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Original research article

ACUTE TOXICOLOGICAL EVALUATION OF PET – ETHER EXTRACT OF *PORTULACA OLERACEA (LINN.)* ON RODENTS.

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ABSTRACT

Introduction: *Portulaca oleracea* is a common plant used in south Indian culinary; recently there is increase in research publication on various biological activities of the medicinal herb. The safety of the medicinal herb well accounted by its widespread accepted use of natives yet scientific evaluation on the safety of the herb is not reported. **Aim** To scientifically evaluate the toxicity profile of the pet - ether extract of *Portulaca oleracea* by standardized methods. **Method** A 24hour acute toxicity study followed by 14 day sub-acute toxicity study with serum haematological, biochemical and histopathological analysis's is evaluated in rodents. **Result** No observable serious side effects are recorded in acute and sub acute toxicity study for 0.5gm/kg and 1gm/kg pet-ether extract of *Portulaca oleracea.* There are statistically significant rising ($p<0.01$) in hemoglobin by 13.25%, 15.42% and 15.04% in *Portulaca oleracea* 0.5gm, 1gm, and 2gm/kg body weight respectively when compared to control 10.56%. *Portulaca oleracea* 2gm/kg dose administration for 14 days has revealed oxalate crystal deposits in the kidney. **CONCLUSION** The pet –ether extracts of *Portulaca oleracea* 0.5gm, 1gm, and 2gm/kg exhibited zero mortality rates in both acute and sub-acute toxicity studies and found to increase haemoglobin, total cholesterol levels in serum which can be seriously evaluated for further research.

Keywords: *Portulaca oleracea (LINN.),* Acute Toxicity, Sub-acute Toxicity, Rodents.

INTRODUCTION

Toxicological research is the primary step in screening chemicals before pharmacological screening for their predictive biological activity. The current research scenario has shown tremendous bioprospecting of compounds from natural origin. Many compounds which are potential drug candidates during preclinical research tend to be rejected in the later stages of research due to unwanted side effects which takes lots of manpower and financial resources, such errors can be avoided by initial toxicological and safety analysis before taking the drug to

pharmacological research. *Portulaca oleracea* is a herb widely used by south Indian population for various ailments and the leaves are widely used in food preperations. This herb belongs to family *Portulacaceae* . The plant is reported to have protective biological effect against bacterial and fungal infection[1]. Towards treatment of infertility and preclinical scientific research on various inflammatory conditions[2]. Despite the uses traditionally, there is much little scientific evidence in established literature on the safety of this plant. Information concerning toxicity of *Portulaca oleracea* from traditional use has also been scarce.The literature search also revealed no scientific evidence available regarding safety of this plant. The present study is an effort to provide preliminary information on the acute and subacute toxicological profile of the Pet - Ether extract of *Portulaca oleracea* in rodents the reports were supported with biochemical,isolated organ and histopathological observations .

MATERIALS AND METHODS

Plant Material: The leaves of *Portulaca oleracea* are used in traditional medicine similarly in the present study the leaves of the plant is collected from botanical garden during the month of May 2009; the collected leaves are authenticated by Department of Botany, Annamalai University, Chidambaram.

Extract Preparation: The leaves of *Portulaca oleracea* were sold dried for seven days and pulverized one kg of coarse powder was filtered through a fine mesh and collected powder is soaked in 4 litres of petroleum-ether for 3 days at room temperature. The collected extract is evaporated to dryness using a rotary vacuum flash evaporator and the yield was stored in airtight container for further research.

Animals : Sprague-Dawley rats weighing 200-250gms were used for toxicity studies. The animals are kept in standard conditions of a 12 hour day and 12 hour night cycles at 22º C room temperature, in polypropylene cages. The animals were fed on standard pellet's (Hindustan Lever Pvt Ltd., Bangalore) and provided tap water ad libitum. To acclimatize to laboratory conditions the animals were housed in polypropylene cages prior to the experiments for one week. The experiment was conducted in Rajah Muthiah Medical college, Department of Forensic medicine and Toxicology and the protocol was approved by the Institutional Animal Ethical Committee (IAEC). All procedures and techniques used in these studies were in accordance with accepted principles for laboratory animal use and care of Annamalai University.

Acute toxicity : Sprague-Dawley rats of either sex were randomly divided into four groups, six animals in each group (n=6). The rats were kept in the experimental environment for an acclimatization period of 1 week before starting the experiment. The animals were fasted overnight with access to water *ad libitum.* The study design included three treatment groups and one control group, the treatment group received orally pet-ether extract of *Portulaca oleracea* in doses of 0.50,1.00, and 2.00 gms/ kg of body weight. The control group received 10 ml/ kg *p.o.* of Normal saline. The rats were observed up to 24 hours for general changes in behaviour and physiological function as well as mortality. The assessment of behaviour and physiological function was carried out by procedures originally4 described by Irwin (1968)[3].

Sub-acute toxicity : Sprague-Dawley rats, 6 per group, were treated orally with *Portulaca oleracea* daily for 14 consecutive days. The study design included four groups. Group -1 the control, received 10 ml kg *p.o.* of Normal saline daily. Group 2, 3 and 4 were treated with daily doses of the extract i.e. 0.5, 1 and 2 gm/ kg respectively. The extract of three different concentrations was prepared such that not more than 2 ml was given orally. The animals were monitored closely for signs of toxicity. Appearance, toxicity signs, cage side observation and behaviour pattern were assessed daily and any abnormalities in food and water intake were registered.

Preparation of serum and isolation of organs: After fourteen days of observation the rats were sacrificed on the fifteenth day by cervical dislocation, the jugular vein was cut and blood samples were collected for hematological assay in vacuum tubes containing 2.5 µg of ethylene diamine tetra acetic acid (EDTA). Hematological parameters including haemoglobin (HGB), red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were determined by an automatic analyzer. Another sample of blood was collected into tubes without anticoagulant to obtain serum was collected and stored at -20°C until assayed for biochemical parameters the next day. Biochemical analyses were performed on serum collected for the determination of the following parameters: fasting blood glucose, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), urea, Blood urea nitrogen (BUN) and Cholesterol. All analyses were carried out using the Automated Clinical Chemistry Analyzer. After collecting blood, the rats were quickly dissected to remove and isolate the organs which were blotted with clean tissue paper and then weighed on a balance.

Effect of extract on body and organ weights in rats : Body weights of the rats in each group were recorded on day 1 and 15. The relative organ weight (ROW) of each organ was calculated as follows:

ROW = Absolute Organ Weight (g) / Rat body weight on sacrifice day X 100.

Histopathological examination : Histopathological examinations were carried out on the tissue obtained from liver, kidney, spleen and stomach of each group. Tissues were fixed in 10 % neutral buffered formalin (pH 7.2) and dehydrated through a series of ethanol solutions, embedded in paraffin and routinely processed for histological analysis. Sections of 2 µm thickness were cut and stained with haematoxylin Eosin for examination.

Analysis of data

The recorded data were statistically analyzed for the presence of significant differences among means of groups using one-way ANOVA followed by Newman-Keuls multiple comparison test.

Data were presented as mean ± SEM (n=6). Graph Pad Prism (GraphPad Software, San Diego, CA, USA) statistical software was used.

RESULTS AND DISCUSSION

Acute toxicity : All animals in each group are observed continuously for first four hours followed by 8th hour and 24th hour. In control and *Portulaca oleracea* 0.5 gm there were no signs of toxicity, whereas the *Portulaca oleracea* 1gm and 2gm exhibited Asthenia, defecation, salivation, urination more than that of control group (Table-1)

Table.1: Acute Toxicity observation of *Portulaca oleracea* Extract.

S.No	Group	Mortality		Toxicity Signs
		D/T	Latency (hrs)	
1	Control N.S 10ml/kg	0/6	-	None
2	*Portulaca oleracea* 0.5gm/kg	0/6	-	None
3	*Portulaca oleracea* 1gm/kg	0/6	-	Asthenia, defecation, salivation, urination
4	*Portulaca oleracea* 2gm/kg	0/6	-	Asthenia, defecation, salivation, urination

N.S-Normal Saline, D/T – Death/Treatment

Sub-acute toxicity

Similar observation as seen in acute toxicity studies was present for initial two days, later Asthenia, increased defecation, salivation, urination were not observed. The cage side observations for 14 days on general behaviour, respiratory pattern, cardiovascular signs, reflexes are normal. The *Portulaca oleracea* 2gm/kg group exhibited decreased motor activity and there were no change in skin and fur. All animals survived for 14 days. The decreased motor activity is probably due to the extract effect on skeletal muscle, a similar observation was made by Parry O et al, and reported to muscle relaxant activity[4]. The analysis of hematological parameter revealed in all treatment groups the haemoglobin ($p<0.001$), RBC ($p<0.05$) counts, packed cell volume ($p<0.01$), mean corpuscular volume ($p<0.01$) and mean corpuscular haemoglobin ($p<0.01$) were significantly increased when compared to control treated rats (TABLE-2).

Table. 2: Hematological parameter of *Portulaca oleracea* Extract after 14 days.

Groups (n=6)	Hb % (gm)	PCV %	MCV(fl)	MCH(pg)	RBC (milli/cu.mm)	WBC (milli/cu.mm)
Control N.S 10ml/kg	10.56 ± 0.25	38.14± 0.56	86.02±3.45	20.83 ±2.25	4.65 ± 0.34	6.65 ± 2.25
Portulaca oleracea 0.5gm/kg	13.25±0.35**	45.24±0.45*	95.24±4.53	28.28 ±0.55**	6.75 ± 1.25*	6.76 ± 0.25
Portulaca oleracea 1gm/kg	15.42±1.24**	44.14±1.26*	94.12±3.56	27.24 ±1.25**	6.60 ± 0.25*	6.50 ± 1.22
Portulaca oleracea 2gm/kg	15.04±0.65**	45.16±2.27*	95.62±2.22	30.65 ±2.20**	7.25 ± 2.24*	6.34 ± 2.24

Values are mean ± S.E.M. *P<0.05;**P<0.01; Compared to Control (one-way ANOVA followed by Newman-Keuls test)

The significant increase in hematological parameter observed with *Portulaca oleracea* 0.5gm and 1gm is dose dependent, were as *Portulaca oleracea* 1gm and 2gm increase in dose did not have an increased effect on parameters clearly establishing the ceiling effect. The leukocyte count was not affected in all three treated groups compared to that of the control group. The leaves of the extract from *Portulaca oleracea* are rich in micronutrient and macronutrient constituting an important source of protein, essential amino acids, mineral elements including free oxalic acids, alkaloids, omega-3 fatty acids, coumarins, flavonoids, cardiac glycosides, anthraquinone, and proteins may have a synergistic effect on the observed rise in haemoglobin levels[5]. The biochemical parameter screening after 14 days did not reveal any significant changes except total cholesterol level in all three treated groups with extract of *Portulaca oleracea* (Table-3)

Table.3: Biochemical parameter of *Portulaca oleracea* Extract after 14 days

Groups (n=6)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	Urea (mg/dl)	BUN (mmol/L)	Glucose mg/dl	Cholesterol mg/dl
Control N.S 10ml/kg	25.23±0.39	42.29±1.90	23.38±2.53	15.20± 2.02	7.52±0.84	83.57±6.97	54.24±2.24
***Portulaca oleracea* 0.5gm/kg**	24.73± 0.39	43.89±2.50	23.45±2.93	16.38 ±2.12	7.52±0.84	83.57±6.97	66.14±3.15*
***Portulaca oleracea* 1gm/kg**	25.35±0.39	42.55±2.34	24.50±1.50	15.45 ±0.25	7.52±0.84	83.57±6.97	64.20±0.26*
***Portulaca oleracea* 2gm/kg**	26.03±0.25	41.25±2.50	24.48± 1.33	16.20 ±2.24	7.52±0.84	83.57±6.97	68.50±3.12*

Values are mean± S.E.M. *P<0.05;**P<0.01; Compared to Control (one-way ANOVA followed by Newman-Keuls test)

Though the all animals in each group were fed with the common diet pattern there a significant increase in total cholesterol level, the total cholesterol level signifies low density lipoproteins, very low density lipoproteins, high density lipoproteins and triglycerides. The probable rise in cholesterol level without a rise in body weight and fat free food could be due to omega 3 free fatty acid. Already there are investigating reports on *Portulaca oleracea* as a rich source of omega 3 fatty acids [6]. The body weight was measured on day 7 and day 14 of sub acute toxicity study did showed increase in body weight in all groups, but when compared to control group the treatment group did not have any statistical difference. The animals in each group after euthanasia were carefully dissected and observed for gross macroscopic tissue/organ pathology. Necropsy observation revealed no gross anatomical abnormalities in all the groups. The organs liver, spleen, stomach and kidney are dissected and checked for relative organ weights (Fig; 1, 2, 3 & 4)

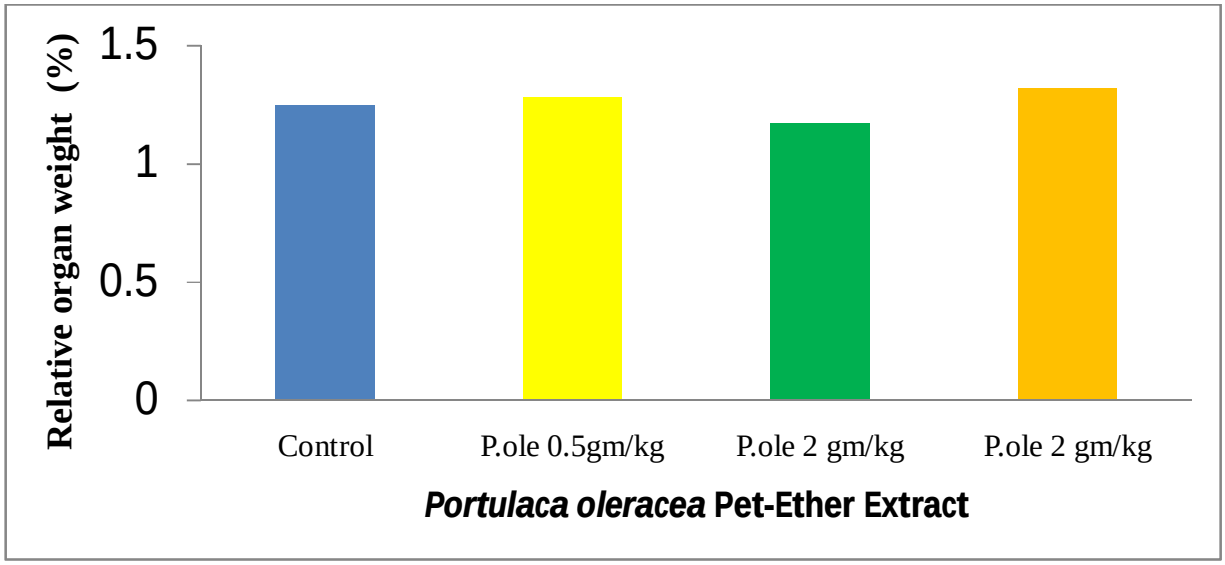

Fig.1: Relative organ weight-Liver

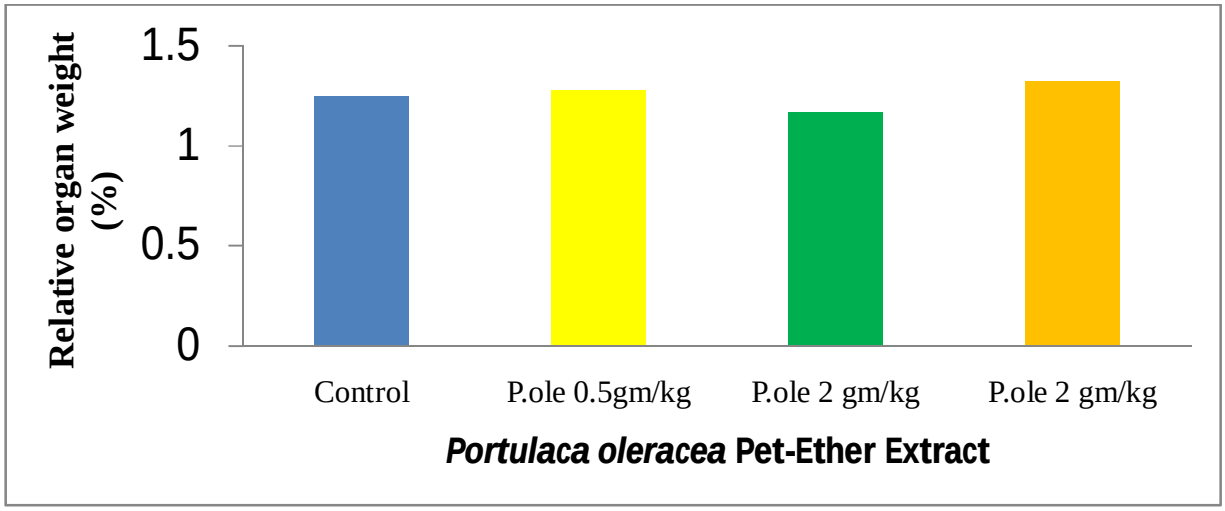

Fig.2: Relative organ weight-Spleen

There was no statistical relative weight difference for spleen, liver and stomach when compared to that of control (fig; 1, 2 & 3.).

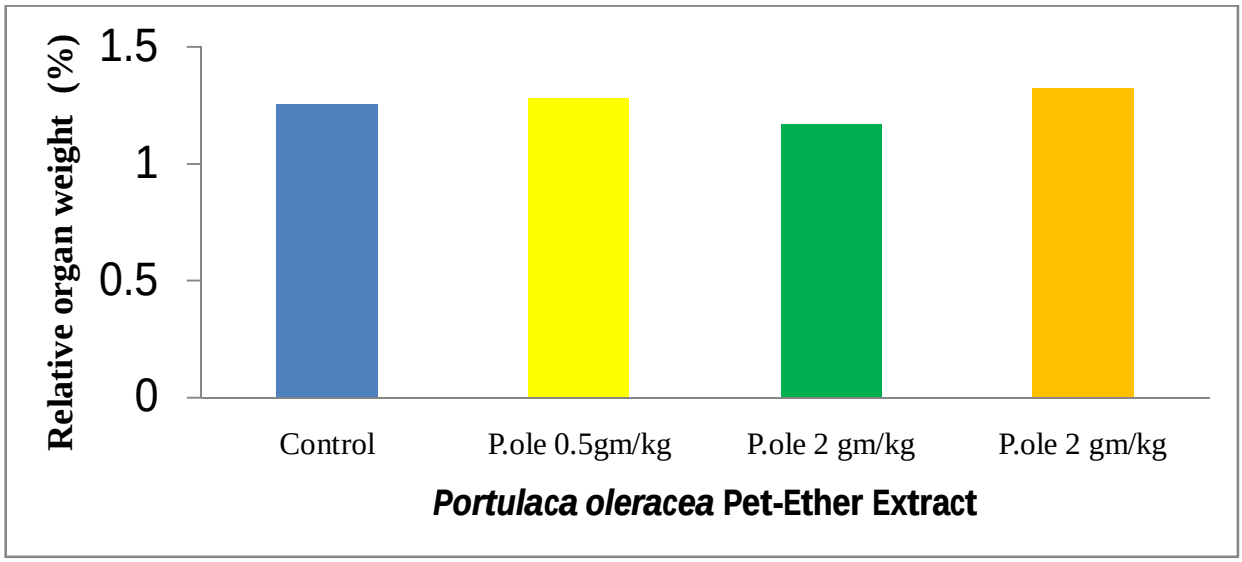

Fig.3: Relative organ weight-Stomach

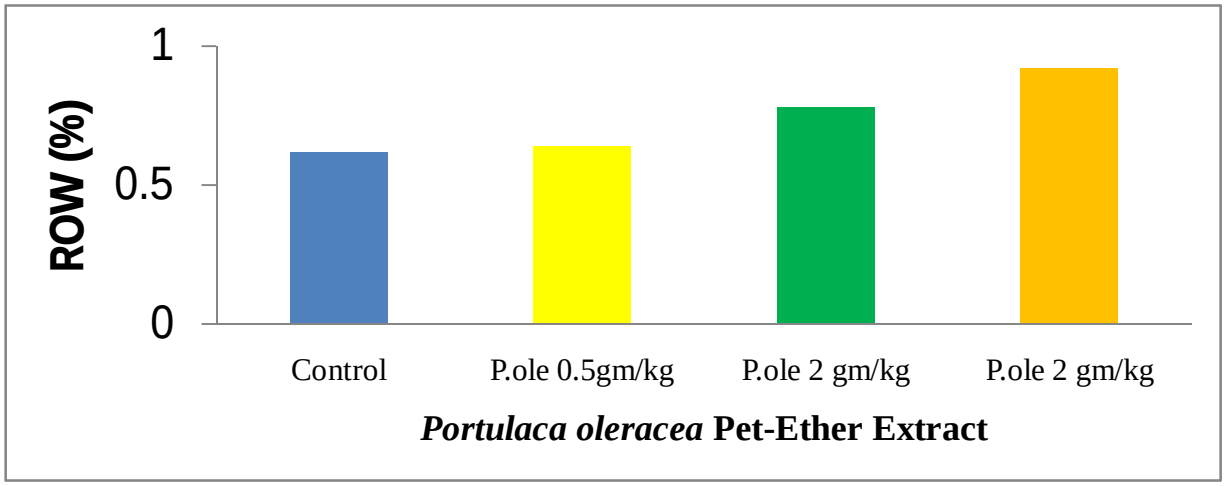

Fig.4: Relative organ weight-Kidney

The weight of the kidney of *Portulaca oleracea* 1gm/kg and 2gm/kg treated group had increased relative weight of organ by 0.2% but not statistically significant (fig 4).

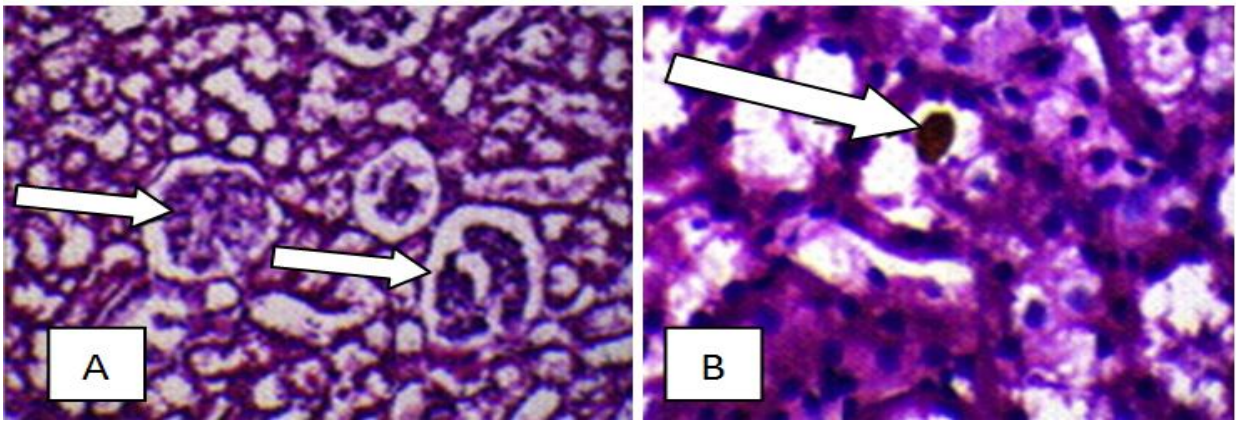

Fig.5. A – Control group with arrow indicating normal glomerular structure and Renal tubules, B – P.oleracea 2gm/kg treated group arrow pinpointing oxalate renal stone and tubular dilation.

Hence all the dissected organs with particular interest of isolated kidney from *Portulaca oleracea* 1gm/kg and 2gm/kg were taken for histopathological studies. The histopathological studies revealed the presence of epithelial inflammation and oxalate stones and hemorrhagic spots (fig; A, B). Earlier studies on nutrition content of *Portulaca oleracea* revealed half a cup of leaves contain 910 mg of oxalate which explains the increase in relative organ weight and histopathalogical appearance of oxalate stones[7]. The phytochemical screening of pet-ether extract of *Portulaca oleracea* can reveal the major active constituents responsible for biological activity of the extract[8].

CONCLUSION

The pet-ether extract of *Portulaca oleracea* 0.5gm/kg, 1gm/kg and 2gm/kg evaluated for acute toxicity and sub-acute toxicity has no observable side effects, except for the renal calculi formation at 1gm/kg and 2gm/kg for 14 days. The study has also provided other important finding such as the ability of the *Portulaca oleracea* pet-ether extract in increasing the hemoglobin and anticipated High density lipoprotein level. The observed finding can be extrapolated for further research of potent hematinic compound.

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