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Hepatic and Renal Biochemical Profile of Albino Rats Exposed to Chloroform and Methanol Leaf Extracts of *Portulaca oleracea* Linn.

Victoria C. Obinna[1*], Hope D. Kagbo[2] and Gabriel O. Agu[1]

[1]Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.
[2]Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria.

Authors' contributions

This study was carried out in collaboration between all authors. Author VCO designed and carried out the study, performed the statistical analysis and wrote the manuscript. Authors HDK and GOA supervised the study, managed the analyses of the study and proofread the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Portulaca oleracea Linn. is among the medicinal plants used globally in the treatment of diseases and management of health challenges. The dearth of information on the long term effect of *Portulaca oleracea* on hepatic and renal toxicity prompted this study. The study investigated the subchronic effect of the oral administration of chloroform leaf extracts of *Portulaca oleracea* (CLEPO) and methanol leaf extracts of *Portulaca oleracea* (MLEPO) on plasma activity of some enzymes (ALT, ALP & AST) and levels of other biochemical parameters such as blood electrolytes, total protein, albumin, bilirubin, urea and creatinine in male albino rats. One hundred and twelve (112) animals were randomly divided into seven (7) groups of sixteen (16) rats each. Group A (Control) received 0.5ml/kg of 20% Tween 80 (vehicle), Groups B, C & D received 125, 250 & 500

**Corresponding author: E-mail: drchiobinna@yahoo.co.uk, chivicobinna@gmail.com;*

mg/kg of CLEPO respectively and Groups E, F & G received 125, 250 & 500 mg/kg of MLEPO respectively for 60 days. On days 14, 28, 42 and 60; four rats from each group were anaesthetized and blood samples were collected for plasma biochemical assay. MLEPO caused a significant ($p<0.05$) decrease in plasma AST and ALP level while CLEPO significantly ($p<0.05$) decreased the plasma AST level. MLEPO produced a highly significant ($p<0.01$) reduction in both total bilirubin and conjugated bilirubin levels as well as significant ($p<0.05$) decline in urea level. CLEPO produced a significant ($p<0.05$) decrease on conjugated bilirubin. Both extracts significantly ($p<0.05$) reduced the chloride level. Oral administration of CLEPO and MLEPO over a 60 day period is neither hepatotoxic nor nephrotoxic.

Keywords: Portulaca oleracea; hepatotoxic; nephrotoxic; biochemical enzymes; subchronic.

1. INTRODUCTION

Although the use of medicinal plants has become quite popular in the world today, some of these plants purported to be safe have side effects or toxicity. *Portulaca oleracea* Lin. (commonly called purslane) a member of the family Portulacaceae, a warm climate green herb with obovate leaves, small yellow flowers, and branched succulent stems that are decumbent near the base Mitich [1] is one of the medicinal plants used globally in the treatment of diseases and management of health challenges. It has different names in various ethnic groups in Nigeria. It is known as "Ntioke", or "Idiridi" in Igbo; "Esan omode" or "Papasan" in Yoruba; "Babbajibji" or "Halshen saniya" in Hausa and "Eferemakara" in Efik Burkill, Iwu [2,3]. It is used in folk medicine for the treatment of dysentery and the enhancement of fertility. It is also used in the treatment of boils, sores, eczema, erysipelas, and insect and snake bites [4]. The leaf extracts have been shown to possess contractile effects on isolated intestinal smooth muscle [5], analgesic and antiinflammatory effects Chan et al. [6], antifungal activity Oh et al. [7], antidiabetic Samarghandian, Bai [8,9], antioxidant effects [10,11] and wound healing properties [12]. Its therapeutic values have been attributed to the presence of many biologically active compounds which include flavonoids (Apigenin, kaempferol, quercetin, luteolin, myricetin, genistein, and genistin), Alkaloids, Coumarins, anthraquinone glycoside, cardiac glycoside, and high content of omega-3 fatty acids [13].

Despite the popular use and numerous therapeutic benefits of *Portulaca oleracea,* little is known about the safety of the plant with regards to its effect on the functional status of the kidney and liver following long term use. This study was therefore designed to investigate the subchronic effect of leaf extracts of *Portulaca oleracea* on hepatic and renal biochemical parameters.

2. MATERIALS AND METHODS

2.1 Plant Material and Authentication

Fresh leaves of *Portulaca oleracea* were collected from Alakahia axis of Port Harcourt, Nigeria, from the months of December, 2017 to February, 2018. The plant was identified at the University of Port Harcourt Herbarium, Department of Plant Science and Biotechnology, with assertion number UPH/V/1,302.]

2.2 Preparation of Plant Extracts

Collected plant leaves were shade-dried at room temperature to constant weight over a period of six weeks. The dried leaves were weighed and ground to fine powder. The powdered leaves (4.5 kg) were extracted by cold maceration using chloroform and 80% aqueous methanol (the ratio of methanol to distilled water is 4:1) in succession. Briefly, the powdered leaves were first soaked in chloroform (500g leaves/ 1.5L solvent) for 72 hours with fresh replacement of solvent every 24 hours. The pooled extract was filtered with the Watman's No. 1 filter paper. The filtrate was concentrated with a rotary evaporator (Model No: RE-52A) at 45°C *in vacuo* and later transferred to an evaporating dish and dried over a water bath (Digital thermostatic water bath, Jinotech instruments). The marc (residue) obtained after extraction with the chloroform was further extracted with the 80% aqueous methanol in a similar manner to obtain the polar extract. The resulting extracts were stored in a desiccator. All reagents used were of analytical grades.

2.3 Acute Toxicity Study

The acute toxicity of the extracts was evaluated according to the method of Lorke [14]. This was done to determine the LD_{50} of the extract which is

vital in identifying its clinical effects following oral administration; and to determine the dosage regimen for the study. For CLEPO, in the first phase, 3 groups of 3 rats each were administered with the extract at doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight by oral gavage and observed for 24hours. In the second phase, 3groups of 3 rats each were administered with the extract at doses of 1600mg/kg, 2900mg/kg, and 5000mg/kg by oral gavage. They were observed for 24hours for mortality and other signs of toxicity. The same procedure was repeated for MLEPO.

2.4 Animals

One hundred and twelve (112) sexually mature male albino rats weighing an average of 200g, procured from the Animal House of the Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria were used in the study. The rats were acclimatized for two (2) weeks before commencing the study. They were fed water and commercially sourced feed (Top Feeds Nigeria Limited) *ad libitum* all through the study.

2.5 Ethical Approval

The study protocols were duly approved by the Research Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt with the Ref. No: UPH/CEREMAD/REC/04. All experimental animals were humanely handled in accordance with the Ethics and Regulation guiding the use of research animals as approved by the University.

2.6 Experimental Procedure

Following acclimatization, the animals were randomly assigned to seven (7) groups of sixteen (16) animals each for treatment as follows:

Group A (Control) received 0.5ml/kg body weight of 20% Tween 80 (vehicle).
Group B received 125 mg/kg body weight of CLEPO
Group C received 250 mg/kg body weight of CLEPO
Group D received 500 mg/kg body weight of CLEPO
Group E received 125 mg/kg body weight of MLEPO
Group F received 250 mg/kg body weight of MLEPO
Group G received 500 mg/kg body weight of MLEPO

Administration of the extract and vehicle were by oral gavage daily for 60 days. The weight of experimental animals was measured weekly and the dose adjusted accordingly. On days 14, 28, 42 and 60; four rats from each group were anaesthetized and blood samples collected into

Plate 1. *Portulaca oleracea* Linn

heparinized bottles by cardiac puncture for determining plasma activity of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP), and levels of total protein, Albumin, total and conjugated bilirubin, creatinine, urea, sodium, potassium, chloride and bicarbonate. The blood was centrifuged at 4000rpm for 10 mins after which the plasma was separated from the coagulated cells and tipped into a separate vial. The vial was placed in microcentrifuge tubes, capped and stored at -20°C until analysis.

The plasma biochemistry determinations were done using commercial test kits. The activities of ALT and AST were measured according to Reitman and Frankel [15] using the diagnostic kits from Randox Laboratories, Northern Ireland. The serum ALP activity was determined by the thymolphthalein monophosphate method according to Roy [16] using Teco Diagnostics kits (1268 N. Lakeview Ave, Anaheim, CA 92807 1-800-222-9880). The plasma total bilirubin and conjugated bilirubin were determined by the Jendrassik-Grof method [17] using the diagnostic kits from Randox Laboratories, Northern Ireland. Plasma total protein (TP), plasma albumin, plasma urea and plasma creatinine was determined by the direct Biuret method [18], the bromocresol green method [19], the Urease-Berthelot method [20] and the modified Jaffe method [21] respectively, using diagnostic kits from Randox Laboratories, Northern Ireland. The plasma potassium (K^+), sodium (Na^+), bicarbonate (HCO^-_3) and chloride (Cl^-) were determined by colorimetric method [22].

2.7 Statistical Analysis

Statistical analysis was done using SPSS 21. All values were expressed as mean ± SEM and data were assessed by one-way ANOVA followed by the Tukey post-hoc test. Significance level was set at $p<0.05$.

3. RESULTS

3.1 Acute Toxicity Study

Acute toxicity test did not show any mortality, morbidity or other apparent signs of toxicity even at the maximum dose of 500mg/kg.

3.2 Hepatic Biochemical Parameters

3.2.1 Aspartate transaminase (AST) assay

Within the CLEPO treatment groups, there were no significant ($p>0.05$) variation in the mean AST levels on day 14, 28, 42 and 60 of treatment except in the 250mg/kg treated rats (group C) which showed a significant ($p<0.01$) decrease on day 28 relative to the control (Table 1). MLEPO caused a significant ($p<0.01$) decrease in the mean AST levels in 500mg/kg treated rats (group G) on day 28 and in both 250 and 500 mg/kg treated rats (group F and G respectively) on day 42 when compared with the control. The mean AST levels of other MLEPO groups were not affected ($p>0.05$) (Table 2)

Generally, both extracts decreased the mean AST levels throughout the treatment.

3.2.2 Alanine aminotransferase (ALT) assay

There was no significant ($p>0.05$) variation in the mean ALT levels within the CLEPO treatment groups on day 14, 28, 42 and 60 in comparison with the control (Table 3).

MLEPO caused a significant ($p<0.05$) increase in the mean ALT levels on day 42 in 250 and 500 mg/kg treated rats (group F and G respectively) relative to control. No significant ($p>0.05$) variation occurred in the other MLEPO treated groups when compared with the control (Table 4).

Table 1. Effect of varied doses of CLEPO on AST

Parameter	AST (U/L)			
Duration / Treatment	14 days	28 days	42 days	60 days
Group A (control)	141.00±6.53	150.00±5.34	145.00±4.49	139.50±5.38
Group B (125 mg/kg)	134.25±5.81	131.75±8.49	130.50±9.51	139.75±5.96
Group C (250 mg/kg)	138.50±3.23	126.75±6.03[b]	141.25±5.63	121.25±7.25
Group D (500 mg/kg)	135.75±4.29	128.00±6.76	121.25±5.56	129.25±4.52

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control)*

Table 2. Effect of varied doses of MLEPO on AST

Parameter	AST (U/L)			
Duration / Treatment	14 days	28 days	42 days	60 days
Group A (control)	141.00±6.53	150.00±5.34	145.00±4.49	139.50±5.38
Group E (125 mg/kg)	123.00±5.05	138.25±3.42	126.50±8.99	132.33±6.33
Group F (250 mg/kg)	127.75±6.41	122.50±10.87	107.00±4.62[b]	114.75±10.66
Group G (500 mg/kg)	124.00±5.79	100.50±4.44[b]	116.25±2.39[a]	112.25±7.09

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

Table 3. Effect of varied doses of CLEPO on ALT

Parameter	ALT (U/L)			
Duration / Treatment	14 days	28 days	42 days	60 days
Group A (control)	31.75±3.75	31.00±0.41	24.00±2.42	33.50±1.19
Group B (125 mg/kg)	28.75±3.47	27.25±3.04	29.25±2.93	28.75±2.69
Group C (250 mg/kg)	23.75±3.12	28.75±4.94	22.75±3.22	27.00±3.24
Group D (500 mg/kg)	29.50±1.71	35.75±3.86	31.50±3.38	28.50±1.85

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control)*

Table 4. Effect of varied doses of MLEPO on ALT

Parameter	ALT (U/L)			
Duration / Treatment	14 days	28 days	42 days	60 days
Group A (control)	31.75±3.75	31.00±0.41	24.00±2.42	33.50±1.19
Group E (125mg/kg)	32.75±2.78	30.50±0.65	27.75±2.56	34.00±3.06
Group F (250mg/kg)	34.25±1.93	34.00±2.65	35.25±1.80[a]	34.50±1.32
Group G (500mg/kg)	36.25±2.06	32.25±2.95	34.50±2.60[a]	32.50±2.60

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control)*

3.2.3 Alkaline phosphatase (ALP) assay

A significant ($p<0.05$) elevation in the mean ALP level was recorded in the 250mg/kg CLEPO treated rats (group C) on day 14 and in 500mg/kg CLEPO treated rats (group D) on day 14 and 60 of treatment relative to the control (Table 5). Other CLEPO treatment groups did not show any significant difference ($p>0.05$) in the mean ALP level. MLEPO treatment significantly decreased ($p<0.05$) the mean ALP levels in the 500mg/kg treated rats (group G) on day 28 and 42, whereas other treated groups showed no significant ($p>0.05$) variation in the mean ALP level relative to the control (Table 6).

3.2.4 Total protein and albumin assay

Tables 7 and 8 show that CLEPO and MLEPO had no significant ($p>0.05$) effect on the mean total protein and albumin levels within the treatment groups in comparison with the control on day 14, 28, 42 and 60 of treatment.

3.2.5 Total and conjugated bilirubin assay

No significant ($p>0.05$) difference was recorded in total and conjugated bilirubin levels within the CLEPO treatment groups throughout the 60 days of treatment except in 125mg/kg treated rats (group B) where a significant ($p<0.05$) decrease

in conjugated bilirubin level was recorded on day 28 (Table 9).

MLEPO caused a significant ($p<0.01$) reduction in total bilirubin and conjugated bilirubin levels in 250mg/kg treated rats (group F) on day 42 and in 500mg/kg treated rats (group G) on day 28 respectively relative to control (Table 10). Other MLEPO treatment groups showed no significant ($p>0.05$) variation in the total and conjugated bilirubin levels when compared with the control.

3.3 Renal Biochemical Parameters

3.3.1 Creatinine assay

CLEPO and MLEPO had no significant ($p>0.05$) effect on the mean serum creatinine level in treated groups in comparison with the control on day 14, 28, 42 and 60 of treatment (Tables 11 and 12).

3.3.2 Urea assay

The mean serum level of all the CLEPO and MLEPO treatment groups showed no significant ($p>0.05$) variation with those of the control throughout the 60-day duration of treatment with the exception of group F (250mg/kg MLEPO treated rats) which showed a significant ($p<0.05$) reduction on day 28 (Tables 11 and 12).

3.3.3 Electrolyte assay

The mean serum level of Na^+, K^+ and HCO_3^- of all the CLEPO and MLEPO did not show any significant ($p>0.05$) variation with those of the control on day 14, 28, 42 and 60 (Tables 13 - 16).

On day 14 of CLEPO treatment, there was a significant ($p<0.05$) increase in the level of mean serum Cl^- in 500mg/kg treated rats (group D) relative to the control. Other CLEPO treatment groups showed no significant ($p>0.05$) variation in the Cl^- level when compared with the control (Table 15). MLEPO produced a significant ($p<0.05$) increase in the mean serum Cl^- level in 500mg/kg treated rats (group G) on day 14 and 60 in comparison with the control (Table 16). Other MLEPO treatments had no significant ($p>0.05$) effect on the Cl^- level throughout the 60 days duration of administration in comparison with the control.

Table 5. Effect of varied doses of CLEPO on ALP

Parameter	ALP (U/L)			
Duration / Treatment	14 days	28 days	42 days	60 days
Group A (control)	68.75±5.71	85.50±3.38	72.00±6.06	63.25±4.48
Group B (125mg/kg)	72.50±3.38	79.25±2.87	74.50±2.36	67.50±2.40
Group C (250mg/kg)	92.00±6.38[a]	62.00±5.48	59.75±5.36	72.00±9.83
Group D (500mg/kg)	93.50±1.55[a]	89.25±4.19	84.50±2.22	93.00±2.52[a]

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control)*

Table 6. Effect of varied doses of MLEPO on ALP

Parameter	ALP (U/L)			
Duration / Treatment	14 days	28 days	42 days	60 days
Group A (control)	68.75±5.71	85.50±3.38	72.00±6.06	63.25±4.48
Group E (125 mg/kg)	70.50±0.96	75.25±2.32	69.00±2.83	68.33±3.53
Group F (250 mg/kg)	69.00±9.15	82.25±5.23	64.50±10.02	80.75±1.49
Group G (500 mg/kg)	50.75±2.87	60.25±7.52[a]	43.00±2.48[a]	60.25±8.09

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control)*

Table 7. Effect of varied doses of CLEPO on total protein and albumin level

Parameter	Total protein (g/L)				Albumin (g/L)			
Duration / Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	70.50±4.17	71.50±2.22	69.75±1.97	74.00±1.68	43.00±4.32	42.50±3.30	38.00±3.24	40.50±3.57
Group B (125 mg/kg)	69.50±1.71	71.25±3.12	69.50±2.10	75.00±1.29	42.50±2.40	40.00±0.82	41.50±2.40	42.00±2.12
Group C (250 mg/kg)	69.50±1.19	65.75±2.06	68.25±0.75	66.25±2.87	45.75±2.63	38.50±1.44	40.00±0.91	37.75±1.89
Group D (500 mg/kg)	69.00±1.29	68.50±0.96	65.75±1.97	68.50±2.60	42.25±1.11	43.75±1.11	38.50±1.32	37.75±1.03

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

Table 8. Effect of varied doses of MLEPO on total protein and albumin level

Parameter	Total protein (g/L)				Albumin (g/L)			
Duration / Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	70.50±4.17	71.50±2.22	69.75±1.97	74.00±1.68	43.00±4.32	42.50±3.30	38.00±3.24	40.50±3.57
Group E (125mg/kg)	67.00±0.82	65.75±3.28	66.50±2.25	65.67±2.73	37.00±1.22	37.50±3.07	39.00±1.91	37.00±3.06
Group F (250mg/kg)	74.50±2.10	70.50±0.50	76.50±2.84	68.50±1.85	44.00±0.82	39.75±2.14	42.25±0.85	38.50±1.26
Group G (500mg/kg)	71.25±1.93	69.25±1.44	69.75±3.30	67.75±2.29	38.75±1.49	38.25±1.18	39.00±3.03	37.50±2.06

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

Table 9. Effect of varied doses of CLEPO on total and conjugated bilirubin level

Parameter	Total bilirubin (µmol/L)				Conjugated bilirubin (µmol/L)			
Duration / Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	23.00±1.68	25.50±1.71	22.50±2.10	18.50±1.32	12.75±1.49	15.50±1.66	12.25±1.31	10.75±0.95
Group B (125mg/kg)	20.75±3.25	17.25±2.84	17.25±2.95	20.50±1.55	9.50±1.19	10.75±2.10	10.50±2.10	12.25±1.11
Group C (250mg/kg)	24.25±1.03	15.00±2.16	15.00±1.63	17.75±2.43	16.25±1.25	7.75±1.25[a]	8.25±1.31	10.00±2.27
Group D (500mg/kg)	15.25±0.85	17.25±3.12	19.75±0.87	18.00±3.34	9.50±1.19	10.75±2.10	12.00±0.71	10.25±2.14

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

Table 10. Effect of varied doses of MLEPO on total and conjugated bilirubin level

Parameter	Total bilirubin (µmol/L)				Conjugated bilirubin (µmol/L)			
Duration / Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	23.00±1.68	25.50±1.71	22.50±2.10	18.50±1.32	12.75±1.49	15.50±1.66	12.25±1.31	10.75±0.95
Group E (125 mg/kg)	25.00±1.73	28.25±0.75	20.50±0.87	21.00±2.08	15.25±1.03	18.00±0.71	12.25±1.11	11.67±0.88
Group F (250 mg/kg)	20.25±1.18	18.50±2.60	14.50±0.96[b]	16.00±2.38	12.25±1.03	10.75±1.89	9.25±0.75	9.00±1.15
Group G (500 mg/kg)	18.75±1.93	15.00±1.41	17.75±0.48	17.50±2.63	11.50±1.19	8.50±0.87[b]	9.50±0.50	11.00±2.12

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

Table 11. Effect of varied doses of CLEPO on creatinine and urea level

Parameter	Creatinine (µmol/L)				Urea (mmol/L)			
Duration / Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	162.00±16.75	152.50±5.97	152.25±11.20	159.00±10.14	7.17±0.86	6.70±0.90	6.05±0.94	6.65±0.73
Group B (125mg/kg)	176.25±7.19	145.50±8.43	190.00±13.72	163.50±7.10	6.43±1.12	5.23±0.40	6.15±0.82	4.43±0.80
Group C (250mg/kg)	167.75±4.77	162.75±20.93	160.75±1.97	151.00±14.55	5.53±0.70	6.03±0.40	5.55±0.50	5.35±14.55
Group D (500mg/kg)	132.25±15.02	157.50±16.88	161.25±6.91	145.50±5.63	5.75±0.25	5.48±1.05	4.93±0.56	6.05±0.73

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

Table 12. Effect of varied doses of MLEPO on creatinine and urea level

Parameter	Creatinine (µmol/L)				Urea (mmol/L)			
Duration / Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	162.00±16.75	152.50±5.97	152.25±11.20	159.00±10.14	7.17±0.86	6.70±0.90	6.05±0.94	6.65±0.73
Group E (125mg/kg)	155.25±19.53	146.00±10.06	148.00±8.96	163.33±25.04	6.30±0.56	6.68±0.49	5.43±0.70	5.90±0.45
Group F (250mg/kg)	177.25±11.22	175.50±10.01	181.75±14.08	164.50±9.19	7.33±0.50	4.30±0.35[a]	7.53±0.35	4.93±0.97
Group G (500mg/kg)	177.75±6.87	169.25±15.64	174.75±5.44	172.25±10.96	5.18±0.22	4.78±0.32	5.73±0.46	5.20±0.36

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

Table 13. Effect of varied doses of CLEPO on potassium and sodium

Parameter	Potassium (mmol/L)				Sodium (mmol/L)			
Duration / Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	4.60±1.23	4.23±1.11	5.33±0.72	3.00±0.58	117.00±7.67	115.00±9.70	102.75±4.99	110.50±5.58
Group B (125 mg/kg)	4.70±0.29	4.10±0.26	5.18±0.76	4.05±0.16	97.00±3.24	106.00±2.89	96.25±3.71	99.50±1.71
Group C (250 mg/kg)	4.33±0.18	4.38±0.30	4.55±0.18	3.75±0.16	108.00±0.82	111.00±2.38	106.75±1.38	109.50±2.53
Group D (500 mg/kg)	4.50±0.29	5.15±0.41	4.20±0.70	4.58±0.58	99.25±6.52	102.00±5.40	97.75±4.59	98.25±6.49

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

Table 14. Effect of varied doses of MLEPO on potassium and sodium

Parameter	Potassium (mmol/L)				Sodium (mmol/L)			
Duration / Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	4.60±1.23	4.23±1.11	5.33±0.72	3.00±0.58	117.00±7.67	115.00±9.70	102.75±4.99	110.50±5.58
Group E (125mg/kg)	5.30±0.20	3.43±0.34	5.15±0.23	4.47±0.29	105.00±2.80	107.50±4.29	102.25±1.93	100.00±4.51
Group F (250mg/kg)	3.70±0.27	4.13±0.37	4.45±0.59	3.55±0.29	101.00±2.42	107.75±1.31	100.25±3.57	103.25±1.11
Group G (500mg/kg)	5.15±0.16	4.90±0.33	4.78±0.23	4.10±0.13	103.00±1.58	96.50±4.57	101.75±1.70	101.75±2.81

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

Table 15. Effect of varied doses of CLEPO on chloride and bicarbonate

Parameter	Chloride (mmol/L)				Bicarbonate (mmol/L)			
Duration / Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	49.00±0.71	52.00±2.04	51.25±1.93	49.75±1.38	25.50±1.26	26.50±2.06	28.50±0.50	24.50±0.96
Group B (125mg/kg)	50.50±0.87	50.50±2.40	50.50±1.71	51.50±1.19	24.00±0.82	25.00±1.29	28.50±0.96	26.50±0.96
Group C (250mg/kg)	51.00±1.29	51.50±1.19	50.50±0.96	49.50±0.87	29.50±0.50	25.00±1.29	29.00±0.58	25.00±0.63
Group D (500mg/kg)	54.00±0.71[a]	47.00±5.21	47.25±4.21	48.47±3.86	24.50±1.50	25.00±2.38	26.00±1.63	26.00±1.41

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

Table 16. Effect of varied doses of MLEPO on chloride and bicarbonate

Parameter	Chloride (mmol/L)				Bicarbonate (mmol/L)			
Duration / Treatment	14 days	28 days	42 days	60 days	14 days	28 days	42 days	60 days
Group A (control)	49.00±0.71	52.00±2.04	51.25±1.93	49.75±1.38	25.50±1.26	26.50±2.06	28.50±0.50	24.50±0.96
Group E (125mg/kg)	52.00±0.71	49.25±2.98	53.50±0.65	50.00±2.08	27.50±1.89	26.00±1.83	25.50±2.06	27.33±1.76
Group F (250mg/kg)	52.50±1.44	56.25±1.38	55.00±3.03	52.75±0.75	25.50±0.96	25.00±1.91	24.50±0.96	28.50±0.96
Group G (500mg/kg)	56.75±3.09[a]	58.75±1.11	57.75±5.51	56.25±1.11[a]	26.00±1.83	29.00±1.00	26.50±1.71	26.00±0.82

** Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a','b' indicate significant difference (at p<0.05, p<0.01 respectively) compared to Group A (Control).*

4. DISCUSSION

The liver and kidney are among the major internal organs of mammals with numerous functions which include metabolism and detoxification. Evaluation of liver and kidney functions are therefore vital in the assessment of the toxicity of drugs and plant extracts because of their role in the survival of animals. Plasma serum biochemistry tests commonly referred to as liver and kidney function tests provide information on the functional state of these organs. The liver function test is used to determine the health status of the liver by measuring the levels of proteins (including albumins), liver enzymes (AST, ALT and ALP) and bilirubin (total and conjugate) in the blood [23]. Aspartate Transaminase (AST) is an enzyme found in different parts of the body such as the kidney, liver, heart, brain and muscle. Alanine Transaminase is also an enzyme found mainly in the liver while Alkaline Phosphatase (ALP) is found in the bone, liver and bile ducts. Injury to or inflammation of the organs where these enzymes are found, result in their increased blood level. Therefore, an increased level of these in the circulation can be an indication of injury or disease of those organs [24]. While AST, ALT and ALP are markers of liver damage or disease, ALT is more specific to the liver because AST and ALP may also be elevated in diseases affecting other organs, such as the heart or muscles [25].

The result of this study shows that MLEPO caused a decrease in plasma AST and ALP levels with an increase in ALT levels. The significantly higher plasma ALT levels on day 42 in 250 and 500 mg/kg groups were due to the reduction in the ALT value of the control on that day (24.00) as opposed to other control values of 31.75, 31.00 and 33.50 for days 14, 28 and 60 respectively. This finding is however an incidental finding. Again, the plasma levels on some days in MLEPO and CLEPO groups were within the range of 35.25 and 34.50; the values at which MLEPO showed significant increase for ALT on day 42. CLEPO decreased the plasma AST level, increased the ALP level and showed no significant effect on ALT level. The elevated plasma ALP level observed in CLEPO test groups as opposed to the reduced level in MLEPO could be associated with the activity of this non polar extract (CLEPO) on other organ(s) outside the liver. Increased blood ALP level is an indication for liver injury or a disorder leading to increased bone cell activity. Since the ALT, total bilirubin, total protein and albumin showed no significant variation; and AST and the conjugated bilirubin were low, it suggests that the increased ALP may not be attributed to the liver.

The decreased activity of these enzymes as recorded in this study agrees with the report of Dkhil et al. [26] that the lowered serum level of ALT, ALP and AST is due to the antioxidant property of *P. oleracea* which plays a protective role against liver injury. Abd El-Aziz et al. [27] also reported that *P. oleracea* extracts administered for 4 weeks decreased the activity of these liver enzymes. Similarly, *P. oleracea* has been reported to possess hepatoprotective effect in rats [28,29]. The relatively low level of the plasma activity of these enzymes in the rats may imply that the extracts have the potential to stabilize the cell membrane which in turn reduces the escape of these enzymes from the cellular sources such as hepatocytes, skeletal myocytes and other depots [30]. Okoye et al. [31] and Anosike et al. [32] have reported this cell membrane stabilizing activity in rats administered methanol extract of *T. occidentalis* (fluted pumpkin) leaf and *S. aethiopicum* (African garden egg) respectively.

The results of this study indicate that both extracts have no significant effect on total protein and albumin levels of test groups throughout the 60-day treatment duration. This implies that the functional integrity of the liver was not compromised by the *P. oleracea* leaf extracts since reduced serum protein is usually implicated in liver damage [33]. The test for total protein measures the total amount of albumin and globulin which are the two types of protein found in the body. These proteins are synthesized by the liver and are also present in the blood. While Albumin proteins prevent the seepage of fluid from blood vessels, globulin proteins are chiefly involved in the immune system. A decrease in blood albumin level is an indication of malfunctioning of liver or kidney, and can also indicate for nutritional deficiency.

The low plasma level of total bilirubin in this study indicates that *P. oleracea* leaf extracts did not cause liver failure or injury, haemolytic anaemia or blockage of bile duct, conditions which would have resulted in an increased level of blood bilirubin. Bilirubin is the end product of heme metabolism. Towards the end of the lifespan of red blood cells when aged red blood cells are lysed, haemoglobin is released. The

heme molecule from the haemoglobin is changed to unconjugated or direct bilirubin which is bound to albumin and transferred to the liver where it attaches to glucuronic acid to form conjugated or indirect bilirubin. Conjugated bilirubin is integrated into bile prior to the storage in the gall bladder. Bile is released into the small intestine where it aids the digestion of fats. It is finally excreted along with faeces. Inability of the liver to process the circulating bilirubin can lead to increased blood bilirubin level.

The low plasma conjugated bilirubin observed in this study implies that the small amount of bilirubin in the circulation was conjugated to glucoronic acid in the liver. This conjugated bilirubin may have been mixed in the bile leaving a little in the circulation. This may account for the reduced plasma level of conjugated bilirubin by *P. oleracea* leaf extracts. MLEPO exhibited more activity than CLEPO in this regard because MLEPO was shown to cause a significant ($p<0.01$) reduction in both total and conjugated bilirubin levels in plasma. This indicates that methanol leaf extracts of *P. oleracea* which may contain more polar phytochemicals than the chloroform leaf extracts elicit greater physiological activity in terms of enhancing metabolism and bilirubin clearance. The methanol leaf extract of *P. oleracea* therefore has greater effect on the functionality of the liver and bile duct.

P. oleracea leaf extracts had no significant effect on the mean plasma creatinine level of treated rats on day 14, 28, 42 and 60 of treatment. While CLEPO did not significantly decrease the mean plasma urea level of treated rats throughout the 60-day treatment, MLEPO significantly reduced the urea level at the dose of 250mg/kg on day 28. The reduced plasma urea level and non significant effect on creatinine level by *P. oleracea* extracts indicate that these extracts are not toxic to the kidneys. Plasma creatinine is an important indicator of renal health because the kidneys maintain a normal range of blood creatinine level. A rise in blood creatinine level is observed in marked renal injury. Blood urea level is also used as a marker of renal function; however, it is inferior to the creatinine marker because blood urea levels are influenced by other factors such as diet and dehydration [34]. Nonetheless, a persistent rise in blood urea and creatinine is a sign of kidney failure. Contrary to our finding, Shafi and Tabassum [35] reported that the ethanolic extract of P.oleracea whole plant increased the serum urea, total protein and albumin level when administered to mice for 14 days at the doses of 200 and 400 mg/kg.

The leaf extracts of *P. oleracea* produced no significant variation in the mean plasma level of sodium, potassium and bicarbonate in all the test groups throughout the duration of the experiment. However, the mean plasma level of chloride was significantly reduced at the dose of 500mg/kg in CLEPO on day 14 and MLEPO on day 14 and 60 respectively. These findings are indication that the plant extracts are non toxic to the kidneys and so did not adversely affect their metabolic function. The kidneys are the major elimination route for most drugs and plant extracts as well as their metabolites. A delay in the excretion can occur as a result of renal impairment, leading to systemic toxicity. The kidneys play vital role in maintaining electrolyte concentrations by filtering fluid and electrolytes from blood while returning some to the blood, and excreting any excess into the urine [36]. The blood electrolytes - sodium, potassium, chloride, and bicarbonate are involved in the regulation of nerve and muscle function. Potassium, an essential intracellular, positively charged ion, is actively “pumped” into the cell from surrounding extracellular fluid, while sodium, an essential extracellular positively charged ion, is pumped out of the cell. This is necessary for proper homoeostasis and the creation of electrical charges across the cell membrane. Elevated level of these blood electrolytes could be an indication of renal failure. According to Eaton and Pooler [37], increased potassium levels could be as a result of damage to the kidneys resulting in extrusion of the ions into the extracellular space.

The result of the acute toxicity test which showed that the animals tolerated up to 5000mg/kg of chloroform and methanol leaf extracts of *Portulaca oleracea* without mortality, morbidity or other apparent signs of toxicity indicates that both extracts were not noxious at the maximum dose.

5. CONCLUSION

Based on the findings of this study, it is therefore concluded that *P. oleracea* leaf extracts as used in this study is neither hepatotoxic nor nephrotoxic, and by implication did not cause systemic toxicity in the experimental animals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study protocols were duly approved by the Research Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt with the Ref. No: UPH/CEREMAD/REC/04. All experimental animals were humanely handled in accordance with the Ethics and Regulation guiding the use of research animals as approved by the University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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