plants (Aesculus hippocastanum and Glycyrrhiza glabra) collected in Kazakhstan, with antigens from the poultry coccidian parasite Eimeria tenella, were evaluated for their potential use in developing a vaccine for control of avian coccidiosis. The results of this study indicated that these ISCOMs were an effective antigen delivery system which may be successfully used, with low toxicity, for preparation of highly immunogenic coccidia vaccine (Berezin VE, et al., 2008).

7] Learning and memory-

Glabridin was isolated from the roots of Glycyrrhiza glabra and its effects on cognitive functions and cholinesterase activity were investigated in mice. Glabridin and piracetam were administered daily for 3 successive days to different groups of mice. Both remarkably reduced the brain cholinesterase activity in mice compared to the control group. Therefore, glabridin appeared to be a promising candidate for memory improvement (Cui YM, et al., 2008).

The study was undertaken 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 of aqueous extract of Glycyrrhiza glabra were administered for 7 successive days in separate groups of mice. Glycyrrhiza glabra showed promise as a memory enhancer in both exteroceptive and interoceptive behavioral models of memory (Parle M, et al., 2004).

Another study investigated the effect of chronic treatment with glabridin on cognitive function in control and streptozotocin (STZ)-induced diabetic rats. The results showed that glabridin prevented the deleterious effects of diabetes on learning and memory in rats due to its combination of antioxidant, neuroprotective and anticholinesterase properties (Hasanein P., 2011).

8] Relaxes smooth muscle-

The tracheal relaxation effects of isoliquiritigenin, was investigated, on guinea-pig tracheal smooth muscle in vitro and in vivo. Result indicated that isoliquiritigenin relaxes guinea-pig trachea through a multiple of intracellular actions, including sGC activation, inhibition of PDEs, and associated activation of the cGMP/PKG signaling cascade, leading to the opening of BKCa channels and [Ca2+]i decrease through PKG-dependent mechanism and thus to tracheal relaxation (Liu B, et al., 2008).

Licochalcone A, a flavonoid found in licorice root (Glycyrrhiza glabra), is known for its anti-microbial activity and inhibition of cancer cell proliferation. This study investigated whether Licochalcone A inhibits rat vascular smooth muscle cell (rVSMC) proliferation. The data provided that Licochalcone A could regulate rVSMC proliferation and suggested that Licochalcone A inhibited the proliferation of rVSMCs by suppressing the PDGF-induced activation of the ERK1/2 pathway and Rb phosphorylation, resulting in cell cycle arrest (Park JH, et al., 2008).

9] Accelerating metabolism processes of the marrow stem cells-

The effect of long-term exposure of vibration and feeding rabbits with liquorice (Glycyrrhiza glabra L) on peripheral blood indicators was studied. It was found that biological active substances of licorice accelerate metabolism processes of the marrow stem cells, enlarge organism compensatory abilities, in that way providing organism resistance to vibration (Minasian SM, et al., 2007).
10] Antiviral effects-

Animal studies demonstrated a reduction of mortality and viral activity in herpes simplex virus encephalitis and influenza A virus pneumonia. In vitro studies revealed antiviral activity against HIV-1, SARS related coronavirus, respiratory syncytial virus, arboviruses, vaccinia virus and vesicular stomatitis virus (Fiore C, et al., 2008).

11] Antifibrotic effects -

Study investigated whether a combination regimen of Salvia miltiorrhiza (S), Ligusticum chuanxiong (L) and Glycyrrhiza glabra (G) exerted in vivo antifibrotic effects on rats with hepatic fibrosis. Fibrosis was induced in rats by dimethylnitrosamine (DMN) administration for 4 weeks. The results showed that SLG exerted antifibrotic effects in rats with DMN-induced hepatic fibrosis (Lin YL, et al., 2008).

12] Antiallergic effects-

In this study, the main components (glycyrrhizin, 18β-glycyrrhetinic acid, isoliquiritin, and liquiritigenin) were isolated from licorice, and their anti-allergic effects, such as antiscratching behavior and IgE production-inhibitory activity, were evaluated both in vitro and in vivo. These components inhibited the production of IgE in ovalbumin-induced asthma mice but liquiritigenin had little effect. The study suggested that the antiallergic effects of licorice were mainly due to glycyrrhizin, 18β-glycyrrhetinic acid, and liquiritigenin, which could relieve IgE-induced allergic diseases such as dermatitis and asthma (Shin YW, 2007).

The study evaluated glycyrrhizin a major constituent of Glycyrrhiza glabra, for its efficacy on asthmatic features in a mouse model of asthma. The results demonstrated that glycyrrhizin alleviated asthmatic features in mice and it could be useful towards developing a better therapeutic molecule in the future (Ram A, et al., 2006).

Glycyrrhiza glabra, Allium cepa and Clerodendrum serratum with hydroalcoholic (50:50) solvent were evaluated for acute toxicity and Anti asthmatic activity. Saponins and flavanoids were the major reason for antioxidant activity as confirmed by DPPH free radical scavenging activity test and were responsible for treating oxidative stress during asthma (Tulsiani Puja, 2012).

13] Cholinesterase-inhibiting activity-

This study estimated the acetyl cholinesterase- inhibiting activity of extracts of Glycyrrhiza glabra, and compared these values with a standard acetyl cholinesterase-inhibiting drug, metrifonate. Aqueous extract of Glycyrrhiza glabra and metrifonate were administered to young male Swiss albino mice. Acetyl cholinesterase enzyme was estimated in brains of mice. Glycyrrhiza glabra and metrifonate significantly decreased acetyl cholinesterase activity as compared with their respective vehicle-treated control groups (Dhingra et al., 2006a).

14] Immunomodulatory properties-

Standardized ethanol extracts of Allium sativum, Glycyrrhiza glabra, Plantago major and Hippophae rhamnoides were assessed for their effects on cellular immunity in laying hens. Birds had blood samples taken and both specific and non-specific immune cell responsiveness were evaluated by a leukocyte proliferation assay, carbon clearance test and SRBC phagocytosis in monocyte-derived macrophage cultures. Licorice and sea buckthorn clearly enhanced the macrophage membrane function. Small concentrations (20 µg/mL) of licorice proved the co-mitogenic potential for both T and B avian lymphocytes (Dorhoi A, et al., 2006).

15] Antidepressant-like activity

This study investigated the effects of aqueous extract of Glycyrrhiza glabra, on depression in mice using forced swim test (FST) and tail suspension test (TST). The dose of 150 mg/kg of the extract significantly reduced the immobility times of mice in both
FST and TST, without any significant effect on locomotor activity of mice. This suggested that antidepressant-like effect of liquorice extract seems to be mediated by increase of brain noradrenaline and dopamine, but not by increase of serotonin (Dhingra D. et al., 2006b).

Other study evaluated the potential of methanolic extract of Glycyrrhiza glabra as an adjuvant in treatment of Parkinson's disease and depression. In acute study Glycyrrhiza glabra extract (30 and 100 mg/kg i.p.) significantly inhibited haloperidol-induced catalepsy in mice in dose dependent manner. Similar inhibitory effect of Glycyrrhiza glabra extract was also observed in chronic study (15 days) which indicated that there was no development of tolerance. The Glycyrrhiza glabra extract reduced duration of catalepsy also. These observations indicated that Glycyrrhiza glabra has a good potential as an adjuvant of anti-Parkinsonian and antidepressant drugs (SB Kasture et al., 2008).

16] Increased resistance to stress-

This study observed the effect of continuous vibration and treatment with licorice root (Glycyrrhiza glabra L.) on peripheral blood red cells in rabbits. Active substances of licorice root accelerated metabolism in cells of the bone marrow erythroid stem, enhanced compensatory reserve of the organism, and increased animal's resistance to stress (Adamyan TI., et al., 2005).

17] Antimutagenic properties-

The antimutagenic effect of the bioactive compounds from fruits of Morus alba L. (MA), Punica granatum L. (PG), Diospyros kaki L. (DK), Cydonia oblonga Mill. (CO) and roots of Glycyrrhiza glabra (GG) were investigated. The antimutagenic effects were studied on mutations induced by genotoxicants (X-rays, N-methylnitrosourea, cyclophosphamide, NaF) and aging in bone marrow cell chromosomes from mice and rats. The antimutagenic properties of the complex mixtures were considerably greater than those of the separate components. More antimutagenic activity of the mixture was revealed when mutagenesis was the result of X-rays and the natural aging processes (Alekperov UK., 2002).

18] Protection of DNA and microsomal membranes-

The radioprotective effect of the root extract of Glycyrrhiza glabra L. on lipid peroxidation in rat liver microsomes and plasmid pBR322 DNA was investigated. The extract was found to protect microsomal membranes, as evident from reduction in lipid peroxidation, and could also protect plasmid DNA from radiation-induced strand breaks (Shetty TK, et al., 2002).

19] Antiulcerogenic effect

Extracts from the plants Iberis amara, Melissa officinalis, Matricaria recutita, Carum carvi, Mentha x piperita, Glycyrrhiza glabra, Angelica archangelica, Silybum marianum and Chelidonium majus, singly and combined in the form of a commercial preparation, STW 5 (Iberogast) and a modified formulation, STW 5-II, were tested for their potential antiulcerogenic activity against indometacin induced gastric ulcers of the rat as well as for their antisecretory and cytoprotective activities. All extracts produced a dose dependent antiulcerogenic activity associated with a reduced acid output and an increased mucus secretion, an increase in prostaglandin E2 release and a decrease in leukotrienes (Khayyal MT, et al., 2001).

20] Hepatoprotective and antihepatocarcinogenic

Liver protective and antihepatocarcinogenic effects of combination use of Gly and Mat (Matrine), a component extracted from Sophora flavescens Ait, Glycyrrhizin (Gly), a major active constituent of licorice (Glycyrrhiza glabra) root, were tested, as compared to effects of Gly or Mat alone. The results showed that compared with Gly or Mat alone, Gly + Mat reduced the mortality of acetaminophen overdosed mice more effectively, attenuate acetaminophen-induced hepatotoxicity, and reduced the
number and area of γ-GT positive foci, thus protecting liver function and preventing HCC from occurring (Xu-ying et al., 2009).

21] Improve efficiency of chemotherapy and surgical treatment-

Experiments on animals with Lewis lung carcinoma and Ehrlich tumor showed that licorice (Glycyrrhiza) extract and glyciram prepared from this plant improved the antitumor effect of cyclophosphamide. Glyciram reduced the toxic effect of the cytostatic on peripheral blood leukocytes. Licorice extract inhibited the growth of Ehrlich tumor and development of metastases in mice with Lewis lung carcinoma. Glyciram administered to mice after removal of Lewis lung carcinoma produced an antimetastatic effect and prevented relapses (Goldberg ED, et al., 2008).

22] Antithrombotic effect-

Here the in vivo effects of Glycyrrhizin upon two experimental models of induced thrombosis in rats are reported. Intravenous administration of Glycyrrhizin caused a dose-dependent reduction in thrombus size on a venous thrombosis model that combines stasis and hypercoagulability. It was observed that Glycyrrhizin doses of 180 mg/kg body weight produced 93% decrease on thrombus weight. Glycyrrhizin was also able to prevent thrombosis using an arteriovenous shunt model. In contrast with heparin, Glycyrrhizin did not potentiate the inhibitory activity of antithrombin III or heparin cofactor II towards thrombin. Altogether, data indicate that Glycyrrhizin is an effective thrombin inhibitor in vivo, which may account for its other known pharmacological properties (Mendes-Silva W, et al., 2003).

23] Reduce ocular hypertension –

This study evaluated the hypotensive effects of glycyrrhizin on a rabbit model of ocular hypertension (OH) induced by triamcinolone acetonide (TA). The administration of Glycyrrhizin could suppress OH induced by TA in rabbits, and improve their electrophysiological parameters. These results indicated that TA-induced ocular metabolism changes could be compensated by Glycyrrhizin (Song Z, 2011).

24] Anticonvulsant Activity -

The anticonvulsant activity of ethanolic extract of roots and rhizomes of Glycyrrhiza glabra in mice was assessed using maximum electroshock seizure (MES) test and pentylenetetrazol (PTZ) using albino mice. The lithium-pilocarpine model of status epilepticus was also used to assess the anticonvulsant activity in rats. The ethanolic extract of Glycyrrhiza glabra did not reduce the duration of tonic hindleg extension in the MES test even in the dose of 500 mg/kg. However, the extract significantly and dose-dependently delayed the onset of clonic convulsions induced by pentylenetetrazol (Shirish D et al., 2002).

25] Restores the impaired production of β-defensins-

In this paper, the decreased production of antimicrobial peptides by EK influenced by Gr-1(+) CD11b (+) cells was shown to be restored by glycyrrhizin. Also, sepsis stemming from P. aeruginosa burn-site infection was not demonstrated in burn mice treated with glycyrrhizin. These results suggested that through the improved production of antimicrobial peptides in tissues surrounding the burn area, sepsis stemming from P. aeruginosa wound infection is controllable by glycyrrhizin in severely burned mice (Tsuyoshi Yoshida, 2010).

26] Modulate rat cardiac performance-

The direct cardiac activity of glycyrrhizin and glycyrrhetinic acid was explored. The effects of synthetic glycyrrhizin and glycyrrhetinic acid were evaluated on the isolated and Langendorff perfused rat heart. The intracellular signaling involved in the effects of the two substances was analyzed on isolated and perfused heart and by Western blotting on cardiac extracts. Under basal conditions, both glycyrrhizin and glycyrrhetinic
acid influenced cardiac contractility and relaxation. Glycyrrhizin induced significant positive inotropic and lusitropic effects starting from very low concentrations, while both inotropism and lusitropism were negatively affected by glycyrrhetinic acid. Both substances significantly increased heart rate (Maria L. Parisella, 2012).

27] Antitumor and antimetastatic effects –

Experiments on mice inoculated with metastasizing Lewis lung carcinoma showed that the antitumor and antimetastatic effects of cyclophosphamid (cyclophosphamide) are potentiated by the extracts of phytopreparations based on Baikal scullcap (Scutellaria baikalensis), rhodiola (Rhodiola rosea), common licorice (Glycyrrhiza glabra), and their principal acting components-baikalin, parasyroso, and glycyrram (Razina TG, 2000).

28] Antitussive activity –

This study analyzed the water-extracted polymeric fraction (WE) of Glycyrrhiza glabra. This arabinogalactan protein enriched fraction, ≥ 85% of which gets precipitated with Yariv reagent, consisted mainly of 3- and 3,6-linked galactopyranosyl, and 5- and 3, 5-linked arabinofuranosyl residues. Peroral administration of this polymer, in a dose of 50 mg/kg body weight decreases the number of citric acid induced cough efforts in guinea pigs more effectively than codeine. It does not induce significant change in the values of specific airway resistance or provoked any observable adverse effects (Saha S et al., 2011).

29] Prevention of hepatorenal damage –

Protective role of Glycyrrhiza glabra rhizomes (roots) at three dose levels (100, 75, & 50 mg/kg/bw) against sublethal dose (300 mg/kg/bw) of acetaminophen (paracetamol) induced hepatorenal damage has been assessed in mice. Parameters of study were glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), bilirubin, alkaline phosphatase (ALP) as liver function tests, creatinine and urea as kidney function tests and histology for pathology. Glycyrrhiza glabra could antagonize acetaminophen induced both, hepato and nephrotoxicity in dose dependent manner. No protection provided by a single dose of Glycyrrhiza glabra (1.5 gm/kg/bw) against lethal dose of acetaminophen (1 gm/kg/bw) (Sharma A et al., 2011).

30] Antiandrogenic activities-

This study was carried out to investigate different aspects of antiandrogenic properties of Glycyrrhiza glabra. Immature male rats, castrated rats without any treatment received only vehicle; castrated rats plus T replacement; three castrated groups with T replacement plus various doses of G. glabra extract (75, 150 and 300 mg/kg). Those receiving the doses of 150 and 300 mg/kg showed a significant reduction in prostate weight, total T and VP epithelium/stroma ratio (V/W). These results in SV and levator ani were shown in response to 300 mg/kg of extract. Increasing in T metabolism, down-regulation of androgen receptors or activation of oestrogen receptors could be involved mechanisms. This study showed that alcoholic extract of Glycyrrhiza glabra has antiandrogenic properties (Zamansoltani F et al., 2009).

CONCLUSION

Yashtimadhu (Glycyrrhiza glabra) still remains a mainstay of Ayurvedic and other traditional medicines. But only some of its uses are supported by clinical data. So it becomes relevant to search the evidence based data and to classify and to analyze it. In last two decades more and more bioactive compounds have been recognized and promising results have been established through various pharmacological studies on animal models as well as clinical studies. Mechanisms of action of its major constituents such as glycyrrhizin (glycyrrhizic acid, glycyrrhizinic acid), isoliquiritigenin, Licorice flavonoid oil (LFO), hydrophobic flavonoids, formononetin, glabridin, hemileiocarpin, hispaglabridin B, 4'-O-methylglabridin, paratocarpin B. Licohalcone A., 18 β-glycyrheetsin acid, isoliquiritin, liquiritigenin, glyciram etc have been discussed in this review which could help the researchers.
REFERENCES


Minasian SM, Adamian TsI, Gevorkian ES. (2007), Changes of morphological indicators of blood from vibration and liquorice effect. Ross Fiziol Zh Im I M Sechenova. 93(9):1035–4


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Conflict of Interest: None Declared
PHARMACOGNOSTICAL EVALUATION OF LEAF OF BLEPHARISPERMUM SUBSESSILE DC. (ASTERACEAE)

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ABSTRACT

Blepharispermum subsessile DC (Asteraceae), a less explored folklore medicinal plant, is a glabrous shrub, found in the forest of Odisha, Karnataka, Madhya pradesh and Maharashtra. It is used as one of the source plants of Rasna. Its root, stem and leaves are claimed to be beneficial in the treatment of Rheumatism. In this study, a detailed pharmacognostical investigation on its leaves, which includes macroscopic and microscopic characters, histochemical tests, quantitative microscopy and preliminary physicochemical analysis, following the prescribed guidelines of Ayurvedic Pharmacopoeia of India. The microscopical study result showed the presence of undifferentiated palisade and spongy tissue, anomocytic stomata, bicollateral vascular bundles, unicellular trichomes, calcium oxalate crystals, oil globules, tannins etc. in the leaves. The quantitative surface microscopy study showed 174 numbers of epidermal cells, each epidermal cell measured about 22.48µm², through veins 29.36µm² and stomatal index 25. The purity test showed loss on drying (9.14%-w/w), total ash (9.68%-w/w), water soluble extractive (30.91%-w/w), alcohol soluble extractive (5.73%-w/w) and pH (6.5).

KEY WORDS:
Asteraceae, Ayurveda, Blepharispermum subsessile DC., Ethnomedicine, Pharmacognosy, Rasna.

Cite this article:
INTRODUCTION

*Blepharispermum subsessile* DC. (Asteraceae), known as *Rasnajhadi* in Odisha, *Naama banta* in Kannada and *Adavi banti* in Telugu, is a glabrous shrub with small close globose cluster head, leaves alternate, entire or toothed with short petiole. It is distributed in Odisha, Karnataka, Madhya Pradesh and Maharashtra. Ethno-botanical studies reports its use in common cold and rhinitis, as wormicidal, as tonic (A.K. Gupta *et al.*, 2004), diarrhoea (Dash *et al.*, 2006), eye troubles, backache and rheumatism (A.B. Prusti *et al.*, 2007), irregular menstruation (Rajesh *et al.*, 2008) and rheumatism (C.P. Khare, 2008). The pharmacognostical characters of its leaf, one of the parts used, is not reported yet. So, the present study deals with the macroscopic and microscopic characters of its leaf including preliminary physicochemical analysis.

MATERIALS AND METHODS

Collection and authentication:

*Rasnajhadi*, identified by local traditional practitioners, growing in Gurudongar medicinal plants conservation area of Nuapada district of Odisha, India, was authenticated by expert taxonomist as *Blepharispermum subsessile* DC., (Asteraceae), on the basis of characters given in Flora of Orissa (Saxena *et al.*, 1990). The fresh plant samples were collected from its natural habitat, Odisha, in the month of October 2011 and voucher specimen has been preserved in the pharmacognosy laboratory of IPGT and RA, vide no 36561. The collected plant samples were shaken to remove adherent soil and dirt. The leaves were separated from the stem, washed with running fresh water and few pieces stored in solution of AAF (Alcohol: Acetic acid: Formalin) in the ratio of (90:5:5) (Johnsen, 1940) to utilize them for microscopic studies. The remaining leaves were shade dried and then powdered with mechanical grinder and passed through mesh no.80# and preserved in an air-tight glass container.

Pharmacognostic studies:

Morphological characters were studied by observing the leaves as such and also with the help of the dissecting microscope. For detailed microscopical observation, free hand thin transverse section passing through the midrib were taken, and cleared with chloral hydrate and observed as such for the presence of any crystals, then were stained with Phloroglucinol and Hydrochloric acid to notice the lignified elements like fibers, vessels etc. of the meristele and other parts (Khandelwal K.R., 2008). Photographs of the section were taken with the help of Canon digital camera attached to Zeiss microscope. Powder characters were observed and histochemical tests carried out, as per the guidelines of Ayurvedic Pharmacopoeia of India, (Anonymous, 1999).

Quantitative microscopy:

Determination of certain leaf constants or diagnostic characters of leaves like micrometric evaluation of stomatal number, stomatal index, stomatal size and epidermal size were taken by mean value (3-5 successive results) carried out as per the method described in Ayurvedic Pharmacopoeia of India (Evans, 2009). [fig 2.g- i] [table-2, 3].

\[ I = \frac{S \times 100}{E + S} \]

(I= stomatal index, S= no. of stomata per unit area, E = no. of epidermal cells in the same unit area).

Phytochemical evaluation:

Preliminary physic-chemical investigations were carried out following standard procedures recommended by API (Anonymous, 1999).

RESULTS AND DISCUSSION

Morphology:

Leaves simple, alternate, stipulate, sessile, measures about 10–12 × 4–6 cm, ovate to elliptic, margin, entire with acute tip and symmetrical base, upper surface dark green, glabrous while lower surface light green in colour, Mid rib strong, 5–7 pairs of veins, venation reticulate. (fig 1-a,c)
Photo plate: 1 Microscopic characters of *Blepharispermum subsessile DC.* leaf

- Fig-1.a leaf arrangement
- Fig-1.b T.S. of leaf through mid-rib

- Fig-1.c individual sub sessile leaf
- Fig-1.d Upper epidermis through veins
- Fig-1.e Upper epidermis with thick cuticle

- Fig-1.f Bicollateral vascular bundle
- Fig-1.g Chlorophyll pigments
- Fig-1.h undifferentiated spongy & palisade tissue

- Fig-1.i. oil globules
- Fig-1.j lower epidermis with stomata
- Fig-1.k lower epidermis with unicellular trichomes
Microscopic description:

T.S. of the leaf passing through the mid rib

1. Midrib convex dorsiventrally with lateral extensions of the lamina on its either side.
2. Epidermis single layered, covered with thick cuticle. Upper epidermal cells wavy walled, large in size than the lower epidermis. Lower epidermis, interrupted with number of stomata along with simple unicellular trichomes aroused from it.
3. The mesophyll consisted of undifferentiated palisade and spongy tissues. Through mid rib both the upper and lower epidermis consisted of thick compactly arranged collenchymatous cells. Vascular bundles located at the centre of the ground tissue. Ground tissue was made up of simple parenchyma cells with many calcium oxalate crystals at the lower side. The meristele, as seen in midrib, in the form of an arc having the xylem towards the upper surface arranged in radiating bands spreading towards the lower surface. Protoxylem being pointed towards the upper side and the phloem bands towards
the lower side. Medullary rays and cambium tissue occasionally was visible in well developed big vascular bundles. Phloem patches could be seen on both sides of the xylem and hence vascular bundle is bicollateral. The vascular bundle was surrounded by parenchymatous bundle sheath.

4. Stomata- Epidermal cells of the leaf in surface view were wavy in outline and stomata were found mostly on the lower surface of the lamina. The stomata were mostly anomocytic in which guard cells were surrounded by 4 subsidiary cells. (fig 1.b, d–k).

**Histochemical test:**

The results of various histochemical tests carried out to detect lignin, crystals of Calcium oxalate and tannin are depicted in table no.1

**Quantitative microscopy**

No. of epidermal cells 174 and 5 veins were passing through in 1 mm (32, 38, 33, 33, 38). In surface study, each epidermal cell measured about 22.48 µm², whereas through veins 29.36 µm². In transverse section lower epidermal cells measured about 134.16 µm², whereas, ground tissue 299.33 µm² measurement of stomata and stomatal index is shown in table 2-3. (fig.2 g–i).

---

**Table no.1: Histochemical test**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Observation</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phloroglucinol + Concentrated HCL(1:1)</td>
<td>Pink colour</td>
<td>Lignin present</td>
</tr>
<tr>
<td>Phloroglucinol + Concentrated HCL(1:1)</td>
<td>Effervescence</td>
<td>Crystals present</td>
</tr>
<tr>
<td>Fecl3 solution</td>
<td>Black colour</td>
<td>Tannin present</td>
</tr>
</tbody>
</table>

**Table no.2: Measurement of stomata**

<table>
<thead>
<tr>
<th>Length (micrometre)</th>
<th>Breadth (micrometre)</th>
<th>Circumference (Square micrometer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.03</td>
<td>14.11</td>
<td>207.1</td>
</tr>
<tr>
<td>17.27</td>
<td>12.63</td>
<td>183.79</td>
</tr>
<tr>
<td>17.37</td>
<td>12.5</td>
<td>186.4</td>
</tr>
</tbody>
</table>

**Table no.3: Stomatal index**

<table>
<thead>
<tr>
<th>Stomata through veinlets</th>
<th>Epidermal cells</th>
<th>Stomatal index (S*100/E+S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>74</td>
<td>222</td>
<td>25</td>
</tr>
<tr>
<td>69</td>
<td>207</td>
<td>25</td>
</tr>
<tr>
<td>82</td>
<td>246</td>
<td>25</td>
</tr>
</tbody>
</table>
Table no-4: Preliminary Physicochemical analysis of *B. subsessile* leaves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreign matter</td>
<td>Nil</td>
</tr>
<tr>
<td>Loss on drying (% w/w)</td>
<td>9.14%</td>
</tr>
<tr>
<td>Total ash content (% w/w)</td>
<td>9.68%</td>
</tr>
<tr>
<td>Water soluble extractive value (% w/w)</td>
<td>30.91%</td>
</tr>
<tr>
<td>Alcohol soluble extractive value (% w/w)</td>
<td>5.73%</td>
</tr>
<tr>
<td>pH</td>
<td>6.5</td>
</tr>
</tbody>
</table>

**Powder microscopy:**

**Organoleptic characters:**

Organoleptic evaluation of leaves of *B. subsessile* revealed its yellowish green colour, aromatic odour and slightly bitter taste. Diagnostic characters of the leaf powder were presence of prismatic crystals of Calcium oxalate, lignified fibers, anomocytic stomata from lower epidermis, spiral and annular vessels from stellar portion, dark yellowish brown content (Tannin), oil globules and many unicellular trichomes. (fig.2 a–f).

**Preliminary Physicochemical analysis:**

While observing the physicochemical characters for purity test, the loss on drying was not more than 9.14% w/w, ash value was not more than 9.68% w/w, water soluble extractive was not more than 30.91% w/w and the alcohol soluble extractive was not more than 5.73% w/w and pH is 6.5. (Table no.4) Total ash content signifies the level of inorganic matter and silica content. The high solubility of the sample in Water denotes that the drug is best suited for extraction with water or water based preparations.

**CONCLUSION**

Leaf of *Blepharispermum subsessile* DC. (Asteraceae) can be identified on the basis of key microscopical characters like undifferentiated palisade and spongy tissue, anomocytic stomata, bicolateral vascular bundles, unicellular trichomes, calcium oxalate crystals, oil globules, tannins etc. The quantitative surface microscopy study showed 174 numbers of epidermal cells, each epidermal cell measured about 22.48µm², through veins 29.36µm² and stomatal index 25. The purity test showed loss on drying (9.14%w/w), total ash (9.68%w/w), water soluble extractive (30.91%w/w), alcohol soluble extractive (5.73%w/w) and pH (6.5).

**ACKNOWLEDGEMENTS**

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REFERENCES


C.P.Khare (2008), Indian medicinal plants, An illustrated medical dictionary, springer references, New Delhi,India. pp103.


Subbiah Arunachalam (1996), Science On The Periphery Enriches Mainstream Science, But At What Cost? The Case of Ethnobotany. Les Sciences Hors D’occidentau Xxe Siecle, Central Electrochemical Research Institute, Karai kudi (India); 6:pp 42


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Conflict of Interest: None Declared
A CLINICAL STUDY ON SHRINGYADI CHURNA WITH AND WITHOUT PRANAYAMA IN THE MANAGEMENT OF TAMAKA SHWASA (BRONCHIAL ASTHMA)

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ABSTRACT

Tamaka Shwasa is very prevalent medical problem in our country. A better and effective therapy is the need of the hour which may be without many side effects and having quick bronchodilator effects and at the same time within the reach of the masses. The present clinical trial was planned to study the effect of an indigenous compound, “Shringyadi Churna” with and without Pranayama in the management of Tamaka Shwasa. After confirming the diagnosis, patients were randomly distributed in three groups, viz. Group I received Shringyadi Churna with Ushnodaka (Warm Water), Group II received the same with Pranayama, and Group III received Placebo with Pranayama. The duration of trial was 12 weeks and the patients were examined weekly for 6 weeks, there after every three weeks up to 12 weeks. The follow up period was of 2 months. The subjective & objective parameters were measured before & after treatment in each group. The result of ‘Shringyadi Churna along with Pranayama’ was found to be significant at the level of P<0.001.

KEYWORDS: Tamaka Shwasa, Shringyadi Churna, Pranayama, Bronchial Asthma
INTRODUCTION

Tamaka Shwasa (Charaka, 200 BC) or bronchial asthma continues to be a distressing and alarming disease of the present world. Inspite of multidimensional development in the field of medical science, it still remained a challenge which is unconquered. Tamaka Shwasa is a yapya vyadhi (Difficult to treat) (Charaka, 200 BC) being paroxysmal in nature and it can prove fatal. Tamaka Shwasa closely resembles to bronchial asthma, which is manifested by widespread narrowing of the air passage and paroxysm of dyspnoea, cough and wheeze. It is also incurable in the opinion of modern medical science (Davidson’s Principal and Practice of Medicine). However, various drug formulations and prescriptions have been advocated to cure Tamaka Shwasa in Ayurvedic Samhitas (Classical texts), Shringyadi Churna is one of the effective medicine for this dreadful disease-advocated by Acharya Chakrapani datta (1100 A.D.) in Chakradatta (A text authored by Chakrapani datta). The term Pranayama, fourth anga of Astanga yoga (8 levels of Yoga technique), has been derived from the root words- Prana and Ayama. Together they mean expansion or prolongation of life force. In the opinion of Maharishi Patanjali, Shwasa and Prashwasa are meant for inspiration and expiration respectively. The vital force of life i.e Prana is manifested externally by breath. Breath is sthoola (Visible) and Prana is Sukshma (Invisible). Thus Pranayama (Maharshi Patanjali, 200 BC) is breath control at the physical or sthoola level and simultaneously Prana is controlled at Sukshma (Invisible) or subtle level. Hence a study was planned to evaluate the efficacy of Shringyadi Churna with and without Pranayama procedures with the help of modern parameters.

MATERIAL AND METHODS:

After confirming the diagnosis by taking history of every case in detail, physical and clinical examinations and laboratory investigation, 44 patients were selected from the O. P. D. & I. P. D. of Dept. of Kayachikitsa, State Ayurvedic College, Lucknow and were enrolled in the series and the patients were randomly distributed in the three groups, out of which 38 patients were completed the trial duration of 12 weeks and 6 were left the treatment in between the treatment. This trial was done after the approval of the Institutional Ethics Committee (IEC) Ref. no. ECL/AY/105/98, State Ayurvedic College, Lucknow.

Group I: Shringyadi Churna was given in the dose of 2 gm (in capsule forma) T.D.S. with Ushnodak (Warm Water)

Group II: The same Shringyadi Churna was given and patients were made to perform 20 round of Pranayama (Nadi Shodhan Pranayam) in morning and 20 rounds in the evening.

Group III: Placebo (Glucose in the dose of 2 gm in capsule form) was given T.D.S. with 20 round of Pranayama in the morning and 20 rounds in the evening.

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Inclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Patient aged between 13–40 yrs</td>
</tr>
<tr>
<td>2</td>
<td>Patient with classical symptoms of Tamaka Shwasa</td>
</tr>
<tr>
<td>3</td>
<td>Patient not taking any other medicine for Tamaka Shwasa</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Patient below age 13 yrs</td>
</tr>
<tr>
<td>2</td>
<td>Pregnant women &amp; lactating mother</td>
</tr>
<tr>
<td>3</td>
<td>Patients with uncontrolled Hypertension/Cardiac problem/ Diabetes mellitus.</td>
</tr>
<tr>
<td>4</td>
<td>Patients already under modern medications for Bronchial asthma</td>
</tr>
</tbody>
</table>
Assessment Criteria:

Clinical Assessment -

Patients were assessed on different parameters for obtaining the effect of therapy some clinical sign and symptoms like Shwasavega (Dyspnoea), Peenasa (Coryza), Ghur-Ghur shabda (Wheezing sound), Kasa (cough), Kastena Shleshma Nirharana (Difficulty in Expectoration), Kanthodhwansa (Hoarseness of Voice), Bhasna Krichata (Difficulty in speech), Parshva avagahana (tightness in chest), Lalat swedata (Sweating on forehead) were assessed on the basis of their presence and absence.

Laboratory Assessment:

a) Blood- T.L.C., D.L.C., Haemoglobin, E.S.R.
   b) Pulmonary function test-PEFR

Trial Drug review

Shringyadi Churna has been described by Chakrapani dutta (1100 AD) in his renowned text “Chakradutta” (Hikka Shwasa Chikitsa 12/9). The contents of drug are Karkata shringi (Pistacia integirma), Triphala [Haritaki (Terminalia chebula) and Amalaki (Phyllanthus emblica)], Trikatu [Shunthi (Zingiber officinale), Pippali (Piper longum) and Maricha (Piper nigrum)], Pushkarmoola (Inula racemosa), Kantakari (Solanum surattense), Bharangi (Clerodendrum serratum) and Panchalavan (five types of salt)

Source of Drug

Shringyadi Churna is prepared in the pharmaceutical division of State Ayurvedic College, Lucknow. All the drugs were taken in equal amount and pulverized and mixed well. Then the prepared drug was filled in capsules.

RESULTS

To observe the effect of the trial drug, 10 major signs and symptoms were considered and the changes were recorded and calculated on thirty eight patients who completed the study period. The facts observed are presented in table no.1.

Pulmonary Function Test

Changes in PEFR (Peak Expiratory Flow Rate)

In this study to measure the bronchodilator effect of drug and for the assessment of effect of Pranayama, PEFR (Peak Expiratory Flow Rate) (Davidson’s Principal and Practice of Medicine) was assessed during the trial period. In group- I, ‘t’ value was found 3.46 and P<0.01, in group-II, ‘t’ value was found 3.55 and P<0.001 and in group-III, ‘t’ value was found 2.29, P>0.05.

Laboratory Investigation

a) Total Leucocyte Count (T.L.C.)

No significant changes after the trial of 12 weeks in all the three groups.

b) Differential Leucocyte Count (D.L.C.)

Polymorphs: No significant changes were observed after the trial of 12 weeks in all the three groups.

Lymphocyte: No significant changes after 12 weeks trial duration in all the three groups.

Eosinophils: All the three groups showed reduction in mean eosinophil count with highest significance for group II. (In Group I ‘t’ value was 2.81 and P<0.05), In Group II ‘t’ value was 2.97 and P<0.01 and In Group III ‘t’ value was 2.3 and P=0.05)

(c) Haemoglobin:

All the three groups showed increase in mean hemoglobin value with highest significance in group II. (In group I t-value = 2.47, P<0.05, in group II t-value = 2.51, P value <0.05, and is group III t-value = 2.16, P=0.05)
(d) Erythrocytic Sedimentation Rate (ESR)

All the three groups showed reduction in mean ESR value, with highest significance in group II. (In group I t-value = 4.57, P<0.001, in group II t-value = 4.76, P value<0.001, and in group III t-value = 3.71, P<0.01)

The clinical study on “Shringyadi Churna with and without Pranayama” has given a relief in the patients of Tamaka Shwasa. On the basis of comparison of before and after trial observations, in the three groups, the fore most symptoms in 44 patients were Shwasa Vega (Paroxysmal dyspnoea) which was noted in 100% patients. After that Peenasa (Coryza), shabda (wheezing sound), sleshma vimokshante sukham (relief on expectoration) and nirbalata (weakness) were reported in 84% cases each.

**Table No.1 STATISTICAL ANALYSIS OF EFFECT OF THERAPY**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Group I</th>
<th></th>
<th></th>
<th></th>
<th>Group II</th>
<th></th>
<th></th>
<th></th>
<th>Group III</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>X²</td>
<td>P value</td>
<td>Mean</td>
<td>X²</td>
<td>P value</td>
<td>Mean</td>
<td>X²</td>
<td>P value</td>
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<td>X²</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shwasvega (Dyspnoea)</td>
<td>100%</td>
<td>76.92%</td>
<td>13.16</td>
<td>.01</td>
<td>100%</td>
<td>80%</td>
<td>16.81</td>
<td>.01</td>
<td>100%</td>
<td>50%</td>
<td>4.27</td>
</tr>
<tr>
<td>Peenas (Coryza)</td>
<td>76.92%</td>
<td>70%</td>
<td>5.54</td>
<td>0.05</td>
<td>86.66%</td>
<td>76.92%</td>
<td>8.69</td>
<td>.01</td>
<td>80%</td>
<td>37.5%</td>
<td>.88</td>
</tr>
<tr>
<td>Ghur Ghur shabda (Wheezing sound)</td>
<td>92.3%</td>
<td>91.6%</td>
<td>15.38</td>
<td>0.01</td>
<td>88.66%</td>
<td>92.3%</td>
<td>18.53</td>
<td>.01</td>
<td>80%</td>
<td>62.5%</td>
<td>3.33</td>
</tr>
<tr>
<td>Kasa (Cough)</td>
<td>84.6%</td>
<td>63.63%</td>
<td>5.67</td>
<td>&lt;.05</td>
<td>80%</td>
<td>75%</td>
<td>13.36</td>
<td>&lt;.01</td>
<td>100%</td>
<td>40%</td>
<td>.20</td>
</tr>
<tr>
<td>Kastena Sleshna Nirharn</td>
<td>84.6%</td>
<td>72.72%</td>
<td>7.58</td>
<td>&lt;.01</td>
<td>86.66%</td>
<td>84.6%</td>
<td>7.58</td>
<td>.01</td>
<td>70%</td>
<td>57.6%</td>
<td>1.80</td>
</tr>
<tr>
<td>(Difficulty in expectoration)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanthodhwansa (Hoarseness of voke)</td>
<td>38.46%</td>
<td>60%</td>
<td>.78</td>
<td>&gt;.05</td>
<td>26.66%</td>
<td>100%</td>
<td>2.6</td>
<td>&lt;.05</td>
<td>40%</td>
<td>75%</td>
<td>1.7</td>
</tr>
<tr>
<td>Bisan (Difficulty in speech)</td>
<td>69.23%</td>
<td>88.88%</td>
<td>7.96</td>
<td>&lt;.01</td>
<td>80%</td>
<td>91.66%</td>
<td>13.57</td>
<td>&lt;.01</td>
<td>40%</td>
<td>75%</td>
<td>1.07</td>
</tr>
<tr>
<td>Parshva avagrahan (Tightness</td>
<td>76.92%</td>
<td>70%</td>
<td>5.54</td>
<td>&lt;.05</td>
<td>60%</td>
<td>77.77%</td>
<td>5.17</td>
<td>&lt;.05</td>
<td>80%</td>
<td>25%</td>
<td>2.4</td>
</tr>
<tr>
<td>of chest)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lalat Swedata (Swelling on</td>
<td>61.5%</td>
<td>100%</td>
<td>8.85</td>
<td>&lt;.001</td>
<td>60%</td>
<td>88.88%</td>
<td>7.35</td>
<td>0.01</td>
<td>60%</td>
<td>66.66%</td>
<td>1.88</td>
</tr>
<tr>
<td>forehead)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ronchi</td>
<td>92.30%</td>
<td>66.66%</td>
<td>5.49</td>
<td>&lt;.05</td>
<td>92.33</td>
<td>85.71%</td>
<td>16.21</td>
<td>&lt;.001</td>
<td>90%</td>
<td>55.55%</td>
<td>1.76</td>
</tr>
</tbody>
</table>

**DISCUSSION:**

Tamaka Shwasa is primarily a disease of Prana vaha Srotas (respiratory system) and is produced by vitiation of Vata and Kapha, in which vitiated Kapha obstructs the Prana vaha Srotas causing hindrance in the path of vayu, which then spreads in different directions causing disorders of respiration. Acharya Charaka specifically mentioned the Samprapti (Pathogenesis) of Tamaka Shwasa has said that “Pratilome vayu”. As a result of airway...
obstruction due to Kapha involving the head and neck. There will be increase in the shleshma secretion and produces Shwasa vega (dyspnoea), Peenaṣa (coryza) and Ghur-ghur Shabda (wheezing sound) (Charaka, 200BC).

Further, five varieties of Shwasa roga namely Maha Shwasa, Urdhwa Shwasa, Chhinna Shwasa, Kshudra Shwasa and Tamaka Shwasa have been described by Acharyas. Tamaka Shwasa can lead to Pratamaka and Santamaka if Pitta Dosha also gets vitiated in these patients (Sushrut, 2000BC). These could be considered as further stages of Tamaka Shwasa. In Ayurvedic texts various methods and Formulations have been described in the management of Tamaka Shwasa roga. Two types of chikitsa i.e. Shodhana and Shamana Chikitsa have been dealt in detail with description. In Ayurvedic literature no description of Prana yama has been mentioned but involvement of Pranvayu (Inhaled Air) in various respiratory disorders has been described.

So, the present clinical trial was planned to study the effect of an indigenous compound, Shringyadi Churna with and without Pranayama in the management of Tamaka Shwasa. The duration of trial was 12 weeks and the patients were examined weekly for 6 weeks, there after every three weeks upto 12 weeks and he follow up period was of 2 months. On the basis of comparison of before and after trial in three groups following point have emerged:

a) 7 (58.85%) cases in Group I, 10 (66.67%) cases in Group II were cured and 4 (30.76%), 3 (20%) and 4 (40%) cases in Group I, II, III respectively showed improvement.

b) No any toxicity or untoward side effects has been noted during the trial period.

c) On the basis of Statistical test, it can be concluded that Pranayama has shown excellent improvement in PEFR of the patients of Group II with maximum no. of patients achieving a PEFR value between 90–100% of their normal predicted value according to height and age.

d) The drug has shown effectiveness in lowering the raised eosinophils and ESR in the patients of Tamaka Shwasa.

e) Statistically the Shringyadi Churna with Pranayam (in group II) was highly significant. (P<0.001). Then the result of treatment with Shringyadi Churna without Pranayama (in group I) was significant (P<0.01). The Placebo with Pranayama (in group III) was less significant (P<0.005) as compare to other groups.

CONCLUSION

The therapeutic efficacy of Shringyadi Churna with and without Prana yama was studied on 38 cases with Tamaka Shwasa in this series. This compound is well accepted, well tolerated, and easily available and does not have any side effect. Therapeutic efficacy of “Trial drug along with Pranayama”, on clinical and pathological investigations which were given above and in table no.1 has shown highly significant improvement. Peak expiratory flow rate assessment before and after trial duration reveals that the best achievement of normal predicted PEFR according to their height and age. The clinical work was a time bound study, so to obtain a better result and satisfactory therapeutic response, the trial must be taken in large number of cases for a long duration of time and the follow up period must be long. Therefore, the drug ‘Shringyadi Churna along with Prana yama’ may prove a valuable contribution from Ayurveda and Yoga-shastra (Gherand Samhita), to the ailing humanity.
REFERENCES


Gherand Samhita (1997), Yogshastram, kemraj srikrishnadas pub., Bombay, Page no. 52, 57


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Conflict of Interest: None Declared
PHARMACOLOGICAL STUDY OF ANTI-INFLAMMATORY ACTION OF HARITAKI PREPARATIONS ON WISTAR RATS IN HEMORRHOIDS (PILES)

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ABSTRACT

The main aim of the present study was to evaluate the anti-inflammatory properties of Ayurvedic preparation of Haritaki (Terminalia Chebula) on wistar rats. Anti-inflammatory activity of Ayurvedic preparations such as Haritaki powder with Anupana (vehicle) buttermilk, Guda Haritaki powder with distilled water and Guda Haritaki powder with anupana (vehicle) butter milk at a dose of 85 mg/kg, orally was evaluated against the standard drug indomethacin at a dose of 10 mg/kg orally. Wistar rats of either sex of five numbers in each group was undertaken for study and evaluated by carrageenan-induced paw edema method. Haritaki powder with Anupana (vehicle) buttermilk and Guda Haritaki powder with distilled water treated groups showed significant reduction in the carrageenan induced paw edema (P< 0.01) when compared to control group rats. The Ayurvedic preparations of Haritaki have potential anti-inflammatory activity and hence could be established by further more studies.

KEYWORDS: Haritaki (Terminalia Chebula), anti-inflammatory, carrageenan-induced paw edema.

Cite this article:
INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury due to any agent. It is a protective and defensive mechanism of body. Inflammation is of two types - acute and chronic. There are various components responsible to an inflammatory reaction such as edema formation, leukocyte infiltration and granuloma formation that can contribute to the associated symptoms and tissue injury (Brooks, 1991).

No matter what the initiating stimulus, the classic inflammatory response includes calor (warmth), dolor (pain), rubor (redness), and tumor (swelling) (Harsh Mohan, 2002). Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (Kumar V, 2004). It can be evoked by a wide variety of noxious agents (e.g., infections, antibodies, or physical injuries). Some of the inflammatory disorders include Atherosclerosis, asthma, irritable bowel syndromes, Nephritis, hepatitis, arthritis, colitis, nephritis etc. Inflammatory responses occur in three distinct temporal phases, each apparently mediated by different mechanisms: (1) an acute phase characterized by transient local vasodilation and increased capillary permeability; (2) a delayed, subacute phase characterized by infiltration of leukocytes and phagocytic cells; and (3) a chronic proliferative phase, in which tissue degeneration and fibrosis occur.

Hemorrhoids are swollen inflamed veins around the anus or in the lower rectum. About 75 percent of people will have hemorrhoids at some point in their lives (Baker H., 2006). Hemorrhoids are most common among adults ages 45–65 as well as in pregnant women (Chong PS, 2008). Various medicinal plant drugs are used in the treatment of Arshas (Hemorrhoids) and Haritaki (Terminalia chebula Retz.) is one of them, which is also cited in Charaka’s “Arshoghna Mahakashaya” (Sastri Satya Narayana, 2002).

The pathophysiology of hemorrhoid disease in producing acute and chronic symptoms is likely multifactorial involving both anatomic and inflammatory components. The inflammatory component from venous stasis and resulting vascular fragility seems to represent a significant clinical component. Therefore anti inflammatory drugs also plays an important role in the conservative management of hemorrhoids (Jon M Hain, 2011).

There are list of complications occurring as a result of taking anti inflammatory drugs like nausea, vomiting, diarrhea, acid peptic disorders etc. To avoid the above complications, it would be better to go with herbal drugs for the management of Hemorrhoids. In Ashtanga Hridayaya, it is advised to use Haritaki along with Takra (Buttermilk) to reduce the doshas which are indulged in the anal region in the patients of Arshas (Tripathi Bramhananda 2007). Some Maharashtrian Vaidyas also have tradition to use Haritaki along with Takra to reduce the pain and swelling in the initial treatment management of Arshas (Ogale, 1921).

Pathya takrena va saha….hrite gudasraye doshe gudaja yanti samkshayam (A. H. Chi 8/58)

When there are symptoms like swelling, spasmodic pain and itching in the rectal area it is advised to use Haritaki along with Guda (Jaggary) in Ashtanga Hridaya (Tripathi Bramhananda 2007).

Gudshwayathushularto… Khaadet guda haritaki (A. H. Chi 8/33)

Therefore the present study has been carried out to investigate the anti-inflammatory properties of Ayurvedic preparation of Haritaki (Terminalia Chebula).
MATERIALS AND METHODS

Materials

In the present study *Terminalia chebula* fruits were used (Seedless) as medicine. Three Types of *Haritaki* i.e. *Rangari*, *Survari* and *Bal* are available in the market of which *Survari* type of *Haritaki* was selected for the present study. Fruits of *Haritaki* were procured from *Chennai* market (N. Shobhakant).

The fruiting season could not be ascertained as the raw material was procured from the market. Fruit pericarp was used in the drug. (Fruits were taken around 10.5 kg) Seeds were separated and removed and the pericarp was powdered and sieved. 9 Kg was the total yield which indicates a loss of 1.5 kgs during the process.

Preparation of *Guda Haritaki*

- *Guda Haritaki* was prepared according to *Bhavprakasha (Arsha chikitsa)*. (Nanal P. G, 1929)
- Equal quantity of *Haritaki* and *Purana guda* were taken (4 kg of *Haritaki* powder and 4 kg *Purana guda* i.e. old jaggary.
- First *Guda* was crushed and made into a powder.
- It was melted over heat after adding little water.
- Then the liquid *Guda* was filtered by using cloth.
- Then *Haritaki* powder was mixed.
- Then mixture was allowed to dry in shade.

Experimental Animals

Adult Albino Wistar rats weighing about 150–200 g of either sex were procured from the animal house of *Krishna Teja College of Pharmacy*, Tirupati, Andhra Pradesh, India. The animals were maintained in a well-ventilated animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with 12:12 hour light/dark cycle in polypropylene cages with 27 ± 2°C temperature. The animals were given standard diet.

Experimental Design

Group I: Buttermilk (3 ml, p.o.) [Control group]

Group II: Indomethacin (10 mg/kg, p.o) [Standard group]

Group III: *Haritaki* powder (85 mg/kg) with *Anupana* (vehicle) buttermilk orally, p.o.

Group IV: *Guda Haritaki* powder (85 mg/kg) with distilled water, p.o.

Group V: *Guda Haritaki* powder (85 mg/kg) with *anupana* (vehicle) buttermilk, p.o.

The anti-inflammatory activity of Ayurvedic preparation of *Haritaki (Terminalia Chebula)* was determined using carrageenan induced rat paw edema assay (Winter CA, 1962). After 30 mins of the treatment, 0.1 ml of 1% carrageenan in saline was injected into the sub plantar region of the left hind paw of each rat to induce edema. The paw volume was measured initially and at intervals of 0, 30, 60, 120, 180 min after carrageenan injection by volume displacement method using Plethysmometer by immersing the paw in mercury cell. The percentage inhibition of paw volume in drug treated group was compared with control group. Indomethacin (10 mg/kg) was used as standard drug. The percentage inhibition of paw edema was calculated by using the following formula;

\[
\text{Percentage of edema inhibition} = \left( \frac{V_c - V_t}{V_c} \right) \times 100
\]

Vc- Volume of paw edema in control group
Vt- volume of paw edema in treated group

Statistical analysis

Results were expressed as Mean ± S.E.M and statistical significance was calculated by applying one way ANOVA followed by dunnett’s test. P<0.05 was considered as significant.
Table 1. Effect of ayurvedic preparation of Haritaki (Terminalia Chebula) on carrageenan induced induced paw edema

<table>
<thead>
<tr>
<th>Treatment &amp; Dose</th>
<th>Paw edema volume (ml)</th>
<th>Percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Group I (Buttermilk 3 ml, p.o.)</td>
<td>74.83 ± 1.222</td>
<td>1.66 ± 0.0175</td>
</tr>
<tr>
<td>Group II (Indomethacin, 10 mg/kg, p.o)</td>
<td>75.5 ± 0.9916</td>
<td>1.07 ± 0.0147</td>
</tr>
<tr>
<td>Group III Haritaki powder with anupana Takra (85mg/kg, p.o)</td>
<td>73.83 ± 1.249</td>
<td>1.54 ± 0.0154**</td>
</tr>
<tr>
<td>Group IV Guda Haritaki powder with distilled H2O (85mg/kg, p.o)</td>
<td>75.83 ± 0.8724</td>
<td>1.43 ± 0.0115**</td>
</tr>
<tr>
<td>Guda Haritaki powder with anupana Takra (85mg/kg, p.o)</td>
<td>73.17 ± 0.8724</td>
<td>1.62 ± 0.0099</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6), *p<0.05; **p<0.01 denotes significance with respect to the control group using one way ANOVA followed by Dunnett’s test.

RESULTS

Anti-inflammatory effect of Haritaki was observed and found to be significant at the level of p<0.01 when compared with the vehicle butter milk (control group) and indomethacin (Standard) (Table 1). The percent inhibition in paw edema after 3 h were recorded 63.49 % in case of indomethacin, 49.74%, 57.14 and 40.74 % in case of Haritaki powder with anupana Takra (buttermilk) (85 mg/kg, p.o), Guda Haritaki powder with distilled H2O (85 mg/kg, p.o) and Guda Haritaki powder with anupana Takra (buttermilk) (85 mg/kg, p.o) respectively.

DISCUSSION

Anti-inflammatory activity was determined by using inhibition of carrageenan-induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan induced edema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins. (Brooks PM 1991) It was observed that ayurvedic preparation of Haritaki possessed significant inhibition against carrageenan induced paw edema in rats. This response tendency of the extract in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the ayurvedic preparation of Haritaki.

CONCLUSION

The present study concluded that Haritaki had a better effect on treating carrageenan induced rat paw edema. Therefore this Haritaki has got definite effect in reducing the inflammatory components. Further, extraction of the active ingredient responsible for the above cited results along with biochemical analyses, evaluating its role in inhibition of various inflammatory mediators like COX, LOX and TNF-α, and subsequent human trials may further elucidate the potential role of Haritaki in treating inflammation.
REFERENCES


Ogale Prabhakar (1921), Chikitsa Prabhakar, Rajesh publication, 1st edn, Gultekdi Pune, pp 442


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SYSTEMIC SCLEROSIS: A CASE STUDY INAYURVEDIC SETTINGS

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ABSTRACT

Systemic sclerosis (SSc) is a multisystem disorder of unknown cause characterized by fibrosis of skin, blood vessels, and visceral organs including the gastrointestinal tract, lungs, heart, and kidneys. There are two distinct clinical presentations viz., Diffuse and limited forms. We are herewith reporting a case of diffuse cutaneous scleroderma in a 21 year old female student. The possible understanding of the case in terms of Ayurveda and a therapeutic protocol with promising result has been discussed.

KEYWORDS: Systemic Sclerosis, Scleroderma, Ayurveda, Vatavyadhi

Cite this article:
INTRODUCTION:

Scleroderma is derived from the Greek words Scleros (hard or indurated) and derma (skin). It was initially defined by Hippocrates, but a detailed description was given by Carlo Curzio (Sapadin AN, Fleischmajer R. 2002). Systemic sclerosis (SSc) is a multisystem disorder of unknown cause characterized by fibrosis of skin, blood vessels, and visceral organs including the gastrointestinal tract, lungs, heart, and kidneys (Fauci AS et.al., 1998). There are two overlapping forms. Limited cutaneous scleroderma is limited to the skin on the face, hands and feet. Diffuse cutaneous scleroderma covers more of the skin, and is at risk of progressing to the visceral organs, including the kidneys; heart, lungs and gastrointestinal tract are affected.

Annual incidence is 19 per million, and prevalence is 19–75 per 100,000. Women are roughly four times more likely than men to develop systemic scleroderma (http://www.clevelandclinicmeded.com). Incidence is twice as high among African Americans, and the Choctow Native Americans in Oklahoma have the highest prevalence in the world (469/100,000). The interval of peak onset starts at age 30–35 and ends at age 50–55 (Bermas BL, 2009 and www.umm.edu) coinciding with the greatest changes in hormone levels (Chifflot H et.al., 2009). There is some hereditary association, some suggestion of immune reaction (molecular mimicry) to a virus, and some cases caused by toxins (Kasper et.al., 2005).

The outstanding feature of SSc is overproduction and accumulation of collagen and other extracellular matrix proteins in the skin and other organs. While the pathogenesis of SSc remains to be further elucidated, the disease process involves immunologic mechanisms, vascular damage, and activation of fibroblasts (Fauci AS et.al., 1998). Clinical manifestations of Reynaud’s phenomenon, skin thickening, telangiectasia are most commonly encountered. Arthralgias, myopathy, esophageal dysmotility, pulmonary fibrosis, pulmonary hypertension are also observed (Fauci AS et.al., 1998).

At present there are no specific diagnostic tests for SSc and the disorder is diagnosed primarily based on the collective appearance of a cluster of clinical symptoms, such as Raynaud’s phenomenon, telangiectasias, esophageal dysfunction with gastro-esophageal reflux, characteristic pigmented changes, or presence of digital ulcers or calcinotic lesions accompanying clinically detectable skin induration. It is well recognized that the presence of specific autoantibodies is one of the most common manifestations of SSc and greater than 90% of SSc patients harbor antinuclear antibodies in their serum. Numerous autoantibodies have been described in SSc patients, some of these are highly specific for SSc, including anti-Scl-70 and anticientromere antibodies, and these have, therefore, been used as diagnostic biomarkers to support or confirm the clinical diagnosis of SSc. Anti-Scl-70 antibodies are directed against DNA topoisomerase I and are almost exclusively present in the sera of patients with the diffuse form of SSc (Castro SV, Jimmenez SA, 2010 and Jimenez SA, Derk CT 2004).

The many complications of scleroderma can have a major impact on a person’s sense of well-being. Patients are greatly concerned about changes in their appearance, particularly those changes caused by tightening of the facial skin. Depression has great impact, along with pain, on reducing patients' ability to function socially (http://www.umm.edu).

Prognosis is difficult to predict until the disease differentiates into recognizable subsets. Patients with limited cutaneous scleroderma have a good prognosis, with 10-year survival of 75%, although <10% develop pulmonary arterial hypertension after 10–20 years. Patients with diffuse cutaneous scleroderma have a 10-year survival of 55%. Death is most often from pulmonary, heart and kidney involvement, although survival has greatly improved with effective treatments for kidney failure. Immunosuppressive drugs are used, although
glucocorticoids have limited application (www.en.wikipedia.org). Various therapeutic modalities are being used for the treatment of localized scleroderma. There is no precise treatment scheme for this disease. A majority of patients can be successfully treated with topical therapy and phototherapy, but the progressive forms of the disease with intensely expressed skin sclerosis sometimes may need even systemic treatment (Braun-Falco O et al., 2004). Hence an attempt is made to explain the condition in Ayurveda and to derive a treatment strategy.

CASE STUDY:

A 21 year old college student was referred from peripheral outpatient department to Kayachikita department of the central hospital on December 26, 2012 with complaints of gradually progressive feeling of tightness of the skin and face with skin thickening. She also had joint pain involving small joints of hands & wrists, elbows, shoulders and knee. There was h/o difficulty in squatting, getting up from squatting position. She also had Raynaud’s phenomenon, digital calcinosis, weight loss since onset of illness with discoloration of the neck region, (Fig. No.1) hair fall and general weakness were also seen. Her ANA Profile for Scl 70 was ++ (strong positive). Pulmonary function tests were technically poor (restriction) and poor patient effort. Hemoglobin 11.4g%, Total leucocyte count 5950/mm³, Differential count was 59% of neutrophils, 38% of lymphocytes, 02% monocytes and 01% eosinophils, ESR was 10 mm/1st hr, routine urine examination was within the normal range.

Fig. No. 1: Clinical features

1.1 Shiny, taut facial skin, 1.2 Digital calcinosis, 1.3 calcinosis in the sole, 1.4 Skin induration, 1.5 Sclerodactyly, 1.6 & 1.7 Discoloration at elbow
Her vitals were stable with Height - 160 cm, Weight - 38 kg, B.P. - 100/70 mm of Hg. On examination, she demonstrated srotodushti (morbidity of channels) of Annava (food transportation) as aruchi (anorexia), Rasavaha srotas (channels carrying food) as Aruchi (anorexia), pandutwa (paleness) and krushangata (leaness). RaktaVaha srotas (blood carrying channel) with Skin lesions, Asthivaha srotas (channels carrying osseous tissue) as Vaivarnya (discolouration), asthisoola (pain in bone), Majjavahasrotas (channels carrying components of bone marrow) as parvaruk (pain in joints) and Swedavahasrotas (channels carrying sweat) as Aswedana (absence of perspiration) and parushyam (roughness).

**DISCUSSION:**

Looking in to the clinical presentation of the case, the following probable diagnoses were contemplated; Vatarakta, Amavata, Kushta (obstinate skin diseases) and Twakgatavata (Vayu located in skin).

The presentation of Reynaud’s phenomenon on exposure to cold, shiny taut skin over extensor surfaces, sclerodactyly and digital calcinosi, the involvement of vitiated Vata (bio-humour) is obvious in terms of shyavaaarunavarna (bluish discoloration) (Acharya YT, 1994) over extremities, an increase of kharatwa (roughness) and rookshatwa (dryness) of Vata. The salt and pepper discoloration pointing towards Charmakushta (Skin disorder characterized by thickening of skin) (Acharya YT, 1994) (Fig.No. 2), resembling the skin of an elephant. The thinning of skin is a feature in TwakgataVata (vayu located in skin) (Acharya YT, 1994). The digital calcinosis progressing in to rat-bite necrosis (Haustein UF, 2002) typically draws attention to the akhuvisha (rat bite poisoning) (Acharya YT, 2003 and Khunte AM, 2002) simile in Vatarakta.

Fig. No.2 Salt and pepper discoloration in the nape (image 2.1) resembling the skin of elephant

Salt and pepper discoloration resembling the skin of elephant (actual unedited patient photograph)

A complex multifactorial etiopathogenesis was finally accepted based on the following logical sequence; the disease has hereditary role, supporting a genetic mutation and auto-immunity, similarly Kushta has beejadosha (genetic) as a factor. The peripheral features of scleroderma resembles the laxanas (signs and symptoms) of Vatarakta (Acharya YT, 1994); of both uttana and gambhira nature at various instances of disease progression. The cutaneous manifestations mimic a particular variety of Kushta especially Charmakhya of Vataja type. The joint manifestations point towards Amavata (Murthy PHC, 2006) or Sandhivata (Khunte AM, 2002) features. However systemic involvement may denote various srotodushti (vitiation of channels) features. Hence, a complex pathological process duly
constituted by vitiated Vata by means of affliction of twak (skin) with the following samprapti ghataka (factors of pathogenesis); Vatapradhana Kaptha are the involved Dosha (bodily humor), Dushya (vitiated bodily tissue) being Twak (skin), Snayu (ligaments), Sira (veins), Kandara (tendons) and Jatharagni (digestive fire) in mandavastha (diminished) and Ama (unprocessed food) born out of it. Many srotas (channels) are involved in the later stages with primary Rasavaha Srotodusti (morbid food carrying channels) of Sanga (obstruction) type followed by Vimargagamana (diversion of flow of the contents to improper channels). The Doshakopa (aggravated bodily humor) begins at Amapakwashaya, moving through Rasayani (circulatory channel) and settling at Twak (skin). Scleroderma being a physical illness, adhisthana (substratum) is Shareera (physical body). The disease has Bahya Jatharagni (external pathological route) involved extending in to Madhyama (median route of pathogenesis). Oil application slightly improving the dryness and tightness of skin may be considered as Upashaya (symptomatic relief). Upadrava like Pulmonary hypertension, involvement of Sandhi (joints), Sira (blood vessels), Snayu (ligaments) are observed as complications.

The conclusion regarding the diagnosis was derived as a complex syndrome dominated by prakupita Vata (vitiated vata) taking ashraya(base) in rasa (circulating fluid tissue), later the samprapti (pathogenesis)extends to deeper tissues especially sira (blood vessels), kandara (tendons), snayu (ligaments) and asthisandhi (bony joints). In therapeutic perspective, the condition is managed with Vataprasamana (pacifying Vata) and raktapasaddana (pacifying Rakta) line of treatment in accordance with the chikitsa (treatment) advocated for Vatavyadh (Acharya YT, 1994 and Khunte AM, 2002) and Vatarakta (Acharya YT, 1994) in classical Ayurvedic literatures.

**TREATMENT PROTOCOL:**

On the day of admission, Agnideepana (improving digestive fire), amapachana line was adopted using Hingvashtaka choorna. Abhyanga (oil massage) was done in the next two days with Yashtimadhu taila and sweda (sudation) using the Nadee sweda apparatus. On the third day, Koshtashodhana was done with Gandharvahastadi eranda taila 30 ml in the morning at 6.30 AM. Avarasudhi was observed. Samsarjana advised for three annakala (time of food intake).

Yogavasti (mediated enema schedule) is planned considering the Samprapti Ghataka with Anuvasana (oil enema) of Yashtimadhu taila and Ksheerabala taila 60 ml with 5 g of saindhava lava Erandamuladi nirooha was carried out as Asthanapanavasti (decoction enema) using Erandamula kwatha-500ml, Ksheerabala taila + yashtimadhu taila-75ml, Madhu (honey)-25ml, Saindhava (rock salt)-10g and Sathupushpaka kalka (Anethum sowa powder)-20 g.

On discharge, she was prescribed with Manjishtadi Kwatha 25ml twice daily,Tablets of Kaishora guggulu 2 with Kwatha, Kamaduqha rasa tablet twice daily. Guggulutikthaka ghrita 10 ml after food as shaman sneha. Yashtimadhu taila was advised for external application.

**RESULTS AND CONCLUSION**

After 3 months of active treatment in the above lines, the patient was happy with relieved tightness over skin of face and limbs, improved ability to perform normal activities and an enhanced overall quality of life. This example may be a silver lining in the horizon for the mankind with Scleroderma.
REFERENCES


http://www.umm.edu/patiented/articles/how_serious_scleroderma_000088_5.htm#ixzz2LWfKH71F


University of Maryland Medical Center. www.umm.edu/ patiented/article/who_gets_scleroderma_000088_4.htm.


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