EVALUATION OF LOCOMOTOR AND DIURETIC ACTIVITIES OF AQUEOUS AND ALCOHOLIC EXTRACTS OF LEAVES OF EPIPREMNUM AUREUM L.

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ABSTRACT

The aim of the present study was to evaluate the locomotor and diuretic activities of both aqueous and alcoholic extracts of leaves of Epipremnum aureum (AQEEa and ALEEa) in experimental animals. Locomotor activity was evaluated by actophotometer and diuretic activity was evaluated using Lipschitz test model in rats. Preliminary phytochemical screening showed the presence of alkaloids, flavonoids, terpenoids and saponins. Intra peritoneal administration of AQEEa and ALEEa at doses 200mg/kg produced significant (P< 0.01) CNS depression by reducing locomotor activity while it showed a significant (P< 0.01) increase in the urine volume when compared to control. Thus, in conclusion the AQEEa and ALEEa have a significant CNS depression activity by reducing locomotor and diuretic action in rats.

Keywords: Epipremnum aureum, Locomotor activity, Diuretic activity, Actophotometer, Lipschitz test.

INTRODUCTION

Epipremnum aureum commonly known as the Golden Pothis, Devil’s Ivy, money plant, silver vine, taro vine etc. Epipremnum aureum is a scrambler shrub and it can climb by means of aerial roots over the trees and plants1. It belongs to family Araceae found throughout the World. This plant removes the indoor pollutants such as formaldehyde, Xylene and benzene2. E. aureum leaf extracts has anti-microbial, anti-oxidant and anti-termites activities3. In general, E. aureum contains the alkaloids, flavonoids, terpenoids, saponins, phenolic and tannins. So, the purpose of the present study was to evaluate the locomotor and diuretic activity of both aqueous and alcoholic extracts of E. aureum leaves.

Anxiety is an extremely dramatic and debilitating multifaceted disorder and it is now becoming clear that without knowledge of clinical and biological aspects of anxiety and depression, it is impossible to offer effective treatment strategies for the patients. Over the past decades, there has been intensive study of a variety of neurobiological aspects of anxiety4. Currently the most widely prescribed medications for anxiety disorders are benzodiazepines. But the clinical applications of benzodiazepines as anxiolytics are limited by their unwanted side effects. Therefore, the development of new pharmacological agents from plant sources is well justified5. The use of herbal medications by physicians in Europe and Asia is becoming more common and researchers are exploring the traditional remedies to find a suitable cure for this mind affecting diseases6.

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug that induced diuresis is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxaemia7. Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue and weakness. Naturally occurring diuretics include caffeine in coffee, tea, and cola, which inhibit Na+ reabsorption and alcohol in beer, wine and mixed drinks, which inhibit secretion of ADH8.9. Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure10. Herbal medicines derived from the plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. A bibliographic survey showed that there are no systematic studies have been reported for locomotor and diuretic activities of E. aureum. This prompted us to investigate the effects of pharmacological activities of E. aureum in experimental model of animals.

MATERIALS AND METHODS

Plant Material Collection
The leaves of Epipremnum aureum was collected from the Geethanjali College in the month of December and was identified and authenticated from Department of pharmacognosy GCOP. The plant material was 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.

Preparation of extracts

Preparation of Aqueous Extract of E. aureum leaves
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 up to 80-100°C for 4-5 hrs. Further the extract was filtered with Whatmatt filter paper. The yield of alcoholic extract was obtained as 5.5% w/w. Moreover, the extract was subjected to preliminary phytochemical screening for the detection of various plant constituents.

Preparation of Alcoholic Extract of *E. aureum* leaves:
Fresh leaves of *E. aureum* were collected and washed under tap water. The leaf extract was prepared by taking 20 gms of finely cut leaves into 250 ml beaker containing 200 ml of alcohol. The contents were mixed well and then the mixture was boiled up to 50-60°C for 4-5 hrs. Further the extract was filtered with Whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The yield of alcoholic extract was obtained as 4.5% w/w. Moreover, the extract was subjected to preliminary phytochemical screening for the detection of various plant constituents.

Phytochemical analysis
Both the aqueous and alcoholic extracts of *Epipremnum aureum* were subjected to preliminary phytochemical screening.

Experimental animals
Healthy adult albino wistar rats weighing 200-250 grams 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. They were fasted overnight before the day of experiment, after 72 hours of fasting from the day of Alloxan introduction. Animals were housed within the departmental animal house and the room temperature was maintained at 27°C. Animal studies had approval of IAEC (CPCSEA) (1648/PO/a/12/CPCSEA/IAEC/05).

Acute Toxicity Studies
The acute oral toxicity of aqueous and alcoholic extracts of *Epipremnum aureum* was determined by using Albino wistar rats (200-250 g) which were maintained under standard conditions. The animals were fasted 12 hours prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract upto 2000 mg/kg and observed for its mortality during 2 days and 7 days study period (short term) toxicity and observed up to 7 days for their mortality, behavioral and neurological profiles.

Assessment of Locomotor Activity in Rats
The locomotor activity can be easily studied with the help of actophotometer. Male Swiss albino rats weighing between 150-200 g were divided into four groups, each group comprising of six animals. Each animal was placed individually and the basal activity score of all the animals were recorded after 30 min of drug treatment. The activity on each mouse was retested for 10 min. The difference in the activity was recorded considering before treatment values and after treatment values. Finally, percentage decrease in locomotor activity was calculated.

Assessment of Diuretic Activity in Rats
The method of Lipschitz was employed for the evaluation of diuretic activity. The male albino rats weighing about 150-200 g were divided into four groups of six rats in each and were fasted and deprived of food and water for 18 hrs prior to the experiment. On the day of experiment, the Group I animals serving as control, received normal saline (25 ml/kg, i.p.); the Group II animals received aqueous extract (200 mg/kg, i.p.); Group III animals also received alcoholic extract (200 mg/kg, i.p.) and the Group IV animals received Furosemide (20 mg/kg, i.p.), in normal saline. Immediately after the administration the animals were kept in metabolic cages (3 per cage) specially designed to separate urine and fecal matter and kept at room temperature (25 ± 0.5°C) throughout the experiment. The total volume of urine was collected at the end of 5 hrs after dosing. During this period no water and food was made available to animals.

Statistical analysis
All data were expressed as Mean ± S.E.M. The results were analyzed statistically by one-way ANOVA followed by Dunnett’s multiple comparisons test. The results obtained were compared with the vehicle control group. *p* < 0.01 was considered to be statistically significant.

RESULTS

Preliminary Phytochemical studies
Preliminary phytochemical screening of the *Epipremnum aureum* revealed the presence of alkaloids, flavonoids, phenolic compounds, glycosides, tannins and saponins.

Acute toxicity studies
There was no mortality amongst the graded dose in groups of animals and they did not show any toxicity or behavioral changes at a dose level of 2000 mg/kg. This finding suggests that AQEEa and ALEEa were safe in or non-toxic to mice up to 2000 mg/kg. Hence, in our study 200 mg/kg doses of extract were selected.

Locomotor activity
The average actophotometer reading in the control group was 245 ± 4.0, after administration of AQEEa and ALEEa 200 mg/kg after 30 min significantly reduced the locomotor activity to 79 ± 3.0 and 69 ± 2.0 respectively when compared to standard 85 ± 5.0. It may be due to the CNS depressant property of the drug (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Locomotor activity (scores) in 10 min</th>
<th>% change in activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>245±4.0</td>
<td>245±4.0</td>
</tr>
<tr>
<td>Standard</td>
<td>30</td>
<td>270±5.0</td>
<td>85±5.0</td>
</tr>
<tr>
<td>AQEEa</td>
<td>200</td>
<td>365±2.0</td>
<td>79±3.0</td>
</tr>
<tr>
<td>ALEEa</td>
<td>200</td>
<td>286±1.0</td>
<td>69±2.0</td>
</tr>
</tbody>
</table>

The results are expressed as means ± S.E.M n=5. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as *p* < 0.01.

Diuretic activity
In this results both the AQEEa and ALEEa (200 mg/kg) showed a significant increase in urine volume (P<0.01) as compared to control. The standard drug (Furosemide) also showed highly significant effect when compared to control. In the evaluation of diuretic activity, Urea treated rats showed a significant effect increase in volume of urine and urinary excretion of sodium, potassium, chloride (P<0.01) as compared with control. AQEEa and ALEEa (200 mg/kg) showed significant change in urine excretion but effective in increasing the sodium ions and much less effect as diuresis and evaluated study is listed in table no.2. All the tests showed significant Lipschitz values listed in table 3.
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. In the present study, no mortality case was observed up to the dose of 2000 mg/ kg of AQEEa and ALEEa (i.p.). Therefore, it may suggest that the extract has no lethal toxicity in rats. Locomotor activity is considered as an index of alertness and can be helpful to confirm the general depressive activity of any drug. However, in the present study the AQEEa and ALEEa was found to have decreased effect on the locomotor activity in actophotometer. The decrease in motor activity gives an indication of the level of excitability of the CNS and this decrease may be related to sedation resulting from depression of CNS. It has been found that flavonoids isolated from plant species such as Eupipremnum aureum showed antidepressant activity. It is reported that GABA, an inhibitory neurotransmitter is involved in the pathophysiology of depression. Moreover, neurochemical research has revealed that the monoamines (5-HT, NA, and dopamine) have a crucial role in the development of the depression syndrome. Thus, it is likely that flavonoids present in AQEEa and ALEEa may be responsible for the observed antidepressant effect. Moreover, triterpenoids (steroidal compounds) are present in the leaves, those are able to cross blood brain barrier (BBB) due to their lipophilic nature and so it can be assumed that such steroidal compounds might also be responsible to elicit antidepressant and other neuropharmacological activities at molecular level in CNS (brain).

The results showed that both aqueous and alcoholic extracts, increases urine output as compared to control group. The diuretic action of furosemide, aquareoside and aquareosidic extract was found to be 5.8, 5.5 and 5.1 respectively as compared with control group. The results show that AQEEa and ALEEa affects urinary electrolyte. In addition, there was no alkalization of urine. But in this study both urinary Na+ and K+ level was increased without any alteration in Na+/K+ ratio. The extracts were also unlikely to be acting as thiazide diuretics: these only increase urinary K+ level and alter the urinary Na+/K+ ratio. Further the urine was slightly acidified. In contrast, the diuresis induced by AQEEa and ALEEa were similar to that of furosemide and accompanied by marked increases in both urinary Na+ and K+ level. These characteristics strongly suggest these extracts are acting as loop diuretic. Loop diuretics inhibit the Na+/K+/Cl- co-transporter system in the thick ascending loop of nephron, thereby increasing natriuresis and k alumu and also cause acidification of urine.

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