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In Vivo Evaluation of Antiarthritic Activity of Sesbania Procumbens Extracts

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KEYWORD

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ABSTRACT:

Systematic evaluation is a crucial one for the herbals used in the traditional healing system that may give more promising data about their medicinal value and may be useful to meet the rising demand for novel agents to combat the infections and diseases. Plants of the genus *Sesbania* have ethno medicinal importance. The plant *S. procumbens* was used traditionally for a variety of medicinal purposes including anti-inflammatory activity. In our previous study, *S. procumbens* was collected, extracted and the anti-inflammatory activity of the extracts were evaluated by *in vitro* successfully. The present study was aimed to evaluate the anti-arthritic activity of the methanol extract of *S. procumbens* by *in vivo* on Wistar albino rats with Complete Freund's adjuvant (CFA) induced arthritis. The institutional animal ethical committee approved the entire study. Initially, LD_{50} of the selected extract was determined and from this the ED_{50} was fixed as 200mg.kg^{-1} for the selected methanol extract of *S. procumbens*. Two doses of extracts (200mg.kg^{-1} & 400mg.kg^{-1}) were subjected to *in vivo* anti-arthritic evaluation in comparison with the standard drug prednisolone (10mg.kg^{-1}). Collectively, results of *in vivo* evaluation strongly supported the anti-arthritic activity of methanol extract of *S. procumbens* particularly, the high dose (400mg.kg^{-1}) of the extract revealed a significant activity comparing with the standard control drug (prednisolone). Our future studies in the direction of toxicity evaluation, compound level studies from this extracts may give significant results valuable for further researches.

INTRODUCTION

Globally, herbal remedies have been widely used to alleviate diverse ailments and disorders since ancient times. Herbal oriented treatment strategies in different traditional healing practices have always inspired and guided the researchers to search for novel therapeutics [1, 2]. Obviously several medicines, notably, antibiotics such as penicillin, antimalarials such as quinine, artemisinin, hypolipidemic such as lovastatin, immunosuppressants such as cyclosporine, anti-cancer agents such as paclitaxel etc., revolutionized the treatment [3]. Certain recent reports predicted that approximately two-thirds of the medicines approved globally are plant originated [4].

The organic molecules otherwise known as secondary metabolites present in the plants are responsible for their definite physiological activity. They include alkaloids, glycosides, flavonoids, essential oils, saponins, resins, phenols etc., which are chemically and taxonomically distinct compounds and present in the different parts of the plants viz., root, stem, flower, fruit, seed and exudates. They can be used to treat different types of chronic as well as infectious diseases [5, 6].

A systematic evaluation is needed for the herbals used in the traditional healing system that may give more promising data about their medicinal value and may be useful to meet the rising demand for novel agents to combat the infections and diseases [7-9]. With this view, *Sesbania procumbens*, a plant with ethnomedicinal importance was selected for our study.

Genus *Sesbania* is a species rich one mainly distributed in tropical and subtropical regions. *Sesbania procumbens* is one among them commonly found in India particularly in Andhra Pradesh, Odisha and Tamil Nadu states. In Tamil Nadu, this plant is commonly found in Kanchipuram, Ramanathapuram and Tirunelveli Districts. It is commonly found in wet places like paddy fields. The

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plant *S. procumbens* was used traditionally by the rural population for a variety of medicinal purposes including anti-inflammatory activity. In our previous study, *S. procumbens* was collected, extracted and the anti-inflammatory activity of the extracts were evaluated by *in vitro* successfully [10]. Now the present study focused on the *in vivo* evaluation of anti-inflammatory activity of *S. procumbens* extracts, an attempt to provide a direction for further studies.

MATERIALS AND METHODS

Plant extract, animal and approval of the study

In our previous study, the whole plant material of *S. procumbens* was collected, authenticated, aerial parts were separated, shade dried for about two weeks and made into a coarse powder by using mechanical grinder. Extraction of powdered material was done by soxhlation using the different solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate and methanol. Dried extracts thus obtained were subjected to *in vitro* anti-inflammatory evaluation by human red blood cell (HRBC) membrane stabilization in normal and different temperature (54°C and −10°C) and tonicity (hypotonic and isotonic) conditions. Based on the results obtained from the *in vitro* evaluations [10] the methanol extract of *S. procumbens* was selected for the *in vivo* evaluation in reference with standard procedure [11, 12] with slight modification. In this study, healthy male Wistar albino rats having 6-8weeks age weighing 160-180gm obtained from the Central animal house, Cape Bio Lab & Research Centre, Marthandam, Kanyakumari, Tamil Nadu, India, was used for the experiments.The study was conducted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The institutional animal ethical committee approved the study (CBLRC/IAEC/02/02-2020).

Acute toxicity study

The LD_{50} of the selected extract was determined as per the guidelines of OECD 423 and in reference with the standard procedure [13, 14]. Required animals were randomly selected, marked facilitating identification, and kept in the standard environmental conditions like ambient temperature (25±1°C), relative humidity (55±5%) and 12h light/dark cycle for five days prior to dosing for adaptation with laboratory conditions. The animals were fed with standard pellet diet and water *ad libitum*. Following the overnight fasting with free access to water, the test extracts were administered in a single dose by gavage using stomach tube. 50mg.kg^{-1} was selected as initial dose and administered to three animals and after 24h, the animals were observed for the number of death. The same procedure was repeated with next doses 300mg.kg^{-1} and 2000mg.kg^{-1}.

Treatment protocol

The selected Wistar albino rats were divided into four groups of six each. Group 1 served as the normal control received diet and water only; Group 2 served as arthritic control received Complete Freund's adjuvant (CFA) only for the induction of arthritis. Group 3 was taken as standard control received prednisolone (10mg.kg^{-1}). Group 4 was experiment group received 200mg.kg^{-1} of selected extract of *S. procumbens.* Group 5 was treated with 400mg.kg^{-1} of the same extract.

Treatment and evaluation

Arthritis was induced in all the selected animals except group 1 by a single intra-dermal injection (0.1 ml) of CFA containing 1.0mg dry heat-killed *M. tuberculosis*/ml sterile paraffin oil into a foot pad of the left hind paw of male rats. A glass syringe (1 ml) with the locking hubs and a 26G needle was used for injection. The rats were anesthetized with ether inhalation prior to and during adjuvant injection. The standard drug and the test extracts were administered orally from the 3rd day of CFA administration and given daily for 21 days. The swelling paws were examined periodically (up to 21 days) in each paw from the ankle using Digital Plethysmometer. Results were expressed as change in paw volume (in ml) by using following formula

$$\text{Increased Volume of edema} = \text{Final paw volume} - \text{Initial paw volume}$$

At the end, blood samples were collected by sublingual route, serum and plasma were separated by centrifugation at 2500rpm for 10min. and stored at –20°C for the investigation of different hematological and biochemical parameters. Different biochemical parameters like Alkaline phosphatase(ALP) marker for bone destruction, Acid Phosphatase (ACP) the lysosomal enzyme activity, Serum glutamate oxalo acetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) were estimated by using ALP, ACP, SGOT and SGPT kit in Auto analyser. For the estimation of Total WBC count blood samples were added with WBC diluting fluid and by the help of Neubauer's chamber total numbers of WBC was calculated by using formula

$$\text{Total WBC count} = \text{Total no. of cells x Volume correction factor x 20}$$

RESULTS AND DISCUSSION

In the present study, methanol extract of *S. procumbens* was selected for the *in vivo* anti-arthritic evaluation. Initially, the acute toxicity evaluation of the extract was done in which the animal death was not observed after the administration of first two doses of the extract. But, two out of three animals died within the 24h of administration of third dose 2000mg.kg^{-1}. So, the third dose was concluded as LD_{50} and from this the ED_{50} was fixed as 200mg.kg^{-1} for the selected methanol extract of *S. procumbens*. Collectively, results of *in vivo* evaluation

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strongly supported the anti-arthritic activity of methanol extract of *S. procumbens* particularly, the high dose (400mg.kg^{-1}) of the extract revealed a significant activity comparing with the standard control drug (prednisolone). Results of *in vivo* anti-arthritic evaluation is shown in Table 1-3.

Table 1. Effect of methanol extract of *S. procumbens* on change in the paw volume of the experimental animals

Days	Normal control	Arthritic control	Standard control (prednisolone)	Test extract (200mg/kg)	Test extract (400mg/kg)
00	0.20±0.03	0.61± 0.04	0.58 ± 0.05	0.60±0.02	0.57 ± 0.03
03	0.22±0.05	0.77 ± 0.01	0.66 ± 0.06	0.69±0.04	0.67 ± 0.05
05	0.21±0.02	0.85 ± 0.03	0.65 ± 0.08	0.73±0.01	0.61 ± 0.04
07	0.23±0.04	0.96 ± 0.05	0.61 ± 0.04	0.69±0.03	0.62 ± 0.06**
09	0.25±0.02	1.05 ± 0.06	0.60 ± 0.03**	0.65±0.05**	0.60 ± 0.04**
11	0.21±0.01	1.08 ± 0.05	0.55 ± 0.06**	0.61±0.01**	0.58 ± 0.03**
13	0.20±0.03	1.11 ± 0.02	0.42 ± 0.04**	0.57±0.04**	0.45 ± 0.02**
15	0.23±0.01	1.12 ± 0.07	0.39 ± 0.05**	0.52±0.03**	0.41 ± 0.06**
17	0.21±0.03	1.15 ± 0.06	0.35 ± 0.07**	0.48±0.01**	0.38 ± 0.05**
19	0.22±0.02	1.18 ± 0.08	0.25 ± 0.08**	0.39±0.05**	0.32 ± 0.06**
21	0.20±0.03	1.20 ± 0.03	0.21 ± 0.03**	0.30±0.02**	0.24 ± 0.05**

All values are expressed as mean ± SEM for 6 determinants; $^{**}P<0.01$, compared to arthritic control

The change in the paw volume of the experimental animals is shown in Table 1. The results clearly indicated that the paw volume was gradually increased, particularly in the first week of observation, animals of all the group except the group 1 (normal control) showed an elevation in the paw volume. In the subsequent days of observation, the Group 3 animals received the standard drug prednisolone showed a significant decline in the paw volume. Similarly, the methanol extract of *S. procumbens* showed a reduction in the paw volume of the group 4 and 5 animals, particularly, in the dose of 400mg.kg^{-1}, the test extract showed (group 5 animals) a significant suppression in the paw volume.

The Table 2 represents the changes in the level of biochemical elements such as SGOT, SGPT, ALP and ACP of the experimental animals. From the results, it was found that the level of all these enzymes were raised after CFA injection. The administration of standard drug reversed the changes in the level. Similarly, the test extract importantly, the high dose (400mg.kg^{-1}) of the extract also revealed a significant change in the level of analyzed parameters.

Table 2. Effect of methanol extract of *S. procumbens* on biochemical parameters of experimental animals

Groups	**SGOT** (U/L)	**SGPT** (U/L)	**ALP** (U/L)	**ACP** (U/L)
Normal control	36.41±0.94	36.80±0.35	72.61±1.80	5.81±0.04
Arthritic control	109.3±2.05*	165.21±0.51*	440.3±2.36*	24.52±1.25*
Standard control	48.52±1.32**	43.61±2.36**	153.1±1.09**	7.25±0.54**
Test extract (Low dose)	65.23±2.01**	67.41±1.32**	220.2±0.59**	15.35±0.41**
Test extract (High dose)	50.61±0.49**	55.72±0.82**	179.5±0.23**	8.35±0.35**

All values are expressed as mean ± SEM for 6 determinants; $^{*}P<0.05$, compared to normal and $^{**}P<0.01$, compared to arthritic control.

The leucocyte count analysis of test animals is presented in Table 3. In this aspect also, the high dose of test extract showed a significant result comparing with the results of standard drug.

Majority of the researchers described that the inhibition of CFA induced arthritis in animal model is an appropriate method to evaluate the anti-arthritic activity since it has close similarity with human arthritis. CFA induced arthritis formed through the cell mediated auto-immunity structural mimicry between mycobacteria and cartilage proteoglycan in rats. The CFA inoculation stimulates the macrophages and lymphocytes or their products like monokines, cytokines, and chemokines may be involved in abnormal lipid and protein metabolism. The CFA administered rats showed soft tissue swelling around the ankle joints during the development of arthritis which was considered as edema of the particular tissues. As the disease progressed, a more diffused demineralization developed in the extremities. Reduction in the paw volume of experimental animals by the test extract may be due to the immunological protection rendered by the test extract [12].

Table 3. Effect of methanol extract of *S. procumbens* on hematological parameter of experimental animals

Groups	**WBC** (Cells/ml×10^{3})
Normal control	10.22±1.41
Arthritic control	14.50±2.25^{a**}
Standard control	11.25±1.70^{b**}

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Test extract (Low dose)	12.55±1.65[b**]
Test extract (High dose)	12.15±1.50[b**]

All values are expressed as mean ± SEM for 6 determinants; [a]** – Values are significantly different from normal control at $P < 0.01$; [b]** – Values are significantly different from arthritic control at $P < 0.01$

CONCLUSION

In the present study, the methanol extract of *S. procumbens* was subjected to *in vivo* evaluation of anti-arthritic activity in Wistar albino rats with CFA induced arthritis. Two doses of extracts (200mg.kg^{-1} & 400mg.kg^{-1}) were evaluated in comparison with the standard drug prednisolone. From the results, it was found that the tested extract in the dose of 400mg.kg^{-1} showed a significant anti-arthritic activity comparing with the standard drug. Our future studies in the direction of toxicity evaluation, compound level studies from this extracts may give significant results valuable for further researches.

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