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# EFFECT OF *SESBANIA GRANDIFLORA* AND *SESBANIA SESBAN* BARK ON CARRAGEENAN INDUCED ACUTE INFLAMMATION AND ADJUVANT-INDUCED ARTHRITIS IN RATS

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## ABSTRACT

Nitric Oxide (NO) can autoregulate its own formation by feedback inhibition of the inducible Nitric Oxide Synthase (iNOS). Modulation of biosynthesis or activity of NO results in amelioration if pathogenesis of experimental arthritis. However, little is known about feedback mechanism on NO generation in response to carrageenan induced paw oedema and adjuvant- induced arthritis. In the present study we have examined the effects of prophylactic administration of extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* on the development of carrageenan induced paw oedema and adjuvant - induced arthritis to assess influence of high NO level in the form of exogenous herbal extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* in the progress of inflammation. Inflammation was assessed by measuring paw swelling. Increased paw oedema of the injected paw measured on 1$^{st}$ to 12$^{th}$ hrs which is feature of carrageenan induced inflammation was significantly reduced after prophylactic administration of petroleum ether, chloroform and methanol extracts of bark of *Sesbania grandiflora* (300mg/kg b.w. p.o.) and *Sesbania sesban* (300mg/kg b.w. p.o.) and arthritis was assessed by measuring primary and secondary paw swelling and changes in thymus, spleen and body weight. Increased swelling of the non injected paw (secondary paw) measured on days 14 and 21, injected paw swelling (primary paw) measured on days 3, 14 and 21, spleenomegaly, thymic involutions and loss in body wt. which are features of adjuvant- induced arthritis were effectively reduced after prophylactic administration of extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* . These data suggests that high NO level in the from extracts of *Sesbania grandiflora* and *Sesbania sesban* may suppress initial stages of immune response to carrageenan and adjuvant injection probably by inhibiting iNOS expression through feedback inhibition mechanism. However, further studies are required to unravel the mechanism involved in these effects of extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* and their clinical implications.

**Key Words:** *Sesbania grandiflora*, *Sesbania sesban,* Inflammation, Arthritis.

## INTRODUCTION

Inflammation is a common clinical conditions and rheumatoid arthritis (RA) is a chronic debiliating autoimmune disorder [1-2], that affects about 1% of the population in developed countries [3] etc.

Nitric Oxide (NO) is a gaseous short lived free radical has been implicated as a mediator of inflammation and modulation of biosynthesis or activity of NO results in amelioration of acute inflammation and experimental arthritis model [4,5]. NO is generated via the oxidation of the terminal guanidino nitrogen atom of L-arginine by the enzyme Nitric Oxide Synthase (NOS). Three major isoforms of Nitric Oxide Synthase (NOS) have been identified. Two expressed constitutively, are calcium/calmodulin-dependent and are classified together as constitutive NOS isoforms (cNOS). The third is a cytokine - inducible, calcium/calmodulin - independent isoform of NOS (iNOS) is regulated in the gene by a variety of inflammatory mediators [6]. Increased NOS activity or NO release have been demonstrated in both acute [7] and chronic [8] models of inflammation and treatment with N-amino ethyl-L-lysine (L-NIL) - specific inhibitor of iNOS effectively reduced these inflammatory changes [9]. Further, administration of L-arginine a precursor for NO synthesis increased the paw swelling in adjuvant arthritis [6].

Several studies have reported acute on prolonged exposure to NO donors inhibited of NOS expression and activity in murine macrophage cell lines J 774 [10], and in rat neutrophils [11], macrophage [12]. These observation serve that NO inhibits NOS activity through a negative feedback mechanism.

The drugs commonly in use for the treatment of inflammation and RA include glucocorticoids eg. cortisone and prednisone etc., NSAIDS e.g. Ibuprofen and naproxen etc., disease-modifying anti-inflammatory and anti-rheumatic drugs e.g. Methotrexate (MTX) and leflunomide etc., biological response modifiers e.g. Tumor necrosis factor, alpha blocking agents. However, besides their high cost, severe adverse reactions and toxicity, including some risk of infections in subsets of patients being treated with biological response modifiers [13]. As a result, alternative treatments based on natural plant products and herbal mixtures belonging to the realm of polyherbal formulations are becoming increasingly popular in the India, US and other countries [14].

*Sesbania grandiflora* consist of dried bark of the plant *Sesbina grandiflora* (L.) Pers (Legumino- sae) commonly called as Agati (SANS) and Hadga (MAR, found and cultivated in many Asian countries e.g. India, Malaysia and Indonesia etc. [15, 16]. All parts of *Sesbania grandiflora* are utilized for medicine in diuretic, emetic, fevers, headaches, smallpox,anemia, bronchitis,inflammation, leprosy, gout, rheumatism, anxiolytic, anticonvulsive, hepatoprotective and potent antidote for tobacco and smoking-related diseases. In a number of cultures the root is used in inflammation, rheumatic swelling and fever, bark is used in smallpox, other eruptive fevers and ulcers, the juice of the leaves is used to treat worms, fever, gout, and leprosy, the flowers are used as emollient, bronchitis, gout and pain, fruits are used for anemia, bronchitis, fever, tumors, pain and thirst [17].

Several reports suggested that the ethanolic extract of the bark of *S. grandiflora* prevented acute gastric injury in rats, the leaf juice of *S. grandiflora* showed significant antiurolithiatic activity [18] .In vivo studies, SF2 (*Sesbania* Fraction 2)administration showed potential anticancer [19], anxiolytic [20], hepatoprotective in rats [21], anti-microbial [22] and analgesic and antipyretic activity was evaluated [23].

*Sesbania sesban* consist of dried bark of the plant *Sesbania sesban (L.) Pers. (Leguminosae)* is found throughout the plains of India and commonly called as Jayanti (SANS) and Shevri (MAR) [24]. Several reports suggested that bark of *Sesbania sesban* is used in diarrhea, spleen enlargement and inflammation. Seeds used in spleen enlargement. Flowers exhibit antifertility activity [25, 26].

However, there is scepticism about polyherbal extracts in the minds of both the public as well as the scientific community, mostly because the mechanisms of action of many of these products are poorly defined, or not at all. Thus, little is known about feedback mechanism on NO generation in response to carrageenan- induced paw oedema and adjuvant-induced arthritis. In the present study, we have examined the effects of prophylactic administration of Petroleum, Chloroform and Methanol extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* on the development of carrageenan induced paw oedema and adjuvant -induced arthritis to assess influence of high NO level in the form of exogenous Petroleum, Chloroform and Methanol extracts of

bark of *Sesbania grandiflora* and *Sesbania sesban* in the progress of inflammation and rheumatic diseases.

## MATERIALS AND METHODS

### Plant Material

In the present study mature plant material were collected in Dec. 2005 and authenticated from Dr. D. A. Patil, Head, Department of Botany, Dhule by studying morphological features (leaf arrangement, bark, flower/ inflorescence arrangement, fruit and seed morphology). The herbarium of the plants specimen has been deposited at K.L.E.S. College of Pharmacy, Belgaum. After authentication plants were dried at room temperature until they become free from moisture. The plant material were powdered and passed through sieve #40, and subjected to standardization with the different parameters

### Preparation of the Extracts

The powdered (# 40 size mesh) crude drug of barks (400gm) of *Sesbania grandiflora* and *Sesbania sesban* were subjected for extraction process by petroleum ether (60-80 ℃), chloroform and methanol at room temperature for 8 days. The extracts were filtered and concentrated to dryness on water bath to avoid the decomposition of natural metabolites. The yield of Petroleum ether, Chloroform and Methanol extracts of bark of *Sesbania grandiflora* 0.35 % w/w, 0.20% w/w, 0.70 % w/w respectively and *Sesbania sesban* 0.37 % w/w, 0.12 % w/w and 2.07 % w/w respectively.

### Chemicals and Drugs

The chemicals used were: Carrageenan Sodium (Sigma Chemical Company, USA), Freund's Complete Adjuvant Injection (Difco Lab. U.S.A.), Petroleum ether 60-80 (Qualligen), Chloroform **(**Qualligen), Methanol (Qualligen), (Eros Pharma. Ltd.), Saline (Wokhardt Laboratories Ltd. Bombay- 400124), Twean 80, Gum Acacia (SBD Chemicals) etc. were purchased. All other chemicals were of analytical grade.

### Experimental Animals

Adults female Albino Wistar rats 120-150 gms ( 8 to 10 weeks old) were kept for one week to acclimate to laboratorical conditions before starting the experiment in standard Macrolon boxes (groups of 4-6 rats/cage) at 20-25$^0$C and maintained on

standard pellet and they were kept in 12/12 h light dark cycle. The animals were starved 24 hrs. prior to the experiment with free access of water for carrageenan induced inflammation and adjuvant induced arthritis study.

The overnight fasted rats were used for the Carrageenan induced inflammation and Freund's adjuvant- induced arthritis study. The studies were conducted in accordance to the international and local Indian guidelines for the animal care.

**Acute Toxicity Studies** [27]

Swiss Albino mice of either sex (20-24 gm weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD and no adverse effects or mortality were detected in the mice starting dose was 300 mg/kg b.w.p.o. to 4000 mg/kg b.w.p.o. during the 24 hr. observation period. Based on the results obtained from this study, the dose for anti-inflammatory activity and anti-arthritic activity of extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* were fixed to be 300mg/kg b.w. p.o. for the study.

**ANTI-INFLAMMATORY ACTIVITY**

The animals were divided into six groups (n = 6). Group I served as Control, received the carrageenan injection. Group II served as Naïve, received the vehicle only (1% Carboxymethylcellulose, CMC, 10ml/kg, b.w., p.o.).Group III served as Standard, received Ibuprofen at dose of 7.2 mg/200mg b.w. p.o. Group IV, Group V and Group VI served as Test, received Petroleum ether, Chloroform and Methanol extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* at dose of 300mg/kg b.w. p.o. of each group respectively.

**Carrageenan induced rat paw oedema** [28]

The animals pretreated with extracts or Ibuprofen or vehicle (1% CMC) one hour before were injected with 0.1 ml of 1% suspension of Carrageenan subcutaneously under sub plantar region in right hind paw. Paw volumes were measured by dislocation of the water column in a Plethysmometer (Ugo Basile, Italy) immediately after Carrageenan application at 0 hr. to 12 hr. Reduction in the paw volume compared to the control animals were considered as anti-inflammatory response.

**ANTI-ARTHRITIC ACTIVITY**

**Freund's adjuvant-induced arthritis** [29]

Rats were injected, 0.1ml of FCA into sub plantar region of the right hind paw. Paw volumes were measured by dislocation of the water column in a Plethysmometer (Ugo Basile, Italy) and body weights were measured on Electronic Balance (Denver Instrument TR-203) on day 0(before administration of FCA) and per day and every days during treatment period ending on day 21. All the animals received either extracts or Ibuprofen or vehicle (1% CMC) orally twice daily, at 8.20 AM and 4.20 PM in 1ml of 0.5% saline vehicle were administered beginning either on the day of immunization with adjuvant (prophylactic regimen) and depending upon their respective grouping for 21 consecutive days from the day of FCA injection. On 21$^{st}$ day rats were anaesthetized using diethyl ether and animals were sacrificed and spleen and thymus were dissected out. Their respective weights were recorded on Electronic Balance (Denver Instrument TR-203).

Arthritis was assessed by measuring primary and secondary paw swelling and changes in thymus, spleen and body weight. Increased swelling of the non injected paw (secondary paw) measured on days 14 and 21, injected paw swelling (primary paw) measured on days 3, 14 and 21, spleenomegaly, thymic involutions and loss in body wt. which are features of adjuvant- induced arthritis were effectively reduced compared to the control animals after prophylactic administration of extracts of bark of *Sesbania grandiflora* (300mg/kg b.w. p.o.) and *Sesbania sesban* (300mg/kg b.w. p.o.) were considered as anti-arthritis response.

**STATISTICAL ANALYSIS** [30]

Results are expressed as mean ± S.E. mean for (n) rats. The results were analyzed by Student's'‘t’ test to determine the significant differences between means. A P-value of <0.001 were considered as to be statistically significant.

**RESULT AND DISCUSSION**

Inflammation is a common clinical conditions and rheumatoid arthritis (RA) is a chronic debiliating autoimmune disorder that affects about 1% of the population in developed countries etc. Extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* has shown the protective effects against the acute and chronic inflammation.

**Clinical characteristics of the Carrageenan induced paw oedema model**

Swelling with erythema was evident within 1hr in the injected hind paw, which peaked on 3 hr and began to decline through 4 hr to 12 hr. (Table1).

**Effect of various extracts of Bark of *Sesbania grandiflora* and *Sesbania sesban* on Carrageenan induced acute inflammation.**

Pretreatment with petroleum ether extract (300 mg/kg b.w. p.o.), chloroform extract (300 mg/kg b.w. p.o.) and methanol extract (300 mg/kg b.w. p.o.) of bark of *Sesbania grandiflora* significantly decreased the mean percentage increase in carrageenan induced paw edema at $2^{nd}$ hr to $12^{th}$ hr, $5^{th}$ hr to $12^{th}$ hr and $10^{th}$ hr to $12^{th}$ hr (Table 1) ($P<0.001$) respectively.

Pretreatment with petroleum ether extract (300 mg/kg b.w. p.o.), chloroform extract ( 300 mg/kg b.w. p.o.) and methanol extract ( 300 mg/kg b.w. p.o.) of bark of *Sesbania sesban* significantly decreased the mean percentage increase in carrageenan induced paw edema at $3^{rd}$ hr to $12^{th}$ hr , $5^{th}$ hr to $12^{th}$ hr ($P<0.001$) and $8^{th}$ hr to $12^{th}$ hr respectively. Ibuprofen, COX-inhibitor at the dose of 7.2 mg/200gm b.w. p.o., also significantly reduced the paw oedema (Table 1) ($P<0.001$).

**Clinical characteristics of the Adjuvant induced arthritis model**

Swelling with erythema was evident within 1 day in the injected hind paw, which peaked on day 3 and began to decline through day 8. Little change in volume was noticed through day 8 in the contralateral, noninjected paw. Rats exhibited a gradual increase in spleen weight compared with naïve rat control whereas thymus weights initially dropped, they partially recovered. Animals lost weight during the first 3 days after immunization, after which weight gain returned to normal upto 8 days.

A secondary chronic phase the inflammatory response began to occur after day 8 after adjuvant injection. During this time, animals exhibited renewed swelling in the phalangeal and tarsal joints of the non-injected paw and swelling in other joint areas such as along the vertebrae of the tail. Also during this time, spleen weight increased more dramatically, thymus weight loss was renewed and total body weight again began to decline. All these changes occuring during the secondary phase of the adjuvant response apperared to reach maximum and level off between days 15 and 22.

**Effect of Prophylactic administration of petroleum ether extracts of Bark of *Sesbania grandiflora* and *Sesbania sesban* on adjuvant induced arthritis.**

Oral administration of petroleum ether extracts of bark of *Sesbania grandiflora* (300mg/kg p.o. b.w.) and *Sesbania sesban* (300mg/kg p.o. b.w.) twice each day during the 21 days of adjuvant induced arthritis showed a significant decrease ( $P< 0.001$) in injected paw oedema from $12^{th}$ day till $21^{st}$ day in petroleum ether extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* and arthritis paw oedema maximum reduction was from $14^{th}$ day till $21^{st}$ day in all above plants extracts (Table 2) . In Non-injected paw all above plants extracts showed decrease in paw oedema was observed in arthritis and maximum decrease was on $12^{th}$ day till $21^{st}$ day (Table3). Body weight, spleen and thymus weight were observed (Table 4).

**DISCUSSION**

The models of Carrageenan induced paw oedema and Freund's Complete adjuvant- induced arthritis in the rats has been used for many years for evaluation of anti-arthritic/ anti-inflammatory agents and are well characterized. In these models, rats develope chronic swelling in multiple joints, with influx of inflammation cells, bone destruction, erosion of joint cartilage and remodeling. These inflammatory changes ultimately, result in the complete destruction of joint integrity and function in the affected animal. Treatments with anti-inflammatory drugs which have been useful for human disease (e.g. COX-2 inhibitor such as indomethacin) ameliorate the joint inflammation in this rat model. The rat carrageenan and adjuvant model has also been useful for development of newer therapeutic agents. Most recently the COX-2 inhibitors. The effects of exogenous administration of Petroleum ether, Chloroform and Methanol extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* in carrageenan induced inflammation model, the result of anti-inflammatory activity of extracts of above plants showed that petroleum ether extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* were having better anti-inflammatory activity as compare to other extracts in carrageenan induced paw oedema in rats (Table1). Therefore, we select petroleum ether extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* for anti-arthritic activity. In FCA induced arthritis rat model, treatment with petroleum ether extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* showed significant

inhibitory effects on injected and non-injected hind paw oedema and maximum inhibition were observed on the 14$^{th}$ and 21$^{st}$ day (Table2 and Table3), loss in body weight, spleenomegaly and thymic involutions (Table4) which are features of adjuvant induced arthritis were effectively reduced after prophylactic administration of petroleum ether extracts of bark of *Sesbania grandiflora* (300mg/kg b.w. p.o.) and *Sesbania sesban* (300mg/kg b.w. p.o.). Therefore, we choose this model to examine the effects of exogenous administration of Petroleum ether, Chloroform and Methanol extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* in carrageenan induced inflammation model and Petroleum ether extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* in FCA induced arthritis in rats at critical time of iNOS activation in response and inflammatory stimuli.

Previous reports have implied that NO is involved in the development of the adjuvant arthritis through activation of T-lymphocyte and / or macrophages. NO generated from iNOS is involved in the initial stages of the immune response to adjuvant arthritis, but do not mediate chronic inflammation, iNOS expression is suppressed by NO donors through feedback mechanism.

Up regulation of iNOS during development of adjuvant induced arthritis is well characterised. In this model Daniel et al have demonstrated a direct parallel between the development of arthritic symptomology in the rat and the appearance and activity of iNOS. Further, they suggested NO level provide an accurate measurement of iNOS activity in this model. The clinical manifestations of disease development in the model of rat adjuvant-induced arthritis have already been described in great detail.

We have confirmed and extended these findings by correlating the timing of inflammatory changes (e.g. paw swelling, body wt., spleenomegaly and thymic involution). With the appearance and enzymatic activity of iNOS. From results, of this study, it appears that high NO level achieved after prophylactic administration of bark of *Sesbania grandiflora* and *Sesbania sesban* may influence initial stages of immune response due to carrageenan and adjuvant injection, possibly by suppressing iNOS expression through feedback inhibition mechanism.

## CONCLUSION

On the basis of these studies, the petroleum ether extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* suggested to exhibit significant anti-inflammatory activity as compared to chloroform and methanol extracts in inflammation. Petroleum ether extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* found to exhibit significant anti-arthritis activity and play negative feedback regulating role on iNOS and therefore influence inflammatory process. However, further studies are required to unravel the mechanism involved in these effects of petroleum ether extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* and their clinical implications.

**TABLE 1 Effect of Prophylactic treatment of petroleum ether chloroform and methanol extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* on paw swelling during carrageenan -induced paw oedema in the rat.**

For the prophylactic treatment of petroleum ether , chloroform and methanol extracts of bark of *Sesbania grandiflora* (SG) (300 mg/kg b.w. p.o.) and *Sesbania sesban* (SS) (300 mg/kg b.w. p.o.) were administered 1 hr before carrageenan injection which were considered 0 hr till 12 hr. Paw volumes were determined at the interval of each hr indicated after immunization. Paw volumes are expressed as the change from 0 hr.

| Treatment | Dose and route | Change in volume (µl) | | | | |
|---|---|---|---|---|---|---|
| | | 1 hr | 3 hr | 6 hr | 9 hr | 12 hr |
| Naive (Saline) | 2ml oral | 0.001 ±0.0004 | 0.002 ±0.0008 | 0.004 ±0.001 | 0.003 ±0.0008 | 0.004 ±0.001 |
| Control (FCA) | 0.1ml s.c. | 42.74 ±2.01 | 85.02 ±2.19 | 76.32 ±0.99 | 71.10 ±0.81 | 66.51 ±0.50 |
| Standard (Ibuprofen) | 7.2mg oral | 26.12 ±1.30 | 47.69** ±4.26 | 31.68** ±1.94 | 21.69** ±1.44 | 15.17** ±1.06 |
| Pet. ether extract (SG) | 300mg oral | 33.09 ±1.76 | 65.93** ±1.09 | 42.74** ±1.48 | 28.08** ±0.94 | 20.85** ±1.19 |
| Chloroform extract (SG) | 300mg oral | 40.08 ±0.60 | 76.73 ±0.77 | 65.60** ±0.67 | 50.60** ±0.67 | 36.14** ±0.636 |
| Methanol extract (SG) | 300mg oral | 38.82 ±0.70 | 83.17 ±0.69 | 75.92 ±0.20 | 65.25 ±0.62 | 38.73** ±0.96 |
| Pet. ether extract (SS) | 300mg oral | 34.60 ±1.45 | 67.70** ±1.35 | 46.67** ±1.71 | 32.70** ±1.08 | 28.70** ±1.29 |
| Chloroform extract (SS) | 300mg oral | 39.88 ±0.77 | 74.43 ±0.79 | 64.43** ±0.55 | 32.84** ±0.64 | 27.29** ±1.36 |
| Methanol extract (SS) | 300mg oral | 40.03 ±0.56 | 79.25 ±0.61 | 70.75 ±0.40 | 59.03** ±1.02 | 37.19** ±0.49 |

**$P < 0.001$ compared with control group.

**TABLE 2 Effect of Prophylactic treatment of petroleum ether extracts of bark of *Sesbania grandiflora* (SG) and *Sesbania sesban* (SS) on primary paw swelling during adjuvant-induced arthritis in the rat.**

For the prophylactic treatment of petroleum ether extracts of bark of *Sesbania grandiflora* (SG) (300 mg/kg b.w. twice daily, p.o.) and *Sesbania sesban* (SS) (300 mg/kg b.w. twice daily, p.o.) were administered 1hr. before adjuvant injection which were considered day 0 till day 21. Paw volumes determined on the days indicated after immunization. Paw volumes are expressed as the change from day 0.

| Treatment | Dose and route | Change in volume (µl) | | | | |
|---|---|---|---|---|---|---|
| | | I Day | III Day | VIII Day | XIV Day | XXI Day |
| Naive (Saline) | 2ml oral | 0.12 ±0.08 | 0.40 ±0.11 | 0.54 ±0.17 | 0.78 ±0.24 | 1.15 ±0.34 |
| Control (FCA) | 0.1 ml s.c. | 41.11 ±0.74 | 56.33 ±1.49 | 39.56 ±0.68 | 72.04 ±2.55 | 86.54 ±2.13 |
| Standard (Ibuprofen) | 7.2 mg oral | 20.83** ±1.53 | 26.96** ±1.53 | 18.79** ±1.77 | 34.96** ±0.90 | 40.80** ±1.00 |
| Pet. ether extract (SG) | 300 mg oral | 34.41** ±0.63 | 38.43**± 0.93 | 33.39 ±1.41 | 47.72** ±1.05 | 52.95** ±1.12 |
| Pet. ether extract (SS) | 300 mg oral | 35.40 ±1.12 | 42.99 ±1.77 | 38.88** ±1.26 | 51.56** ±1.21 | 55.78** ±1.55 |

**P < 0.001 compared with control group.

**TABLE 3 Effect of Prophylactic treatment of petroleum ether extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* on secondary paw swelling during adjuvant-induced arthritis in the rat.**

For the prophylactic treatment of petroleum ether extracts of bark of *Sesbania grandiflora* (SG) (300 mg/kg b.w. twice daily, p.o.) and *Sesbania sesban* (SS) (300 mg/kg b.w. twice daily, p.o.) were administered 1hr. before adjuvant injection which was considered day 0 till day 21. Paw volumes determined on the days indicated after immunization. Paw volumes are expressed as the change from day 0.

| Treatment | Dose and route | Change in volume (µl) | | | | |
|---|---|---|---|---|---|---|
| | | I Day | III Day | VIII Day | XIV Day | XXI Day |
| Naive (Saline) | 2ml oral | 0.12 ±0.08 | 0.46 ±0.07 | 0.42 ±0.08 | 0.80 ±0.11 | 1.18 ±0.15 |
| Control (FCA) | 0.1ml s.c. | 9.36 ±0.48 | 14.89 ±0.48 | 6.39 ±1.02 | 19.24 ±0.63 | 28.34 ±1.30 |
| Standard (Ibuprofen) | 7.2mg oral | 3.78** ±0.30 | 5.30** ±0.45 | 3.27 ±0.35 | 7.93** ±0.44 | 10.45** ±0.57 |
| Pet. ether extract (SG) | 300mg oral | 2.49** ±0.18 | 4.23** ±0.38 | 3.31 ±0.26 | 8.44** ±0.93 | 12.14** ±1.10 |
| Pet. ether extract (SS) | 300mg oral | 5.03** ±0.25 | 7.91** ±0.29 | 6.15 ±0.34 | 12.80** ±0.41 | 17.69** ±0.55 |

**$P < 0.001$ compared with control group.

**TABLE 4 Effect of Prophylactic treatment of petroleum ether extracts of bark of *Sesbania grandiflora* and *Sesbania sesban* on whole body and organ weights during adjuvant-induced arthritis in the rat.**

For the prophylactic treatment of petroleum ether extracts of bark of *Sesbania grandiflora* (SG) (300 mg/kg b.w. twice daily, p.o.)and *Sesbania sesban* (SS) (300 mg/kg b.w. twice daily, p.o.) were administered 1hr. before adjuvant injection which was considered day 0 till day 21. Body weights are compressed as the change from day 0 and Spleen and Thymus weights are measured after 21st days.

| Treatment | Dose and route | Change in Body weights (gm) | | | | | Spleen (mg) | Thymus (mg) |
|---|---|---|---|---|---|---|---|---|
| | | I Day | III Day | VIII Day | XIV Day | XXI Day | XXI Day | XXI Day |
| Naïve (Saline) | 2ml oral | 168.50 ±5.36 | 176.30 ±5.05 | 186.80 ±5.81 | 204.50 ±5.94 | 226.50 ±3.51 | 293.90 ±1.83 | 91.87 ±1.45 |
| Control (FCA) | 0.1ml s.c. | 175.50 ±4.53 | 160.33 ±3.51 | 173.00 ±3.94 | 154.70 ±2.71 | 158.83 ±3.98 | 570.80 ±3.01 | 77.86 ±1.17 |
| Standard (Ibuprofen) | 7.2mg oral | 160.30 ±3.54 | 165.20 ±4.05 | 170.50 ±4.60 | 183.20 ±4.52 | 199.20 ±3.37 | 294.90** ±1.87 | 92.14** ±1.28 |
| Pet. Ether extract (SG) | 300mg oral | 167.70 ±2.32 | 151.30 ±1.85 | 171.80 ±2.38 | 197.70** ±3.45 | 213.50 ±2.71 | 342.40** ±3.84 | 86.73 ±1.69 |
| Pet. ether extract (SS) | 300mg oral | 157.70 ±2.45 | 146.00 ±2.06 | 159.30 ±2.34 | 185.70** ±2.17 | 202.30** ±3.92 | 342.10** ±4.08 | 87.63 ±1.70 |

**P < 0.001 compared with control group.

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