Research Article

Anti-inflammatory and Anti-Arthritic Activity of *Smithia sensitiva*

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ABSTRACT

Smithia sensitiva belonging to the family Fabaceae is a plant used as an anti inflammatory and anti oxidant drug by tribal peoples in kerala. The whole plant is traditionally used as Refrigerant, Galactogogue and as lotion in headaches. Preliminary phytochemical screening of the extract showed the presence of alkaloids, sugars and Carbohydrates, steroids, tannins and flavanoids[6,] The acute toxicity studies of the extracts showed that there was no lethality or any toxic reactions found at any dose selected untill the end of the study period. LD_{50} was found to be more than 2000mg/kg. The methanolic extract at 200mg/ml produced 29.8 % inhibition in the hypotonicity induced HRBC membrane lysis. The % inhibition of inflammation produced in standard was more on day 21 and methanolic extract showed significant antiarthritic effects. The effect of extract was represented as follows methanol> chloroform> pet.ether.

Keywords: *Smithia sensitiva*, Anti-inflammatory activity, HRBC membrane stabilization.

INTRODUCTION[1, 2]

Inflammation is the immediate defensive mechanism or reaction to an injury, which may be caused by infection, chemical or physical agent. It involves pain, heat, redness and swelling and loss of function of affected part. This mechanism is a useful function protecting against attack, however the inflammatory response can also be determined as it is non specific and may lead to the development of inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, psoriasis etc. Reactive oxygen species and free radicals are thought to act indirectly as cellular messengers and elicit an inflammatory response. Anti-inflammatory drugs are broadly classified in to two categories ie. Steroidal and Non steroidal anti-inflammatory agents (NSAIDs). Steroidal Drugs act on the inflammatory cells and the inflammatory mediators. Non Steroidal anti-inflammatory drugs acts by inhibiting cox-1 and cox-2. The major limitations of currently used synthetic anti-inflammatory agents are gastrointestinal irritation and ulceration. Natural products devoid of these side effects will be promising group for treating inflammatory disorders.

Smithia sensitiva belonging to the family Fabaceae is a plant used as an anti inflammatory and anti oxidant drug by tribal peoples in kerala. This effect is due to the property of any of the constituents present in the plant. Our aim is to extract the dried plant with methanol and study the anti-inflammatory and antiarthritic activity. *Smithia sensitiva* is a low growing annual herb 30-90cm long and it is distributed widely in Hilly areas. The whole plant is traditionally used as Refrigerant, Galactogogue and as lotion in headaches.

MATERIALS AND METHODS

Collection and identification of plant:

The plant Smithia sensitiva collected from Western Ghats and identified by Subrahmanya Prasad.K, Research Scholar, Dept. Of PG Studies and Research in Botany, Sir Sayed College Taliparamba.

Preparation of extracts[3]

The whole plant is dried and powdered and is subjected to successive extraction

with Petroleum ether, Chloroform and methanol. The crude extract were subjected to preliminary phytochemical screening and showed the presence of alkaloids, sugars and Carbohydrates, steroids, tannins and flavanoids.

Acute Toxicity Studies[5, 6]

Healthy adult wistar albino rats of either sex weighing 150-200gm were used for the study. The starting dose level of the extracts was 2000mg/kg body weight. Animals were starved overnight. After dosing, the animals were closely observed for first 4 hours for any abnormal activity and intermittently for the next 24 hours. The number of animals dead was noted after 24 hours. (CPCSEA No. 282/CADD/11).

In vitro anti- inflammatory activity by HRBC membrane stabilization method[6, 7]

Human Red Blood Corpuscles (HRBC) membrane stabilizing method was used for the determination of anti-inflammatory avtivity. Extract were made into dose of 1 mg/kg body weight with 2% sodium carboxy methyl cellulose . Diclofenac sodium was used as standard. The reaction mixture(4-5 ml) consist of 2ml of hypotonic saline (0.25% w/v NaCl) , 1 ml of 0.15 M phosphate buffer (pH 7.4) and 1 ml of test solution (1 mg/ml) in normal saline ,0.5 ml of 10% HRBC in normal saline was added . For control, 1 ml of isotonic saline was used instead of test solution. The mixtures were incubated at 56^{o}c for 30 min. Then they were cooled at running tap water, centrifuge at 3000 rpm for 20 min. The absorbance of supernatant was read at 560 nm.

Percent membrane stabilizing activity was calculated as follows:-

% Membrane stabilization = 100 – OD of Drug/OD of test control ×100

Antiarthritic activity by formalin induced arthritic inflammation[8, 9]

Healthy adult wistar albino rats of either sex weighing 150-200gm were divided in to five groups of three animals. Control recieves 1% CMC, 10ml/kg,p.o and standard was given with Prednisolone 5mg/kg body weight/p.o. Arthritis was induced in rats by using 2% w/v of formalin solution. The inflammation was produced by sub aponeurotic injection of 0.1 ml of the 2% w/v formalin solution in the left hind paw of the rats on the first and third day. The paw volume of all the animal groups was measured by vernier calipers at 0, 7, 14, 21 days after the injection of formalin solution.

The percentage inhibition of Paw edema= [(Control - test)/ Control] x100.

Statistical analysis

Results obtained were evaluated by Student's't' test, values of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the extract showed the presence of alkaloids, sugars and Carbohydrates, steroids, tannins and flavanoids[4,] The acute toxicity studies of the extracts showed that there was no lethality or any toxic reactions found at any dose selected untill the end of the study period. LD_{50} was found to be more than 2000mg/kg (Table 1). The extract did not produce any characteristic behavioral changes. The methanolic extract at 200mg/ml produced 29.8 % inhibition in the hypotonicity induced HRBC membrane lysis. The maximum inhibition was produced for methanolic extract at 180 mts. (Table 2). The extracts significantly inhibited the formalin induced arthritis in rats, supporting the use of the plant in arthritis. The % inhibition of inflammation produced in standard was more on day 21 and methanolic extract showed significant antiarthritic effects (Table 3). All the extracts exhibited dose dependant response. This effect may be due to the presence of steroids, alkaloids, tannins and flavonoids present in various fractions. The effect of extract was represented as follows methanol> chloroform> pet.ether.

CONCLUSION

Acute toxicity study of the extracts showed that a dose upto 2000mg/kg per b.w was nontoxic. The extract did not produce any characteristic behavioral changes. The compounds significantly inhibited the formalin induced arthritis in rats,

supporting the use of the plant in arthritis. The methanolic extract at 200mg/ml produced 29.8 % inhibition in the hypotonicity induced HRBC membrane lysis. The In vitro studies on *Smithia sensitiva* showed the presence of significant anti-inflammatory and anti-arthritic activity. The methanolic extract shows more anti inflammatory and anti-arthritic activities. The Activity may be due to the presence of steroids, flavonoids and alkaloid. Our future aim is to isolate the chemical constituents responsible for the anti inflammatory and anti-arthritic activities.

ACKNOWLEDGEMENT

The authors are thankful to the management authoritories of CCOPS for providing necessary facilities to carry out this study.

Table 1: Acute toxicity studies

S. No.	Treatment group (Extract)	Dose	Wt: of animal in grams		Signs of toxicity	Onset of toxicity	Reversible or Irreversible	Duration
			Before test	After test				
1	Methanolic	2000mg/kg	170	180	No signs of toxicity	Nil	Nil	14 days
	Chloroform	,,	169	176	,,	,,	,,	,,
	Pet ether	,,	172	179	,,	,,	,,	,,
2	Methanolic	500mg/kg	170	175	*	*	*	,,
	Chloroform	,,	176	185	,,	,,	,,	,,
	Pet ether	,,	175	182	,,	,,	,,	,,
3	Methanolic	50mg/kg	180	190	*	*	*	,,
	Chloroform	,,	172	179	,,	,,	,,	,,
	Pet ether	,,	174	185	,,	,,	,,	,,

Table 2: Anti-inflammatory activity

Group	Drug	Phosphate buffer	Hyposaline	Distilled water	HRBC suspension	O.D at 560 nm	% Inhibition of haemolysis
Control	--------	1 ml	2 ml	1 ml	0.5 ml	1.475	-----
Blank	--------	1 ml	-----	2 ml	0.5 ml	1.650	-----
Standard	Diclofenac (50 mg/ml)	1 ml	2 ml	-----	0.5 ml	0.832	43.59
Test-1,Pet ether	Extract (200 mg/ml	1 ml	2 ml	-----	0.5 ml	1.112	21.89
Test-2, Methanolic	200 mg/ml	1 ml	2 ml	-----	0.5 ml	1.034	29.89
Test-3, Chloroform	200 mg/ml	1 ml	2 ml	-----	0.5 ml	1.113	24.54

Table 3: Antiarthritic activity

Group	Treatment	Wt of animals in grams	Thickness of linear cross section in mm		Diff: in thickness in mm	Mean+S E	% Inhibition	P Value
			1'st day	21'st day				
Control	1% CMC, 10 ml/kg, p.o.	200 250 150	5.67 5.47 5.19	8.50 8.94 8.36	2.83 3.47 3.17	3.31±0.142	-----	-----
Standard	Prednisolone, 5mg/kg, p.o.	200 200 250	5.82 5.52 5.04	6.54 6.26 6.17	0.72 0.74 1.13	0.938± 0.095	71.66	‹0.001
Test-1	Pet ether (200mg/kg)	300 250 150	5.46 5.84 5.26	8.09 8.32 8.02	2.63 2.48 2.76	2.7± 0.068	18.42	‹0.02
Test-2	chloroform (200mg/kg)	300 250 200	5.10 5.89 5.72	7.52 8.24 8.02	2.42 2.35 2.30	18± 0.088	26.94	‹0.01
Test-3	methanolic (200mg/kg)	300 250 200	5.69 5.74 5.82	8.03 7.81 8.24	2.34 2.07 2.42	2.248± 0.054	32.08	‹0.001

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