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IN VIVO ANTI INFLAMMATORY AND ANTIMICROBIAL EFFECT OF EXTRACT OF HERBAL PLANT *SWERTIA CHIRATA* L.

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ABSTRACT

Inflammation is a reaction of a living vascularised tissue to an injury. Conventional or synthetic drugs used in the treatment of inflammatory diseases are inadequate, it sometimes has serious side effects. So, number of herbal medicines is recommended for the treatment of inflammation that has no side effects. Hence our study focused to investigate the phytochemical analysis, quantification of bioactive compounds, *in vitro* antimicrobial activity and anti-inflammatory activity (Carrageenan-induced paw edema model) of hydroalcoholic extract of aerial parts of *Swertia chirata* which has boundless medicinal properties. Qualitative analysis of various phytochemical constituents, quantitative analysis of total phenolics (Folins ciocalteau reagent method) and flavonoids (Aluminium chloride method), and *in vitro* antimicrobial activity were determined by the well-known test protocol available in the literature. For anti-inflammatory activity, wistar albino rats were used and divided into four groups of six animals each group and Group 1 was treated as manage (formalin (0.2 ml of 2% v/v freshly prepared formalin resolution in distilled water), group 2 was received diclofenac sodium 30mg/kg, p.o. group 3 were dealt with extract (100mg/kg, p.o.). group 4 were handled with extract (200mg/kg, p.o.). The thickness was measured earlier and after injecting the formalin every day at a fixed time for seven consecutive days utilizing a vernier caliper. Phytochemical analysis revealed the presence of phenols, flavonoids, carbohydrates, and saponins. The total phenolics content of hydroalcoholic extract of *Swertia chirata* was (0.748mg/100mg), followed by flavonoids (1.087mg/100mg). This plant also exhibits better anti-inflammatory activity. From the present observation, it is evidenced that *Swertia chirata* would be an effective plant for the treatment of inflammatory reactions.

Keywords: Inflammation, *Swertia chirata*, *in vitro* antimicrobial activity, carrageenan-induced paw edema model, phytochemical analysis.

INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infections, burns, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as etiological factor for many chronic illnesses [1]. Non-steroidal anti-

inflammatory drugs (NSAIDS) are widely used in the treatment of pain and inflammation. Currently available NSAIDS are associated with unwanted side effects and have their own limitations. About 34-46% of the users of NSAIDS usually sustain some gastrointestinal damage due to inhibition of the protective cyclooxygenase enzyme in gastric mucosa [2]. Hence there is a need for anti-inflammatory drugs with fewer side effects. Plants have been an important source of medicine for 1000's of years. Herbal medicine is still the mainstay of therapy for about 75-80% of the whole population in developing countries for primary health care [3]. This is because of better cultural acceptability, affordability, better compatibility with the human body and fewer or no side effects, in addition, the last few years have seen a major increase in the use of herbal remedies in developed countries [4]. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation, has demonstrated the safety and efficient value of traditional medicine [5]. World Health Organization encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases [6]. Many Indian ethno botanic traditions propose a rich repertory of medicinal plants used by the population for the treatment, management and/or control of different types of pain [7]. However, there were not enough scientific investigations on the anti-inflammatory and analgesic activities conferred to these plants.

Swertia chirata is known as Chirayata in India. In Hindi the herb is called Chiretta and in Sanskrit it is called Bhunimba or Kirata tikata [8]. This annual herb is found in the Himalayas majorly between the heights of 1200 to 1500 meters and grows up to the height of 1.5 meters [9]. *S. chirata* is a beneficial bitter tasting tonic which is used as a laxative and also an appetizer. It corrects the nutrition disorders in the body and helps in bringing normality into the system. The herb is used widely to stimulate the appetite of people suffering from anorexia and other such problems. It helps in relieving acidity, nausea and biliousness. It used as a laxative, vermifuge, sedative and alterative. It has the properties to relieve cough, bronchial infections, malaria and asthma. The entire plant is used in medicines for over centuries [10]. Hence, in the present study, antimicrobial and anti-inflammatory activities of hydroalcoholic extract of aerial parts of *Swertia chirata* were evaluated using Carrageenan-induced paw edema model.

MATERIALS AND METHODS

Plant material

Aerial parts of *Swertia chirata* were collected from rural area of Bhopal (M.P), India in the months of December, 2019. Plant materials selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature.

Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Dried plant material was packed in air tight container and stored for Phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade. Diclofenac Sodium (Themis Pharmaceuticals, Mumbai), Carrageenan (Sigma Chemical Co, St Louis, MO, USA) were used in present study.

Extraction

The 56.7 gm of shade dried of *Swertia chirata* were coarsely powdered and proceed to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material. After that aerial parts powdered of *Swertia chirata* has been extracted with hydroalcoholic solvent (ethanol: water; 80:20) by maceration method for 48 hrs, filtered and dried using vaccum evaporator at 40°C and stored in an air tight container free from any contamination until it was used. Finally, the percentage yields were calculated of the dried extracts [11].

Qualitative phytochemical analysis of plant extract

The *Swertia chirata* extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [12-13]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, saponins, alkaloids, diterpenes and protein.

Total Phenol Determination

The total phenolic content was determined using the method of Olufunmiso et al [14]. A volume of 2 ml of extract or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total Flavonoids Determination

The total flavonoid content was determined using the method of Mishra *et al* [15]. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 10 min at room temperature;

the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

In vitro antimicrobial activity

The well diffusion method was used to determine the antimicrobial activity of the extract prepared from *Swertia chirata* using standard procedure of Bauer et al [16]. The drugs used in standard preparation were ciprofloxacin and Ofloxacin of IP grade. The antimicrobial activity was performed by using 24 hr culture of *E. Coli* and *S. Mutans*. There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antibiogram studies. Its essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculum. The plates were incubated at 37^{o}C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug. The diameter of zone of inhibition of each wall was recorded.

In vivo anti-inflammatory activity

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.

Acute oral toxicity

Healthy adult male albino rats were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the hydroalcoholic extract of *Swertia chirata* were administered to each group of rats (Each group carries 6 rats) and they were observed continuously for 1 hour and then at half-hourly intervals for 4 hours, for any gross behavioural changes and further up to 72 hours, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 425[17]. The hydroalcoholic extract of *Swertia chirata* was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for antiulcer evaluation was 100 and 200 mg/kg respectively [18].

Experimental designs

Carrageenan-induced paw edema model paw edema was induced [19] by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat. Rats were divided into four groups; each group consisting of six animals.

Experimental designs

Group –1: Carrageenan control

Group –2: Diclofenac sodium (10 mg/kg) as standard reference

Group –3: Hydroalcoholic extract of *Swertia chirata* (HESC) (100mg/kg, p.o.)

Group –4: Hydroalcoholic extract of *Swertia chirata* (HESC) (200mg/kg, p.o.)

The paw thickness was measured before injecting the carrageenan and after 60, 120, 180, 240 min. using vernier caliper. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group [20].

The percentage (%) inhibition of edema is calculated using the formula;

$$\text{Percentage Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc- Edema volume of control group

Vt- Edema volume of test group

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 DISCUSSIONS

The % yield of petroleum ether and hydroalcoholic extracts of *Swertia chirata* were 5.62 and 7.44 % w/w respectively. Preliminary phytochemical screening of hydroalcoholic extract of *Swertia chirata* revealed the presence of various components such as phenols, flavonoids, carbohydrates, and saponins and the results are summarized in Table 1. The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. TPC and TFC of hydroalcoholic extract of *Swertia chirata* were found 0.748 and 1.087 respectively. Results are provided in Table 2. Table 5 showed there is a significant ($P < 0.05$) percentage inhibition of paw edema,

at doses of 100 and 200mg/kg, respectively, at 3rd hour by Hydroalcoholic extract of *Swertia chirata* (HESC).

Table 1: Result of phytochemical screening of aerial parts of *Swertia chirata*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Hager's test	-ve
3.	**Flavonoids**	
	Lead acetate	-ve
	Alkaline test	+ve
4.	**Phenolics**	
	$Fecl_3$	+ve
5.	**Proteins**	
	Xanthoproteic test	-ve
6.	**Carbohydrates**	
	Fehling's test	+ve
7.	**Saponins**	
	Foam test	+ve
8.	**Diterpenes**	
	Copper acetate test	-ve

Table 2: Total phenolic and total flavonoid content of aerial parts of *Swertia chirata*

S. No.	Extract	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	Hydroalcoholic extract	0.748	1.087

Table 3: Antimicrobial activity of *standard drug* against selected microbes

S. No.	Name of drug	Microbes	Zone of inhibition		
			10 µg/ml	20 µg/ml	30 µg/ml
1	Ciprofloxacin	*S. Mutans*	12±0.15	15±0.13	17±0.19
2	Ofloxacin	*E. Coli*	16±0.86	21±0.57	28±0.5

Table 4: Antimicrobial activity of hydroalcoholic extract of *Swertia chirata* against selected microbes

S. No.	Name of microbes	Zone of inhibition Hydroalcoholic extract		
		25mg/ml	50 mg/ml	100mg/ml
1.	*S. Mutans*	7±0.86	9±0.47	10±0.5
2.	*E. Coli*	7±0	8±0.94	12±0.57

Table 5: Effect of Hydroalcoholic extract of *Swertia chirata* (HESC) at doses of 100 and 200 mg/kg, and diclofenac sodium as compared to carrageenan control group at different hours in carrageenan-induced paw edema model using vernier caliper

Groups	Dose of extract (mg/kg) p.o.	Change in paw thickness (mm) ± SD (% inhibition)			
		1st h	2nd h	3rd h	4th h
Carrageenan control (0.1 ml of 1% w/v)	-	1.35 ± 0.10	2.40 ±0.15	3.70± 0.147	3.30 ± 0.16
Carrageenan control (0.1 ml of 1% w/v) + Hydroalcoholic extract of *Swertia chirata* (HESC)	100	1.15 ± 0.10 (14.81%)	1.78 ± 0.20[a] (25.83%)	2.60 ± 0.106[a] (30.3%)	1.99 ± 0.10[a] (40%)
Carrageenan (0.1 ml of 1% w/v) + Hydroalcoholic extract of *Swertia chirata* (HESC)	200	1.05 ± 0.15 (22.22)%	1.60 ± 0.16[a] (33.33%)	1.89 ± 0.10[a] (49.2%)	1.50 ± 0.10[a] (55%)
Carrageenan (0.1 ml of 1% w/v) + diclofenac sodium	10	0.61 ± 0.10[a] (74%)	0.90 ± 0.121[a] (62.50%)	1.16 ± 0.15[a] (68.64%)	1.0 ± 0.15[a] (70%)

All values are expressed as mean ± SD; [a]$P < 0.05$ v/s carrageenan control

CONCLUSION

Phytochemical analysis revealed the presence of phenols, flavonoids, carbohydrates, and saponins. The total phenolics content of hydroalcoholic extract of *Swertia chirata* was (0.748mg/100mg), followed by flavonoids (1.087mg/100mg). This plant also exhibits better anti-inflammatory activity. From the present observation, it is evidenced that *Swertia chirata* would be an effective plant for the treatment of inflammatory reactions.

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