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Characterization of Anti-Inflammatory Activities and Antinociceptive Effects of Papaverine from *Sauropus androgynus* (L.) Merr

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Abstract: The present study was aimed to investigate the anti-inflammatory activity of papaverine from *Sauropus androgynus* L. Merr. (Phyllanthaceae) using experimentally induced inflammatory models in rats. *In vitro* anti-inflammatory activity by Inhibition of albumin denaturation, Membrane stabilization test, Heat induced hemolytic activity, Protein inhibitory action and *In vitro* anti-inflammatory activity by Carrageenan Induced Rat Paw Edema Method, Antinociceptive activity, Hot-Plate Test. *Sauropus androgynus* showed significant reduction when compared with the standard dose of 100 mg/kg b.wt Papaverine, Morphine which exhibited stronger inhibition than aminopurine in acetic-acid induced abdominal stretching at 100 mg/kg dose. Papaverine also showed antinociceptive activity at 100 mg/kg dose in tail-flick test. However, of *Sauropus androgynus* at doses of 200 mg/kg and 400mg/kg b.wt showed a significant reduction with 59.2 % and 51.5 %, respectively in rat paw oedema induced by carrageenan against the reference anti-inflammatory drug Phenylbutazone 100 mg/kg of 48.2% in 1hr. This study shows significant reduction in paw edema and significant increased reaction time in tail flick test were observed at a dosage of 400 mg/kg/body weight.

Key words: *Sauropus androgynus* · Papaverine · Analgesic Activity · Anti Inflammatory Activity

INTRODUCTION

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical and microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove irritant and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells [1]. It is a complex process mediated by activation of inflammatory and immune cells. Acute or chronic inflammation plays a critical role in the initiation or development of many human diseases such as septic shock, rheumatoid arthritis, asthma, or cancer [2-4]. It is the central communication network and regulatory process that senses and controls threat, damage, containment and healing, which are all critical aspects in the maintenance of the integrity of an organism [5]. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses [6, 7].

Sauropus androgynus Merrill of family Euphorbiaceae which is also known as *Katuk* in Indonesia. It is widely used as tonic, antioxidant and febrifuge. The leaves are used as antitussive, tonic and soothing lungs and to relieve internal fever. The dark-green leaves provide a rich source of chlorophyll which is a valuable blood building element, cell rejuvenator and beneficial to the circulation [8, 9]. *Sauropus androgynus* leaves can increase the quantity of milk production in mice [10]. It was also reported that the *Sauropus androgynus* leaf extract can increase the mother's breast milk production without decreasing the quality of the breast milk [11]. *Sauropus androgynus* can be useful as a dye in food colouring a delicious hot weather green vegetable, widely considered to be nutritious, approximately 10% protein content, the roots and leaves are used as medicine [12].

Alkoloids are diverse group of nitrogenous compound found in 20% of plant. The benzyl isoquinoline class consists of several important medicinal compounds including morphine, codeine, papaverine and berberine [13]. Papaverine is also a pharmacological compound being used as muscle relaxant and vasodilator. Therefore, the physiological properties of the molecule and the interaction of papaverine with other biomolecules were extensively studied [14-16]. Papaverine, a potent

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non-selective phosphodiesterase inhibitor, has shown itself to be effective in pulmonary arterial hypertension [17, 18]. This study therefore seeks to examine *Sauropus androgynus* for anti-inflammatory activity and analgesic effects since pain is one of the cardinal signs of inflammation.

MATERIALS AND METHODS

Plant Material Preparation: Mature *Sauropus androgynus* leaves were obtained in herbal garden of University. The mature leaves were picked. The leaves of the plant were air dried at room temperature for 5days. The dried material was pulverized with an electric blender.

Extraction and Isolation Procedure: The dried and ground to a fine powder before extraction then they were subjected to maceration with ethanol at room temperature. Extract was filtered from filter paper and then concentrated under low pressure. With addition of 5% HCl pH of the extract was calibrated to pH 3. Acidic extract was stored in the cold at night to collapse resinous substances and they were removed by filtration. Filtered extract was subjected to extraction and concentrated under low pressure. Concentrated extract pH was calibrated to pH 8 with 25% NH_4OH addition. Then extraction with chloroform was repeated. Cream colored precipitate was formed during concentration procedure and this precipitate was obtained by filtration.

Animals: The protocol for the study was approved by the Ethical Committe. Male and female albino rats of Wistar strain approximately 150-200gm were used in this study. All of them were maintained in the single cage. The animals were obtained from the animal house of Pharmacy College, Thanjavur. All animals were housed in standard cages at room temperature 20±2°C, with artificial light at night and provided with pelleted foods. Prior to administration of the drugs, the rats of the anti-inflammatory activity groups were fasted for 12 hrs with free access to tap water. Food was withdrawn 12hrs before and during the experimental hours. The first and second groups received a dosage of mature *Sauropus androgynus* leaf extracts.

Drugs and Chemicals: Lambda-carrageenan (Type IV) and indomethacin were obtained from Sigma Aldrich Chemicals, Mumbai. Papaverine obtained from Merck, Mumbai. The *Sauropus androgynus* by using the procedure as described in extraction and lambda-carrageenan were dissolved in isotonic saline solution (0.9% NaCl, w/v) and indomethacin were dissolved in ethyl alcohol (96%).

***In vitro* Anti-Inflammatory Activity**

Inhibition of Albumin Denaturation: Methods of Sakat *et al*. [19] followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C Hcl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

% inhibition= [{Abs control- Abs sample}/Abs control] x 100,

Membrane Stabilization Test

Preparation of Red Blood Cells (RBCs) Suspension: Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline Sakat *et al*. [19].

Heat Induced Hemolytic Activity: The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula mentioned in the foregoing Sakat *et al.* [19].

Protein Inhibitory Action: The test was performed according to the modified method of Sakat *et al.* [19]. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH7.4) and 1 ml test sample of different concentrations. The reaction mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (W/V) casein was added. The mixture was inhibited

for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated.

In vitro Anti-Inflammatory Activity

Anti-Inflammatory Activity by Carrageenan Induced Rat Paw Edema Method: The rat paw edema was induced by carrageenan (100 µg/paw), which was injected into the right hind paw plantar surface to groups of five animals each [20]. Saline solution (0.9%, 0.1ml) was injected into the left paw as the control reference for the tested paw. The measurement of foot volumes was carried out following the plestimographic method [21]. It was done by recoding the rat paw volume at 1, 2, 3 and 4 hrs after the carrageenan injection. The extract was administered at 200 and 400 mg/kg body weight. Phenylbutazone 100 mg/kg body weight was used as standard anti-inflammatory agent.

Antinociceptive Activity: The writhing test in mice was carried out using the method of Koster *et al*. [22]. The writhes were induced by intraperitoneal injection of 0.6% acetic acid (v/v) (80 mg/kg). Three different doses (100, 200 and 300 mg/kg b.wt.) of *Sauropus androgynus* were administered orally to groups of five animals each, 30 min before chemical stimulus. The number of muscular contractions was counted over a period of 20 min after acetic acid injection. The data represent the total number of writhes observed during 20 min and are expressed as writhing numbers. Percentage of protection against acetic acid induced writhing was calculated using the formula:

Percentage protection = (Wc-Wt)/Wc) x 100

where,

Wc = Mean values of number of writhing in control group

Wt = Mean values of number of writhing in the test groups.

Hot-Plate Test: The hot plate test in rats was performed by the method of Woolfe and Mc Donald [23], which was adapted for rats. T he evaluated parameters were the latency time for paw licking and jumping responses on exposure to the hot plate surface, kept at 55±1°C. The animal was kept on the hot plate until it lifted one of its hind paws. Three different doses (100, 200 and 300 mg/kg) of *Sauropus androgynus* extract were administered orally to groups of five animals, 30 min before the thermal stimulus and the response was determined every 30 min, during 120 min. The data represent the mean reaction time for the animals. Latency time was recorded and the results are expressed as hot plate analgesic index [24].

RESULTS AND DISCUSSION

The investigation denaturation is a mechanism of the anti inflammation activity for the ability of *Sauropus androgynus* extract protein denaturation, membrane stabilization and Proteinase inhibition was studied. In denaturation it shows effective in inhibiting heat induced albumin denaturation is mentioned in Table 1. Dried leaf extract showed 77.61±1. In the fresh leaf extract the albumin denaturation of about 78.26±0.34 showed inhibition. Papaverine and Phenylbutazone act as a standard anti-inflammation drug showed the maximum inhibition 79.49±1.25 and 75.05±1.01 at the concentration of 200 µg/ml. Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of different ethanolic extract of *Sauropus androgynus* inhibited the heat induced hemolysis of RBCs to varying degree Table 1. The inhibition was recorded as 68.22±1.12 in dry extract and 67.43±0.05 in fresh extract. The *Sauropus androgynus* extract exhibited significant antiproteinase activity was observed from dried leaf extract is 73.11±0.32 and fresh leaf extract is 78.33±0.25 Table 1.

Phytochemical screening of the ethanolic extract of *Sauropus androgynus* leaves revealed the presence of anti-inflammatory effect of the extract on carrageenan induced oedema right hind paw volume in rats is shown in Table 2. There was a gradual increase in the oedema paw volume in the distilled water treated control group throughout the period of the experiment. The extract at 200 and 400 mg/kg body weight as well as 100 mg/kg Papaverine and 100 mg/kg Phenylbutazone significantly reduced the oedema paw volume in a manner that was not dose dependent. There was also substantial inhibition against the oedema induced paw volume in the extract and drug treated animals. The injection of carrageenan to the hind paw volume of the negative control increased significantly throughout the 4hrs experimental period. In contrast, the leaf extract of *Sauropus androgynus* reduced the carrageenan induced right hind paw volume in a manner that was not dose-dependent. The Phenylbutazone treated animals produced the highest inhibition of carrageenan induced oedema.

Table 1: Effect of Ethanolic extracts of *Sauropus androgynus* on albumin denaturation, membrane stabilization and proteinase inhibitory activity percentage inhibition

Sample	Albumin Denaturation	Membrane stabilization	Proteinase inhibition
Control	81.55±2.01	70.41±1.21	70.21±2,05
Dry Extract	77.61±1.22	68.22±1.12	73.11±0.32
Fresh extract	76.26±0.34**	67.43±0.05**	78.33±0.25**
Papaverine (200 µg/ml)	79.49±1.25**	68.62±1.06**	74.53±1.03**
Phenylbutazone (200 µg/kg)	75.05±1.01**	62.11±1.25**	71.32±0.12**

**p* values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test); ***p*<0.001. All values are mean of individual data obtained from six rats (n=6)

Table 2: Anti-inflammatory activity of crude extract of *Sauropus androgynus* by carrageenan induced rat paw edema

	% Increase in Paw Volumes (ml x 1000) ± SEM (Percent inhibition)			
Group	1h	2hrs	3hrs	4hrs
Control	72.5±2.10	93.1±1.2	108.5±2.31	115.2±3.51
Sauropus androgynus (200 mg/kg)	59.2±1.15** (18.34)	71.2±1.95** (23.52)	72.6±3.56** (33.10)	84.2±2.6** (26.91)
Sauropus androgynus (400 mg/kg)	51.5±2.75** (28.97)	64.5±1.85** (30.72)	70.5±3.05** (35.02)	73.2±3.01** (36.46)
Papaverine (100 mg/kg)	49.3±1.02	60.7±1.51	63.2±1.91	73.1±2.05
Phenylbutazone (100 mg/kg)	48.2±1.52** (33.51)	58.2±2.75** (37.49)	62.1±1.6** (42.76)	72.5±3.06** (37.07)

p* values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test);*p*<0.001. All values are mean of individual data obtained from six rats (n=6) Phenylbutazone

Table 3: Effect of crude extract on acetic acid induced writhing response in mice

Group	Dose (mg/kg)	Writhing	%Inhibition
Sauropus androgynus	-	18.20±1.52	-
	200	10.56±0.82**	41.97
	400	8.35±0.68**	54.12
Papaverine	100	8.20±0.55	55.10
Aminopyrine	50	7.25±0.83**	60.16

1h after treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1 ml/10g); 10 minutes after the injection, the number writhing was counted for 10 min

Table 4: Effect of crude extract[a] on radiant heat tail-flick response in mice

		Reaction time (sec)[c]		
Group	Dose	30 min (% elongation)	60 min (% elongation)	120 min (% elongation)
Control		4.60±0.17	4.69±0.15	5.01±0.20
Morphine[b]	100mg/kg	8.45±0.18** (83.70)	7.20±0.20** (53.53)	6.35±0.30** (26.75)
Papaverine[b]	100mg/kg	8.05±0.22	7.11±0.10	6.05±0.03
Sauropus androgynus[a]	200mg/kg	6.45 ± 0.19** (40.21)	6.11 ± 0.25 ** (30.28)	5.73 ± 0.27** (14.38)
	400mg/kg	7.35 ± 0.35** (59.78)	6.85 ± 0.26** (46.06)	5.95 ± 0.25** (18.76)

[a]Per oral administration of vehicle and crude extract, radiant heat intensity was 5 amp

[b]Papaverine and Morphine was administered sub-cutaneously

[c]values are mean ± SEM (n =6)

The *Sauropus androgynus* extract decreased the acetic acid induced number of licks in the first phase 30min in a manner that was inversely proportional to the doses that decrease in the same parameter in the second phase 30-60 min and 60-120 min was dose dependent is mentioned in Table 3. The carrageenan treated animals produced the greatest reduction in the number of licks as well as inhibitory effect on the acetic acid induced pain in the animals. The administration of *Sauropus androgynus* leaf extract elevated body temperature in rats is shown in Table 4. Whereas the distilled water control group remained throughout the experimental period, the extract and Papaverine treated animals had their body temperature lowered. The lowering of elevated body temperature for 100 mg/kg body weights manifested after half an hour. The anti inflammatroy effect was sustained throughout the remaining period of the experiment in a manner similar to Phenylbutazone. Papaverine is used to inhibit phosphodiesterase activity, through this action it increases cellular cAMP level and alters cellular function.

It has been shown to reduce arterial and cerebral vasospasm [25, 26]. The results from the present study show that the leaf extract of *Sauropus androgynus* exhibited activities in various degrees against inflammation, pain and fever etc. By activating the cyclooxygenase, the levels of prostaglandin, especially PGE2, increases markedly and its production provokes inflammation, pain and fever [27]. Therefore, we assume that some active metabolites of the extract in this study could inhibit cyclooxygenase activity.

Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation. Similar results were observed from many reports from plant extract [19]. Papaverine is also a pharmacological compound being used as muscle relaxant and vasodilator. Therefore, the physiological properties of the molecule and the interaction of papaverine with other biomolecules were extensively studied [14-16]. The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage [28]. The precise mechanism of this membrane stabilization is yet to be elucidated, it is possible that the *Sauropus androgynus* produced this effect surface area of the cells, which could be brought about by an expansion of membrane or the shrinkage of cells and an interaction with membrane proteins. Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play important role in the development of tissue damage during in inflammatory reactions and significant level of protection was provided by proteinase inhibitors [29].

The most widely used primary test to screen anti-inflammatory agent is to measure the ability of a compound to reduce local oedema induced in rat paw following the injection of irritants such as carrageenan [20]. The first phase of 30min involves the release of serotonin and histamine while the second phase of the next 1h is mediated by prostaglandin [30]. The significant reduction as well as inhibitory effect of the extract on the carrageenan-induced oedema paw volume is an indication of the anti-inflammatory potentials of the plant. Centrally acting analgesics such as narcotics inhibits both phases equally while peripherally acting drugs, such as steroids and non-steroidal anti-inflammatory drugs (NSAIDS) like aspirin suppresses mainly the late phase [31, 32]. Although, acetic acid extract did not inhibit both phases equally, it may still be logical to assume that it produced analgesic effect on the two phases. This may thus suggest that the extract is a centrally acting analgesic. The tail flick or tail immersion model is an index that is used to evaluate acute pains in animals [33]. The present results show that the extract of *Sauropus androgynus* leaves possesses significant elevation of body temperature in rats. The reduction induced fever by the extract in this study suggests some influence on the prostaglandin biosynthesis since it is believed to be a regulator of body temperature [34]. The result of this study confirmed that *Sauropus androgynus* leaves could be beneficial in the management of inflammations, pains and fever. These activities may be due, in part, to the presence of phytochemicals such as flavonoids, alkaloids, steroids or terpenes. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the antioxidant and anti-inflammatory activity of many plants [35]. The results of the pharmacological screening indicated bioactive compounds possess significant analgesic activity associated with NSAIDs properties [36]. The efficiency of most herbal remedies is attributed to various active constituents in combination. The analgesics and anti-inflammatory activity may be due to the presence of phyto-constituents like flavonoids, tannins, saponins, triterpenes and coumarins as reported in phytochemical investigation. Therefore it is possible that the antinociceptive and anti-inflammatory effects observed with the plants attributes to the components that are present in abundance which provide scientific basis for the traditional medicinal uses of these plants for analgesics and anti-inflammatory activities [37, 38].

The *Sauropus androgynus* extracts inhibited the heat induced albumin denaturation and proteinase activity and stabilized the red blood cells membrane. These reports provide a basic scientific evidence to support its traditional medicinal uses. In this study might suggest a possible use of natural anti-inflammatory agent. The result concluded that the ethanolic extract of *Sauropus androgynus* shows anti-inflammatory activities and it shown dose dependent activities. The results support the traditional use of this plant in inflammatory conditions and suggest the presence of biologically active components which may be worth further investigation and elucidation.

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