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Analgesic Effects of the Aqueous Extracts of Plant *Ipomea pes-tigridis* Studied in Albino Mice.

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Abstract: *Ipomoea pes-tigridis* , is a herb traditionally used by the tribes of kerala as a single drug remedy to treat painful conditions like headache etc, swellings, poisonous stings and snake bites. The ethanolic extract of *Ipomoea pes-tigridis,* Family: (Convolvulaceae) was evaluated for analgesic property using plate reaction time. *I. pes-tigridis* extract showed a dose dependent significant reduction of the number of writhes ($P<0.05$) with 100 mg/kg body weight dose giving the highest reduction. The extract showed an insignificant elongation of the hot plate reaction time ($P>0.05$).The preliminary studies shows that the ethanolic leaf extract of *I. pes-tigridis* has significant analgesic activity and needs further investigations.

Key words : *Ipomoea pes-tigridis* · Analgesic Activity · Tail Flick Response Time · Reaction Time.

INTRODUCTION

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs present well known side and toxic effects. Moreover synthetic drugs are very expensive to develop since, for the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested where the cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs [1]. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [2, 3].

Ipomoea pes-tigridis, is a herb traditionally used by the tribes of kerala as a single drug remedy to treat painful conditions like headache etc, swellings, poisonous stings and snake bites[4]. *Ipomoea pes-tigridis* is considered to be the plant source for the ayurvedic drug "Vyaghrapada" mentioned in the Ayurvedic text. Thus the traditional use of this plant for medicinal purpose has evoked renewed interest in the plant. However, no scientific data is available to validate the traditional claim. Therefore, the study was undertaken to evaluate the analgesic and anti-inflammatory activities using acetic acid induced writhing and radiant heat tail-flick test in mice models.

MATERIALS AND METHODS

The whole plants used for this study were collected from the Veli hills region of Kanya Kumari District, Tamil Nadu and was identified with the authentic specimens maintained as Voucher specimen collections maintained at Department of Botany, Government Arts College, Salem, Tamil Nadu, India. The shade-dried plants were reduced to all pieces and kept in desiccators until they were used for extraction.

The shade-dried leaves were reduced to powdery form and 200 g of the powdered sample was extracted with Ethanol (analytical grade) for 3 days. The macerated mixture was filtered and evaporated in a carefully regulated water bath maintained at 45°C to yield a green solid extract weighing 8 g. The extract was stored in a refrigerator at 4°C and dilutions of the extract were made in normal saline to determine their effects.

The dried fine powder of *I. pes-tigridis* was mixed with 10 times its quantity of sterile distilled water in a round-bottomed flask and the suspension was kept at 4°C for 72 h. The aqueous extract was decanted, clarified by filtration through a muslin cloth and evaporated in a flat-bottomed porcelain dish at 40°C. The dried extract was again suspended in polyethylene glycol (20% v/v) and

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distilled water evaporated to get the final concentrate. Appropriate dilutions were made to prepare lower doses for administration according to the body weight of mice.

Tail Flick Response Method: The analgesic activity was determined by radiant heat tail-flick method in mice. Mice are held in suitable restrainer with tail protruding out. Radiant heat is applied over the tail on a spot with the help of suitable device such as analgesiometre. The time taken by the animal to withdraw the (flick) tail is taken as reaction time. Tail-flick latency was assessed by the analgesiometer (Inco, India). The strength of the current passing through the naked nicrome wire was kept constant at 5 ampere. The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. Cut-off reaction time was 10 sec to avoid any tissue injury during the process.

Standard Drug: Paracetamol was used as the standard drug .400mg tablets of paracetamol was powdered and dissolved in 40ml of distilled water in a conical flask, so that 1.0ml of the solution contains 10mg of paracetamol. Paracetamol was administered in the dose of 0.1ml per 100gm body weight of albino mice in group I. The drug paracetamol used as a control for tail flick experiment is administered orally with the help of a cannula.

Selection of Animals: For tail flick experiment 15 albino mice weighing between 150-250 gms were selected and grouped into three, each group consisting of five mice. Before selecting animals the basal reaction time was taken by placing the tip of the tail on the radiant heat source. Normally a mouse withdraws its tail within 3-5 seconds [flicking response]. Any animal failing to withdraw its tail in 3-5 seconds is rejected from the study. All the mice subjected to experimentation were marked for their individual identification.

METHOD

An analgesiometer was used to record the flicking time of tail [reaction time] of the animals using the heated nichrome wire as the source of heat stimulus. At a certain point the tail of each rat is marked with ink. Prior to experiment the initial reaction times of all the mice were noted for three times with an interval of 15 minutes. Those animals failed to show any response were eliminated from the experiment.

Then the animals of each group were administered with the respective drugs orally. The initial tail flicking time of each group was considered as the control readings.

Group I was taken as the standard group and the standard drug paracetamol was given to these animals orally. Group II received the test drug [ethanol extract] and the Group III received the test drug [aqueous extract]. The doses were determined on the basis of body weight of the animals. Thus the animals received 0.26ml, 0.20ml, 0.17ml, 0.26ml as per 250gm, 200gm, 200gm, 175gm and 250gm body weight of albino mice respectively. Then the tail flicking time or reaction time of each group was recorded at intervals of 30 minutes till 180 minutes.

The time was noted down when the rat tried to escape the heat stimulus by flicking its tail. The time interval between the onset of stimulus and the flicking time was taken as the reaction time.

Acetic Acid - Induced Writhing in Mice: For the mouse writhing assay mice were divided into five groups comprising of five mice per group. Writhings were induced by the method of Koster *et al.* [5]. The test groups were administered 50, 100and 200 mg/kg of *I. pes-tigridis* extracts i.p, while the control group received 0.3ml normal saline. The reference group received 100 mg/kg Aspirin, i.p. The animals were fasted for 16 h prior to the treatments. One hour after treatment, the mice were injected i.p with 0.3 ml of 6 % acetic acid solution to induce the writhing. The number of abdominal constrictions (writhing) and stretching with a jerk of the hind limb was counted between 5 and 15 minutes after acetic acid injection. The response of the extract and aspirin treated groups were compared with those of the animals in the control group (0.3ml saline). Percentage protection against writhing movement (% inhibition of writhing) was taken as an index of analgesia and it was calculated as follows:

$$\text{Percentage Inhibition} = \frac{\text{Wt (Control)} - \text{Wt (test group)}}{\text{Wt (Control)}}$$

Wt = Mean number of writhing

RESULTS

The tail flicking time before administering the drug and that at thirty- minute intervals after administering it, were recorded up to 180 minutes.

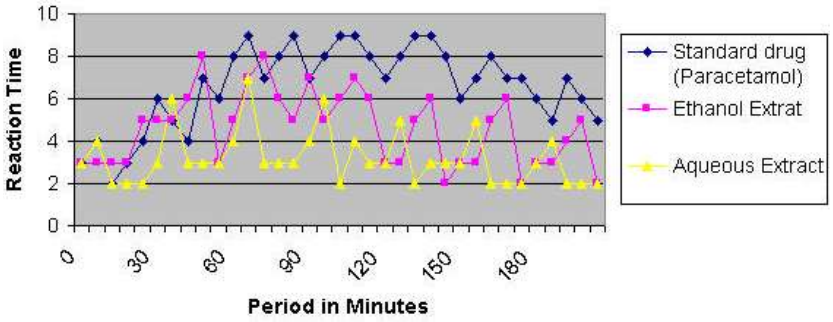

Fig. 1: Reaction Time of standard drug (paracetamol), Ethanol and Aquoeus extracts of plant Ipomea pes-tigridis

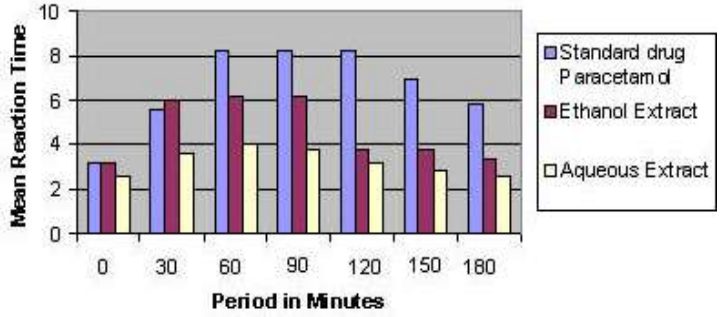

Fig. 2: comparasion of RT of ethanol and aqueous extract of plant with standard drug (paracetamol)

In the standard group [group-I] the mean initial tail flicking time [IFT] was 3.2 seconds. The R.T increased gradually, after administration of the drug and a mean of 8.2 seconds was reached at the end of 120 minutes, which was maintained through 180 minutes. Thereafter RT receded gradually and the values fluctuated around 5 seconds at the end of 180 minutes. Fig. 1

In the group II where the test drug [ethanol extract] was given the mean IFT was 3.2 seconds. After administration of the drug the mean RT gradually increased to reach a maximum mean value of 6.2 seconds at the end of 90 minutes and then decreased gradually to reach a mean of 3.4 seconds at the end of 180 minutes Fig.1

In the group III where the test drug [aqueous extract] was given the mean IFT was 2.6 seconds. After the administration of the test drug the mean RT gradually increased to reach a maximum of 4 seconds at the end of 60 minutes and the gradually decreased to reach 2.6 seconds at the end of 180 minutes Fig. 1.

Statistical analysis of the data obtained in this study show the following results. In group I the mean IFT of albino mice were computed before starting the experiment and at 30 minutes intervals after the administration of the drug till 180 minutes. The IFT before the administration of drug demonstrated a mean increase of 2.4 seconds. The student -t test showed that the difference is statistically significant ($P < 0.01$).The reaction time (RT) increased to 8.2 at 60 minutes after administration of the drug , the mean increase being 5 seconds ($P < 0.001$).The mean increase in RT remained constant for a short time and showed a declining trend thereafter.. By 180 minutes the reaction time came down to 5.8 seconds. However invariably in all cases, the mean increase recorded was highly significant statistically Table 1.

Animals of group II showed a mean IFT of 3.2 seconds initially. After administration of drug, the mean RT showed an ascending trend. The mean RT was highest 6.2 at 60 and 90 minutes interval and later it showed a declining trend Statistical analysis showed that the increase was highly significant ($P < 0.001$) till 90 minutes. The result shows that the effect of test drug (ethanol extract) in a dose of 0.1 ml/ 100gm body weight of albino mice is equivalent to that of standard drug paracetamol. Table 1.

In group III (aqueous extract) the mean IFT was recorded as 2.6 seconds. The mean RT showed a slight increase after the oral administration of the drug. The mean RT was found to be higher at the end of 60 minutes ($P < 0.5$), but showed a sudden decline thereafter. Further increase in time minimized the effect of drug and the mean RT is not at all statistically significant Table 1.

Table 1: Table showing the increase in Reaction Time of Albino Mice of Group I (Standard Drug) (G-I), Group-II (Ethanol Extract)(G-II) and Group-II (Aqueous Extract) (G-III)

	Mean R.T								
	IFT			After administration of drug			MeanIncrease in R.T		
Period in Minutes	G-I	G- II	G-III	G-I	G- II	G-III	G-I	G- II	G-III
30	3.2	3.2	2.6	5.6 ±1.14	6.0± 2.12	3.6±1.34	2.4	2.8*	1.0
60	3.2	3.2	2.6	8.2 ±0.83	6.2± 1.30	4±1.73	5	3*	1.4
90	3.2	3.2	2.6	8.2 ±0.83	6.2± 1.30	3.8±1.3	5	3*	1.2
120	3.2	3.2	2.6	8.2±0.83	3.8±1.64	3.2±1.09	5	0.6	0.6
150	3.2	3.2	2.6	7 ±0.70	3.8±1.64	2.8±1.60	3.8	0.6	0.2
118	3.2	3.2	2.6	5.8 ±0.83	3.8± 0.92	2.6±1.70	2.6	0.2	

*P<0.001

Table 2: The effect of *I. pes-tigridis extract* on acetic acid - induced writhings in mice

Treatment group	No of writhes(per 30 min)	Percentageinhibition
Control (0.3ml normalsaline)	65.0 ± 1.34	--
I. pes-tigridis (50 mg/kgb.w)	29.0 ± 1.41*	55.38
I. pes-tigridis (100mg/kg b.w)	26.2 ± .49*	59.69
I. pes-tigridis (200mg/kg b.w)	24.4 ± 3.1	62.4
Aspirin (100 mg/kg b.w)	30.2 ± 1.8*	53.53

Values are mean number of writhes ± SEM. (n = 5 per group).
* *P<0.05* significantly different from control group.

Comparisons of Group I , Ii & Iii: Comparison of increase in the mean tail flicking time of Albino Mice of Group II and Group III with the standard drug at 30 minute intervals after the oral administration of test drug provides with the following results. At 30 minute intervals after administration of drug, the mean increase of RT in group I and group II was 2.4 seconds and 2.8 seconds respectively. The differences between the readings of these two groups are not statistically significant till the end of 90 minutes. In other words, though there was slight improvement in RT numerically in the standard group compared to group II till 90 minutes and it was only due to sampling variation and in fact the effect of test drug is very much identical till 90 minutes. However after the time duration group II showed less effectiveness in the mean RT. The difference in the readings between these two groups was statistically significant ($P < 0.001$). Therefore the study shows that the effective dose of the alcohol extract is similar in its effect to the effect of paracetamol for a period of 90 minutes. Comparison of mean RT values of standard drug , ehtanol extract and aqueous extracts were depicted in Fig.2.

Acetic Acid Induced Writhing Assay: The effect of *I. pes-tigridis extract* on acetic acid - induced writhings in mice is presented in Table 1. In acetic acid induced writhing model the extract showed 55.38 , 59.69 and 62.04 % inhibition of writhing response at 50, 100 mg and 200 mg/kg, respectively. The results were found to be highly significant ($P <0.001$) in comparison to the control.

DISCUSSION

Pain is a condition which is regularly dealt with in daily clinical practice. Hence, any attempt to contribute an easily available analgesic drug from the available flora is always accepted without any reluctance. *Ipomea pes-tigridis* has been traditionally used by the tribals of middle kerala to cure specific ailments. This attempt is to prove the efficacy of the plant extract as a potential analgesic drug and to demonstrated a positive result. Search for safe herbal remedies with potent antipyretic activity received momentum recently as the available antipyretic, such as paracetamol, aspirin, nimusulide etc. have toxic effect to the various organs of the body [6].

The result obtained from using the models show that *Ipomea pes-tigridis* extract can effectively reduce inflammation.The analgesic properties were also studied using sensitive models that could provide different grades of noxious stimuli (in thermal stimulus and chemically induced tissue damage). In the present study the thermal test was selected because of several advantages including the sensitivity to strong analgesics and limited tissue damage. Furthermore, the acetic acid induced writhing test was selected because of several advantages including the ability to mimic human clinical pain conditions, sensitivity to mild analgesics, production of tonic stimulus and sensitivity to non-steroidal anti-inflammatory drugs [7-10]. The observed effects in this study (Tables 1 and 2) have shown that *Ipomea pes-tigridis* can significantly inhibit the responses to thermal

stimulus and acid induced writhing. Although the inhibition of formalin-induced pain was dose-dependent and the effect of thermal stimulus was not dose-dependent. However, it is not unusual for variation of this nature to occur. For example there are reports where responses to acid induced writhing test were strong while there were no significant responses to thermal test [9, 11].

CONCLUSION

Studies show that the *Ipomea pes-tigridis* extract is capable of inhibiting non-inflammatory reactions as well as inflammatory pain. The clinical applications of these findings must await further studies. Although the mechani involved was not determined in the present study. This is likely to be the focus of the forthcoming studies. Pharmacodynamics studies should be undertaken to establish the mechani of action of the plant extracts. Phytochemical investigation is also proposed in order to isolate the active fraction and eventually the pure compound.

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