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Toxicological Investigation of Ethanolic Extract of *Epipremnum aureum* in Rodents

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ABSTRACT

The present study was aimed to explore the acute and sub chronic toxicity studies with orally administered ethanolic leave extract of *Epipremnum aureum*. For the acute toxicity study, the animals were divided into four groups and each group receives a dose of (50, 500, 2000) mg/kg except control group which receives only 1% CMC. They were observed for 14days for signs of toxicity. In case of sub chronic toxicity, the Sprague dawley rats were fed with ethanol extract (100, 600, and 1000) mg/kg per day for 28 days. The parameters measured include organ weight, biochemical test, haematological test and histopathological observations. Acute oral administration of *Epipremnum aureum* did not show any mortality, CNS and ANS toxicities. Similarly in subchronic toxicity studies, *Epipremnum aureum* did not show any visible signs of toxicity. There were also no significant differences between the control and extract treated groups in terms of their organ weight, haematological and biochemical parameters. Histopathological examination did not reveal any remarkable and treatment related changes. A no-observed adverse-effect level for extract is 2000 mg/kg for rats under the conditions of this study. Hence, the extracts could be considered safe at the doses administered since they did not provoke toxic effect on the key organs examined and also did not alter any biochemical and haematological parameters.

INTRODUCTION

Since the beginning of the human race plants were the preferable source of medicines. Medicinal plants are often used without satisfactory demonstration of their pharmacological activities. Moreover, many people believe that traditional medicines have no adverse effects. During the past few years it is observed that, the adverse effects of phytomedicines, as well as its adulteration, toxicity, and drug interaction are common problems related to public health (Saude-Guimaraes *et al.*, 2012). The recognization and acceptability of herbal medicines has been limited due to lack of defined chemical characterization, dose regimen and adequate toxicity data to determine their safety (Gautam *et al*., 2014). The indiscriminate increase in the use of plant extract is further aggravated by the belief that herbs are safe simply because they are natural in origin. Bioactives derived from plant acts as defence mechanism but at the same time can be toxic in nature which may not compensate the therapeutic index. According to available data many plants are regarded as toxic due to the presence of some nonliving inclusions for example raphides and calcium oxalate crystals. The presence of these substances causes irritations and vomiting and stomach upset. *Epipremnum aureum* belonging to the family Araceae is commonly known as money plant having indoor air pollution removing capacity. Araceae is a large family comprising of many therapeutically active medicinal plants. This plant is widely known in Malaysia and Singapore and has a reputation as a traditional anticancer preparation as well as a remedy for skin diseases (Chan *et al*., 1998). A decoction of the fresh leaves with meat or eggs or as tea was reported to be a common practice among the locals. Aerial roots and leaves of *Epipremnum aureum* show great potential for antimicrobial activity (Srivasatava *et al*., 2011). The phytochemical investigation of *Epipremnum aureum* reveals the presence of potent phytoconstituents which are responsible for the major pharmacological activities. It is reported that *Epipremnum aureum* is considered toxic due to the presence of oxalates which causes irritations in animals. As to the best of our knowledge, there is no reference about the toxicological profile of *Epipremnum aureum* in traditional medicine so it was considered worthwhile to

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evaluate the acute and sub chronic toxicity (biochemical, haematological and histopathological) toxicity studies in rodents respectively with ethanolic extract of *Epipremnum aureum* extract with the aim to obtain information and guidance for selecting a safe dosage.

MATERIALS AND METHODS

Collection and authentication of Plant

The fresh whole plant of *Epipremnum aureum* was collected from Kepong district, Malaysia. The plant was identified by Miss Tan Ai Lee, Research officer, Natural products, Forest Research Institute Malaysia. The voucher specimen (No. SBID: 001/15) was prepared and deposited in the Faculty of Pharmacy, Lincoln University College, Malaysia for imminent reference.

Preparation of Ethanol extract

The authenticated leaves were washed with fresh water and dried under shade of sunlight for 5 days. The dried plant leaves were coarsely powdered with the help of mechanical grinder. The powder was stored in an airtight container for further use. The ethanolic extract was provided by the method of hot percolation using soxhlet apparatus and 90% ethanol. After completion of extraction the resulting extract was concentrated using rotary evaporator and stored in desiccator (Pandey *et al*., 2011).

Animal used

Adult Sprague dawley rats (125-130 g) were selected for study. They were kept in the departmental animal house at 26±2°C and relative humidity 44-56%, light and dark cycles of 12hr respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 24 hr before the experiment, though water was allowed ad libitum. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, Lincoln University College, Malaysia.

Acute toxicity

The acute oral toxicity of the *Epipremnum aureum* ethanol extract was evaluated in Sprague dawley rats as per the OECD guideline 423 (OECD, 2001). Four group of rats of both sexes equal in number (n = 6) which have been fasting overnight. The control animals received 1% Carboxymethyl Cellulose (CMC) suspension in distilled water and extract was administered in CMC suspension at doses 50, 500 and 2000 mg/kg. All of the experimental animals were maintained under close observation for any signs of toxicity and mortality immediately after dosing, at 4 h and at 24 h and intervals, and twice daily for 14 days. Additional observations include change in skin and fur, eyes and mucous membranes and also somatomotor activity and behaviour pattern. Attentions were given to observations of tremors, colonic convulsions, salivation, diarrhoea, sleep and coma and death.

Sub-Chronic toxicity

The Sprague dawley rats of either sex weighing between 125-130 g were used for this study and evaluation of sub-acute toxicity was performed as the methods described by OECD guidelines 407 (OECD, 1995). The animals were permitted free access to standard pelleted food and water ad libitum. The animals were grouped into four groups of 10 animals (either sex) each. The Group I rats served as control, Group II, III and IV received *Epipremnum aureum* extract at doses of 100, 600 and 1000 mg/kg respectively for 28 days. Toxic manifestations and mortality were monitored daily for 28 days. At the end of the experiment, all the rats were anaesthetized using ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively) and blood samples were collected via cardiac puncture (Manaharan *et al*., 2014). The collected blood samples were assayed for biochemical and haematological parameters. All the animals were sacrificed by cervical dislocation. The vital organs mainly liver, kidney, heart and pancreas was removed, cleaned with saline, weighed and preserved in 10% formalin for further histopathology observation.

Observation

Clinical signs were observed at least once a day through the 28 days of dosing. Body weight, food intake and weight gain were measured once a week. Sensory and motor activity was observed during the 4th week of dosing for all the groups. Motor activity was measured by using animal actophotometer.

Effects on vital organs

Following the sacrifice, qualitative data on the weights of vital organs (heart, lungs, liver, kidneys and testes) were assessed by carefully dissecting each organ from sacrificed animal into 10 % formal saline contained in a Petri dish. The isolated organs were dried with cotton wool and weighed on a sensitive balance. Each weighed organ was standardized for 100 g body weight of each rat (Mbaka *et al*., 2010).

Biochemical and haematological analysis

Blood analysis was performed to determine both biochemical and haematological parameters. After 28 days of extract administration, the rats were fasted overnight prior to blood collection. On the 29th day, after overnight fast, the animals were sacrificed under mild diethyl ether anaesthesia and blood was obtained via cardiac puncture into fluoride oxalate, heparinized and EDTA containers. The blood collected with fluoride oxalate tube was centrifuged within 5 min of collection at 4000 g for 10 min and plasma obtained was used to determine the blood glucose level. The heparinized blood was used for a haematological study which included Haemoglobin concentration (Hb), Packed cell volume (PVC), Red blood cell count (RBC), White blood cells (WBC), platelets (PLT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), differential WBC count Neutrophils (N), Lymphocytes (L), Eosinophils (E), Monocytes (M)] Lipoprotein (HDL-cholesterol) levels and other biochemical

parameters were estimated with heparinized blood using precipitation and modified enzymatic procedures from Sigma Diagnostics (Ogbonnia *et al*., 2010). The non-heparinized blood was allowed to coagulate before being centrifuged and the serum separated. The serum was assayed for Total protein, albumin, globulin, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, urea nitrogen, urea, creatinine, total billirubin, calcium, phosphorus, sodium and potassium.

Histopathological observation

Small blocks of tissues were collected from the harvested organs. The specimens for histopathology were fixed in 10% neutral, buffered formalin for 18 h at 4°C. The fixed tissues were then dehydrated with 100% ethanol solution and embedded in paraffin. In each specimen of liver, kidney, heart, lung and stomach, 3-4 μm in thickness were obtained and stained with Hemotoxylin and eosin stains (Chang *et al*., 2015). The stained sections were examined under microscope for any cellular damage or change in morphology of that particular tissue.

Statistical analysis

All data are expressed as mean ± SD. Each value represents a minimum of ten rats (n = 10) in a group. Data were analyzed using Student's paired t-test and one-way ANOVA (SPSS version 16). A $p < 0.05$ was considered statistically significant.

RESULTS

Body weight measurement

The body weight of rats were measured weekly until the end of acute and sub chronic toxicity studies. There were no significant ($p > 0.05$) differences in the body weight changes between the control and *Epipremnum aureum* ethanol extract treated rats as shown in Fig.1

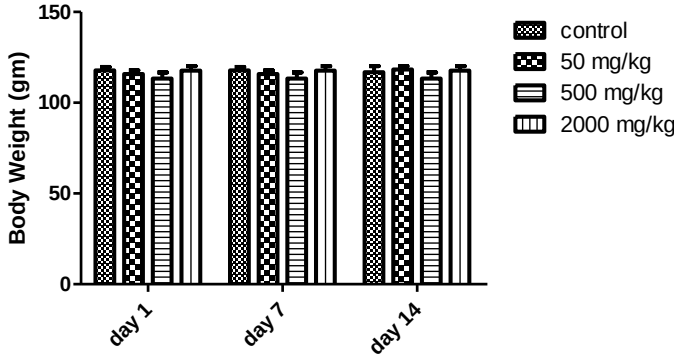

Fig. 1: Body weight of control and *Epipremnum aureum* extract treated rats in the acute toxicity(for 14 days).

No death was recorded in the 14 days of observation period in the animals given up to 2000 mg/kg p.o. of the given ethanol extract. The animals did not show any changes in the general appearance during observation period, except at a dose of 2000mg/kg showed increased motor activity.

Table 1: Parameters observed during acute toxicity study for 14 days.

Parameters Observed	Control	Treatment		
	1% CMC	50mg/kg	500mg/kg	2000mg/kg
Diarrhea	-	-	-	-
Muscle Relaxation	-	-	-	-
Paw Licking	-	-	-	-
Sedation	-	-	-	-
Tremors	-	-	-	-
Muscle Spasm	-	-	-	-
Motor activity	-	-	+	+

Table 2: Organ weight of control and treated rats at doses 100, 600, 1000 mg/kg b.wt. in the sub-chronic toxicity study for 28 days.

Organs	Control	*Weight of organs after treating with Epipremnum aureum* ethanol extract mg/kg		
		100	600	1000
Liver	6.39±0.28	6.87±0.34	6.91±0.14	6.69±0.23
Kidney	1.38±0.11	1.43±0.21	1.38±0.17	1.46±0.16
Heart	0.62±0.02	0.59±0.03	0.61±0.03	0.65±0.01
Pancreas	0.91±0.11	0.84±0.15	0.89±0.13	0.94±0.21
Adrenals	0.03±0.08	0.03±0.01	0.03±0.03	0.03±0.02
Lungs	1.26±0.16	1.31±0.13	1.46±0.33	1.34±0.15

The weight of individual organs reveals that *Epipremnum aureum* ethanol extract has no effect on the weight of heart, liver, kidney adrenals, lungs and pancreas when compared to control group**.**

DISCUSSION

The herbal preparations are administered without any standard dosage due to lack of adequate scientific data on their safety. This issue has raised concerns regarding the toxicity of herbal preparations. Epipremnum species have been evaluated for various pharmacological activities. *Epipremnum aureum* is of more interest to researchers because it contains bioactives and it is easily available. As the safety profile of this plant in acute and sub chronic tests are not determined yet, this research showed the safety of this plant in two models of toxicity assessment. It is deemed important to evaluate the toxicity effect of a medicinal plant extract in order to increase the confidence in their safety to humans, particularly for use in the development of nutraceuticals and pharmaceuticals. To our best knowledge, this is the first study reported the toxicity effects of *Epipremnum aureum* in sprague dawley rats. The acute toxicity study does not show any toxic symptoms, changes in behaviour or mortality at 2000 mg/kg doses. Through-out the 14 day periods all animals were found to be healthy with no changes in their skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system and as well as somatomotor activity and behavioural patterns. On the basis of above observations, this extract has anticipated having an LD50 higher than 2000 mg/kg bodyweight which is not hazardous in acute doses. The high dose

of *Epipremnum aureum* ethanol extract (1000 mg/kg/day) in the sub chronic 28-days study was applied because human exposure indicates the use of a high dose level in accord with the sub chronic 28 days guidelines. A lower dose of 600 mg/kg day was used to determine dose related toxic effects. In a sub chronic toxicity study, it appeared that the *Epipremnum aureum* ethanol extract at the doses (100mg/kg, 600 mg/kg, & 1000mg/kg) did not produce any marked changes in both male and female rats, as evidenced by the absence of toxic symptoms, no changes in water/food ingestion, or weight gain. Normal organ weight revealed that the extract did not produce organ swelling, atrophy or hypertrophy. The biochemical parameters level indicates physiological condition. The increase and decrease of biochemical parameters can convey indications regarding toxicity of specific organs. In the present study, biochemical parameters were estimated particularly ALT, ALP, AST, creatinine, total cholesterol and total protein were tested and the analysis showed there were no significant differences in parameters level of the rats treated with *Epipremnum aureum* ethanol extract compared to the control in sub chronic toxicity studies as shown in table 3. Although there is a slight decrease or increase in the level of biochemical parameters in then treated rats compared to the control, these values were still within the normal range. The biochemical findings suggested that the administration of *Epipremnum aureum* did not cause any toxicological effect. Morphological examination on the vital organs, liver, kidney, heart and pancreas as well revealed no treatment-related changes due to the administration of *Epipremnum aureum* ethanol extract in the animals as shown in fig 4. The liver and kidney were studies extensively for histopathology observation because of their primary function to expel toxins that results from body's metabolism of food, drug or any other substances that was being consumed [12]. In sub-chronic toxicity studies, the rats treated with the extract showed normal architecture of the liver and kidney. There is no evidence of lesion due to toxic effect of extract in the liver and kidney as shown in fig 2 and fig.3.

Table 3: Effect on haematological and biochemical parameters after 28 days oral administration of *Epipremnum aureum* extract.

Parameters assayed	Control group	100mg/kg	600mg/kg	1000mg/kg
RBC (million/mm^3)	9.08±0.20	8.20±0.48	8.98±1.05	9.25±0.67
Hb (g/dL)	12.16±0.16	12.79±0.16	12.29±0.64	12.30±0.47
WBC (million/mm^3)	8.47±0.24	8.96±0.12	9.65±0.61	9.12±0.32
Neutrophils %	24.57±0.45	24.27±0.16	23.57±0.35	25.17±0.32
Basophils %	0.21±0.39	0.26±0.21	0.28±0.34	0.29±0.17
Eosinophils %	2.81±0.45	2.63±0.61	2.91±0.37	3.12±0.42
Lymphocytes %	74.31±0.75	74.82±0.15	74.50±0.43	75.31±0.27
Monocytes %	3.44±0.87	3.12±0.77	3.99±0.63	4.49±0.57
AST (U/L)	195.47±1.21	196.12±1.02	195.75±1.58	196.51±1.32
ALT (U/L)	84.57±1.74	84.89±1.74	85.45±1.47	86.45±1.96
ALP (U/L)	234.42±1.34	234.13±1.51	235.11±1.23	235.85±1.55
Creatinine (mg/dL)	0.94±0.21	0.94±0.11	0.95±0.42	0.95±0.32
Albumin (g/dL)	2.71±1.41	2.57±1.1.87	3.41±1.84	3.74±1.73
Total protein (g/dL)	7.46±1.74	7.85±1.85	8.75±1.90	8.75±1.80
Glucose (mg/dL)	96.74±1.14	96.18±1.71	67.24±1.87	67.51±1.81
Total cholesterol (mg/dL)	132.43±1.03	132.25±1.74	133.12±1.92	133.74±1.14
Bilirubin Total (mg/dL)	1.31±1.20	1.97±1.15	2.41±1.22	2.45±1.12
Bilirubin Direct (mg/dL)	0.79±1.14	0.78±0.27	0.79±0.32	0.79±0.23
Calcium (mg/dL)	8.41±1.10	8.32±1.47	9.74±1.41	9.54±1.49
Sodium (mg/dL)	140.79±1.27	140.14±1.21	141.41±1.30	141.44±1.23
Phosphorous (mg/dL)	5.31±1.17	5.62±1.11	5.41±1.21	5.84±1.14

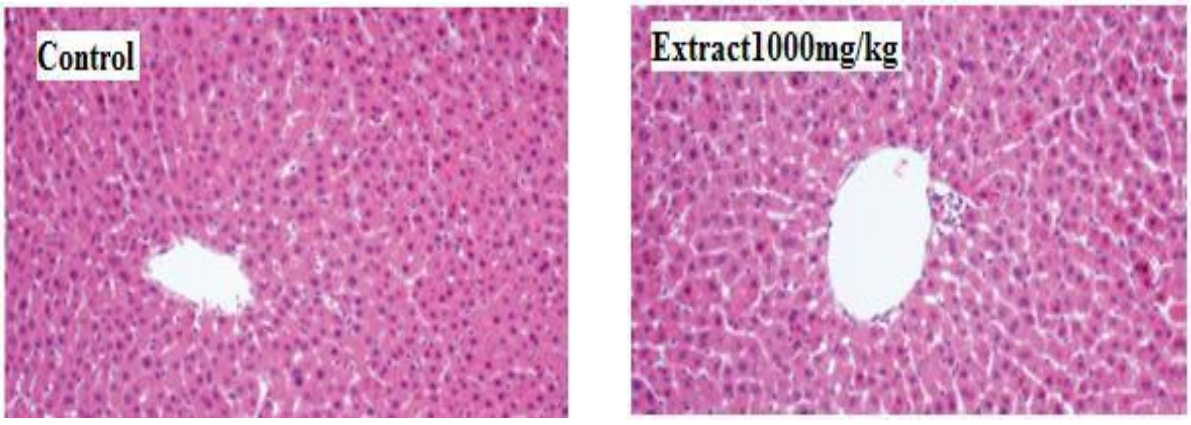

Fig. 2: Photomicrograph from liver control (Fig 1) and *Epipremnum aureum* extract treated rats (Fig 2).

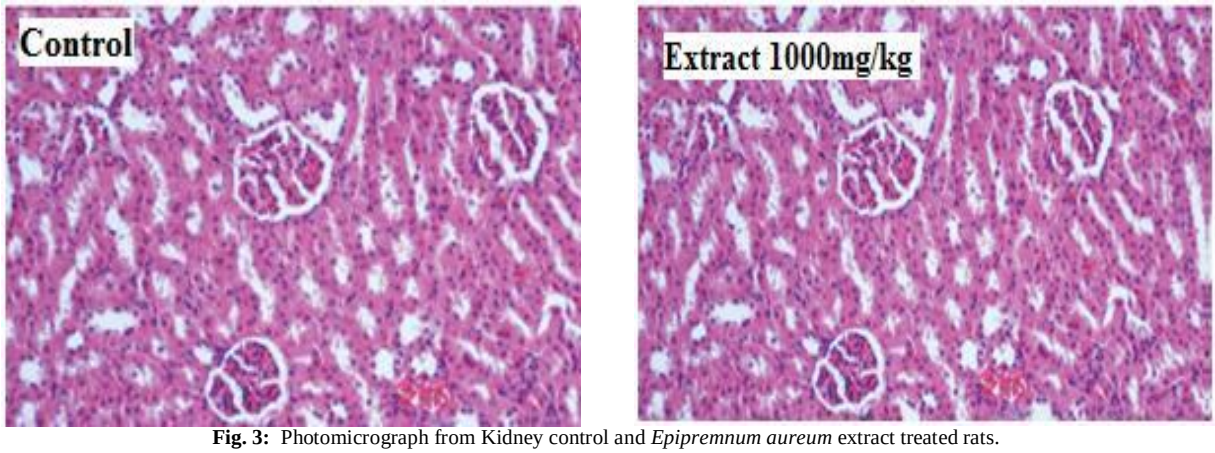

Fig. 3: Photomicrograph from Kidney control and *Epipremnum aureum* extract treated rats.

Fig. 4: Morphological structure of organs after treating with *Epipremnum aureum* extract in sub chronic toxicity study after 28 days.

CONCLUSION

The *Epipremnum aureum* ethanol extract was found to be nontoxic when oral acute and sub chronic 28-days toxicities in rats were performed. Based on the above investigation it can be concluded that the LD 50 of the extract was above 2000mg/kg. No signs of toxicity were observed in the histopathological studies. However mutagenicity and carcinogenicity studies are necessary to further support the safe use of this plant product and before developing a pharmaceutical product.

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