International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-7, Issue-2, 2016, pp621-633
http://www.bipublication.com

Research Article

Activity of *Sauropus androgynus* L. leaf extract against inflammation and its immunomodulatory effect in Swiss albino mice.

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[Received-15/04/2016, Accepted-12/05/2016, Published-17/06/2016]

ABSTRACT

In the present study, we have evaluated the anti-inflammatory and immunomodulatory potential of the methanol extract of *Sauropus androgynus* leaf. The *Sauropus androgynus* leaf extract (SALE) were ingested orally (p.o) in the form of suspension in 0.5% tween 80 in two different doses, 250 and 500 mg/kg body weight. The anti-inflammatory activity of SALE treatment was checked in acute and chronic inflammation in mice model induced by carrageenan and formaldehyde respectively. Immunomodulatory activity was checked both at cellular and humoral levels. Cellular immunity was evaluated by Delayed type Hypersensitivity (DTH), neutrophil adhesion test and carbon clearance assay, whereas, humoral immunity was analyzed by heamagglutination antibody (HA) titer assay. Following total WBC count, body weight and relative immune organ weight were analyzed. Acute oral toxicity study concluded that SALE was safe at 2500 mg/kg. The results showed that administration of SALE (500 mg/kg bw) significantly reduced oedema formation, the reduction was similar to diclofenac. The same dose of SALE caused a significant increase in HA titer in response to sheep red blood cells (SRBC) when compared to control group, and a decrease in DTH (footpad thickness) in response to challenge with SRBC sensitized hosts and also caused increases in WBC counts. Administration of SALE enhanced the relative organ weight and showed a significant ($p \leq 0.01$) increase in percentage neutrophil adhesion to nylon fibers and phagocytic activity. Based on the results, we conclude that SALE has a potent immunomodulatory potential but also could significantly impact upon inflammation reactions.

Key words: *Sauropus androgynus*, inflammation, immunomodulation, delayed type hypersensitivity, antibody titer.

INTRODUCTION

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain [1,2]. However, persistent orover-inflammation leads to tissue damage and possibly the failure of organs. Pro-inflammatorycytokines (e.g., TNF-a, IL-6, and IL-1ß) are produced in large quantities by activatedmacrophages/monocytes that stimulate cellular responses via increasing prostaglandins (PGs) andreactive oxygen species (ROS). Inflammation is either acute or chronic inflammation. Acute inflammation may be an initial response of the body to harmful stimuli. In chronic inflammation, the inflammatory response is out of proportion resulting in damage to the body[3]. The immune system is a complex system consisting of a network of interacting

cells, tissues and organs. Immunomodulators of natural or synthetic origin can modify the activity of the immune system. Most immunomodulants have biphasic effects, i.e. some tend to stimulate immune system at low doses but suppress host defense parameters at high (er) levels [4].Several medicinal plants are considered immunomodulatory as after they are ingested in whole or in part, they impart a variety of anti-inflammatory, anti-microbial and anti-tumor effects [5]

Recent screening with plants has revealed many compounds (e.g. alkaloids, flavonoids, quinones, terpenoids) with pronounced antioxidant, antineoplastic, antiulcer, anti-inflammatory and immunostimulating potential [6]. Drugs employed in conventional medicine are formulated to act as chemical strait jackets, preventing the cells of the body from performing some function that has become hyperactive. The adverse drug effects are a direct extension of their actions and may be fatal. The interest on plant based products to treat disease is gaining much significance due to the side effects caused by synthetic drugs [7]. Plants have been used as a folk remedies and ethno botanical literature have described the usage of plants extracts to treat human diseases since time immemorial [8]. *Sauropus androgynus* L., belonging to the family Euphorbiaceae and it is a perennial shrub, cultivated in India, Sri Lanka, Thailand, Laos, Malaysia, Indonesia and Southeast Asia. In India found growing in Sikkim, Khasi Hills, Western ghats and south India. The exact origin of *Sauropus* is unknown.It is commonly known as star goose berry (Tropical asparagus, Chekkurmanis), multivitamin, multigreen plant. In Tamil known as Thavasaimurungai and in Malayalam Madhuracheera. *Sauropus* is highly nutritious for its substantial vitamin content. Decoction is given in stricture of the bladder and in fevers also used as diuretic. Fresh leaves are an excellent source of provitamin A, Carotenoids, vitamins B and C, protein and minerals. Mature leaves have more nutrients than young leaves. Vitamin C content was high in raw and cooked *Sauropus androgynus*. SA has gained popularity as a weight reducing vegetable[9]. Hence, the present study evaluates the immunomodulatory and anti-inflammatory activity of SALE in experimental mice model.

MATERIALS AND METHODS

Plant material

The leaves of the plant *Sauropus androgynus*(SA) were collected from Maruthancode, KanyaKumari district (TamilNadu, India). The plant sample were further identified at the Botanical survey of India Coimbatore, receipt specimens of the SA sample (BSI/SRC/5/23/2011-12/Tech/1129) have been deposited in the herbarium of the department. The Institutional Animal ethics committee, School of biotechnology and health sciences (IAEC/KU/BT/12/020) approved the study.

Extract Preparation

The leaves of the plant *Sauropus androgynus* were shade dried and then crushed to make a coarse powder. The dried powder was weighed and solvent extraction using methanol was performed at a 10% concentration. The extraction of the powder was performed using soxhlet extractor. The extract was dried in an evaporator at 45°C and stored at 4°C until ready for use. The yield of the extract was found to be 12% [w/w].

Animals

Swiss albino Male mice (4-6 weeks old) were purchased from Sri Venkateswara Enterprises, Bangalore, Reg No- 237/99/CPCSEA. Swiss albino Male mice were used for both inflammation and immunomodulation study. The animals were kept in well-ventilated cages in a facility maintained at 22 (± 1) °C, with a 40 (± 10) % relative humidity, and a 12-hr light-dark during the experimental period. Mice were provided access to normal mouse chow (Sai Feeds, Bangalore, India) and filtered water *ad libitum*. All the animal experiments were carried

out according to the rules and regulation of the Institutional Animal Ethics Committee and approved by Committee for the purpose of control and supervision on experiments on animals (CPCSEA).

Chemicals and reagents

Carrageenan and Drabkin's solution was purchased from Hi-Media, Diclofenac from Biochem pharmaceutical, Mumbai. Septilin from Himalaya products, Indian ink, camel India Pvt.Ltd. All other chemicals and solvents used were of analytical reagent grade.

Acute toxicity study

Acute toxicity of SALE was performed on male Balb/c mice, according to OECD (Organization of Economic Cooperation and Development) Guideline 423[10]. Six groups of six mice each were used for the study. Group I served as control and received distilled water. Group II to VI received single oral dose of SALE (50, 150, 300, 750, 2500 mg/kg b.wt.). The animals were observed at short intervals of time for 24 h and then daily for 14 days for mortality and physical/behavioral changes.

Carrageenan Induced Acute Inflammation

Acute inflammation was studied utilizing carrageenan- induced mice paw oedema model as previously described by Winter*et al.,* 1962 [11]. Swiss albino mice were divided into four groups and each groups contains six mice. Group I received distilled water (10 ml/kg, p.o) and was kept as control, group II received standard drug Diclofenac (10 mg/kg, p.o) and group III and IV received SALE (250 and 500 mg/kg, p.o) respectively. Subsequently 1 hr after treatment 0.1 ml of 1 % suspension of carrageenan in normal saline was injected into the sub-planter region of the right hind paw to induce inflammatory oedema.

The paw size was measured initially before carrageenan injection and then after carrageenan injection at 1 hr intervals for 5 hrs using Vernier caliper. The percentage of inhibition of paw thickness was calculated.

Formaldehyde Induced Chronic Inflammation

Formaldehyde induced sub chronic inflammatory test was conducted based on the method of Turner, 1965 [12].

Swiss albino mice were divided into four groups and each groups contains six mice. Group I received distilled water (10 ml/kg, p.o) and was kept as control, group II received standard drug Diclofenac (10 mg/kg, p.o) and group III and IV received SALE (250 and 500 mg/kg, p.o) respectively. Subsequently 1 hr after treatment 0.1 ml of 1 % suspension of formaldehyde in normal saline was injected into the sub-planter region of the right hind paw to induce inflammatory oedema. The paw size was measured initially before carrageenan injection and then after carrageenan injection at 24 hr intervals for 5 days using Vernier caliper. The percentage of inhibition of paw thickness was calculated.

Preparation of SRBC and Host Sensitization

Healthy sheep blood was collected from a local butcher house. The material was mixed with sterile Alsever's solution (1:1) and then centrifuged at 3000 rpm for 5 min. The supernatant was discarded and the SRBC pellets washed with sterilized PBS (pH 7.2) three times. The SRBC were re-suspended in PBS and adjusted the desired cell concentration (1 x 10^8 cells).

Determination of the Effect of SALE on Total WBC Count for immunomodulatory activity

Blood was collected from the tail vein and total WBC count was recorded on the 14th day. The total WBC count was carried out based on the method described by Benjamin, 1985 [13]. Briefly, 0.38 ml of diluting fluid was mixed with 0.02 ml of blood and loaded in to the hemocytometer counting chamber and the cells were allowed to settle at the bottom of the chamber for 2 minutes.

Four large squares were counted under a microscope with 10 x objective.

Determination of the Relative Organ Weight for immunomodulatory activity

Body weight was taken at the end of experiment to calculate relative organ weight. Six mice from each group were sacrificed at the end of the experiment (on 14th day) by cervical dislocation and lymphoid organs such as thymus and spleen were excised out. The organs of each mouse were weighed and were expressed as relative organ weight by using the formula:

Relative organ weight = organ weight/ body weight x 100

Determination of Effect of SALE on HA titer

The haemagglutination antibody titer was determined by the method of Puri*et al.,* 1994 [14]. The male Swiss albino mice were divided into five groups of six animals each. Gropu I received distilled water (10 ml/kg, p.o) and was kept as control, groupII was injected with 1x10^8 cell i.p. SRBC and kept as SRBCcontrol, group III received standard drug Septilin (10 ml/kg, p.o) and group IV and V received SALE (250 and 500 mg/kg, p.o) respectively from 0th day to the end of the experiment (14th day). On the day 7, the mice were immunized with 0.1 ml of 1x10^8 SRBC i.p. Blood samples were collected on day 14 from the retro orbital plexus under mild ether anaesthesia of individual animals. Blood was centrifuged to obtain serum, normal saline (0.9%) was used as a diluent and SRBCs count was adjusted to 1%. Each well of the microtiter plate was filled initially with 25 µl of saline and 25 µl of serum was mixed in the first well of the microtiter plate. Subsequently the 25 µl diluted serum was removed from first well added to the next well to get twofold dilutions of the antibodies present in the serum. Further twofold dilutions of this diluted serum were similarly carried out till the last well of the second row (24th well), so that the antibody concentration of any of the dilutions is half of the previous dilution. 25 µl of 1x10^8 SRBC were added to each of these dilutions and the plates were incubated at 37°C for one hour and then observed for haemagglutination. The highest dilution giving haemaggutination was taken as the antibody titer and the mean of different groups were compared for statistical significance. Antibody titre obtained on 14th day after immunization (on 7th day) with SRBCs was considered as humoral immune response.

Determination of Effect of SALE on DTH

The assessment of the SALE on the cellular immune response was measured by delayed type hypersensitivity as described by Doherty, 1981 [15]. The drug treatment was exactly the same as described above for HA titer. On 7th day of the study all the groups I to V were immunized with 0.1 ml of 1x10^8 SRBC i.p. in normal saline. On day 14th all animals from all groups were challenged with 0.05 ml of 1x10^8 SRBCs in the subplantar region of right hind paw and normal saline in left hind paw in same volume. Foot thickness was measured 24 hr after the challenge using Vernier caliper and the difference in the thickness of the right hind paw and left hind paw was used as a measure of DTH reaction and expressed in mm and % inhibition was calculated.

Determination of Effect of SALE on Neutrophil adhesion test

The neutrophil adhesion assay was determined by the method of Wilkinson, 1978[16]. The male Swiss albino mice were divided into four groups of six animals each. Gropu I received distilled water (10 ml/kg, p.o) and was kept as control, groupII received standard drug Septilin (10 ml/kg, p.o) and group III and IV received SALE (250 and 500 mg/kg, p.o) for 14 consecutive days. On the 14th day 1 h after the treatment, blood was collected by retro-orbital puncture into vials pre-treated by disodium EDTA and analyzed for total and differential leukocyte counts. After the initial analysis, the samples were incubated with nylon fibers (80 mg/ml) at 37 °C for 15 min. The samples were then analyzed for total and differential count and the

neutrophil index and neutrophil adhesion percentage were calculated as follows,

Neutrophil adhesion = $(NI_u - NI_t)/(NI_u) \times 100$ where, $NI_u$are Neutrophil Index before incubation with nylon fiber, and NI_t are Neutrophil Index after incubation with nylon fiber.

Determination of Effect of SALE on Phagocytic Index

Phagocytic index was determined by carbon clearance test. The treatment was exactly the same as with the neutrophil adhesion test as described above. On 14th day 3 hours after the last dose of the extracts, all the animals were injected with 0.1 ml of Indian ink via the tail vein. Blood samples were withdrawn from retro-orbital plexus at 0 and 15 min after injection. A 50 µl of blood sample was mixed with 0.1% sodium carbonate (5 ml) and the absorbance were measured at 660 nm. The phagocytic index K was calculated using the equation K= (In OD 1 – In OD 2)/(t2-t1) where, OD 1 and OD 2 are the optical densities at time t1 and t2 respectively.

Statistical analysis

All data were expressed as mean (±SD). The statistical analysis was done using one-way analysis of variance (ANOVA) followed by a Dunnett's test (using GraphPadInStat version 3.00 for Windows 95 (GraphPad Software, San Diego California USA). P-value ≤0.05 was considered significant.

RESULTS

Acute oral toxicity study

In acute toxicity study, SALE treated animals did not show any change in their behavioral pattern. No toxic effect was reported up to 5 and 10 times of effective dose of the extract and there were no deaths in any of these groups. Thus, it was concluded that SALE was safe at 2500 mg/kg. one tenth and one fifth of the maximum tolerated dose of the extract (2500 mg/kg) tested for acute toxicity was selected for the study *i.e*., 250 and 500 mg/kg.

Effect of SALE on Carrageenan Induced Paw Thickness

Effect of SALE during carrageenan induced paw oedema is shown in Figure 1. The paw oedema induced by carrageenan in mice was significantly ($p \leq 0.01$) inhibited by the SALE 250 mg/kg bw (Gr-III) and more efficiently by SALE 500 mg/kg bw (Gr-IV) when compared with carrageenan alone induced control mice. As shown in Table 1, the SALE have reduced the paw oedema by 58.33 ± 0.40% as compared with carrageenan control group and is closer with 75 ± 0.80% reduction produced by Diclofenac.

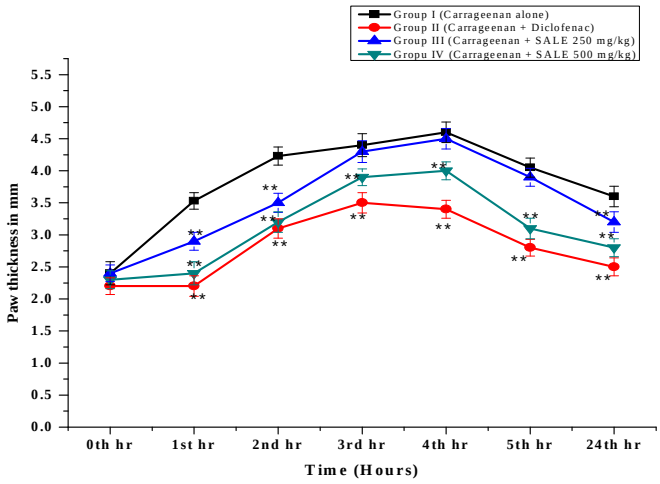


Figure 1. Effect of SALE on carrageenan induced paw oedema.Each value is expressed as mean ± SD of 6 animals. ** $p \leq 0.01$ and * $p \leq 0.05$ when compared to corresponding values of the Carrageenan alone. (Abbreviation: SALE-*Sauropus androgynus*Leaf Extract; mm- milli meter).

Table 1. Effect of SALE on Carrageenan injected mice model on percentage inhibition of inflammation
All values are mean ± SD of 6 animals.

Groups	Difference in paw size (mm) on 24thhr	Percentage (%) inhibition
Carrageenan alone injected	1.2 ± 0.05	-
Carrageenan + Standard drug (Diclofenac)	0.3 ± 0.10 **	75 ± 0.80
Carrageenan + SALE 250 mg/kg	0.8 ± 0.15 **	33.33 ± 0.10
Carrageenan + SALE 500 mg/kg	0.5 ± 0.20 **	58.33 ± 0.40**

** Significant ($p \leq 0.01$) when compared with the Carrageenan alone group.

Effect of SALE on Formaldehyde Induced Paw Thickness

The intraplantar injection of 1% formaldehyde into the mice right hind paw produced an intense oedema. The paw oedema produced by formaldehyde was significantly ($p \leq 0.01$) reduced by the treatments by SALE 250 and 500 mg/kg bw and by diclofenac in Figure 2. The SALE 250 and 500 mg/kg bw reduced the paw oedema by 52.63 ± 0.07% and 63.15 ± 0.04% respectively and 78.94 ± 0.06% reduction was produced by standard drug Diclofenac in Table 2.

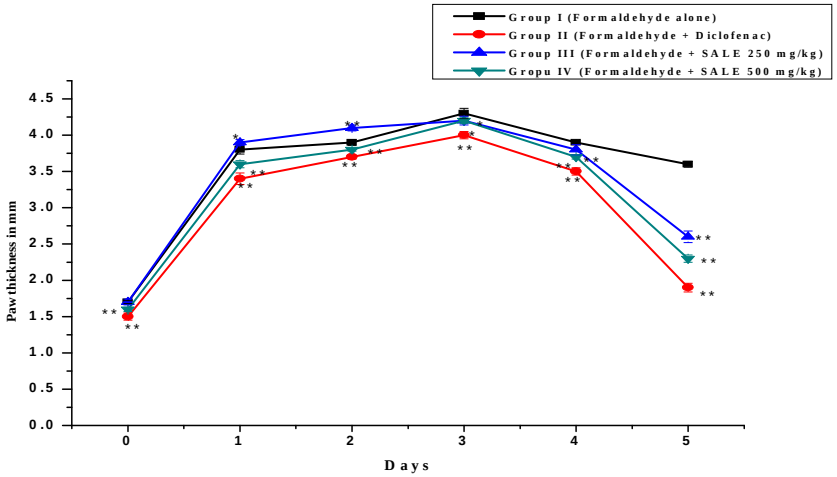


Figure 2. Effect of SALE on Formaldehyde induced paw oedema.Each value is expressed as mean ± SD of 6 animals. ** $p \leq 0.01$ and * $p \leq 0.05$ when compared to corresponding values of the Formaldehyde alone. (Abbreviation: SALE- *Sauropus androgynus*Leaf Extract; mm- milli meter).

Groups	Difference in paw size (mm) on 5th day	Percentage (%) inhibition
Formaldehyde alone injected	1.9 ± 0.05	-
Formaldehyde + Standard drug Diclofenac)	0.4 ± 0.08 **	78.94 ± 0.06
Formaldehyde + SALE 250 mg/kg	0.9 ± 0.03 **	52.63 ± 0.07
Formaldehyde + SALE 500 mg/kg	0.7 ± 0.02 **	63.15 ± 0.04**

Table 2. Effect of SALE on Formaldehyde injected mice model on percentage inhibition of inflammation
All values are mean ± SD of 6 animals.
** Significant ($p<0.01$) when compared with the Formaldehyde alone group.

Effect of SALE on Total WBC Count for immunomodulatory activity

Administration of SALE 250 and 500 mg/kg bw and standard drug Septilin decreased the total WBC count which was raised during SRBC Challenging in mice was found in Figure 3. The WBC counts in SRBC induced mice was found to be 11.84 x 10^3 ± 0.17 cells/mm^3 and with treatment with SALE 250 and 500 mg/kg bw and standard drug Septilin were 10.84 x 10^3 ± 0.17 cells/mm^3, 9.92 x 10^3 ± 0.15 cells/mm^3 and 9.45 x 10^3 ± 0.13 cells/mm^3 respectively at the end of the experiment.

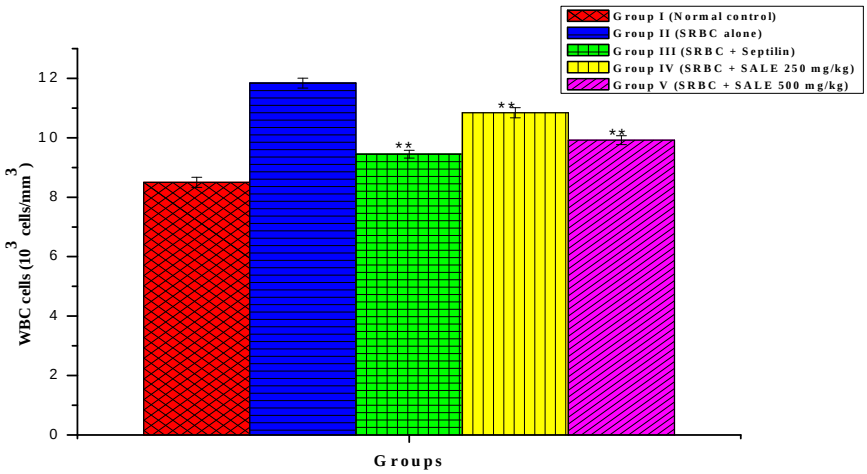
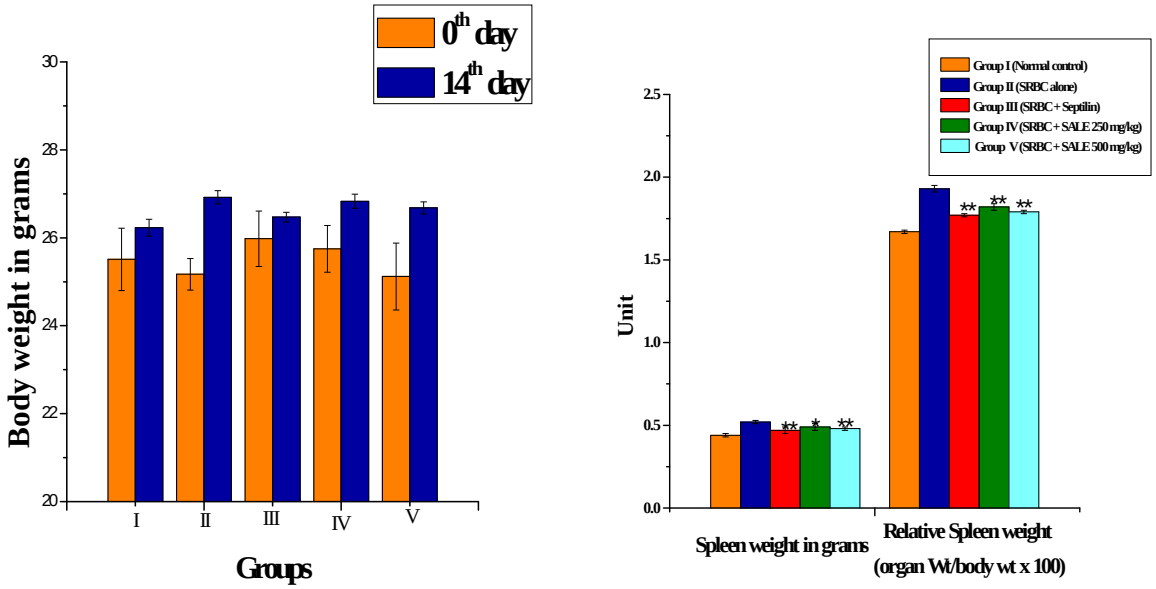


Figure 3. Effect of SALE on Total WBC count.Each value is expressed as mean ± SD of 6 animals. ** p≤0.01 when compared to corresponding values of the SRBC alone. (Abbreviation: SALE- *Sauropus androgynus*Leaf Extract; SRBC – Sheep red blood cells; mm- milli meter).

Effect of SALE on Relative Organ Weight for immunomodulatory activity

The effect of SALE 250 and 500 mg/kg bw and standard drug Septilin on body weight and organ weight was measured on 14th day. The relative weight of spleen was decreased in SALE 250 mg/kg treated mice (0.49±0.02 g/100g BW) and SALE 500 mg/kg treated mice (0.48±0.01 g/100g BW) and Standard drug Septilin treated mice (0.47±0.02 g/100g BW) compared to SRBC induced mice (0.52±0.01 g/100g BW). The relative weight of thymus was also significantly decreased SALE 250 mg/kg treated mice (0.29±0.03 g/100g BW) and SALE 500 mg/kg treated mice (0.27±0.01 g/100g BW) and Standard drug Septilin treated mice (0.26±0.01 g/100g BW) compared to SRBC induced mice (0.34±0.03 g/100g BW) Figure 4



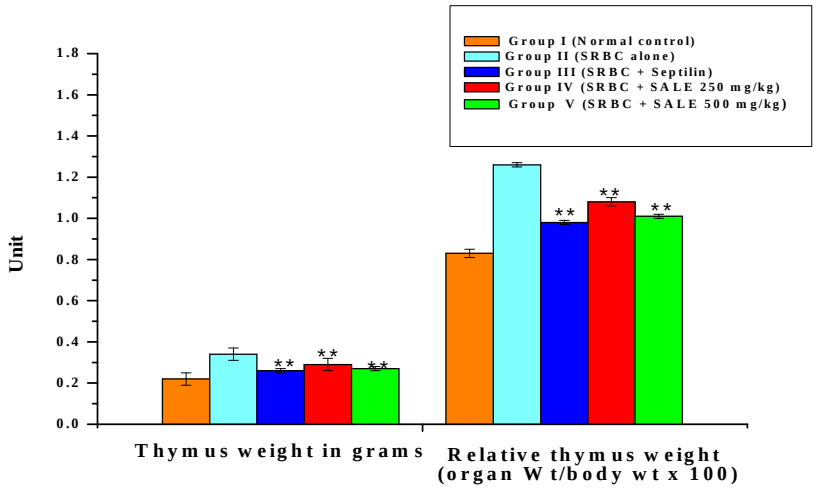


Figure 4. Effect of SALE on Body weight, Relative organ weight spleen, Relative organ weight thymus.Each value is expressed as mean ± SD of 6 animals. ** p≤0.01, * p≤0.05 when compared to corresponding values of the SRBC alone. (Abbreviation: SALE- *Sauropus androgynus*Leaf Extract; SRBC – Sheep red blood cells; mm- milli meter).

Effect of SALE on HA titer assay

The effect of SALE 250 and 500 mg/kg bw and standard drug Septilin on HA titer assay is shown in Figure 5. The antibody response on day 14 in 250 and 500 mg/kg bw and standard drug Septilin treated group with normal immune status showed significant increase (p≤0.01) (7.52 ±0.63)(6.42 ± 0.48) (5.56 ± 0.26)in HA titer when compared with control group (4.66 ± 0.36).

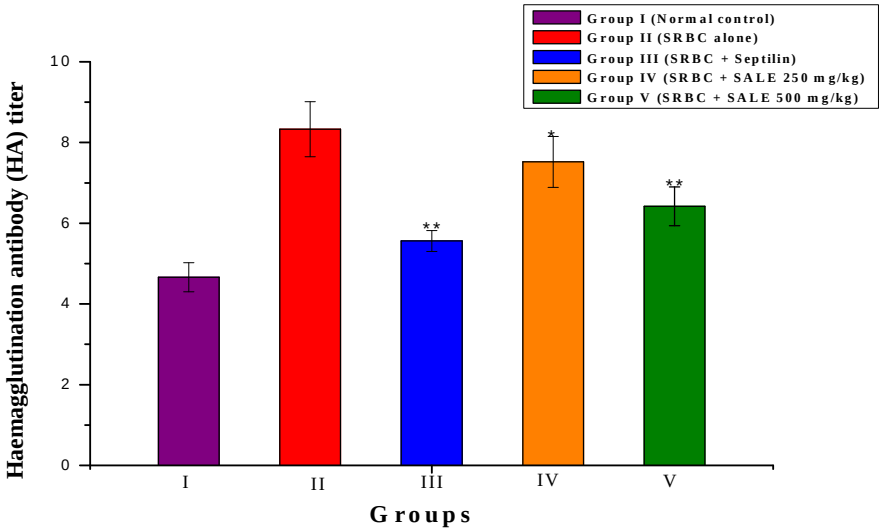


Figure 5. Effect of SALE on HA titer assay.Each value is expressed as mean ± SD of 6 animals. ** p≤0.01, * p≤0.05 when compared to corresponding values of the Normal control. (Abbreviation: SALE- *Sauropus androgynus*Leaf Extract; SRBC – Sheep red blood cells; mm- milli meter).

Effect of SALE on DTH Reaction.

The effect of SALE 250 and 500 mg/kg bw and standard drug Septilin on delayed type hypersensitivity is given in Table 3. The SALE 250 and 500 mg/kg bw and standard drug Septilin were found to inhibit the

delayed type hypersensitivity reaction. The maximum inhibition of DTH was observed in 250 mg/kg (30.35±1.10%) and 500 mg/kg (48.21±1.50%) and standard drug Septilin (58.92±1.20%) treated mice.

Table 3. Effect of SALE treatment on cell mediated response by delayed type hypersensitivity induced footpad oedema

Groups	Difference in paw size (mm)	Percentage (%) inhibition
Normal mice received normal saline	0.34 ± 0.24	-
SRBC alone injected (1 x 10^8 cells IP).	0.56 ± 0.13	-
SRBC injected + standard drug (Septilin)	0.23 ± 0.11**	58.92±1.20%
SRBC injected + SALE 250 mg/kg	0.39 ± 0.10	30.35±1.10%
SRBC injected + SALE 500 mg/kg	0.29 ± 0.12*	48.21±1.50%

All values are mean ± SD of 6 animals.

** Significant ($p \leq 0.01$) when compared with the SRBC alone group.

* Significant ($p \leq 0.05$) when compared with the SRBC alone group.

Effect of SALE on Neutrophil adhesion test

The effect of SALE 250 and 500 mg/kg bw and standard drug Septilin on neutrophil adhesion test is given in Table 4. The percentage neutrophil adhesion was significantly ($p \leq 0.01$) increased by SALE 250 mg/kg (36.08 ± 0.46) and 500 mg/kg (44.87 ± 0.36) and standard drug Septilin (62.24 ± 0.67) when compared with control group.

Table 4. Effect of SALE on Neutrophil activation by Neutrophil adhesion test

Treatment	% Neutrophil adhesion
Normal Control	30.25 ± 0.63
Standard drug Septilin	62.24 ± 0.67 **
SALE 250 mg/kg	36.08 ± 0.46 **
SALE 500 mg/kg	44.87 ± 0.36 **

All values are mean ± SD of 6 animals.

** Significant ($p \leq 0.01$) when compared with the Normal control group.

* Significant ($p \leq 0.05$) when compared with the Normal control group.

Effect of SALE on Phagocytic Index

The effect of SALE 250 and 500 mg/kg bw and standard drug Septilin on the phagocytic activity by the carbon clearance test is shown in Table 5. The phagocytic activity of the reticulo endothelial system (RES) is generally measured by the rate of removal of carbon particles from the blood stream. In carbon clearance test, SALE 250 and 500 mg/kg bw and standard drug Septilin treated animals exhibited significantly ($p \leq 0.01$) increase in phagocytic index compared to the control group. This indicates the stimulation of reticulo endothelial system.

Table 5. Effect of SALE on Phagocytic index by carbon clearance test

Treatment	Phagocytic index
Normal Control	0.035 ± 0.002
Standard drug Septilin	0.024 ± 0.002 **
SALE 250 mg/kg	0.028 ± 0.006 *
SALE 500 mg/kg	0.025 ± 0.001 **

All values are mean ± SD of 6 animals.

** Significant ($p \leq 0.01$) when compared with the Normal control group.

* Significant ($p \leq 0.05$) when compared with the Normal control group.

DISCUSSION

Acute inflammation is a vital response to infection that is initiated within seconds of pathogen detection [17]. 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 [11]. The first phase initially involves the release of serotonin and histamine while the second phase of the next 1h is mediated by prostaglandin [18]. In formaldehyde test also there are two inflammation episode progressed, the first neurogenic phase there is a direct chemical stimulus occurring in the nociceptive afferent fibres, chiefly C fibres, and the release of substance P [19]. During the inflammatory second phase, there would be release of inflammatory mediators such as prostaglandins, histamine, bradykinin, and serotonin at the inflamed affected area [20]. In chronic inflammatory model, the inhibition of formalin induced paw oedema in animal is considered as one of the most suitable test to screen anti-inflammatory agents.

Elevated level of TNF-α, IL-1, and IL-6 are mostly destructive and are also concerned with some of the pathologic responses that occur in chronic inflammatory diseases [21].

Most of the studies concerning the evaluation of immunomodulatory activities of the plants have been undertaken utilizing the crude extracts [22, 23]. Phytochemical investigation of the leaves of *S. androgynus* reveals that they contained sterols, resins, tannins, saponins, alkaloids, flavonoids, terpenoids, glycosides, phenols, catechol, cardiac glycosides, and acidic compounds [24]. *S. androgynus* leaves were found to have highest content of flavonoids and bioactive compounds among 11 vegetables from Indonesia with 142.64 mg per 100 gram of fresh weight with quercetin, myricetin, luteolin, apigenin and kaempferol which were detected by HPLC analysis [25, 26].

Immunomodulators are biologic response modifying compounds that affect the immune response in either a positive or negative fashion. If it results in an enhancement of immune reaction is named as, “immunostimulation” and primarily implies stimulation of non-specific system i.e. stimulation of the function and efficiency of granulocytes, macrophages, complement, certain T-lymphocytes are different effector substances. Immunosuppression implies mainly to reduce resistance against infection, stress and may be because of environmental or chemotherapeutic factors. Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence immunostimulating agents and immunosuppressing agents have their own standings and search for better agents exerting activity is becoming the field of major interest all over [27].

Those with weakened immunity are more susceptible to infections and diseases. The need to restore or maintain a healthy defense mechanism had led researchers to many sources of substances that are capable of interacting with specific targets of the host response mechanisms to up regulate or down regulate [28]. In this study we found that methanolic extract of SALE possesses immunomodulatory activity in experimental models of cellular and humoral immunity. The extract was found to be most effective at high dose (500mg/kg, p.o), whereas low dose (250 mg/kg, p.o) of SALE was moderately effective in modulating immune system. The variety of plant products can modulate immune reaction either by stimulation or suppression and may assist as a supportive therapy along with conventional drugs in immune compromised patients [29].

Administration of SALE 250 and 500 mg/kg bw and standard drug Septilin decreased the total WBC count which was raised during SRBC Challenging in mice was found.The organ weight

is an important indicator of the physiological and pathological state in humans and other animals. The relative organ weight is fundamental to diagnose whether the organ was exposed to an injury or not [30]. The spleen and thymus are an important organ of the immune system and so the level of immune competence of an organism is closely correlated with the developmental and functional integrity of those organs [7]. The increase in spleen and thymus weight observed in our experiments may be partly due to the stimulatory effect of the SALE on the immune organ.

B lymphocytes and antibodies secreting plasma cells are the key elements involved in humoral immune responses [31]. The enhanced antibody titer indicates the improved humoral response mediated by T and B lymphocytes involved in the antibody synthesis [32]. The hemagglutinating antibody titer was determined to evaluate the effect of SALE on humoral immune response. The results obtained with the current investigation revealed that SALE significantly stimulated the humoral immunity by enhancing the antibody produced against the SRBC, which remain sustained even after treatment. An increase in the number of antibody producing cells against particular antigen indirectly signifies the stimulatory effect on the humoral immunity [33]. The present study revealed that humoral immunity plays a vital role in the immunostimulation achieved by SALE.

The delayed type hypersensitivity reaction was measured as an indicator of T-cell mediated immunity [34]. DTH requires the specific recognition of a given antigen by activated T-lymphocytes which subsequently proliferate and release cytokines. The significant difference in the DTH response observed in our experimental animals indicates that the SALE has a stimulatory effect on lymphocytes and accessory cells required for the expression of the reaction and thus increases the cell mediated immunity.

Cell adherence property of neutrophils is one of the earliest responses of both immunological and physical injury [35]. In the neutrophil test both doses of SALE and standard drug Septilin showed significant effect on neutrophil adhesion. The carbon clearance test was used valuate the effect on reticulo endothelial cell mediated phagocytosis [36, 37]. When ink containing colloidal carbon is injected intravenously, the macrophages engulf the carbon particles of the ink. The rate of clearance of ink carbon particles from the blood is known as the phagocytic index. The SALE produced an increase in the phagocytic index, suggesting its effect on reticuloendothelial system.

CONCLUSION

Based on the results, we conclude that SALE has a potent immunomodulatory potential but also could significantly impact upon inflammation reactions. Further studies to identify the active moieties and elucidation of the mechanism of action are recommended.

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