INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

ANTI-INFLAMMATORY ACTIVITY OF *SESBANIA SESBAN* (L) MERR.

Shaikh Sajid.R[1]*, Pawar Vijay T[2], Md. Rageeb Md. Usman[3]
[1]Oriental College of Pharmacy,Sanpada, Navi-Mumbai, India
[2] Bharati Vidyapeeth College of Pharmacy, Kolhapur, India
[3]Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda, India

Article Received on: 10/11/11 Revised on: 27/12/11 Approved for publication: 09/01/12

*E-mail: sajiddash@yahoo.co.in

ABSTRACT
The current study was conducted to obtain three different extracts from *Sesbania Sesban* (L) MERR. leaves. To achieve crude extracts, leaves were dried in shade under normal environmental conditions and then subjected to size reduction to coarse powder. Performing successive hot soxhlet extraction using petroleum ether (60-80^0), Chloroform and methanol. Then study detail phytochemical investigation and pharmacological screening of all extracts. Methanolic extract showed significant activity. Methanolic extract was subjected to thin layer chromatography, which showed three spots i.e 1, 2 and 3. The methanolic extract was then subjected to column chromatography for separation and isolation of the constituents present in it. The constituents were collected in fractions. The elution of the column yielded three compounds. All the three separated constituents were subjected to evaluation of anti-inflammatory activity. Constituent no. 2 showed significant activity.

KEY WORDS: Sesbania Sesban, Methanolic extract, Anti-inflammatory activity.

INTRODUCTION

Throughout human history, people have relied on natural products and plants to promote and maintain good health, to fight sickness, pain and disease[1]. Use of plant products, as medicine is inherent in Ayurveda, the ancient Indian system of health care[2]. *Sesbania sesban* (L.) Merr has a long history of use in India, grows in a wide range of soils from loose sands to heavy clays. It is found widely in tropical Asia and Africa up to an altitude of 1200m[3]. *Sesbania sesban* (L.) Merr (Papilionaceae) has synonyms such as Common sesban, Sevari, Shewari, Jayanti, Jait, Jaya, Jayantika etc[4,5]. The plant is an erect, branched, a quick-growing, short lived shrub or small tree up to 6 m. tall with soft wood and leaves are 7.5-15 cm long including rachis, paripinnate; stipules 3-7 mm long, linear, acute, leaflet 7-28 pairs opposites, 0.6-2.5 cm long and 0.3-0.6 cm wide, linear-oblong, glabrous, margins entire, apex obtuse and often faintly apiculate. Flowers are 1.2-1.5 cm long, borne in 3-20 flowered, calyx 5mm long,campanulate,5 nerved, teeth deltoid, shorter than the tube, yellow colour corolla. Fruits are 12-23 cm long and 2.5-3.8 mm in diameter, cylindrical, twisted, Shortly beaked. Seeds are sub cylindrical, 3-4.5 mm x 2 mm x 2 mm, Olive-green or brown, usually mottled. 20-30 seeds are there in fruit, septet between the seed[6]. It has chemical constituents such as Protein, Sterol, Saponin, Flavonoid, Sesbanine, Sesbanimide Phosphorus, Glycoside, Kampferol, Fat and good source of Vitamin[7]. The plant is used as Astringent[8], Anti-inflammatory[8], Carminative, Fever, Ulcers, Purgative, Demulcent, Anthelmintic[9], Antifertility[10], antibacterial[11], antifungal[12] ,antimicrobial[13] and use in pain.

MATERIALS AND METHODS

Animals Selection

The wistar strain albino rats (150-200g) were used for anti-inflammatory model. Rats were kept in polypropylene cages and oil extracted groundnut feed was given. The animals were exposed to 12 cycles of darkness and light. The bedding material of cages was changed every day. Rats were divided into five groups. Each group contained six animals.

Material used

Extracts used

1. Petroleum ether extract
2. Chloroform extract
3. Methanol extract

Iboprofen**:** (The Reelif Pharmaceutical, Ahmednager, Maharashtra)
Carrageenin: (Sigma Chemicals, USA)
Plethysmometer : (PTH /707, Medicaid.)

Methodology

Anti-inflammatory Activity[14]

The subcutaneous subplantar region injection of carrageenin produce inflammation in rats. A decrease in inflammation can be achived by administration of compounds with anti-inflammatory activity.

Groups of Rats

The animals were divided into different groups and each group consists of 6 animals.
Group-1**:** Control Group, supplied with carrageenin.
Group-2: Standard Group- treated with Ibuprofen (50mg/kg).
Group-3: Treated with suspension of petroleum ether extract orally (250mg/kg).
Group-4: Treated with suspension of Chloroform extract orally (250mg/kg).
Group-5: Treated with suspension of Methanolic extract orally (250mg/kg). The suspension of each extract was prepared in saline with the help of Tween80 (0.3% w/v)

Procedure[15]

1. Different extracts were suspended in 0.9% saline and dose (250mg/kg) administered orally.
2. The injection of 0.1ml of freshly prepared solution of carrageenin (1%) in physiological saline was injected into subplantar region of the left hind paw of each rat.
3. One group has been treated with Ibuprofen as standard (50 mg/kg).
4. A mark was put on the leg at the malleolus to facilitate uniform dipping.
5. The paw edema volume was measured with the help of plethysmometer.

6. The same procedure was repeated at 1h, 2 h and 3 h. The percentage (%) inhibition of paw edema in the various treated groups was then calculated by using the formula;

Percentage inhibition (%) = (1 – Vt/Vc) X 100 .... equation (1)

Where, Vt = mean relative change in a paw volume in test group.

Vc = mean relativechange in paw volume in control group

Sample preparation

Leaves of *sesbania sesban* (L.) Merr were dried in shade under normal environmental conditions and then subjected to size reduction to coarse powder. Powdered material was stored in air tight polythene bags. The coarse powder material was charged into the soxhlet extractor and hot continuous successive extraction was carried out using different solvents, a) Petroleum ether (60-80^0) b) Chloroform c) Methanol. Each time before extracting with the next solvent, the powdered material was air-dried and each extract was concentrated by distilling off the solvent to obtain the crude extractive. The drug was extracted with each solvent till completion of extraction (40 cycles).

RESULTS AND DISCUSSION

The color, consistency and percentage extractive values of each extract are shown in Table 1.The results of preliminary phytochemical screening of different extracts of leaves of *Sesbania sesban* are given in Table 2. The acute toxicity study of each extract showed signs of toxicity at 2500 mg/kg. 1/10th of the same dose for all these extracts was taken as therapeutic dose i.e. 250 mg/kg. Extracts obtained were subjected to evaluation of anti-inflammatory activity by carrageenin induced rat paw edema method. Ibuprofen (50mg/kg) was taken as standard drug. Methanolic extract showed significant decrease in paw edema of rat, while petroleum ether extract & chloroform extract did not show significant decrease in paw edema of rat as compared to standard drug. Results are given in Table 3 and in fig. 1.The methanolic extract significantly reduced rat paw edema with respect to corresponding control, but the petroleum ether extract and chloroform extract did not show any significant reduction in rat paw edema. The methanol extract was subjected to thin layer chromatography to know the number of constituents present in it. Various solvent systems were tried. The most suitable solvent system was Toluene: chloroform: Methanol (1:1:0.8). The spots were detected using ultra-violet light. The methanolic extract showed three spots, which indicated the number of constituent present in methanol extract. The R_f value of these spots are given in Table 4. The three constituents were separated by column chromatography using gradient elution technique. The constituents separated were collected in fractions. The fractions having similar pattern of TLC were mixed and the solvent was evaporated. The various solvents and their combinations used for separation and isolation along with the compounds obtained are given in Table5. The separated constituents were evaluated for the anti-inflammatory activity at a dose of 250 mg/kg. It was observed that constituent 1 and 3 did not show significantly decrease in paw edema volume while the constituent 2 significantly reduced paw edema volume as compared to standard drug. Results are given in Table 6 and in fig.2

CONCLUSION

Sesbania sesban (L.) Merr. is commonly known as Sevari or Jayanti. *Sesbania sesban*(L.)Merr has a long history of use in India; the plant grows in a wide range of soils from loose sands to heavy clays. *Sesbania sesban* (L.)Merr is reported to possess astringent (bark), anthelmintic, anti-inflammatory (bark and leaves), ulcers and diarrhea (seed), antifertility and antimicrobial activity, while anti-inflammatory activity of leaves is still not scientifically investigated.

In the present work attempts were made to study detail phytochemical investigation and pharmacological, particularly anti-inflammatory activity of leaves of *Sesbania sesban* belonging to family papilionaceae .The percentage extractive values of leaves of *Sesbania sesban* obtained as in petroleum ether (60-80^0) 2.4% w/w, in chloroform 3.2% w/w and in methanol 3.5% w/w. The extracts after the preliminary phytochemical investigation had shown the presence of following active principles. Methanol: Sterols, saponin , flavonoids, Petroleum ether (60-80^O) : Fats and oil. Chloroform: Sterols, alkaloids, flavonoids. The acute toxicity study of extracts of leaves of *Sesbania sesban* were showed 50% mortality at a dose (2500 mg/kg). Hence 1/10th of the same dose for all these extract were taken as therapeutic dose i.e. 250 mg/kg. The methanol extract showed significant reduction in paw edema as compared to control group, where as petroleum ether (60-80^0) extract and chloroform extract showed comparatively less reduction in paw edema volume. Methanolic extract was subjected to thin layer chromatography by using solvent system Toluene: Chloroform: Methanol (1:1:0.8), which showed three spots, having Rf values 0.74, 0.58 and 0.43. The methanolic extract was then subjected to column chromatography for separation and isolation of the constituents present in it. The constituents were collected in fractions. The elution of the column yielded three compounds. All the three separated constituents were subjected to evaluation of anti-inflammatory activity. Constituent no.2 showed significant reduction in paw edema as compared to standard. Constituent no. 1 and 3 showed comparatively less reduction in paw edema.

ACKNOWLEDGMENT

The team acknowledges Bharati Vidyapeeth College of Pharmacy, Kolhapur, and Oriental College of Pharmacy,Sanpada, Navi-Mumbai for providing the necessary facilities to carry out this work.

REFERENCES

1. Fetrow W, Avoila I. The Complete Guide to Herbal Medicine. Spring House Corporation Springhouse 2000; 1:11-13.
2. Parle M, Bansal N, Harmless of Herbal Remedies 2005; 12(2):532.
3. Parrota J A. Healing Plants of Peninsular Indian. CABI Publishing, Wallingfoard United kingdom 2001; 53:413 -414.
4. Anonymous. Wealth of India A dictionary of Indian Raw Materials and Industrial Product. NISCAIR (CSIR) New Delhi 2003; 4: 298-301.
5. Kirtikar KR,Basu BD. Determination of nutritive value and analysis of mineral element for some medicinally valued plants from Uttranchal. Indian Medicinal Plants 1933; 9: 1026.
6. Rastogi RP ,Mehrotra BN. Compendia Indian Medicinal Plants PID:New Delhi 1995 ; 4: 672.
7. Chatterjee A, Pakrashi S. The Treatise of Indian Medicinal Plants. Deep Publication. New Delhi 1992; 2:121.
8. Yusuf M, Chowdhury J, Wahab M, Begum J. Medicinal Plants of Bangladesh, Bangladesh Council of Scientific & Industrial Research (BCSIR).Dhaka,Bangladesh 1994;223.
9. Ibrahim A M. Anthelmintic activity of some sudanese medicinal plants. Phytotherapy Research 1992; 6(3): 155-157.
10. Singh S P. Fertility control of female through Sesbania susban seeds. Journal of Research and Education in Indian Medicine 1990; 9(4): 27-32.
11. Jayalaxmi S, Shrivastava R, Kohali D. Antibacterial activity oil isolated from the sesbania sesban. Indian Hosp.Pharm 1881;19(2): 67-69.
12. Tatiya AU, Patil UK, Nikam DD, Surana SJ. Evalutation of antibacterial and antifungal activity *Sesbania sesban* leaves. Indian Drugs 2008;45(1):44-47.

13. Hossain A, Chaudhary S. Antimicrobial activity of methanolic extract of *sesbania sesban*. Journal of Pharmaceutical Science 2007; 6 (1):61-63.
14. Vogel HG. Drug Discovery and Evaluation, Pharmacological Assays. Springer-Verlag, Berlin Heidelberg: New York 2002; 2: 759.
15. Winter CA. Risely E, Proceedings of the Society for Experimental Biology and Medicine 1962; 111,544 .

Table 1 The Color, Consistency and Percentage Extractive Values of Extracts

Sr.No.	Solvents	Color	Consistency	Extractive Value (% w/w)
1	Petroleum ether (60-80^0)	Yellowish green	Semisolid	2.4%
2	Chloroform	Green	Solid	3.2%
3	Methanol	Dark brown	Solid	3.5%

Table 2 Preliminary Phytochemical Screening

Test	Pet. Ether extract	Chloroform extract	Methanol extract
1. Tests for Carbohydrates:			
a) Molisch's Test	-ve	-ve	-ve
b) Fehling's Test	-ve	-ve	-ve
c) Benedict's Test	-ve	-ve	-ve
d) Barfoed's Test	-ve	-ve	-ve
e) Bial's Orcinol Test	-ve	-ve	-ve
f) Aniline Acetate test	-ve	-ve	-ve
g) Phloroglucinol Test	-ve	-ve	-ve
h)Seliwinoff's Test	-ve	-ve	-ve
i)Tollen's Phloroglucinol Test for galactose	-ve	-ve	-ve
j) Cobalt-chloride Test	-ve	-ve	-ve
2. Test for Gums:			
Fehling's test	-ve	-ve	+ve
Benedict's test	-ve	-ve	+ve
3. Test for Mucilage:			
a) Powdered drug + ruthenium red.	-ve	-ve	+ve
b) Powdered drug + water or aqueous KOH.	-ve	-ve	+ve
4. Tests for Proteins:			
a) Biuret test (General Test):	-ve	-ve	+ve
b) Millon's test	-ve	-ve	+ve
c) Xanthoproteic test	-ve	-ve	+ve
d) Test for proteins containing sulphur	-ve	-ve	-ve
5. Tests for Amino Acids:	-ve	-ve	-ve
a) Ninhydrin test	-ve	-ve	-ve
b) Test for tyrosine	-ve	-ve	-ve
c) Test for tryptophan	-ve	-ve	-ve
d) Test for cysteine	-ve	-ve	-ve
6.Test for Fats and Oils:			
a) Solubility Test:	+ve	-ve	-ve
b) Saponification Test	+ve	-ve	-ve
7.Test for Sterols and Triterpenoids:			
a) Salkowaski test	-ve	+ve	+ve
b) Liebermann-Burchardt test	-ve	+ve	+ve
c) Liebermann test	-ve	+ve	+ve
8. Tests for Cardiac Glycosides:			
a) Legal's Test	-ve	-ve	-ve
b) Keller-Killiani Test	-ve	-ve	-ve
9.Tests for Anthraquinone Glycosides:			
a) Borntrager's Test	-ve	+ve	+ve
b) Modified Borntrager's Test for C-Glycosides	-ve	+ve	+ve
10.Tests for Cynogenetic Glycosides:			
Grignard reaction or Sodium Picrate test	-ve	-ve	-ve
11. Tests for Coumarin Glycosides:			
a) Aomatic odor.	-ve	-ve	-ve
b) Alkaline test	-ve	-ve	-ve
12. Tests for Alkaloids:			
a) Mayer's test	-ve	+ve	-ve
b) Wagner's test	-ve	+ve	-ve
c) Hager's test	-ve	+ve	-ve
d) Dragendorff's test	-ve	+ve	-ve
13.Tests for Saponins:			
a) Foam test	-ve	-ve	+ve
b) Haemolysis test	-ve	-ve	+ve
14. Tests for Flavonoids:			
a) Ferric-chloride test	-ve	+ve	+ve
b) Shinoda test	-ve	+ve	+ve
c) Zinc-HCL reduction test	-ve	+ve	+ve
d) Alkaline reagent test	-ve	+ve	+ve

e) Lead acetate solution test	-ve	+ve	+ve
15.Test for Tannins and Phenolic Compounds:			
a) Ferric-chloride test	-ve	-ve	-ve
b) Gelatin test	-ve	-ve	-ve
c) Lead Acetate Test	-ve	-ve	-ve
d) Potassium Dichromate Test	-ve	-ve	-ve
e) Bromine Water Test	-ve	-ve	-ve
f) acetic acid test	-ve	-ve	-ve
g) Iodine test	-ve	-ve	-ve
h) Nitric acid test	-ve	-ve	-ve
i) NH4OH & K-ferricyanide test	-ve	-ve	-ve

Table 3 : Anti-inflammatory Activity of Extracts

Group No.	Treatment	Dose	0 h	1h	2h	3h
1.	Carrageenin	0.1mL. 1% sol.	0.98	1.31±0.04	1.40±0.07	1.50±0.05
2	Ibuprofen	50mg/kg	0.99	0.55±0.03 (58.02%)	0.58±0.06 (58.58%)	0.62±0.05 (58.95%)
3	Pet. Ether extract	250mg/kg	1.00	0.83±0.04 (36.65%)	0.87±0.07 (37.86%)	0.92±0.03 (38.67%)
4	Chloroform extract	250mg/kg	0.97	0.92±0.07 (29.78%)	0.98±0.09 (30.00%)	1.00±0.05 (33.34%)
5	Methanol extract	250mg/kg	0.96	0.75±0.02 (42.75%)	0.78±0.04 (44.29%)	0.82±0.03 (45.34%)

n = 6, Values are mean± standard deviation,
P<0.03 with respect to corresponding control.

Table 4 : Thin Layer Chromatography of Methanolic Extract

Sr.No.	Solvent System	No. of Spots in UV-light	Colors of spot	R_f value
1	Toluene: Chloroform : Methanol (1:1:0.8)	I	Fluorescent green	0.74
2		II	Pink	0.58
4		III	Fluorescent green	0.43

Table 5: Column Chromatography of Methanolic Extract

Solvent	Fraction No. (25 mL)	Constituents	R_f value
Pet. Ether	1 - 5	No spot	-----
Pet. Ether : Benzene 4:1	6-10	No spot	-----
3:2	11-15	No spot	-----
2:3	16-20	I	0.77
1:4	21-25	I	0.77
Benzene	26-30	I and II	----
Benzene: Acetone 4:1	31-35	II	0.61
3:2	36-40	II	0.61
2:3	41-45	II	0.61
1:4	46-50	II	0.61
Acetone	51-55	II and III	----
Acetone : Methanol 4:1	56-60	III	0.40
3:2	61-65	III	0.40
2:3	66-70	III	0.40
1:4	71-72	No spot	----

Table 6 : Anti-inflammatory Activity of Separated Constituents

Group No.	Treatment	Dose	0h	1h	2h	3h
1.	Carrageenin	0.1mL. 1%	0.97	1.30±0.05	1.42±0.03	1.51±0.02
2	Ibuprofen	50mg/kg	0.99	0.56±0.03 (56.93%)	0.58±0.05 (58.58%)	0.61±0.08 (58.79%)
3	Chem. Constituent.1	250mg/kg	0.95	0.80±0.02 (38.47%)	0.85±0.06 (39.29%)	0.88±0.04 (39.47%)
4	Chem. Constituent.2	250mg/kg	0.98	0.62±0.03 (52.31%)	0.66±0.07 (52.86%)	0.68±0.04 (54.06%)
5	Chem. Constituent.3	250mg/kg	0.99	0.91±0.09 (30.77%)	0.93±0.05 (34.29%)	0.94±0.03 (35.14%)

P<0.05 with respect to corresponding control,
n = 6, Values are mean± standard deviation.

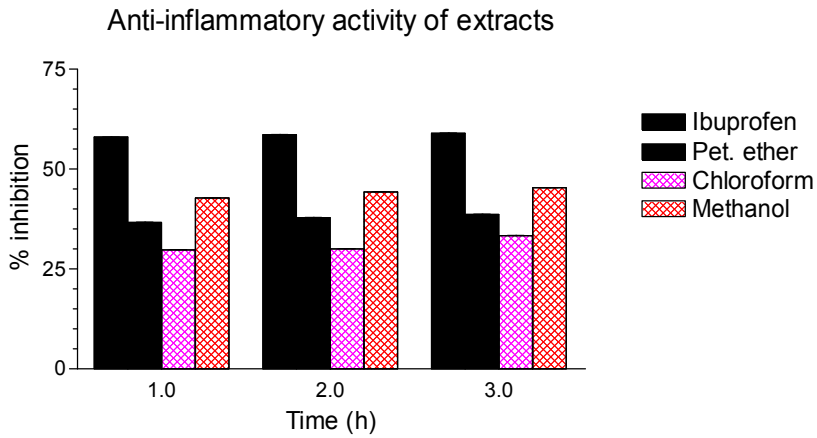

Fig 1. Anti inflammatory activity of extract

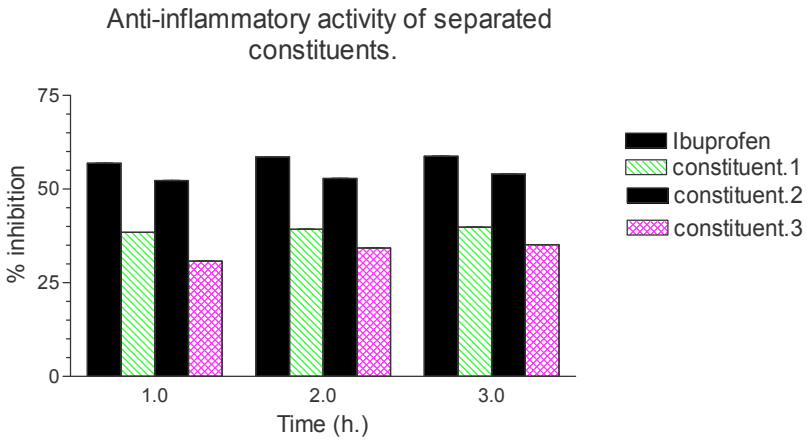

Fig 2. Anti inflammatory activity of separated constituents

Source of support: Nil, Conflict of interest: None Declared