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Original Research Article

Evaluation of anti-inflammatory effects of the aqueous extract of *Stephania rotunda* in experimental animals

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ABSTRACT

Background: The objective of the current study was to analyse the anti-inflammatory effects of the aqueous extract of *Stephania rotunda* in experimental animals.
Methods: It was an experimental study conducted in the experimental laboratory with 30 acclimatized healthy albino rats and mice divided into 5 groups namely A, B, C, D, and E fed with the aqueous extract of *Stephania rotunda* in laboratory conditions to assess the anti-inflammatory property using Carrageenan induced rat paw oedema for acute inflammation, granuloma pouch for sub-acute inflammation and Formaldehyde induced arthritis for chronic inflammation from 17th December 2019 to 22nd January 2021. Aspirin was taken as the standard drug. Data was analysed using Chi-square test.
Results: In assessment for acute inflammation, the aqueous extract of *Stephania rotunda* in the doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg for groups B, C and D respectively produced 17.12%, 17.12% and 18.78% inhibition of paw oedema which was statistically significant when compared to 22.65% inhibition produced by 100mg/kg of the standard drug aspirin in group E. The groups B, C and D with the extract doses of 500mg/kg, 1000mg/kg and 2000mg/kg produced 43%, 60% and 77% inhibitions of exudate formation respectively which statistically was significant as compared to the Standard aspirin of group E which produced 62% inhibition of exudate formation. In chronic inflammation testing, both the extract and standard drugs produced highly significant inhibition of paw oedema when compared to inhibition produced by the Control.
Conclusions: The aqueous extract of *Stephania rotunda* was found to be a potent anti-inflammatory drug when compared with Aspirin. Further tests are required in a larger scale so as to ascertain the effects for human consumption.

Keywords: Aqueous, Anti-inflammatory, Acclimatized, Carrageenan, Aspirin

INTRODUCTION

The use of medicinal plants by traditional healers for various ailments is an age-old method continued by health-care providers in various parts of the world. The Indian system of medicine including Unani-Tibbi and Homeopathy is practiced particularly among the people of rural and remote places of the country.[1,2] Treatment with medicinal plants is considered very safe with no or minimal side effects. This is the fact that the use of this type of plant is considered by the ancient healers for curing a number of health-related problems and diseases. Therefore, the treatment with herbs and medicinal plants is growing in popularity across the globe.[3,4] Medicinal plants thus play a key role in health care as more than 80% of the global population depends on traditional treatment for primary health care. Despite the rapid development in the field of allopathy, the medicinal plants and their

derived products are still used in modern medicine. In India more than 7300 plant species are used in traditional health care systems for the treatment of different disorders.[5] Interestingly, the market demand for the medicinal herbs is likely to remain high because many of the active ingredients in medicinal plants cannot be prepared synthetically.[6-8] The codified herbs and medicinal plants around the world have not been screened and investigated yet for their medicinal activities and properties. Therefore, it is very important to encourage researchers and clinicians to study on properties and activities of herbs and medicinal plants along with identification of the main active ingredients that produce the desired effects.[9,10] With the above background, the medicinal plant *Stephania rotunda* of Stephania family (Menispermaceae) locally known as Ayanglei was selected for the present study.

Objectives

The present study was undertaken to investigate the anti-inflammatory (acute, sub-acute, chronic) property of the aqueous extract of the plant, *Stephania rotunda* in experimental animals.

METHODS

The present experimental study was conducted in the post graduate experimental laboratory of the Department of Pharmacology, JNIMS, Porompat, Imphal, Manipur.

Study location and duration

The whole study was conducted in the post-graduate experimental laboratory of the department of pharmacology, Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Manipur from June 2019 to March 2021.

Selection of plant materials

The fresh whole plant of *Stephania rotunda* was collected in the month of June, 2019. The plant was identified and authenticated by the department of life sciences of Manipur university (L.S.D., M.U. 000245).

Preparation of plant extract

The collected plant was cleansed with water and air-dried in shade for several days. The shaded dried plant was powdered by using a mechanical grinder. Preparation of aqueous extract was done by the method of Miranda et al.[11] The powdered material was then put into Soxhlet extractor with roughly ten times its volume of distilled water. The water was then heated to boil and subjected to extraction for 30 minutes. On evaporation of water from filtrate, a deep brown residue was obtained which was filtered, evaporated, shade-dried, scraped out, weighed and stored in glazed porcelain jar at 4^0C for future use. The aqueous thus obtained was investigated for its anti-inflammatory properties in healthy animals (albino rats).

Figure 1: Carrageenan induced paw oedema in albino rat.

Selection and grouping of animals

Health Albino rats are most commonly used because of its small size and great sensitivity to most drugs. It is also the most standardized of all laboratory animals. It can be bred to obtain pure and uniform strains and is found to be very sturdy to withstand long periods of experimentation under anaesthesia. The rodents do not vomit due to lack of vomiting center, no tonsil or gall bladder, diffuse pancreas. They are omnivorous and resemble human beings nutritionally. There are two types: Wistar rat and Sprague-Dawley rat. Wistar rat is selected for this study.[12] 30 healthy albino rats of either sex weighing 200-250 gms were recruited from the Animal House of JNIMS, Imphal, kept in the departmental polypropylene cages and acclimatized for 10 days at the departmental laboratory. The animals were fed with standard laboratory diet and water *ad libitum* and reared at 24-28^{0}C temperature with 12 hrs dark-light cycle-maintained room. The animals were fasted for 18 hrs prior to the experiment and maximum care was taken for prevention of coprophagy.

For all the tests, the animals were divided into five groups having six animals in each group and drugs and the extract were administered to the different groups. Group A: Control (2% gum acacia in distilled water 10 ml/kg), Group B: Dose 1 (500 mg/kg of extract of *Stephania rotunda*), Group C: Dose 2 (1000 mg/kg of extract of *Stephania rotunda*), Group D: Dose 3 (2000mg/mg of extract of *Stephania rotunda*) and Group E: Standard (100 mg/kg of Aspirin).

Acute toxicity testing

Acute oral toxicity study for the test extract of the plant was carried out using OECD/OCED guideline 425.[13] The test procedure minimizes the number of animals required to estimate the oral toxicity. Six healthy young adult albino rats were used for this study. Animals were fasted (water provided prior to dosing). Body weight of each animal was determined and the dose (mg/kg) was calculated according

to the body weight of the animal. The aqueous extract of *Stephania rotunda* was utilised during the experiment for the investigations of acute inflammation, sub-acute inflammation and chronic inflammation as described below.

Figure 2: Granuloma pouch formation in Albino rat.

Acute inflammation

The animals were starved overnight with water being provided *ad libitum.* Treatment groups of rats were pre-treated with the test drugs and Aspirin orally and control group received gum acacia suspension one hour before carrageenan injection Freshly prepared carrageenan (1%) in 0.9% sodium chloride solution was injected in a volume of 0.1ml into sub-plantar region of the hind paw of the rat as seen in Figure 1. The foot volume was measured in un-anaesthesized rats by a Plethysmometer immediately after and again at three hours after carrageenan injection and the 'volume of oedema' being recorded as the difference between the two readings.[14,15] The data thus obtained was analysed and the mean comparison was done using ANOVA via Dunnett's T test and p value less than 0.05 was taken to be statistically significant. The method of Marak et al was followed to study the inflammatory effect with some modification.[16] The percentage of anti-inflammatory activity was then calculated by the formula of Tewari et al and Diniz et al.[17,18]

$$Percentage\ of\ inhibition = Vc - Vt/Vc \times 100$$

Where V_c = mean increase in paw volume in control group and V_t = mean increase in paw volume in drug treated group.

Sub-acute inflammation

Rats were anaesthesized with ether and a subcutaneous dorsal pouch will be prepared in between the shoulder blades by injecting 20 ml of air. Then, 0.5 ml of turpentine oil was injected into the pouch. Treated groups of rats were treated with the test drugs. Aspirin and control group received 2% gum acacia suspension orally for 6 days beginning from the day of pouch formation as seen in Figure 2. On the seventh day, the pouch was opened under ether anaesthesia and the exudate was sucked out and the amount measured. The percentage inhibition was then calculated for the different groups of drugs as compared to the control group with reference to Tewari et al and Diniz et al.[17-19] The data thus obtained was analysed and the mean comparison was done using ANOVA via Dunnett's T test and p value less than 0.05 was taken to be statistically significant.

Figure 3: Formaldehyde arthritis in Albino rat.

Chronic inflammation

A subcutaneous injection of 0.1 ml of 2% formalin was given under the plantar aponeurosis of the right hind foot of the rat as seen in Figure 3. The paw volumes were measured plethysmometrically for 13 days to assess the degree of inflammation. Groups of animals as described before were treated with test drugs, gum acacia and Aspirin orally for 13 days. The data thus obtained was analysed and the mean comparison was done using ANOVA via Dunnett's T test and p value less than 0.05 was taken to be statistically significant.[20,21]

RESULTS

Acute toxicity test of Stephania rotunda

The result on the acute toxicity testing with the aqueous extract of *Stephania rotunda* in the experimental animals was observed and no significant effect up to the dose of 2000mg/kg body weight p.o for a period of 14 days was elicited. However, the subjective effects like nausea, headache and insomnia were not seen significantly.

Anti-inflammatory activity

Paw oedema (acute inflammation): Paw oedema was induced by injecting freshly prepared carrageenan (1%) in 0.9% sodium chloride in a volume of 0.1 ml into the sub-plantar region of the hind paw of the rat. The increase in paw volume was measured using Plethysmometer (Table 1). The mean increase in paw volume of the group A (Control) was 1.81±0.06. For group B (500 mg/kg), it was 1.50±0.07 ($p<0.01$). Then for group C (1000 mg/kg), it was 1.50±0.02 ($p<0.01$).

Table 1: Acute anti-inflammatory activity of the aqueous extract of *Stephania rotunda*.

Groups	Drug dose	Mean increase in paw volume (ml) After 3 hours	% of inhibition of paw oedema
A	10ml/kg	1.81±0.06	0
B	500	1.50±0.07*	17.12
C	1000	1.50±0.02*	17.12
D	2000	1.47±0.03*	18.78
E	100	1.40±0.01*	22.65

One way ANOVA F=10.52, df=4, 25, p<0.01, (n=6 in each group, values were mean±SEM, p<0.01)

Table 2: Sub-acute inflammation of aqueous extract of *Stephania rotunda*.

Group	Dose	Mean volume of exudate (mean±SEM) in ml	% of inhibition
A	10 ml/kg	2.25±0.13	0
B	500	1.28±0.11	43
C	1000	0.88±0.07	60
D	2000	0.51±0.06	77
E	100	0.85±0.04	62

One way ANOVA F=54.34, df=4, 25, p<0.05, (n=6 in each group, values were mean±SEM, p<0.05)

For group D (2000 mg/kg), it was 1.47±0.03 (p<0.01) and finally for group E (Standard aspirin 100 mg/kg), it was 1.40±0.01 (p<0.01). The aqueous extract of *Stephania rotunda* in the doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg produced 17.12%, 17.12% and 18.78% inhibition of paw oedema when compared to 22.65% inhibition produced by 100mg/kg of the standard drug aspirin. The increase in the doses of the extract produced increase in the percentage of inhibition of paw oedema. Both the extract and standard drug produced highly significant (p<0.01) inhibition of paw oedema in comparison with the inhibition produced by the Control group.

Granuloma formation (Sub-acute inflammation): A subcutaneous dorsal pouch was prepared in between the shoulder blades by injecting 20 ml of air. 0.5 ml of turpentine oil was then injected again in the same pouch so as to induce sub-acute inflammation. The animals were observed for 6 days after which the exudates were sucked out from the pouch and measured (Table 2). The volume of exudates in the group A (Control) was 2.25±0.13 (p<0.05). For group B, it was 1.28±0.11 (p<0.05). Then for group C, it was 0.88±0.07 (p<0.05). For group D, it was 0.51±0.06 (p<0.05) and finally for GROUP E it was 0.85±0.04 (p<0.05). The groups B, C and D with the extract doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg produced 43%, 60% and 77% inhibitions of exudate formation respectively as compared to the Standard aspirin of group E which produced 62% inhibition of exudate formation.

Table 3: Anti-inflammatory activity of the aqueous extract of *Stephania rotunda*.

Group	Dose	Mean increase in paw volume (ml)				
		3rd	5th	9th	11th	13th
A	10 ml/kg	0.52±0.01	0.56±0.01	0.62±0.02	0.64±0.01	0.64±0.02
B	500	0.37±0.01*	0.34±0.01*	0.23±0.2*	0.20±0.02*	0.18±0.02*
C	1000	0.56±0.02	0.49±0.01	0.35±0.01*	0.29±0.01*	0.22±0.01*
D	2000	0.51±0.03	0.43±0.02*	0.29±0.02*	0.21±0.01*	0.23±0.00*
E	100	0.41±0.01*	0.37±0.03*	0.21±0.01*	0.16±0.01*	0.10±0.01*

One way ANOVA F=13.13, 14.25, 60.09, 118.39, 14.81 df=4, 25, p<0.05.

The increasing dose of the extract produced increased inhibition of exudate formation. Both the extract and the standard drug produced significant inhibition (p<0.05) of exudate formation in comparison to the inhibition produced by the group A which was the control group.

Development of arthritis (Chronic inflammation)

Arthritis was induced by injecting 0.1ml of 2% formalin under the plantar aponeurosis of the right hind foot of the rat. The paw volume was measured using Plethysmometer for 13 days. The findings are shown in the (Table 3). The mean increase in the paw volumes is as: group A (Control) were 0.52±0.01, 0.56±0.01, 0.62±0.02, 0.64±0.01, 0.64±0.02 on the 3rd, 5th, 9th, 11th and 13th day respectively. On pre-treatment with the aqueous extract of *Stephania rotunda*, the mean increase in paw volume for the 3rd, 5th, 9th, 11th and 13th days for the different groups were: group B: 0.37±0.01 (p<0.05), 0.34±0.01 (p<0.05), 0.23±0.2 (p<0.05), 0.20±0.02 (p<0.05) and 0.18±0.02 (p<0.05) respectively. Group C: 0.56±0.02, 0.49±0.01, 0.35±0.01 (p<0.05), 0.29±0.01 (p<0.05) and 0.22±0.01 (p<0.05) respectively. Group D: 0.51±0.03 (p<0.05), 0.43±0.02 (p<0.05), 0.29±0.02 (p<0.05), 0.21±0.01 (p<0.05) and 0.23±0.00 (p<0.05) respectively. Aspirin (100 mg/kg) pre-medication showed mean increase in paw volume of 0.41±0.01 (p<0.05), 0.37±0.03 (p<0.05), 0.21±0.01 (p<0.05), 0.16±0.01 (p<0.05) and 0.10±0.01 (p<0.05) on the 3rd, 5th, 9th, 11th and 13th days respectively. Both the extract and standard drugs produced highly significant inhibition of paw oedema when compared to inhibition produced by the Control. The result shows that the Control group and the aqueous extract of *Stephania rotunda* produced significant reduction in the paw volume.

DISCUSSION

Stephania species particularly rotunda and its extract are used for many ailments and also as an analgesic and anti-inflammatory agent for the pain, wound, fever, headache, arthritis etc. The present study of the extract of *Stephania rotunda* was focused on its anti-inflammatory activities. The anti-inflammatory activity of the extract was investigated on the status of acute, sub-acute and chronic nature. The carrageenan induced paw oedema in albino rats was the standard model for testing of anti-inflammatory drugs. The standard drug which was used in this study was Aspirin (100 mg/kg). The elevated mean of the paw volume of the control was 1.81±0.06. The doses 500 mg/kg, 1000 mg/kg and 2000 mg/kg of the extract produced 17.12%, 17.12% and 18.78% inhibition of paw oedema ($p<0.01$) when compared to the inhibition 22.65% ($p<0.01$) produced by Aspirin (100 mg/kg). Aspirin was found more effective than the extract in the inhibition of paw oedema. The mean increased in paw volume (ml±SD) of the standard drug was 1.40±0.01. These findings are in line with those of Ahmed et al, Udegbunam et al.[22,23] Carrageenan induced oedema is a biphasic response. The 1st phase is mediated through the release of histamine, 5-HT and Bradykinin; the 2nd phase is with the release of PGs and slow reacting substances.[24,25] It is suggested that the anti-inflammatory activity of the extract of *Stephania rotunda* is due to the inhibition of the mediators of inflammatory action. The experiment producing the granuloma pouch was a model of Sub-acute type of inflammation. The mean volume of exudate in the control group was 2.25±0.13. The doses of the extract 500 mg/kg, 1000 mg/kg and 2000 mg/kg and Aspirin (100 mg /kg) produced 43%, 60%, 77% and 62% inhibition of exudate formation respectively. The mean volume of exudate of the standard Aspirin was 0.85±0.04. Therefore, the different doses of the extract produced highly significant inhibition ($p<0.05$) of exudate which was greater than or similar to that of the standard. The paw oedema following Arthritis with formaldehyde was then to be measured as paw volume. The mean of the paw volume which was increased among the control group was from 0.52±0.01 on 3rd day to 0.64±0.02 on the 13th day. The extract with the doses 500 mg/kg, 1000 mg/kg and 2000 mg/kg produced the changes in the paw volume from 0.37±0.01, 0.56±0.02 and 0.51±0.03 to 0.18±0.02, 0.22±0.01 and 0.23±0.11 respectively from 3rd to 13th day.

Aspirin in the dose of 100 mg/kg also produced the changes of paw volume from 0.41±0.01 on 3rd day and 0.10±0.01on 13th day. It is observed that the extract with doses 500 mg/kg, 1000 mg/kg and 2000 mg/kg produced significant inhibition of paw oedema induced by formaldehyde. The dose 2000 mg/kg of the extract was observed to produce similar inhibition and efficacy to that of the dose (100 mg/kg) of aspirin. The findings are supported by the results of Shan et al and Fan et al.[26,27] Many previous investigators induced arthritis with formalin as an accepted model for evaluation of Anti-Arthritic and Anti-Inflammatory agents with probable anti-proliferative activity. This is associated with two phases -early neurogenic component followed by tissue mediated response.[28-30] However further studies are needed to isolate the active compound responsible for the observed effect and also to establish the possible mechanism of action responsible for the anti-inflammatory activity of the selected plant *Stephania rotunda*.

Limitations

The present study could not isolate the active component responsible for eliciting the anti-inflammatory effects of *Stephania rotunda.* Also, subjective findings like nausea and vomiting could not be elicited. Hence, further prospective study assessing the pharmacokinetic and pharmacodynamic propereties of *Stephania rotunda* is essential.

CONCLUSION

In a nutshell, the present study which was carried out in the experimental animals for evaluating anti-inflammatory of the aqueous extract of *Stephania rotunda* yielded positive findings to the claims of having significant anti-inflammatory properties which was also supported by the findings of other investigators in different settings. With more precise studies, the principal active constituent of *Stephania rotunda* could be isolated and made available for human consumption in the long run with hopefully lesser side-effects.

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