ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF METHANOL EXTRACT OF SCINDAPSUS OFFICINALIS ROOT IN EXPERIMENTAL ANIMALS.

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Summary

The methanol extract of the root of Scindapsus officinalis (200, 400 and 600 mg/kg) was evaluated for analgesic and anti-inflammatory activities using acetic acid-induced writhing, hot plate, carrageenan-induced inflammation and cotton pellet-induced granuloma method. Phytochemical analysis of methanol extract of Scindapsus officinalis indicated the presence of glycosides, flavonoids, phenolics, triterpenoids, steroids and tannins. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional
Introduction

Most of the herbal medicines derived from the plant extracts have been used for centuries in the treatment of wide variety of diseases. Chronic inflammatory diseases remain one of the world’s major health problems (1). Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. As a result of adverse effects such as gastric lesions caused by non-steroidal anti-inflammatory drugs (NSAID), tolerance and dependence induced by opiates the use of these
drugs as anti-inflammatory and analgesic agents have not been successful in all cases (2). The study of plants that have been traditionally used as pain killers should still be seen as fruitful and logical research strategy in the search of new analgesic drug and pain mechanism (3). Inflammation is the result of host response to tissue injuries or pathogenic challenges and ultimately leads to the restoration of a normal tissue structure and function. Acute inflammation is a limited beneficial process, particularly in response to infectious pathogens, whereas chronic inflammation is an undesirable persistent phenomenon that can lead to the developments of inflammatory diseases (4). An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses (5). Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases (6). Attention is being focused on the investigation of the efficacy of plant-based drugs used in the traditional medicine because they are cheap, have little side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies (7). 

*Scindapsus officinalis* (Roxb.) Schott. (Family: Araceae) commonly known as “Gajapippali” in India, is an epiphytic climber clinging to trees and rocks by its adventitious aerial roots having obliquely ovate-oblong, cuspidate leaves (8). The plant possesses antioxidant activity (9). The ethanolic extract of fruit is reported to have anti-inflammatory and analgesic activity (10). It also has significant application in treating bronchitis and helminthiasis (11,12).

The objective of this investigation was to ascertain the scientific basis of its use in treatment of pain and inflammation. The present investigation reports the analgesic and anti-inflammatory activity of the methanol extract of *Scindapsus officinalis* root on which previous data are not available.
Materials and Methods

Collection of plant material

The fresh roots of *Scindapsus officinalis* (*S. officinalis*) were collected in the month of November from the Trisulia forest, Nayagarh, Odisha and were authenticated by senior taxonomist Dr. P. C. Panda, Regional Plant Resource Centre, Bhubaneswar, Odisha. A voucher specimen of the herbarium has been deposited at the Department of Pharmacognosy, School of Pharmaceutical Sciences, Siksha ‘O’ Anusandhan University, Bhubaneswar, Odisha, India.

Preparation of methanol extract

*S. officinalis* roots were cut into small pieces and were allowed to dry in the shade. About 100 g of the dried material was coarsely powdered and was defatted by using pet. ether and subsequently extracted with methanol in soxhlet extractor for 24 h. The extract was filtered through Whatman (No. 1) filter paper and concentrated by a rotator evaporator under low pressure. Dark-brown residue (yield-15.42 %, w/w) obtained was stored in glass container and kept in a refrigerator (4ºC) until use.

Preliminary phytochemical screening

Methanol extract of *S. officinalis* (MESO) root was subjected to various qualitative tests for the identification of various plant constituents present in this plant (13).

Experimental animals

Wistar albino rats (150–200 g) and Wistar albino mice (20–25 g) of both sexes were obtained from the experimental animal facility of Siksha ‘O’ Anusandhan University, Bhubaneswar, Odisha. Before and during the experiment, rats were fed with standard diet. After
randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature (25 ± 2º C), relative humidity (35-60%) and dark/light cycle (12/12h). Animals described as fasted, were deprived of food and water for 16 h ad libitum. The conditions in the animal house and at the study, protocol were approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) vide registration no. 1171 / C/ 08 / CPCSEA.

Chemicals and drugs

Carrageenan, acetic acid and Tween 80 all from Sigma-Aldrich Chemie Gmbh, Steinheim, Denmark. The standard drugs used were diclofenac sodium and histamine also from Sigma-Aldrich. All the chemicals and drugs used were of analytical grade.

Acute toxicity studies

Healthy adult albino mice of either sex, starved overnight were divided into four groups (n = 6) and were orally fed with the methanol extract of S. officinalis in increasing dose levels of 100, 500, 1000, 3000 and 5000 mg/kg body weight (14). They were observed for 2 to 72 h and two weeks period for morbidity or mortality and changes in behavior were recorded.

Analgesic activity

Acetic acid induced writhing in mice

The acetic acid induced writhing in mice was carried by previous workers (2). A group of mice were injected intraperitoneal (ip) with 0.1ml/10mg of 0.3 % (v/v) acetic acid. The mice exhibiting the writhing movements (stretching of hind limbs and bending of trunk) were selected for the study. These mice were randomly divided into 5 groups (n=6). Group I served as
control which received normal saline (2ml/kg, b. w.) and group II received Diclofenac sodium (10 mg/kg, b. w.) by oral route of administration. The suspensions of methanol extract of the plant at the dose of 200, 400 and 600 mg/kg body weight were administered to Gr. III, IV and V respectively 1-h prior to acetic acid injection. The numbers of writhing movements were counted for 30 minutes following acetic acid injection.

Table 1: Analgesic effect of MESO on acetic acid-induced writhing in mice.

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg)</th>
<th>Number of writhing</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-10 min</td>
<td>10-20 min</td>
</tr>
<tr>
<td>Vehicle</td>
<td>22.41±1.51</td>
<td>24.56±0.768</td>
</tr>
<tr>
<td>Diclofenac sodium (10)</td>
<td>8.25±1.35</td>
<td>12.26±1.56**</td>
</tr>
<tr>
<td>MESO (200)</td>
<td>14.45±0.525</td>
<td>18.56±1.12*</td>
</tr>
<tr>
<td>MESO (400)</td>
<td>12.85±1.88</td>
<td>16.65±0.772*</td>
</tr>
<tr>
<td>MESO (600)</td>
<td>9.16±1.42</td>
<td>14.67±0.587**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). P values less than 0.05 were considered significant. *: p <0.05; **: p <0.01 when compared to vehicle treated group.

Hot plate assay

The analgesic activity was evaluated by using hot plate method (15). The animals were divided into six groups of 6 animals each. Group I served as control which received normal saline (2ml/kg, b. w.). Group II served as standard and were injected diclofenac sodium (10 mg/kg) intraperitonially. Group III, IV and V were treated orally with methanol extract of 200, 400 and 600 mg/kg body weight respectively. The animals were
individually placed on the hot plate maintained at 55±2°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds.

Table-2: Analgesic effect of MESO on latency to hot plate test in mice.

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg)</th>
<th>Mean latency (s) before and after drug administration(s)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.65±1.76</td>
<td>2.96±1.24</td>
</tr>
<tr>
<td>Diclofenac sodium (10)</td>
<td>1.98±1.52</td>
<td>6.24±1.78**</td>
</tr>
<tr>
<td>MESO (200)</td>
<td>2.34±0.886</td>
<td>3.88±1.52*</td>
</tr>
<tr>
<td>MESO (400)</td>
<td>2.75±1.65</td>
<td>4.72±0.766*</td>
</tr>
<tr>
<td>MESO (600)</td>
<td>2.86±0.826</td>
<td>5.22±1.88**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). P values less than 0.05 were considered significant. *: p <0.05; **: p <0.01 when compared to vehicle treated group.

Anti-inflammatory activities

Carrageenan-induced rat paw oedema

Thirty rats were used in this study and they were divided into five groups of six per group. Each group one of the following treatment: methanol extract of S. officinalis (200, 400 and 600 mg/kg body weight), diclofenac sodium (10 mg/kg body weight) and vehicle control (0.9% normal saline in 3% Tween 80 [2ml/kg]), which were administered orally. Acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% carrageenan in normal saline that contained
Tween 80 in the right paw of rats. The paw volume was measured at 0, 1, 2 and 3 h after carrageenan injection using a micrometer screw gauge. Increases in the linear diameter of the right hind paws were taken as an indication of paw oedema. Oedema was assessed in terms of the difference in the zero time linear diameter of the injected hind paw and its linear diameter at time t (i.e. 60, 120, 180 min) following carrageenan administration. The anti-inflammatory effect of the extract was calculated by the following equation:

Anti-inflammatory activity (%) = (1-D/C) X 100, where D represented the percentage difference in paw volume after the extract was administered to the rats and C represented the percentage difference of volume in the control groups. The percentage inhibition of the inflammation was calculated from the formula:

% inhibition = D0-Dt/D0 X 100 where D0 was the average inflammation (hind paw oedema) of the control group of rats at a given time; and Dt was the average inflammation of the drug treated (i.e. extracts or reference diclofenac sodium) rats at the same time (16).

Table 3: Anti-inflammatory effect of MESO on carrageenan induced paw oedema in rats

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg)</th>
<th>Mean increase in paw volume (ml)</th>
<th>% inhibition of paw oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.37±0.185</td>
<td>0.68±0.127</td>
</tr>
<tr>
<td>Diclofenac sodium (10)</td>
<td>0.17±0.165</td>
<td>0.24±0.085**</td>
</tr>
<tr>
<td>MESO (200)</td>
<td>0.28±0.257</td>
<td>0.44±0.158*</td>
</tr>
<tr>
<td>MESO (400)</td>
<td>0.24±0.316</td>
<td>0.35±0.174**</td>
</tr>
<tr>
<td>MESO (600)</td>
<td>0.19±0.241</td>
<td>0.26±0.128**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). P values less than 0.05 were considered significant. *: p <0.05; **: p <0.01 when compared to vehicle treated group.
Cotton pellet granuloma

The rats were divided into five groups (n=6). After shaving the fur, the rats were anesthetized with ether and 20 mg of sterile cotton pellets were surgically inserted in the groin region. The MESO (200, 400 and 600 mg/kg), diclofenac sodium (10 mg/kg, p.o.) and vehicle was administered for 7 consecutive days from the day of the cotton pellet implantation. The animals were anesthetized on the 8th day and the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37° for 24 h and dried at 60° for constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation (17).

Table-3: Anti-inflammatory effect of MESO on cotton-pellet granuloma in rats

<table>
<thead>
<tr>
<th>Treatment and dose (mg/kg)</th>
<th>Weight of cotton pellet (mg)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>145.46±1.87</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac sodium (10)</td>
<td>87.5±1.34**</td>
<td>39.84</td>
</tr>
<tr>
<td>MESO (200)</td>
<td>127.56±2.41*</td>
<td>12.30</td>
</tr>
<tr>
<td>MESO (400)</td>
<td>104.42±1.05**</td>
<td>28.21</td>
</tr>
<tr>
<td>MESO (600)</td>
<td>93.25±2.17**</td>
<td>35.64</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). P values less than 0.05 were considered significant. *: p <0.05; **: p <0.01 when compared to vehicle treated group.

Results

Phytochemical screening

The phytochemical screening of methanol extract of *S. officinalis* root revealed the presence of glycosides, flavonoids, phenolics, triterpenoids, steroids and tannins.
Acute toxicity study

The acute toxicity study result of the methanol extract of *S. officinalis* on mice showed no mortality and no significant gross behavioural changes observed even at a higher dose level of 5 g/kg b. w.

Acetic acid-induced writhing in mice

Table 1 shows the effects of the extracts of MESO on acetic acid-induced writhing in mice. The methanol extracts and diclofenac sodium induced significant decrease in the number of writhes when compared to the control. The extract at 200, 400, 600 mg/kg and diclofenac sodium at 10 mg/kg body weight exhibited inhibition of writhing by 42, 49.2, 58.8 and 62 respectively in the reaction time of 20-30 minutes indicating that the diclofenac sodium has slightly higher antinociceptive than the test drug used in this study.

Hot plate method

Results of hotplate test are presented in Table 2 for the methanol extracts of *S. officinalis*. The extracts of the plants were found to exhibit a dose dependent increase in latency time when compared with control. At 90 minutes, the percent inhibition of three different doses of *S. officinalis* (200, 400 and 600 mg/kg body weight) was 57.59, 71.96 and 77.38 while diclofenac sodium at the dose level of 10 mg/kg exhibited percent inhibition of 79.63. The results were found to be statistically significant (p<0.001).

Carrageenan induced paw oedema

The results obtained as increase in paw volume and % age inhibition are shown in table -3. After 3 h of administration of carrageenan, the percentage inhibition of paw oedema by MESO in dose (200, 400 and 600 mg/kg) were 42.9, 53.1 and 66.4 respectively. The reference drug (10 mg/kg) showed 70.5% inhibition of paw oedema. The anti-inflammatory effect of the extract
and the reference drug increased with time. This was found to be dose-dependent for the test extract.

**Cotton pellet granuloma**

The results obtained as percentage inhibition of granuloma formation are shown in table-2. The results shown percentage inhibition of granuloma formation with 200, 400 and 600 mg/kg dose MESO are (12.30, 28.21 and 35.64%) respectively, while diclofenac sodium (10mg/kg) showed 39.84% inhibition of granuloma formation.

**Discussion**

Any injury or tissue damage is associated with pain and inflammation. Analgesics can act on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptors site of pain, while centrally acting analgesics not only raise the threshold for pain, but also alter the physiological response to pain and suppress the patient's anxiety and apprehension. Pain and inflammation are an essential prelude to the repair process(18).

Acetic acid induced writhing response in mice is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve ending. Acetic acid is also known to increase PGE$_1$ and PGE$_2$ peripherally (19).

NSAIDs can inhibit COX in peripheral tissues and therefore interfere with the mechanism of transduction of primary afferent nociceptors (20). The mechanism of analgesic activity of MESO could be probably due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings.
similar to that of diclofenac sodium and NSAIDs. Thus, the reduction in the number of writhing indicates that MESO might exert anti-nociceptive activity by inhibition of prostaglandin synthesis or action of prostaglandin.

In the hot plate model, nociceptive reaction toward thermal stimuli in mice is a well-validated model for detection of opiate analgesics as well as several types of analgesics drugs from spinal origin (21).

Carrageenan-induced oedema involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leukotrienes and serotonin, all of which also cause pain and fever (22). Inhibitions of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorate the inflammation and other symptoms. This study has shown that the methanol extract of the root of *S. officinalis* possessed a significant anti-oedematogenic effect on paw oedema induced by carrageenan. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation (23). Carrageenan oedema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic; the first phase (1h) involves the release of serotonin and histamine while the second phase (over 1h) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins (24). Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation (25), the results of this study are an indication that *S. officinalis* can be effective in acute inflammatory disorders.

In granuloma method inflammation is always accompanied by increase vascular permeability and collagen formation at the sight of injury (26). Therefore the decrease in granuloma weight indicates suppression of the granuloma weight indicates suppression of the
collagen formation, which was effectively inhibited by MESO in the present study. Efficacy of MESO in this model might be due to an increase in the synthesis of collagen and mucopolysaccharides and increase in the number of fibroblasts during granuloma tissue formation.

The antinociceptive and anti-inflammatory activities of the methanol extract could be attributed to the presence of phytosterols (27) that were detected and isolated (28). Similarly, terpenoids that were reported to be present in the extract (29) could possibly be responsible. The flavonoids have potent anti-inflammatory activity by inhibiting prostaglandin synthesis (30). So anti-inflammatory activity of S. officinalis can be attributed to bradykinin and PG synthesis inhibition property of flavonoids.

Conclusion

In conclusion, since the plant extract reduced significantly the formation of oedema induced by carrageenan as well as reduced the number of writhes in acetic acid induced writhing models, the root of S. officinalis exhibited anti-inflammatory and analgesic activities which was found to be the most effective at higher concentrations employed. The study has thus provided some justification for the folkloric use of the plant in several communities for conditions such as pain and inflammations. However, a more extensive study is necessary to determine the exact mechanism(s) of action of the extracts and its active compound(s).

Acknowledgement

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