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EVALUATION OF *INVIVO* ANTI-INFLAMMATORY ACTIVITY OF HYDROALCOHOLIC LEAVES EXTRACT OF *SACCOPETALUM TOMENTOSUM*

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ABSTRACT

The present work showed phytochemical screening, anti-inflammatory activities and sub-acute toxicity of hydro-ethanolic extract of leaves of *Saccopetalum tomentosum.* The total flavonoid content (TFC) was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y=0.032X + 0.018, R^2=0.998, where X is the quercetin equivalent (QE) and Y is the absorbance. The total alkaloid content (TAC) was expressed as mg/100mg of Atropine equivalent of dry extract sample using the equation obtained from the calibration curve: Y=0.007X+ 0.024, R^2=0.995, where X is the atropine equivalent (AE) and Y is the absorbance. Total alkaloid and flavonoids (mg/100mg) was found in hydroalcoholic extract of *Saccopetalum tomentosum* 1.635 *and* 0.957 mg/100mg respectively. Evaluation of the anti-inflammatory activity of the Hydroalcoholic Extract of leaves of *Saccopetalum tomentosum* was performed using the formalin-induced rat paw oedema model using diclofenac sodium as the reference drug. Mean changes in paw oedema thickness of animals treated with the tested compounds from induction of inflammation was measured, together with the inhibition percent of oedema by the tested extracts at 2 dose level 100mg/kg and 200mg/kg. Results shown that the tested extract 200mg/kg was found more active.

Key words: *Saccopetalum tomentosum*, anti-inflammatory, formalin-induced, quantitative

INTRODUCTION:

Inflammation is a mechanism of great benefit for maintaining homeostasis in the body. The inflammatory response may be appropriate, physiologic and necessary in the presence of an infection and cellular damage or stress. Conversely, it may be inappropriate, altered homeostasis, pathologic and damaging when it is reacting out of proportion causing undesired effects (Miller et al., 2009; Abdelmagid et al., 2011) and contribute to diseases. In fact, inflammation is implicated in osteoarthritis, heart disease, Alzheimer's, age-related macular degeneration, chronic obstructive pulmonary disease, multiple sclerosis, stroke and cancer (McCarty et al., 1999; Azeem et al.,

2010; Medzhitov, 2010; Kumar, 2011). Inflammation is frequently associated with the increase in vascular permeability and mediator release (Vane et al., 1998), increase of protein denaturation and membrane alteration (Umapathy et al., 2010). Further, leucocyte infiltration, oedema and granuloma formation represent typical features of inflammation (Gorzalczany et al., 2011). Moreover, the host response has been considered to be mediated mainly by B and T lymphocytes, neutrophils and monocytes/macrophages. These cells are triggered to produce inflammatory mediators, including cytokines, chemokines, arachidonic acid metabolites and proteolytic enzymes, which collectively contribute to tissue degradation by activation of several distinct host degradative pathways (Birkedal-Hansen et al., 1993; Hernandez et al., 2011). Steroidal and non-steroidal anti-inflammatory drugs are known to treat inflammation and pain. However, their prolonged use often leads to serious side-effects such as gastrointestinal tract dyspepsia, peptic ulceration, haemorrhage and perforation leading to death in some patients (Griffin, 1998). Many medicines of plant origin have been used since long time without any adverse effects, and new medicinal plants are introduced to develop analgesic and anti-inflammatory drugs. Therefore, the present study was designed to investigate anti-inflammatory activities of hydro-ethanolic extract of leaves of *Saccopetalum tomentosum* by using Carrageenan-Induced Rat Paw Edema model.

Material and Method

Material

Diclofenac Sodium (Themis Pharmaceuticals, Mumbai), Carrageenin (Sigma Chemical Co, St Louis, MO, USA) were used in present study.

Methods

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs:

Defatting of plant material

Leaves of *Saccopetalum tomentosum* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Dried powdered leaves of *Saccopetalum tomentosum* has been extracted with hydroalcoholic solvent (ethanol: water: 80:20) using maceration process for 48 hrs, filtered

and dried using vacuum evaporator at 40°C [Mukherjee, 2007; Kokate, 1994).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods [Roopashree et al., 2018; Obasi et al., 2010).

Total flavonoids content estimation

Principle: Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Total alkaloids content estimation

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

Formalin-induced Anti- inflammatory activity of leaves of *Saccopetalum tomentosum*

Materials and methods:-

Animals:-

Wistar rats (150–200 g) were group housed (n=6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics

Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity study

Preliminary experiments were carried out on rats (n=6). Hydroalcoholic Extract of leaves of *Saccopetalum tomentosum* were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (OECD; 2001). Animals were kept fasting providing only water, extract were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-inflammatory effect [Audu et al.2007; OECD, 2001; Singh et al., 2010).

Anti-inflammatory activity

Experimental designs

Group –1: Control

Group –2: Diclofenac Sodium (Standard)

Group –3: Hydroalcoholic Extract of leaves of *Saccopetalum tomentosum* (HEST) (100mg/kg, p.o.)

Group –4: Hydroalcoholic Extract of leaves of *Saccopetalum tomentosum* (HEST) (200mg/kg, p.o.)

Formalin-induced paw edema model

The animals were divided into four groups of six animals each and were fasted for a period of 24 h prior to the study. Group 1 was treated as control (formalin (0.2 ml of 2% v/v freshly prepared formalin solution prepared in distilled water), Group 2 was received Diclofenac Sodium 30mg/kg, p.o. Group 3 were treated with Hydroalcoholic Extract of leaves of *Saccopetalum tomentosum* (HEST) (100mg/kg, p.o.). Group 4 were treated with Hydroalcoholic Extract of leaves of *Saccopetalum tomentosum* (HEST) (100mg/kg, p.o.). The thickness was measured before injecting the formalin and after injecting the formalin everyday at a fixed time for seven consecutive days using a vernier caliper (precision) (Singh *et al.*, 2010).

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

Results and Discussion

Small portion of the dried extracts was subjected to the phytochemical tests using standard methods. Phytochemical screening reveals that presents of various phytoconstituents such as alkaloids, glycosides, saponins, flavonoids carbohydrates and proteins separately for hydroalcoholic extract of *Saccopetalum Tomentosum*. The total flavonoid content (TFC) was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y=0.032X + 0.018, R^2=0.998, where X is the quercetin equivalent (QE) and Y is the absorbance. The total alkaloid content (TAC) was expressed as mg/100mg of Atropine equivalent of dry extract sample using the equation obtained from the calibration curve: Y=0.007X+ 0.024, R^2=0.995, where X is the atropine equivalent (AE) and Y is the absorbance. Total alkaloid and flavonoids (mg/100mg) was found in hydroalcoholic extract of *Saccopetalum tomentosum* 1.635 *and* 0.957 mg/100mg respectively. Evaluation of the anti-inflammatory activity of the Hydroalcoholic Extract of leaves of *Saccopetalum tomentosum* was performed using the formalin-induced rat paw oedema model using diclofenac sodium as the reference drug. Mean changes in paw oedema thickness of animals treated with the tested compounds from induction of inflammation was measured, together with the inhibition percent of oedema by the tested extracts at 2 dose level 100mg/kg and 200mg/kg. Results shown that the tested extract 200mg/kg was found more active.

Table 1: % Yield of *Saccopetalum tomentosum (Leaves)*

S. No.	Solvents	% Yield
1.	Pet ether	4.28
2.	Hydroalcoholic	6.79

Table 2: Phytochemical screening of extract *of Saccopetalum tomentosum*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Dragendroff's test Hager's test	 -ve +ve
2.	**Glycosides** Legal's test	 +ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
4.	**Phenol** Ferric chloride test	 -ve
5.	**Proteins** Xanthoproteic test	 +ve
6.	**Carbohydrates** Fehling's test	 +ve
7.	**Saponins** Foam test	 +ve
8.	**Diterpenes** Copper acetate test	 -ve

Table 3: Estimation of total flavonoids and alkaloid content of *Saccopetalum tomentosum*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	total alkaloid content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.957	1.635

Table 4: Effect of different extracts on paw oedema induced by formalin in rats

Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	Percentage of Inhibition (%)
Control	0.2 ml of 2% v/v	4.80±0.20	--
Diclofenac	30	3.50±0.19*	96.0
MEGL	100	4.10±0.21	84.00
MEGL	200	3.82±0.22	87.00

Conclusion

Evaluation of the anti-inflammatory activity of the Hydroalcoholic Extract of leaves of *Saccopetalum tomentosum* was performed using the formalin-induced rat paw oedema model using diclofenac sodium as the reference drug. Mean changes in paw oedema thickness of animals treated with the tested compounds from induction of inflammation was measured, together with the inhibition percent of oedema by the tested extracts at 2 dose level 100mg/kg and 200mg/kg. Results shown that the tested extract 200mg/kg was found more active.

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