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EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OFAQUEOUS AND ALCOHOLIC EXTRACTS OF LEAVES OF *Epipremnum aureum.linn* IN RATS

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ABSTRACT

Background: *Epipremnum aureum* (Family Araceae) commonly known as Money plant is a vigorously growing in India. It is a common indoor plant generally used for ornamental purposes having indoor air pollution removing capacity. The preliminary phytochemical studies of the aqueous and alcoholic extracts of the leaves showed the presence of various phytochemical constituents such as alkaloids, tannins, flavonoids, triterpenoids, and saponins. **Aims and Objectives:** The aim of the present study was carried out with the objective of phytochemical screening and to evaluate the anti-inflammatory and analgesic of aqueous and alcoholic extract of *E.aureum* in albino rats. **Materials and Methods:** Albino Wistar rats (100–150 g body weight) were used in this study. Aqueous and alcoholic extract of *Epipremnum aureum* was used to evaluate acute anti-inflammatory and analgesic activity by plethysmometer and hot plate method by oral administration at doses of 200 mg/kg body weight in healthy albino rats. **Results:** In acute studies, the aqueous and alcoholic extract showed anti-inflammatory activity by significant reduction in the paw edema volume, and significantly increased the latency of paw licking in hot plate method in a dose-dependent manner when compared with the control and standard drug. Statistical analysis was carried out by one-way ANOVA test. **Conclusion:** Thus, the positive results suggest that *Epipremnum aureum* extracts should be further studied to determine the bioactive chemical compounds as well as to understand the possible mechanism of action and evaluate their toxicity looking towards pharmaceutical actions.

KEY WORDS: *Epipremnum aureum*, Anti-inflammatory; Analgesic; Carrageenan -induced Paw Edema, Hot plate.

INTRODUCTION:

Epipremnum comprises 15 species of slender to gigantic root-climbing Iianes[1]. All these herbaceous evergreens are native to South East Asia and Solomon islands[2]. Variegated clones of *E. aureum* are extremely popular as cultivated plants worldwide, perhaps constituting the most commonly cultivated aroid, and the golden variegated form of this species is frequently met with as an escape from horticulture throughout the tropics[3]. Plants used for interiors cape purposes such as pedestal plants, totems, hanging baskets, dish gardens and small desk plants usually have heart- shaped leaves that rarely exceed 6 inches in length[4].

Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammatory diseases are very common throughout the world. Rheumatoid arthritis is one of the oldest known diseases of mankind affecting the majority of population no substantial progress has been made in achieving a permanent cure and different types of rheumatic diseases are a major cause of morbidity of the working force. [5]Inflammation results in the liberation of endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc. These mediators even in small quantities can elicit pain response. Anti-inflammatory drugs make up about half of analgesics, relieving pain by reducing inflammation as opposed to opioids which affect the central nervous system[6]. Based on phytochemical investigation presence of Saponins and flavonoids are the constituents which are used for and other complication by providing anti-inflammatory activity[7].

Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause[8]. Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect new compounds with improved pain management capacity and fewer side effects are being sought with urgency[9]. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine. However, scientific evaluation is needed to provide evidences of their safety and efficacy. Based on phytochemical screening *E.aureum* leaves of both aqueous and alcoholic extracts shows the presence of some flavonoids and terpenoids are used for the investigation of analgesic activity hot-plate method.[10]

MATERIALS AND METHODS

Plant Material Collection

The leaves of E. aureum were collected from the Geethanjali College in the month of December. The taxonomic identification of the plant was confirmed and processed for further investigations. The leaves were cleaned, reduced to small fragments, air dried under shade at room temperature, and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

Drugs and Chemicals

Drugs and chemicals used in this study were of analytical grade and highest purity procured from standard commercial sources in India. carrageenan (Essel Fine Chem, Mumbai), diclofenac sodium (Ranbaxy laboratories Ltd, New Delhi.), Alcohol (ChangshuYangyuan Chemicals, China).

Experimental animals

Healthy adult albino Wistar rats, weighing 200-250 g of either sex, were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. Animals were housed within the departmental animal house, and the room temperature was maintained at 27°C. Animal studies had the approval of the Institutional Animal Ethics Committee (IAEC) of the committee for the purpose of control and supervision on experiments on animal [11] (CPCSEA) (1648/PO/a/12/CPCSEA/IAEC/06).

Preparation of Plant Extract

Preparation of Aqueous Extract:

Fresh leaves of *E.aureum* were collected and washed under tap water. The leaf extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of distilled water. The contents were mixed well and then the mixture was boiled upto 80-100^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities[12].

Preparation of Alcoholic Extract:

Fresh leaves of *E.aureum* were collected and washed under tap water. The leaf extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of alcohol. The contents were mixed well and then the mixture was boiled upto 50-60^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities[12].

Preliminary phytochemical analysis of the extracts

Both the aqueous and alcoholic extracts of *E. aureum* were subjected to preliminary phytochemical screening.[13]

Selection of dose for animal study

The dose considered for the experiment on rats was obtained from conversion of human dose of *E. aureum* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats (Ghosh 1984). Hence, the calculated dose for the rats (considering human dose 5 g/kg) is 200 mg/kg. Thus, analgesic and anti-inflammatory activity was done at dose of 200 mg/kg body weight. Acute toxicity was done at dose of 2000 mg/kg body weight[14].

Acute oral toxicity

The acute oral toxicity of aqueous and alcoholic extracts of *E. aureum* was determined using Albino Wistar rats (100-150 g), which were maintained under standard conditions. The animals were fasted 12 hr before the experiment, up and down procedure OECD Guideline No. 423.[15]

Assessment of Anti-Inflammatory Activity in Rats

Carrageenan-Induced Paw Edema[16]

Carrageenan-induced paw edema is a suitable experimental animal model for evaluating an anti edematous effect. Edema developed following injection of carrageenan serves as an index of acute inflammatory changes, was and can be determined from differences in the paw volume measured immediately after carrageenan injection and then every hour for 6 hours. Edema induced by carrageenan is believed to be biphasic: the first phase (1 h) involves the release of serotonin and histamine and the second phase (over 1 h) is mediated by prostaglandins, cyclooxygenase products. Continuity between the two phases is provided by kinins.

Animals:

Albino wistar rats (100-150 g) were divided into four groups containing of 3 animals of each group-1 received standard drug, group-2 received test drug aqueous extract of *E.aureum* and group-3 received test drug alcoholic extract of *E.aureum* and they were housed under standard conditions of temperature (25±2ºC) and 12 hr/12 hr light/dark cycles. The animals were kept under laboratory conditions for one week before start of the experiments and allowed food and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical committee.

Procedure:

The anti-inflammatory activity was determined using a carrageenan-induced paw edema model, rats (100-150 g) either sex, were randomly divided into 4 groups and fasted overnight before the experiment with free access to water. Treatments administered at their body weight to rats for one hour before subcutaneous injection of carrageenan (1% in NSS) into the plantar surface of the left hind paw.

After the carrageenan injection, the paw volumes were measured at 15, 30, 60&120min using a Plethysmometer (Dolphin, India). The difference between the intial and subsequent readings gave the actual edema volume Edema was expressed as the mean increase in paw volume relative to control animals. The percentage inhibition of edema was calculated by the following equation:

$$\% \text{ inhibition of edema} = 100\ (1-Vt/Vc),$$

Where Vc is the edema volume in the control group and Vt is the edema volume in tested group.

Assessment of Analgesic Activity in Rats

Hot Plate Method[17]

Animals

Albino wistar rats (100-150 g) were divided into four groups containing of 3 animals of each group-1 received standard drug, group-2 received test drug aqueous extract of *E.aureum* and group-3 received test drug alcoholic extract of *E.aureum* and they were housed under standard conditions of temperature (25±2ºC) and 12

hr/12 hr light/dark cycles. The animals were kept under laboratory conditions for one week before start of the experiments and allowed food and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical committee.

Procedure

Take the basal reaction-time by observing hind paw licking or jumping response in animals when placed on the hot-plate maintained at constant temperature (55^0C). Normally animals show such response in 6-8 sec. a cut-off period of 15sec i.e observed to avoid damage to the paws. Inject the standard drug to group-2 and test drugs to group-3&4 and note the reaction time of animals on the hot plate at 15, 30, 60 and 120min after the drug administration. Calculate the percentage increase in reaction time at each time interval.

RESULTS

Preliminary phytochemical analysis of the extracts

Phytochemical investigation of aqueous and alcoholic extracts of E. aureum revealed the presence of alkaloids, tannins, saponins, terpenoids, and flavonoids as secondary metabolites.

Acute toxicity testing

Acute toxicity studies revealed that the aqueous and alcoholic extracts of E.aureum were safe up to 2000 mg/kg of body weight, and approximate LD50 is more than 2000 mg/kg. No lethality or any toxic reactions were observed up to the end of the study period. This is not surprising as E. aureum is used as a decoration plant.

CARRAGENAN INDUCED PAW OEDEMA IN RATS

In carrageenan induced paw oedema activity, the paw volumes and percentage of inhibition of the control, standard and test compounds are shown in Table No: 1. The tests compounds are compared with diclofenac as a standard at a dose of 40mg/kg for anti-inflammatory activity. Presently diclofenac showed inhibition of inflammation at 2 hours when compared to control.

Aqueous and Alcoholic extracts of *Epipremnum aureum.linn* leaves (200 mg/kg) shown significant inhibition of inflammation at 2 hours when compared with control. The results of test compounds were found to be statistically significant at value$P<0.05$.

Table No: 1. Effect of extracts of *E.aureum* on paw oedema volume.

GROUPS	Dose (mg/kg)	Change in paw volume (ml) mean±SEM & Percentage inhibition									
		0min		15min		30 min		60 min		120min	
		R	L	R	L	R	L	R	L	R	L
Control	--	0.2± 0.1	0.3± 0.2	0.2± 0.1	0.5± 0.3	0.2± 0.1	0.3± 0.2	0.2± 0.1	0.3± 0.1	0.2± 0.1	0.3± 0.2

Std (Diclofenac Sodium)	**10**	0.2± 0.1	0.3± 0.2	0.2± 0.1	0.4± 0.2	0.2± 0.1	0.3± 0.1	0.2± 0.1	0.3± 0.1	0.2± 0.1	0.2± 0.1
AQEEa	**200**	0.3± 0.1	0.5± 0.2	0.3± 0.2	0.5± 0.2	0.2± 0.1	0.4± 0.2	0.1± 0.2	0.3± 0.1	0.2± 0.1	0.2± 0.1
ALEEa	**200**	0.2± 0.1	0.3± 0.1	0.2± 0.1	0.3± 0.2	-	0.2± 0.1	0.2± 0.1	0.2± 0.1	0.1± 0.2	0.2± 0.1

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

Hot plate method:

The analgesic activity of AQEEa and ALEEa was assessed using Hot plate method in Swiss albino rats were illustrated in Table No: 2. AQEEa and ALEEa showed significant analgesic activity at 200 mg/kg, i.p. Analgesic activity was comparable with the standard drug Diclofenac. Both two doses of 200mg/kg showed maximum analgesic activity at reaction time is slightly lower than the standard drug Diclofenac.

Table No: 14. Effect of extracts of *E.aureum* on Analgesic activity.

Groups	**Dose**	**Basal reaction time(sec)**		**After drug administration(sec)**							
		0min		**15min**		**30min**		**60min**		**120min**	
		Paw licking(P)	**Jumping(J)**	**P**	**J**	**P**	**J**	**P**	**J**	**P**	**J**
Control	**10**	-	9	4	6	5	3	3	-	5	-
Standard	**40**	9	-	10	-	6	-	14	-	-	10
AQEEa	**200**	14	-	5	-	12	-	8	-	12	-
ALEEa	**200**	12	10	4	10	-	10	-	12	-	5

The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

DISCUSSION:

The phytoconstituents are known to play an important role in bioactivity of medicinal plants. In qualitative phytochemical analysis reveals the presence of alkaloids, flavonoids, tannins, terpenoids and saponins have associated with various degree of anti-microbial, anti-bacterial, anti-fungal, anti-oxidant and anti-termites. Therefore, anti-inflammatory and analgesic activities were observed in this study may be due to the presence of chemical constituents in both aqueous and alcoholic extracts of *Epipremnum aureum.*

It is believed that current anti-inflammatory drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases because of their side effects and low potency. As a result, search for other alternatives became necessary and imperative. Therefore, the present study was aimed at evaluating the scientific basis for the traditional use of *Epipremnum aureum* leaves using carragenan induced rat paw edema for anti-inflammatory models. Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. Carrageenan induced rat paw edema is a suitable model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation. Carrageenan-induced hind paw edema in rat is a biphasic event. The early phase (90 - 180 min) of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase (270– 360 min) is associated with the activation of kinin-like substances, i.e., prostaglandins, proteases and lysosome. The aqueous and alcoholic extracts of *Epipremnum aureum* leaves inhibited the carrageenan induced rat paw edema formation, at both early and later phase. This result tends to suggest that the inhibitory effect of the extract on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibitors due to its COX-dependent mechanism; thus, it is suggested that the AQEEa and ALEEa may possess arachidonate COX inhibitory property.

The extracts increased reaction latency to thermal pain induced by the hot plate method in rats, which is a specific central antinociceptive test. Inhibition of histamine or kinin pathway may reduce pain. The results of the present study also showed that extract exhibited a comparable magnitude of antinociceptive activity in hot plate method of pain which suggested that the phytochemical constituents are responsible for the analgesic effect. The analgesic activity of some flavonoids and terpenoids already has been reported suggesting that these or similar constituents may be responsible for the analgesic effect of the extract. The results of the present study indicated that the aqueous and alcoholic extracts of *E.aureum* might contain constituents capable of relieving or modifying responses to pain caused by either thermal or chemical stimulation of the nociceptors mediated by both central and peripheral mechanisms.

CONCLUSION

E.aureum is a natural plant and it has anti-termites, anti-bacterial, anti-microbial, anti-fungal and anti-oxidant activities. The phyto-chemical constituents present in the leaves of two different extracts of *E.aureum* may vary. Among these studies it could be concluded that leaves of *E.aureum* have shown great potential of anti-inflammatory and Analgesic activity. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific drugs. The pharmacological activities of the present studies support the folkoric usage of plant and suggest that *E.aureum* extracts may also posses antipsychotic, hypolipidemia, anti-convulsants etc., can be studied further. we have concluded that, in this study, both aqueous and alcoholic extract of *E.aureum* (200mg/kg, p. o.) significantly reduced edema induced by carrageenan in all the phases. aqueous and alcoholic extract of *E.aureum* significantly increased the latency of paw licking in hot plate method.

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