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A study of the analgesic activity 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 analgesic property of the aqueous extract of *Stephania rotunda* in experimental animals.

Methods: It was an experimental study conducted in the experimental laboratory with 30 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 analgesic property using thermal (Tail-flick) method and chemical (writhing test) method from 26st December 2019 up to 22nd February 2021. Aspirin was taken as the standard drug. Data was analysed using chi-square test.

Results: In the assessment of analgesic property using thermal (tail-flick) method, the aqueous extract of *Stephania rotunda* in the doses of 500mg/kg, 1000mg/kg and 2000mg/kg for Group B, C and D respectively produced dose dependent increase in reaction time at various intervals which was statistically significant. In the chemical (writhing test) method, the extract in the doses of 500mg/kg, 1000mg/kg and 2000mg/kg for the GROUPS B, C and D produced the percentages of inhibition 27%, 39% and 44% respectively for the writhing movements which was also statistically significant.

Conclusion: The aqueous extract of *Stephania rotunda* was found to be a potent analgesic drug when compared to Aspirin. Further tests are required on a larger scale to ascertain the pharmacokinetic and pharmacodynamic effects for human consumption.

Keywords: Aqueous, Analgesic, Acclimatized, Aspirin, Thermal, Writhing

Introduction

Medicinal plants have been associated with human race since time immemorial and its use for treating various ailments is still continued by health-care providers in various parts around the globe. Unani-Tibbi and Homeopathy is practiced particularly among the people of rural and remote places of the country.[1,2]

Treating various ailments with medicinal plant is considered very safe with no or minimal side effects. Therefore, the treatment with herbs and medicinal plant 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 depend on traditional treatment for the primary health care. Despite the current globalization in all spheres including the field of allopathy, 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 treatment

of different disorders .[5] Interestingly, the market demand for the medicinal herbs is likely to remain high as many of the active ingredients in medicinal plants cannot be prepared synthetically till now .[6,7,8]

According to WHO, it has been documented that medicinal plants would be the best source to get a variety of drugs. Following which, about 80% of people of the developed countries consider medicinal plants as their primary need for their day-to-day health care.[9]

AYUSH (Ayurveda, Yoga, Naturopathy, Unani, Siddha and Homeopathy) system in India codified 8000 (approx.) herbal remedies for the health care providers. Among the systems, Ayurveda and Unani medicines are the most developed system in India. Multiple factors viz., increased population, inadequate supply of drugs, prohibitive cost of treatment, side effects of several synthetic drugs and development of resistance to currently used drugs have led to an increased emphasis on the use of plant materials as a source of medicine for a wide variety of human ailments.[10]

Hence, it is very important to encourage the researchers and clinicians to study on the properties and activities of the herbs and medicinal plants along with identification of the main active ingredients which produce the desired effects.[11]

Thus, the medicinal plant of Stephania family (Menispermaceae) locally known as Ayanglei in Manipur was selected from among the currently used medicinal plants for the present study.

Objective:

The present study was undertaken to evaluate the analgesic property of the aqueous extract of the plant, *Stephania rotunda* in experimental animals.

Materials And Methods

The study was conducted in the post graduate experimental laboratory of the Department of Pharmacology, Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Porompat, Imphal, Manipur.

Ethical approval of the Institutional Animal Ethics Committee (RIMS) was taken for the study (Registration no. 1596/GO/a/12/CPCSEA dated 12/12/2019).

Materials

1. Dried *Stephania rotunda* plant(whole)
2. Soxhlet apparatus
3. Distilled water
4. Albino rats
5. Albino mice
6. Carrageenan
7. Needle with syringe
8. 2% gum acacia
9. Aspirin
10. Acetic acid
11. Analgesio meter
12. Beaker

Methods

Study Location And Duration:

The whole study was conducted in the post-graduate experimental laboratory of the department of Pharmacology, JNIMS, Porompat, Imphal, 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 (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 using a mechanical grinder. Preparation of aqueous extract was done by the method of Miranda et al.[12] 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 analgesic property in healthy animals (albino mice and rats).

Selection and grouping of animals:

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Health Albino rats and mice are most commonly used because of their small size and great sensitivity to a wide variety of drugs. They are the most standardized of all laboratory animals, can be bred to obtain pure and uniform strains and are found to be very sturdy to withstand long periods of experimentation under anaesthesia. The rodents do not vomit due to lack of vomiting centre. They are also deficit in tonsil or gall bladder and diffuse pancreas. They are omnivorous and resemble human beings nutritionally. There are two types: Wistar rat and Sprague-Dawley rat. Wistar rat was selected for this study. 30 healthy albino rats of either sex weighing 200-250 gms and 30 healthy albino mice of weight 25-30gms were recruited from the Animal House of JNIMS, Imphal and kept in the departmental polypropylene cages and acclimatized for 10 days at the departmental laboratory. The animals were fed with standard laboratory diet, 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 to which drugs and the extract were administered.

1. GROUP A Control (2% gum acacia in distilled water 10ml/kg)
2. GROUP B Dose 1(500mg/kg of extract of *Stephania rotunda*)
3. GROUP C Dose 2(1000mg/kg of extract of *Stephania rotunda*)
4. GROUP D Dose 3(2000mg/mg of extract of *Stephania rotunda*)
5. GROUP E Standard (100mg/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]*(OECD guidelines for the testing of chemicals (Acute oral toxicity – up and down procedure). Adopted 23rd march 2006. [Cited 2008 Mar 20]; Available from: URL: www.oecd.org.)*. 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 accordingly.

The aqueous extract of *Stephania rotunda* was utilised during the experiment for the investigations of the analgesic effect as described below:

1) Thermal method:

Tail-Flick method (Chakraborty et al[14])

Albino rat with normal reaction time of 3-4sec was used and drugs autoclaved at 121^{0}C for 30 mins were then administered intraperitonially with the volume kept constant at 1ml/kg body weight of the animals. The current intensity passing through the naked nichrome wire was maintained at 6 Ampere, the distance between the heat source and the tail skin at 1.5 cm and the site of application of the radiant heat in the tails at 2.5cm measured from the root of the tail for all the rats as shown in figure-1. The time taken by the animal to withdraw (flick) the tail from the hot wire was taken as the reaction time and was assessed by an Analgesio meter. The cut-off reaction time was fixed at 10 sec to avoid any tissue damage. The reaction time was noted at 30,60 and 120 minutes aftert the drug administration.

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Figure-1: Tail-flick method

2) Chemical method:

Acetic acid induced writhing test (Saha A et al[15])

Albino mice of either sex weighing 25-30 gms were screened (animals which failed to exhibit writhing within 10 mins was discarded) and drugs was suspended in 2% gum acacia and administered orally. The volume of the medicaments was kept constant at 10ml/kg body weight of the animals. Writhing was induced 20 mins later in each mouse by intraperitoneal (I.P) administration of 10ml/kg body weight of 3% acetic acid in distilled water. Each mouse was put into a beaker and total number of writhes for the treated animals for 20 minutes was counted starting after 5 minutes of injection. For scoring, a writhe was indicated by stretching the abdomen with simultaneous stretching of at least one hind limb. Effects derived from the drug-treated animals were compared with an equal number of Controls

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.

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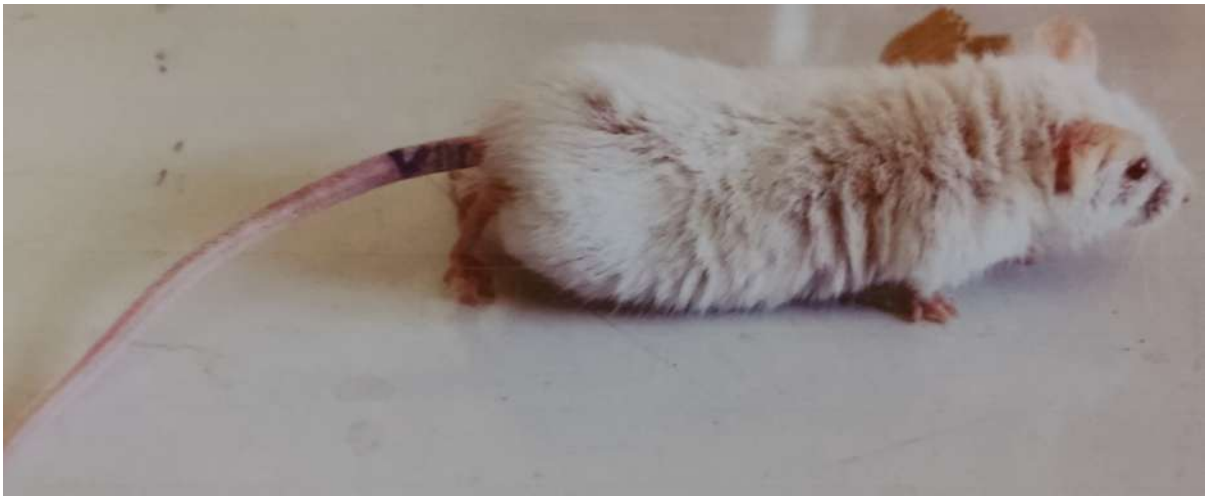
Figure-2: Acetic Acid Induced Writhing Of Albino Mice



The percentage of protection at each dose level was calculated as follows

% protection= (1-No. of writhes in treated group/ No. of writhes in Control group) × 100

Results And Observations

The results and observations of the present study are described as below:

A. Acute toxicity test of *Stephania rotunda*:

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

B. Analgesic Activity:

1. Thermal Method: Tail-Flick Method

The tail flick method was selected for the present study as it is the standard and well-known method which has been adopted by various investigators and researchers in the past. The findings are presented in table-1.

Table-1. Analgesic effect of the aqueous extract of *Stephania rotunda*

GROUP	DRUG DOSE (mg/kg, p.o)	PRE-DRUG REACTION TIME(SECONDS)	REACTION TIME (IN SECONDS) AFTER TREATMENT		
			30 MINS	60 MINS	120 MINS
A	10ml/kg	3.55 ± 0.12	3.6 ± 0.12	3.58 ± 0.18	3.43 ± 0.17
B	500	4.63 ± 0.20	6.13 ± 0.09*	7.26 ± 0.15*	7.70 ± 0.08*
C	1000	4.27 ± 0.15	7.46 ± 0.16*	7.66 ± 0.19*	8.20 ± 0.05*
D	2000	3.75 ± 0.18	7.90 ± 0.10*	8.86 ± 0.09*	9.20 ± 0.11*
E	100	3.63 ± 0.15	8.03 ± 0.12*	8.90 ± 0.23*	9.16 ± 0.28*

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ONE WAY ANOVA	F	7.82	217.84	147.21	214.86
	df	4,25	4,25	4,25	4,25
	*	p<0.05			

(n=6 in each group, values are Mean ± SEM, p<0.0001

The reaction time in this investigation method was calculated in seconds. (Time period between current/heat transfer to tail and withdrawal of tail)

The observation showed that there was no significant difference between the mean pre-drug reaction time of the different groups (p>0.05). 30 mins after the injection of various drugs, there was significant increase in reaction time for the test and standard drugs when compared to the pre-drug reaction time.

The mean reaction time in seconds for GROUP B in 30 mins, 60 mins and 120 mins after consumption of the extract in the dose of 500mg/kg were 6.13±0.09(p<0.05), 7.26±0.15(p<0.05) and 7.70±0.08(p<0.05) respectively.

For GROUP C after consumption of the extract with the dose of 1000mg/kg, the mean reaction time in seconds in 30 mins, 60 mins and 120 mins were 7.46±0.16(p<0.05),7.66±0.19(p<0.05) and 8.20±0.05(p<0.05) respectively.

The mean reaction time in seconds for GROUP D after consumption of the extract with the dose of 2000mg/kg in 30 mins, 60 mins and 120 mins were 7.90±0.10(p<0.05), 8.86±0.09(p<0.05) and 9.20±0.11(p<0.05) respectively.

The mean reaction time in seconds for the standard drug ASPIRIN i.e., GROUP E (100mg/kg) in 30 mins, 60 mins and 120 mins were 8.03±0.12(p<0.05), 8.90±0.23(p<0.05) and 9.16±0.28(p<0.05) respectively.

It is observed that the aqueous extract of *Stephania rotunda* in the doses of 500mg/kg, 1000mg/kg and 2000mg/kg produced dose dependent increase in reaction time at various intervals of the observations.

2. Chemical Method: Writhing Test

Writhing test was performed by administering acetic acid I.P in albino mice. The resultant findings are shown in table-2. The analgesic activity was investigated by observing the number of writhes with the various treated groups of the experimental animals.

Table-2: Analgesic effect of aqueous extract of *Stephania rotunda* on acetic acid induce writhing test in albino mice.

GROUP	DRUG DOSE (mg/kg) p.o	NO. OF WRITHING MOVEMENT (MEAN±SEM)	%OF PROTECTION
A	10 ml/kg	9.17±0.30	0
B	500	6.67±0.33*	27
C	1000	5.67±0.33*	39
D	2000	5.17±0.30*	44
E	100	4.67±0.33*	49

ONE WAY ANOVA	F	30.39	
	df	4,25	

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* P<0.05

(n=6 in each group, values were Mean ± SEM, p<0.05)

The mean number of writhing movements in the Group A(Control) was 9.17±0.30. For GROUP B, it was 6.67±0.33 (P<0.05). Then for GROUP C, it was 5.67±0.33 (P<0.05). For GROUP D, it was 5.17±0.30 (P<0.05) and finally for that of the GROUP E with the standard drug ASPIRIN, it was 4.67±0.33 (P<0.05). The extract in the doses of 500mg/kg, 1000mg/kg and 2000mg/kg for the GROUPS B, C and D produced the percentages of inhibition 27%, 39% and 44% respectively for the writhing movements.

The standard drug Aspirin in the dose of 100mg/kg produced 49% inhibition of writhing movement. The number of writhing was significantly produced in both the extract and standard group in comparison to the control group.

Discussion:

Stephania species particularly Rotunda and its extracts are used as an analgesic for pain, wound, fever, headache, arthritis etc. The present study of the extract of Stephania rotunda was focused on its analgesic activities.

Two tests i.e., Tail flick for screening of centrally acting analgesic and Acetic acid induced writhing test for screening of centrally and peripherally acting analgesic was carried out.

The tail flick test is the standard test for evaluating analgesic activity of drug in experimental animals like albino rats. In this investigation, the number of tail flicks was counted for 10 seconds and the reaction time was recorded at 30, 60 and 120 mins after the administration of the extract. Aspirin was used as the standard drug. There was significant difference between the pre drug reaction time of different group. The reaction time of 3-4 second was considered as normal reaction time and the reaction time was recorded at 30mins after the extract and standard were administered. The doses of the extract 500mg/kg, 1000mg/kg and 2000mg/kg were able to increase the pain threshold significantly (p<0.05) at the interval of 30 mins of administration in a dose dependent manner when compared to the pain threshold produced by the control group at the respective time interval. The standard drug aspirin (100mg/kg) increased the pain threshold significance at the interval of 30 mins, 60 mins and 120 mins of observations. Such type of observations was reported by previous investigators Mao Hsien Wang et al[16] and Deepak Kumar Semwal et al[17]. Therefore, the results of the present study support their findings.

The increase in pain threshold may be due to the association of the effect of enzyme COX-1 and 2 as the analgesic action of the Standard aspirin goes through COX-1 and COX-2 and involvement of the varieties of PGs indicating the central and peripheral action.[18]

The observations on writhing test with acetic acid showed that the aqueous extract of *Stephania rotunda* produced significant decrease in the number of writhes in a dose dependant manner. The doses of the extract when given orally exhibited the decrease in writhing movement by 27%, 39% and 44% respectively while the standard drug aspirin has shown an inhibition of 49%. The number of writhing movements during 20 min of observation in the control group was 9.17±0.30 per min. The observations were almost similar to the findings of M Habibur Rahman et al[19], Bishwajit Bokshi et al[20] and S.M.Naim Uddin et al[21].

The extract when given orally at the doses of 500mg/kg (p<0.0001) 1000mg /kg (p<0.0001)) 2000mg/kg (p<0.0001) significantly inhibited the acetic acid induced writhing in mice. The writhing paradigm following acetic acid is produced with the localised inflammatory response through the release of free Arachidonic acid from tissue phospholipid via COX-1 and COX-2. This therefore causes inflammation by increasing capillary permeability. Any substance which can inhibit writhing will have analgesic effect preferably by inhibition of PG synthesis. The abdominal constriction of the animal is related to sensitisation of nociceptive receptors.[22,23]

The IP acetic acid can produce an abdominal writhing response due to sensitization of chemo-sensitive nociceptive by PGs[24]. The suggested mechanism of action of the extract as an analgesic agent is either due to action on visceral receptor sensitive to acetic acid inhibition of production of allogeneic substance or inhibition of the release of

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neurotransmitter at the central level for transmission of painful messages.

The present study can suggest the probable MOA of the extract as an analgesic agent through peripheral and central neurotransmitter and autacoids.

Limitations:

The present study could not isolate the active component responsible for eliciting the analgesic effects of the aqueous of *Stephania rotunda*. Subjective effects like nausea and vomiting could not be demonstrated. Hence, further prospective studies for accounting the pharmacokinetic and pharmacodynamic properties of *Stephania rotunda* is required.

Conclusion:

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

Ethical approval: The study was approved by the Institutional Ethics Committee vide Registration no. 1596/GO/a/12/CPCSEA dated 12/12/2019.

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