



## Acute, Sub-acute and Chronic Toxicity Evaluation of Aqueous Extract of the Aerial Parts of *Leonurus cardiaca* in Normal Non-pregnant Female Wistar Albino Rats per OECD 425 TG

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### Authors' contributions

This study was carried out in collaboration among all authors. All authors read and approved the Final manuscript.

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### ABSTRACT

This study graded the acute, sub-acute, and chronic toxicity of aqueous extract of the aerial parts of *Leonurus cardiaca* in non-pregnant female wistar rats. Fifty wistar albino rats weighing between 180 and 200g were used for this study. The rats were grouped into 10 groups of five rats per group. Group 1 served as control while group 2-11 were orally administered aqueous extract of *Leonurus cardiaca* at 166, 250, and 500mg/kg for 7, 14, and 21 days. All haematological and biochemical parameters were determined based on standard methods. The plasma RBC, Hb, MCV, and WBC of the control were  $5.33 \pm 0.01 \times 10^{12}/L$ ,  $13.54 \pm 0.01$  g/dl,  $42.16 \pm 0.01$  fl, and  $1.83 \pm 0.01 \times 10^9/L$  respectively. The plasma RBC, Hb, MCV, and WBC of the rats treated with 500mg/kg of the extract for 21 days were  $10.93 \pm 0.01 \times 10^{12}/L$ ,  $19.24 \pm 0.01$ g/dl,  $28.33 \pm 0.01$ fl, and  $1.41 \pm 0.01 \times 10^9/L$  respectively and were significantly different from the control at  $p \leq 0.05$ . The plasma  $Na^+$ ,  $K^+$ ,  $Cl^-$ ,  $HCO_3^-$ , creatinine, and urea levels treated with extract at 500mg/kg for 21 days were  $167.97 \pm 0.01$  01mmol/l,  $7.85 \pm 0.01$ 01mmol/l,  $164.24 \pm 0.01$ 01mmol/l,  $40.84 \pm 0.01$ 01mmol/l,  $1.16 \pm 0.01$ 01mmol/l, and  $5.68 \pm 0.02$  01mmol/l respectively, were significantly different from the control at  $p \leq 0.05$ . The plasma ALT, ALP, and AST activities treated with extract at 500mg/kg for 21 days were  $146.24 \pm 0.02$ U/L,  $77.33 \pm 0.01$ U/L, and  $168.71 \pm 0.01$ U/L respectively, were significantly different from the control at  $p \leq 0.05$ . Enhancement on architecture of the heart, kidney and liver tissues occurred after treatment

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with 500mg/kg of the extract for 21 days in comparison to the control. The significantly improved effects observed on all assayed parameters were expressive that the extract of the aerial parts of *Leonurus cardiaca* is safe at 500 mg/Kg.

**Keywords:** HPLC; G.C; heart; kidney; liver; *Leonurus cardiac*; plasma.

## 1. INTRODUCTION

Medicinal plants elicit several therapeutic virtues, but they are not free from any danger of intoxication. Researchers have pointed out the potential toxicity, as well as the risks associated with the use of certain species of plants and vegetables [1]. Some medicinal plants that toxic cause damage to vital organs such the liver, kidney, heart, and the brain, which has made studies on the safety and effectiveness of medicinal plants of great concern in order to guarantee their usages [2].

Toxicity can be considered as complete indication of poisonous impact which present the state of adverse effects facilitated by the interaction of toxicants with the cellular components of an organism. Perniciousness can occur within or around cell membranes on the periphery of tissues including at the extracellular matrix [3].

*Leonurus cardiaca* (Motherwort) is known as a uterine stimulant, which is where its name originated from. It is used for the treatment of painful menstruation, delayed ovulation and menstrual flow. *Leonurus cardiaca* is also acknowledged to ease symptoms of menopause [4]. Preparations from the leaves of *Leonurus cardiaca* is used by traditional healers for treatment of heart failure. In Latin, the plant is called lion's heart, purporting the term motherwort and it is consumed as a cardiovascular tonic in Latin traditional system of medicine [5]. It strengthens the heart and is used to treat heart palpitations and irregularities, especially the ones that are associated with anxiety and tension [4]. However, there are no existing literature regarding the toxicological implication of *Leonurus cardiaca*, hence the need to investigate on acute, sub-acute and chronic toxicity effects of the aerial parts of the plants on rat model becomes imperative.

## 2. MATERIALS AND METHODS

### 2.1 Chemical/Reagents

All chemical/reagents used for this study were purchased from commercial industries and the

manufacturers' standard methods and procedure were strictly followed with regard to this study.

### 2.2 Source and Identification of Plant Material

The fresh aerial parts of *Leonurus cardiaca* (LNC) were harvested from Idema Community, in Ogbia Local Government Area of Bayelsa State, Nigeria. The plant sample was identified and authenticated by Dr. Ekeke Chimezie at the Herbarium Unit of the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt. The sample was registered with Voucher Number UPH/P/203.

### 2.3 Aqueous Extraction of the Aerial Parts of *Leonurus cardiac*

The fresh aerial parts of *Leonurus cardiaca* were harvested, washed with clean running tap water and air-dried under shade for five weeks. The dried aerial parts of the plant were pulverized into coarse powder. One hundred and five grams (105 g) of the powdered sample was macerated in 500 ml of distilled water at room temperature for 72 hours. The mixture was filtered using a Whatzman filter paper grade 1 (542 mm), the filtrate was condensed and evaporated to dryness using a rotary evaporator (RM2235 Leica Biosystems USA) and water bath at 50°C. The extract which weighed 79 g was stored in air-tight containers in a refrigerator until when required for treatments.

### 2.4 Preliminary Phytochemicals Screening

The aqueous extract of the aerial parts of *Leonurus cardiaca* were screened for the presence of various phytoconstituents according to the procedure described by Khandelwal [6].

### 2.5 Source of Experimental Wistar Albino Rats

Fifty (50) adult non-pregant Wistar rats (*Rattus norvegicus*) weighing 180 and 200g were purchased from the Biochemistry Animal House,

University of Nigeria Nsukka (UNN) and be acclimatized for two weeks, giving free access to rat feed and distilled water (DW). The rats were kept in clean plastic cages in well ventilated room, fed with standard animal feeds produced by Grand Cereals and Oil Mills Ltd., Port Harcourt, and water *ad libitum*. The animals were handled with care, according to the principles and standard protocols for the use of laboratory animals for experiments.

## 2.6 Determination of Acute Oral Toxicity and Median Lethal Dose (LD<sub>50</sub>) of Aqueous Extract of the Aerial Parts of *Leonurus cardiaca*

The LD<sub>50</sub> values of aqueous extract of the aerial parts of *Leonurus cardiaca* was determined using the up and down method of Organization of Economic Cooperation and Development (UPD/OECD 425) (1998), which received regulatory approval by the regulatory body (PISC, 2017). Following this method, twenty (20) female, non-pregnant wistar albino rats were obtained and grouped into four groups, five rats per group. The wistar albino rats in each group were sequentially dosed one at time, using four graded doses (1250, 2500, 3750 and 5000 mg/kg b.wt) of aqueous extract of the aerial parts of *Leonurus cardiaca*. Rats in group one were first administered the extract at 1250 mg/kg b.wt once and observed for two days for signs of toxicity and mortality. After two days of observation, toxicity and mortality did not occur, hence the initial dose was doubled (2500 mg/kg b.wt) and administered to rats in group two and same continued until 5000mg/kg. B.wt, based on the up and down method approved by Peta International Science Consortium Ltd in 2017 reported by Enevide et al. [7] and Earnest et al. [8]. The median lethal dose was calculated using the formula as explained by Enevide et al. [7] shown below:

$$LD_{50} = \frac{[M_0 - M_1]}{2}$$

Where M<sub>0</sub> = Highest dose of test substance that gave no mortality,  
M<sub>1</sub> = Lowest dose of test substance that gave mortality.

## 2.7 Animals and Approval from Animal Ethical Committee

Healthy nulliparous and non-pregnant female wistar albino rats (180-200g) between 8 and 10 weeks were used for all the experiments in this

present study. The animals were maintained under standard husbandry conditions in the animal house of 'College of Pharmaceutical Sciences, University of Port Harcourt, Nigeria (temperature 25 ± 2 °C) in a natural light-dark cycle and fed with standard rodent diet and water *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of UPH/CEREMAD/REC/MM72/035 .Ref. No. G2018/PHD/BCH/FT/009).

## 2.8 Experimental Design

Fifty (50) wistar albino rats weighing between 180 and 200g were used for this study. They were be purchased from the Biochemistry Animal House, University of Nigeria Nsukka (UNN) and be acclimatized for two weeks, giving free access to rat feed and distilled water (DW). The rats were then be grouped on basis of body weight into ten groups five rats per group and treated as follows:

- Group 1: Normal control: Feed + H<sub>2</sub>O.
- Group 2: 166 mg/kg/b.wt Extract+ feed+ H<sub>2</sub>O for 7days
- Group 3: 166 mg/kg/b.wt Extract+ feed+ H<sub>2</sub>O for 714 days
- Group 4: 166 mg/kg/b.wt Extract+ feed+ H<sub>2</sub>O for 21 days
- Group 5: 250 mg/kg/b.wt Extract+ feed+ H<sub>2</sub>O for 7days
- Group 6: 250 mg/kg/b.wt Extract+ feed+ H<sub>2</sub>O for 14 days
- Group 7: 250 mg/kg/b.wt Extract+ feed+ H<sub>2</sub>O for 21 days
- Group 8: 500 mg/kg/b.wt Extract + feed+ H<sub>2</sub>O for 7 days
- Group 9: 500 mg/kg/b.wt Extract + feed+ H<sub>2</sub>O for 14 days
- Group 10: 500 mg/kg/b.wt Extract + feed+ H<sub>2</sub>O for 21 days

Exactly, 24 hours after the last day of oral treatment with the extract, the rats were humanly sacrificed through cervical dislocation, blood sample were be collected for biochemical assays and estimation of haematological profile. The heart, kidney and liver were harvested and were cut into two equal halves for each organ. Each half of the organs were homogenized for estimation of oxidative biomarkers for heart and kidney, while the other halves were subjected to histological examination.

## 2.7 Biochemical Analysis

Different biochemical parameters were measured using ELISA and Randox kits in a biochemical analyzer. The parameters observed for liver function parameters observed are aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate, bilirubin, albumin and, and total protein. Plasma cardiac markers were cardiac troponin T (CTn-T), cardiac troponin I (CTn-I), interleukin 6 (IL-6) and C-reactive protein (CRP). The plasma enzymes biomarkers of cardiac were creatine kinase (CK), alanine amino transferase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST) and lactate dehydrogenase (LDH) while the Kidney homogenate biomarkers were sodium ion ( $\text{Na}^+$ ), potassium ion ( $\text{K}^+$ ), chloride ion ( $\text{Cl}^-$ ), bicarbonate ion ( $\text{HCO}_3^-$ ), creatinine and blood urea nitrogen (BUN). The oxidative plasma cardiac and kidney homogenate biomarkers determined were malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (PDx), superoxide dismutase (SOD) and catalase (CAT). Lipid Profile determined were total cholesterol, triglyceride, high density lipoprotein (HDL), very low density lipoprotein (VLDL), and low density lipoprotein (LDL) were observed.

## 2.8 Haematological Analysis

Blood samples from non-pregnant female wistar albino rats (both treated and vehicle control groups) were collected into EDTA containing tubes for haematological study. Hemoglobin (Hb), total RBC, packed cell volume (PVC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), hematocrit, white blood cells (WBC) count were determined.

## 2.9 Histopathological Analysis of Heart, Kidney, and Liver Tissues

The heart, kidney, and liver isolated from sacrificed non-pregnant wistar albino rats were fixed in 10% formalin, then after processing embedded in paraffin wax. Paraffin sections were made at 5mm and stained with hematoxylin and eosin. The slides were studied under a light microscope and captured the magnified images of tissues structure for further study.

## 2.10 Statistical Analysis

All Data are represented as means  $\pm$  standard deviation ( $M \pm S$ ) and were analyzed using

Statistical Package for Social Sciences (SPSS) for window version 17.0 USA. Descriptive statistics was done by one way analysis of variance (ANOVA) and multiple comparison was done using Turkey Post hoc at ( $p \leq 0.05$ ) confidence interval.

## 3. RESULTS AND DISCUSSION

Medicinal plants have been used many years ago to treat various diseases. One of such medicinal plant is *Leonurus cardiaca*. The plant is used in Latin and Nigeria by traditional healers as curative agent. There may be toxicological implication associated with the plant's constituents. Hence, this study was designed to grade and evaluate the toxic effect of the plant by oral route through modification of 425 toxicity guidelines in order to achieve the purpose the study.

The medicinal properties and toxicity effects engendered by medicinal plants are attributed to the presence of bioactive ingredients in the roots and aerial parts of such plants [10]. In this study, phytochemical screening and quantification of the aerial parts of *Leonurus cardiaca* showed the presence of terpenoids, phenolic acids, anthraquinones, anthocyanins, saponins, tannins, phytates, flavonoids, cynogenic glycosides, alkaloids, glycosides, and essential oils as shown in Table 1, which could be responsible for the medicinal properties of the reported by [11-14].

Toxic and mortality outcomes can be assessed by the clinical signs and symptoms among other toxicity markers [15]. In this present study, aqueous extract of the aerial parts of *Leonurus cardiaca* did not caused mortality even at 5000 mg/kg b.wt based on the OECD 225 TG guidelines. Also, the wistar albino rats exposed to the extracted at four graded doses particularly at 166, 250, and 500 mg/kg b.wt, did not lead to mortality in any of the rats tested in each group as shown in Table 2 to 4. Behavioral studies like respiration, increased somatomotor activity and itching were been observed during the first 30 min after the aqueous extract of the aerial parts of *Leonurus cardiaca* was orally administered. These results are suggestive that the extract did interfere with the normal physiological and biochemical processes within the rats, which agrees with the report of [15-17].

**Table 1. Preliminary phytochemical and essential oil characterization of aqueous extracts of the aerial parts of *Leonurus cardiac***

Phytochemical	Concentration (mg/100 g)
Terpenoids	26.19 x 10 <sup>-1</sup>
Phenolic acids	506.33
Saponins	62.33
Cyanogenic glycosides	118.03
Glycosides	16.17
Anthocyanins	56.53
Alkaloids	1.31
Flavonoids	7.31
Sterols	5.91
Tannins	426.49
Phytate	69.12
Essential oils	100%

**Table 2. Dose-dependent effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on toxicity parameters in normal non-pregnant female wistar albino rats for 7 days of exposure**

Parameter	166 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
Coma	A	A	A
Convulsion and tremor	A	A	A
Eye and bleeding	N	N	N
Faeces consistency	N	N	N
Itching	N	N	P
Mucous membrane	N	N	N
Mortality	A	A	A
Respiration	N	N	N
Salivation	N	P	P

A=absent, N=Normal, P= present

**Table 3. Dose-dependent effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on toxicity parameters in normal non-pregnant female wistar albino rats for 14 days of exposure**

Parameter	166 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
Coma	A	A	A
Convulsion and tremor	N	A	A
Eye and bleeding	N	N	N
Faeces consistency	N	N	N
Itching	N	N	P
Mucous membrane	N	N	N
Mortality	A	A	A
Respiration	N	P	N
Salivation	N	P	P

A=absent, N=Normal, P= present

**Table 4. Dose-dependent effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on toxicity parameters in normal non-pregnant female wistar albino rats for 21 days of exposure**

Parameter	166 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
Coma	A	A	A
Convulsion and tremor	A	P	P
Sleep	N	N	N
Faeces consistency	N	N	N
Itching	N	P	P
Mucous membrane	N	N	N
Mortality	A	A	A
Respiration	N	P	N

A=absent, N=Normal, P= present

**Table 5. Effect of aqueous of the aerial part of *Leonurus cardiaca* on the plasma haematological parameters of normal wistar albino rats**

Treatment	RBC (X 10 <sup>12</sup> /L)	Haemoglobin (g/dl)	Haematocrit (l/L)	MCV (fl)	MCH (pg)	WBC (X 10 <sup>9</sup> /L)
Normal control	5.33±0.01	13.54±0.01	0.55±0.01	42.16±0.01	50.24±0.01	1.83±0.01
166mg/kg for 7 days	5.65±0.01 <sup>a</sup>	13.84±0.02 <sup>a</sup>	0.86±0.01 <sup>a</sup>	42.01±0.01 <sup>a</sup>	50.44±0.01 <sup>a</sup>	1.80±0.00 <sup>a</sup>
166mg/kg for 14 days	5.95±0.01 <sup>a</sup>	14.85±0.01 <sup>a</sup>	0.93±0.02 <sup>a</sup>	41.94±0.01 <sup>a</sup>	50.94±0.01 <sup>a</sup>	1.77±0.01 <sup>a</sup>
166mg/kg for 21 days	6.25±0.02 <sup>a</sup>	15.05±0.01 <sup>a</sup>	1.04±0.01 <sup>a</sup>	41.72±0.01 <sup>a</sup>	51.44±0.01 <sup>a</sup>	1.72±0.01 <sup>a</sup>
250mg/kg for 7 days	7.33±0.01 <sup>a</sup>	16.45±0.01 <sup>a</sup>	1.64±0.01 <sup>a</sup>	38.83±0.01 <sup>a</sup>	62.43±0.01 <sup>a</sup>	1.65±0.01 <sup>a</sup>
250mg/kg for 14 days	7.85±0.02 <sup>a</sup>	16.94±0.01 <sup>a</sup>	1.85±0.02 <sup>a</sup>	38.44±0.01 <sup>a</sup>	62.93±0.01 <sup>a</sup>	1.61±0.01 <sup>a</sup>
250mg/kg for 21 days	8.34±0.01 <sup>a</sup>	17.85±0.01 <sup>a</sup>	2.15±0.02 <sup>a</sup>	38.13±0.01 <sup>a</sup>	63.44±0.01 <sup>a</sup>	1.57±0.01 <sup>a</sup>
500mg/kg for 7 days	10.24±0.01 <sup>a</sup>	18.44±0.02 <sup>a</sup>	2.65±0.01 <sup>a</sup>	28.84±0.01 <sup>a</sup>	69.24±0.01 <sup>a</sup>	1.51±0.01 <sup>a</sup>
500mg/kg for 14 days	10.65±0.01 <sup>a</sup>	18.95±0.02 <sup>a</sup>	2.85±0.01 <sup>a</sup>	28.53±0.01 <sup>a</sup>	69.82±0.01 <sup>a</sup>	1.46±0.01 <sup>a</sup>
500mg/kg for 21 days	10.93±0.01 <sup>a</sup>	19.24±0.01 <sup>a</sup>	3.06±0.02 <sup>a</sup>	28.33±0.01 <sup>a</sup>	71.04±0.01 <sup>a</sup>	1.41±0.01 <sup>a</sup>

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values with similar superscript letters indicate significant differences ( $p \leq 0.05$ ) when compared with the control group

Hematological parameters are perceptive indicators of the physiological changes in retort to any toxic stress or environmental pollutant in animals [18]. Blood platelet plays a vital role in Haemostasis [19]. In this investigation, remarkable increases occurred on the plasma RBC, Hb, MCV, MCH, haematocrit, and WBC, after exposure to the extract at 166, 250, and 500 mg/kg b.wt for 7, 14 and 21 days as shown in Table 4. The remarkable increases on the haematological profile of the extract treated rats are evocative that enhancement on haematopoiesis pathway in the extract treated rats, thus agreeing with the reports of [18 and 19].

Treatment with aqueous extract of the aerial parts of *Leonurus cardiaca* at 166mg/kg b.wt for 14 and 21 days, resulted in significant decreases on the CTn-T, CTn-I, IL-6 and c-reactive protein in group 4-5 when compared to the positive and negative control (Table 6.).

Significant decreases ( $p < 0.05$ ) on the CTn-T, CTn-I, IL-6 and c-reactive protein occurred in rats after treatment with *Leonurus cardiaca* extract at 250mg/kg b.wt. Treatment with the extract 250mg/kg b.t yielded more significant decreases ( $p < 0.05$ ) on the CTn-T, CTn-I, IL-6 and c-reactive protein when compared with the control (Table 6). However, treatment at 500mg/kg b.wt significantly resulted in decreases ( $p < 0.05$ ) on the plasma CTn-T, CTn-I, IL-6 and c-reactive protein when compared to the control. The significant decreases observed after treatment with aqueous extract of *Leonurus cardiaca* were reflective of the ability of *Leonurus cardiaca* to enhance cardiac functioning through improvement on the neuro-cardiac protein and hormones. The significant improved effects elicited by *Leonurus cardiaca* extract agrees with the report of Kaksha et al. [17] on evaluation of cardioprotective effect of aqueous extract of *Garcinia indica* Linn. fruit rinds on isoprenaline-induced myocardial injury in Wistar albino rats.

Significant increases ( $p < 0.05$ ) were observed after treatment with the extract at 166mg/kg b.wt dose for 7, 14, and 21 days in comparison to the control (Table 6), which is reflective that the extract at 166mg/k elicit improved effect n kidney function in the rats. However, significant increases on kidney plasma sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ), bicarbonate ( $\text{HCO}_3^-$ ), creatine and urea concentrations were observed at 250mg/kg b.wt treatment for 7, 14,

and 21 days in comparison to the control values (Table 7). More so, a more significant increases ( $p < 0.05$ ) on the plasma sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), chloride ( $\text{Cl}^-$ ), bicarbonate ( $\text{HCO}_3^-$ ), creatine and urea concentrations were observed at 500mg/kg b.wt treatment for 7, 14, and 21 days in comparison to the control values (Table 7). The significant improvement or increases observed following treatment with aqueous extract of the aerial parts of *L. cardiaca* is suggestive that *Leonurus cardiaca* extract enhanced renal function by improving on the markers of renal function parameters of wistar rats. This findings were agreement with the report of [19] on the effects of ethanolic extracts of leaf, seed and fruit of *Datura metel* on amelioration of cisplatin-induced nephrotoxicity by ethanolic extract of *Bauhinia purpurea*: An in vivo study in rats on kidney function of male albino rats.

Also, oxidative damage is aggravated by the decrease in antioxidant enzymes activities such as superoxide dismutase, catalase (CAT), and glutathione peroxidase (GPx) which acts as a free radical scavengers in conditions associated with oxidative stress [20]. The decreases observed on the heart homogenate CAT, SOD, and GPx activities were pointers to oxidative stress condition which is in line with the report of [21]. Significant increases on the heart and kidney CAT, SOD, and GPx activities occurred after 7, 14, and 21 days after treatment with *Leonurus cardiaca* extract at 166, 250 and 500mg/kg in comparison to the control values (Table 8 and 9). The significantly increased plasma CAT, SOD and GPx activities noticed in both the heart and kidneys after exposure to *Leonurus cardiaca* extract are suggestive of the antioxidant properties of the aerial parts of *Leonurus cardiaca* against oxidative stress in rats. This findings confirm the report of [21] on the immunomodulatory potential of *Leonurus cardiaca* extract in relation to endothelial cells and platelets.

Treatment with aqueous extract of *Leonurus cardiaca* at 166, 250 and 500mg/kg b.wt for 7, 14, and 21 day resulted in a significantly decreased CK, ALT, ALP, AST, and LDH activities when compared with the control (Table 10). A more improved effects occurred at 250 and 500mg/kg b.wt treatment with the extract. The decreases noticed after treatment with *Leonurus cardiaca* extract is indicative of the potential capacity of the plant in enhancing cardiac performance through enhancement of

markers of cardiac protein parameters in rats. *hydasypica* R. Parker on histological and [22] reported similar results in their work on biochemical changes induced by cisplatin in the evaluating the protective potency of *Acacia* cardiac tissue of rats.

**Table 6. Effects of Aqueous extract of the aerial Parts *Leonurus cardiaca* on plasma cardiac markers in Normal Wistar Albino Rats**

Treatment	CTn-T (pg/mL)	CTn-I (pg/mL)	IL-6 Level (pg/dl)	CRP (mg/ml)
Normal control	291.14±0.01	172.24±0.01	27.34±0.01	14.28±0.02
166mg/kg b.t for 7 days	279.87±0.01 <sup>a</sup>	167.96±0.01 <sup>a</sup>	27.24±0.01 <sup>a</sup>	14.19±0.01 <sup>a</sup>
166mg/kg b.t for 14 days	279.02±0.01 <sup>a</sup>	164.92±0.02 <sup>a</sup>	27.15±0.01 <sup>a</sup>	14.06±0.01 <sup>a</sup>
166mg/kg b.t for 21 days	272.74±0.01 <sup>a</sup>	157.65±0.02 <sup>a</sup>	27.05±0.02 <sup>a</sup>	13.86±0.02 <sup>a</sup>
250mg/kg b.t for 7 days	251.71±0.01 <sup>a</sup>	141.75±0.01 <sup>a</sup>	25.87±0.01 <sup>a</sup>	13.66±0.03 <sup>a</sup>
250mg/kg b.t for 14 days	241.96±0.01 <sup>a</sup>	137.67±0.01 <sup>a</sup>	25.12±0.02 <sup>a</sup>	13.41±0.01 <sup>a</sup>
250mg/kg b.t for 21 days	241.22±0.01 <sup>a</sup>	133.73±0.01 <sup>a</sup>	22.74±0.01 <sup>a</sup>	13.21±0.01 <sup>a</sup>
500mg/kg b.t for 7 days	231.07±0.02 <sup>a</sup>	128.86±0.02 <sup>a</sup>	22.12±0.02 <sup>a</sup>	11.72±0.03 <sup>a</sup>
500mg/kg b.t for 14 days	210.77±0.01 <sup>a</sup>	128.04±0.01 <sup>a</sup>	19.84±0.01 <sup>a</sup>	11.40±0.01 <sup>a</sup>
500mg/kg b.t for 21 days	195.23±0.01 <sup>a</sup>	125.45±0.01 <sup>a</sup>	19.05±0.01 <sup>a</sup>	11.26±0.02 <sup>a</sup>

Values are reported as mean ± standard error of mean (M±SEM) (n=5). Values with similar superscript letters indicate significant differences (p≤ 0.05) control group

**Table 7. Effect of aqueous extract of the aerial parts of *Leonurus cardiaca* plasma kidney biomarkers concentrations in normal wistar albino rats**

Treatment	Na <sup>+</sup> (mmol/l)	K <sup>+</sup> (mmol/l)	Cl <sup>-</sup> (mmol/l)	HCO <sub>3</sub> (mmol/l)	Creatinine (mmol/l)	Urea (mmol/l)
Normal control	153.24±0.01	3.45±0.01	81.34±0.01	26.45±0.04	0.36±0.04	2.35±0.01
166mg/kg for 7 days	155.04±0.01 <sup>a</sup>	3.77±0.02 <sup>a</sup>	87.45±0.01 <sup>a</sup>	26.81±0.02 <sup>a</sup>	0.36±0.04	2.86±0.01 <sup>a</sup>
166mg/kg for 14 days	158.23±0.03 <sup>a</sup>	3.97±0.00 <sup>a</sup>	91.45±0.01 <sup>a</sup>	27.12±0.02 <sup>a</sup>	0.52±0.02	3.06±0.01 <sup>a</sup>
166mg/kg for 21 days	158.72±0.01 <sup>a</sup>	4.13±0.01 <sup>a</sup>	101.65±0.01 <sup>a</sup>	23.18±0.01 <sup>a</sup>	0.60±0.00 <sup>a</sup>	3.55±0.01 <sup>a</sup>
250mg/kg for 7 days	161.84±0.01 <sup>a</sup>	4.95±0.01 <sup>a</sup>	112.07±0.01 <sup>a</sup>	34.23±0.02 <sup>a</sup>	0.80±0.00 <sup>a</sup>	4.03±0.02 <sup>a</sup>
250mg/kg for 14 days	162.39±0.02 <sup>a</sup>	5.09±0.00 <sup>a</sup>	112.79±0.02 <sup>a</sup>	34.82±0.02 <sup>a</sup>	0.80±0.00 <sup>a</sup>	4.36±0.01 <sup>a</sup>
250mg/kg for 21 days	162.54±0.01 <sup>a</sup>	5.35±0.02 <sup>a</sup>	113.05±0.01 <sup>a</sup>	35.09±0.01 <sup>a</sup>	0.80±0.00 <sup>a</sup>	4.75±0.01 <sup>a</sup>
500mg/kg for 7 days	165.93±0.02 <sup>a</sup>	7.05±0.02 <sup>a</sup>	143.35±0.01 <sup>a</sup>	40.08±0.03 <sup>a</sup>	0.72±0.18 <sup>a</sup>	4.94±0.01 <sup>a</sup>
500mg/kg for 14 days	167.34±0.01 <sup>a</sup>	7.43±0.06 <sup>a</sup>	163.94±0.01 <sup>a</sup>	40.48±0.02 <sup>a</sup>	1.01±0.00 <sup>a</sup>	5.14±0.02 <sup>a</sup>
500mg/kg for 21 days	167.97±0.01 <sup>a</sup>	7.85±0.01 <sup>a</sup>	164.24±0.01 <sup>a</sup>	40.84±0.01 <sup>a</sup>	1.16±0.01 <sup>a</sup>	5.68±0.02 <sup>a</sup>

Values are reported as mean ± standard error of mean (M±SEM) (n=5). Values with similar superscript letters indicate significant differences (p≤ 0.05) when compared to the control group



**Table 8. Effect of aqueous of the aerial parts of *Leonurus cardiaca* on plasma oxidative stress cardiac biomarkers activities in normal wistar albino rats**

Treatment	MDA (mmol/l)	SOD (mg/g)	GPx (IU/g)	Catalase ( $\mu\text{mol.H}_2\text{O}_2\text{mg/pro/min}$ )
Normal control	4.95±0.01	6.00±0.00	56.24±0.01	84.78±0.59
166mg/kg b.t for 7 days	4.85±0.01 <sup>a</sup>	6.13±0.01 <sup>a</sup>	56.55±0.02 <sup>a</sup>	87.45±0.02 <sup>a</sup>
166mg/kg b.t for 14 days	4.33±0.01 <sup>a</sup>	6.73±0.01 <sup>a</sup>	56.84±0.01 <sup>a</sup>	89.03±0.01 <sup>a</sup>
166mg/kg b.t for 21 days	4.15±0.02 <sup>a</sup>	6.93±0.01 <sup>a</sup>	87.12±0.01 <sup>a</sup>	89.72±0.01 <sup>a</sup>
250mg/kg b.t for 7 days	3.83±0.02 <sup>a</sup>	7.27±0.01 <sup>a</sup>	91.05±0.02 <sup>a</sup>	96.75±0.02 <sup>a</sup>
250mg/kg b.t for 14 days	3.44±0.01 <sup>a</sup>	7.82±0.01 <sup>a</sup>	91.54±0.01 <sup>a</sup>	100.26±0.01 <sup>a</sup>
250mg/kg b.t for 21 days	3.84±0.01 <sup>a</sup>	8.02±0.01 <sup>a</sup>	100.04±0.01 <sup>a</sup>	100.72±0.02 <sup>a</sup>
500mg/kg b.t for 7 days	2.76±0.01 <sup>a</sup>	9.39±0.02 <sup>a</sup>	100.76±0.01 <sup>a</sup>	101.55±0.01 <sup>a</sup>
500mg/kg b.t for 14 days	2.54±0.01 <sup>a</sup>	9.84±0.01 <sup>a</sup>	101.23±0.01 <sup>a</sup>	101.89±0.02 <sup>a</sup>
500mg/kg b.t for 21 days	2.15±0.01 <sup>a</sup>	10.02±0.01 <sup>a</sup>	101.74±0.01 <sup>a</sup>	102.13±0.01 <sup>a</sup>

Values are reported as mean  $\pm$  standard error of mean ( $M\pm SEM$ ) ( $n=5$ ). Values with similar superscript letters indicate significant differences ( $p\leq 0.05$ ) when compared to the control group

**Table 9. Effect of aqueous of the aerial parts *Leonurus cardiaca* on the oxidative stress kidney biomarkers activities in normal wistar albino rats**

Treatment	MDA (mmol/l)	SOD (mg/g)	GPx (IU/g)	Catalase ( $\mu\text{mol H}_2\text{O}_2$ C/mg/pro./min)
Normal control	4.62±0.01	8.05±0.02	51.14±11.20	87.57±0.01
166mg/kg b.t for 7 days	2.45±0.01 <sup>a</sup>	8.23±0.01 <sup>a</sup>	62.94±0.01 <sup>a</sup>	87.64±0.01 <sup>a</sup>
166mg/kg b.t for 14 days	2.35±0.01 <sup>a</sup>	8.66±0.01 <sup>a</sup>	78.53±0.01 <sup>a</sup>	87.95±0.01 <sup>a</sup>
166mg/kg b.t for 21 days	2.22±0.01 <sup>a</sup>	93.06±0.01 <sup>a</sup>	81.53±0.01 <sup>a</sup>	88.77±0.01 <sup>a</sup>
250mg/kg b.t for 7 days	1.01±0.01 <sup>a</sup>	100.77±0.02 <sup>a</sup>	94.47±0.01 <sup>a</sup>	104.05±0.01 <sup>a</sup>
250mg/kg b.t for 14 days	0.87±0.01 <sup>a</sup>	100.95±0.01 <sup>a</sup>	100.45±0.01 <sup>a</sup>	104.83±0.01 <sup>a</sup>
250mg/kg b.t for 21 days	0.75±0.01 <sup>a</sup>	101.35±0.01 <sup>a</sup>	100.96±0.01 <sup>a</sup>	104.97±0.01 <sup>a</sup>
500mg/kg b.t for 7 days	0.34±0.01 <sup>a</sup>	142.08±0.01 <sup>a</sup>	132.33±0.01 <sup>a</sup>	116.66±0.01 <sup>a</sup>
500mg/kg b.t for 21 days	0.26±0.01 <sup>a</sup>	142.86±0.01 <sup>a</sup>	132.93±0.01 <sup>a</sup>	116.95±0.01 <sup>a</sup>

Values are reported as mean  $\pm$  standard error of mean ( $M\pm SEM$ ) ( $n=5$ ). Values with similar superscript letters indicate significant differences ( $p\leq 0.05$ ) when compared to the control group

**Table 10. Effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on plasma Cardiac biomarkers activities in normal non-pregnant wistar albino rats**

Treatment	C-kinase (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	LDH (U/L)
Normal control	192.27±0.02	63.25±0.01	72.05±0.01	185.87±0.01	178.24±0.01
166mg/kg for 7 days	188.24±0.02 <sup>a</sup>	162.73±0.01 <sup>a</sup>	67.25±0.01 <sup>a</sup>	156.46±0.02 <sup>a</sup>	161.69±0.02 <sup>a</sup>
166mg/kg for 14 days	165.24±0.02 <sup>a</sup>	158.65±0.01 <sup>a</sup>	61.88±0.01 <sup>a</sup>	142.85±0.01 <sup>a</sup>	155.33±0.01 <sup>a</sup>
166mg/kg for 21 days	157.10±0.01 <sup>a</sup>	145.76±0.01 <sup>a</sup>	57.08±0.01 <sup>a</sup>	132.05±0.01 <sup>a</sup>	144.23±0.01 <sup>a</sup>
250mg/kg for 7 days	150.66±0.01 <sup>a</sup>	121.07±0.01 <sup>a</sup>	53.87±0.01 <sup>a</sup>	119.53±0.01 <sup>a</sup>	132.34±0.02 <sup>a</sup>
250mg/kg for 14 days	146.31±0.01 <sup>a</sup>	105.64±0.01 <sup>a</sup>	50.14±0.01 <sup>a</sup>	100.64±0.01 <sup>a</sup>	114.06±0.02 <sup>a</sup>
250mg/kg for 21 days	125.43±0.01 <sup>a</sup>	89.53±0.01 <sup>a</sup>	47.26±0.01 <sup>a</sup>	94.03±0.02 <sup>a</sup>	102.06±0.02 <sup>a</sup>
500mg/kg for 7 days	102.85±0.01 <sup>a</sup>	73.08±0.01 <sup>a</sup>	45.76±0.01 <sup>a</sup>	87.02±0.01 <sup>a</sup>	97.02±0.02 <sup>a</sup>
500mg/kg for 14 days	97.24±0.01 <sup>a</sup>	63.66±0.01 <sup>a</sup>	41.25±0.01 <sup>a</sup>	83.03±0.02 <sup>a</sup>	92.76±0.01 <sup>a</sup>
500mg/kg for 21 days	94.05±0.03 <sup>a</sup>	61.76±0.01 <sup>a</sup>	35.96±0.01 <sup>a</sup>	74.04±0.01 <sup>a</sup>	85.66±0.01 <sup>a</sup>

Values are reported as mean  $\pm$  standard error of mean ( $M\pm SEM$ ) ( $n=5$ ). Values with similar superscript letters indicate significant differences ( $p\leq 0.05$ ) when compared to the control group

**Table 11. Effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on plasma liver biomarkers activities**

Treatment	TP (g/L)	Albumin (mmol/L)	Total bilirubin ( $\mu$ mol/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	GGT (U/L)
Normal control	55.24 $\pm$ 0.01	27.30 $\pm$ 0.01	10.45 $\pm$ 0.01	146.24 $\pm$ 0.02	77.33 $\pm$ 0.01	168.71 $\pm$ 0.01	9.56 $\pm$ 0.00 <sup>a</sup>
166mg/kg for 7 days	59.76 $\pm$ 0.19 <sup>a</sup>	29.05 $\pm$ 0.02 <sup>a</sup>	10.24 $\pm$ 0.01 <sup>a</sup>	131.84 $\pm$ 0.02 <sup>a</sup>	72.65 $\pm$ 0.01 <sup>a</sup>	162.54 $\pm$ 0.02 <sup>a</sup>	9.43 $\pm$ 0.01 <sup>a</sup>
166mg/kg for 14 days	63.24 $\pm$ 0.01 <sup>a</sup>	35.74 $\pm$ 0.01 <sup>a</sup>	10.04 $\pm$ 0.01 <sup>a</sup>	124.44 $\pm$ 0.02 <sup>a</sup>	67.23 $\pm$ 0.01 <sup>a</sup>	153.70 $\pm$ 0.02 <sup>a</sup>	9.35 $\pm$ 0.01 <sup>a</sup>
166mg/kg for 21 days	67.89 $\pm$ 0.02 <sup>a</sup>	39.53 $\pm$ 0.02 <sup>a</sup>	9.94 $\pm$ 0.01 <sup>a</sup>	117.24 $\pm$ 0.01 <sup>a</sup>	62.62 $\pm$ 0.01 <sup>a</sup>	149.68 $\pm$ 0.02 <sup>a</sup>	9.29 $\pm$ 0.0 <sup>a</sup>
250mg/kg for 7 days	73.12 $\pm$ 0.01 <sup>a</sup>	43.03 $\pm$ 0.02 <sup>a</sup>	9.53 $\pm$ 0.01 <sup>a</sup>	97.65 $\pm$ 0.01 <sup>a</sup>	56.34 $\pm$ 0.01 <sup>a</sup>	143.24 $\pm$ 0.02 <sup>a</sup>	9.03 $\pm$ 0.01 <sup>a</sup>
250mg/kg for 14 days	75.03 $\pm$ 0.01 <sup>a</sup>	48.65 $\pm$ 0.01 <sup>a</sup>	9.03 $\pm$ 0.01 <sup>a</sup>	92.03 $\pm$ 0.02 <sup>a</sup>	50.64 $\pm$ 0.02 <sup>a</sup>	137.22 $\pm$ 0.01 <sup>a</sup>	8.73 $\pm$ 0.01 <sup>a</sup>
250mg/kg for 21 days	75.96 $\pm$ 0.01 <sup>a</sup>	52.14 $\pm$ 0.01 <sup>a</sup>	8.44 $\pm$ 0.01 <sup>a</sup>	85.05 $\pm$ 0.02 <sup>a</sup>	44.93 $\pm$ 0.01 <sup>a</sup>	124.22 $\pm$ 0.01 <sup>a</sup>	8.24 $\pm$ 0.02 <sup>a</sup>
500mg/kg for 7 days	84.26 $\pm$ 0.02 <sup>a</sup>	64.04 $\pm$ 0.01 <sup>a</sup>	7.63 $\pm$ 0.01 <sup>a</sup>	73.35 $\pm$ 0.02 <sup>a</sup>	31.21 $\pm$ 0.01 <sup>a</sup>	115.64 $\pm$ 0.02 <sup>a</sup>	8.02 $\pm$ 0.01 <sup>a</sup>
500mg/kg for 14 days	85.93 $\pm$ 0.01 <sup>a</sup>	68.31 $\pm$ 0.07 <sup>a</sup>	16.85 $\pm$ 0.01 <sup>a</sup>	157.36 $\pm$ 0.02 <sup>a</sup>	27.21 $\pm$ 0.01 <sup>a</sup>	100.85 $\pm$ 0.01 <sup>a</sup>	7.52 $\pm$ 0.01
500mg/kg for 21 days	87.14 $\pm$ 0.02 <sup>a</sup>	72.04 $\pm$ 0.01 <sup>a</sup>	16.08 $\pm$ 0.02 <sup>a</sup>	147.33 $\pm$ 0.01 <sup>a</sup>	24.64 $\pm$ 0.02 <sup>a</sup>	96.03 $\pm$ 0.02 <sup>a</sup>	7.13 $\pm$ 0.02
							5.44 $\pm$ 0.02

Values are reported as mean  $\pm$  standard error of mean (M $\pm$ SEM) (n=5). Values with similar superscript letters indicate significant differences ( $p \leq 0.05$ ) when compared to the control group

**Table 12. Effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on the plasma lipid profile of normal wistar albino rats**

Treatment	Total Cholesterol (mg/dl)	HDL-CHOL (mg/dl)	LDL-CHOL (mg/dl)	VLDL-CHOL (mg/dl)	Triglyceride (mg/dl)
Normal control	56.65 $\pm$ 0.01	36.75 $\pm$ 0.01	76.89 $\pm$ 0.01	10.65 $\pm$ 0.01	52.14 $\pm$ 0.01
166mg/kg for 7 days	56.92 $\pm$ 0.01 <sup>a</sup>	36.87 $\pm$ 0.01	76.43 $\pm$ 0.01 <sup>a</sup>	10.05 $\pm$ 0.02 <sup>a</sup>	52.44 $\pm$ 0.02 <sup>a</sup>
166mg/kg for 14 days	57.44 $\pm$ 0.01 <sup>a</sup>	36.94 $\pm$ 0.01	76.04 $\pm$ 0.01 <sup>a</sup>	9.76 $\pm$ 0.02 <sup>a</sup>	52.85 $\pm$ 0.01 <sup>a</sup>
166mg/kg for 21 days	57.94 $\pm$ 0.02 <sup>a</sup>	37.25 $\pm$ 0.01 <sup>a</sup>	75.94 $\pm$ 0.01 <sup>a</sup>	9.74 $\pm$ 0.02 <sup>a</sup>	53.25 $\pm$ 0.01 <sup>a</sup>
250mg/kg for 7 days	61.01 $\pm$ 0.01 <sup>a</sup>	43.55 $\pm$ 0.01 <sup>a</sup>	73.85 $\pm$ 0.02 <sup>a</sup>	9.15 $\pm$ 0.02 <sup>a</sup>	53.85 $\pm$ 0.01 <sup>a</sup>
250mg/kg for 14 days	61.73 $\pm$ 0.01 <sup>a</sup>	43.94 $\pm$ 0.01 <sup>a</sup>	73.24 $\pm$ 0.01 <sup>a</sup>	8.25 $\pm$ 0.01 <sup>a</sup>	54.24 $\pm$ 0.01 <sup>a</sup>
250mg/kg for 21 days	73.25 $\pm$ 0.01 <sup>a</sup>	48.13 $\pm$ 0.02 <sup>a</sup>	67.83 $\pm$ 0.01 <sup>a</sup>	8.05 $\pm$ 0.01 <sup>a</sup>	58.23 $\pm$ 0.01 <sup>a</sup>
500mg/kg for 7 days	83.24 $\pm$ 0.02 <sup>a</sup>	53.84 $\pm$ 0.01 <sup>a</sup>	61.44 $\pm$ 0.02 <sup>a</sup>	7.23 $\pm$ 0.01 <sup>a</sup>	64.74 $\pm$ 0.01 <sup>a</sup>
500mg/kg for 14 days	87.05 $\pm$ 0.02 <sup>a</sup>	55.24 $\pm$ 0.01 <sup>a</sup>	58.76 $\pm$ 0.01 <sup>a</sup>	7.08 $\pm$ 0.04 <sup>a</sup>	68.04 $\pm$ 0.01 <sup>a</sup>
500mg/kg for 21 days	93.09 $\pm$ 0.02 <sup>a</sup>	60.02 $\pm$ 0.01 <sup>a</sup>	53.74 $\pm$ 0.01 <sup>a</sup>	6.84 $\pm$ 0.01 <sup>a</sup>	57.82 $\pm$ 0.01 <sup>a</sup>

Values are reported as mean  $\pm$  standard error of mean (M $\pm$ SEM) (n=5). Values with similar superscript letters indicate significant differences ( $p \leq 0.05$ ) when compared to the control group

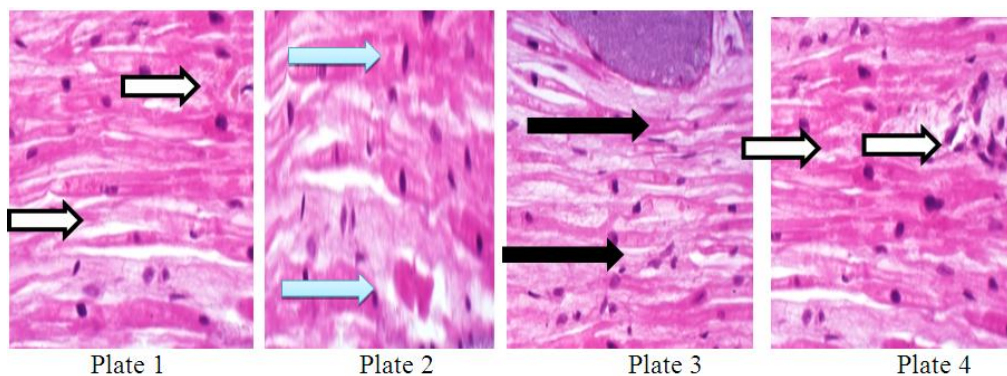
Total protein, albumin, and total bilirubin are also biomarkers that can be used to predict the health status of the liver. Significant decreases on serum or plasma total protein and albumin concentration as well as increases in bilirubin levels and AST, ALP, GGT, and ALT activities are pointers to liver compromise and hepatotoxicity [23]. However, the total protein and albumin were significantly increased while bilirubin level was observed to be significantly decreased ( $p < 0.05$ ) at 166mg/kg b.wt *Leonurus cardiaca* exposure for 7, 14, and 14 days treatment in comparison to the control values (Table 11). The ALT, ALP, and AST activities were significantly decreased ( $p < 0.05$ ) at 166mg/kg b.wt of the extract for same periods of treatment. Treatment with the extract at 250 and 500mg/kg b.wt for 7, 14, and 21 days resulted in significantly increased and decreased plasma total protein, albumin and bilirubin concentrations respectively in comparison to the control values (Table 11). The significant improvement observed following exposure to the aqueous extract particularly at doses 250 and 500 mg/kg b.wt are expressive of the capacity of the extract to enhance hepatic performance in rats. These results conformed to the report of [24] on the effects of *Azadirachta indica* leaf aqueous extract on the antioxidant enzymes in paracetamol-induced hepatotoxicity in Wistar rats.

Table 11 shows the effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on the

plasma concentrations and enzymes activities of normal wistar rats.

Significant increases ( $p < 0.05$ ) on the total cholesterol, HDL, VLDL, and triglyceride concentrations were observed after treatment with *Leonurus cardiaca* extract at 166, 250, and 500mg/kg b.wt for 7, 14, and 21 days in comparison to the control values (Table 12). More significant increases on at 500mg/kg exposure to the *Leonurus cardiaca* extract. The dose-dependent increases on the lipid profile observed after treatment with extract were expressive of the plant is a rich source of good cholesterol and can be used in treating hypolipidaemic conditions. However, the *Leonurus cardiaca*-treated showed intact renal and hepatic histoarchitecture (Fig . 2-11, 13-22, and 24-30).

Improvement on the heart tissues was observed after treatment with aqueous extract of the aerial parts of *Leonurus cardiaca* for 7, 14, and 21 days when compared to the control heart, kidney and liver tissues (Plates 1-10). Appearance of prevascular inflammation and normal myocytes (blue arrows), mild perivascular inflammation and normal myocytes (black arrows), and improved perivascular inflammation and normal myocytes (Plates 1-10). The improvement and mild inflammation after treated with *Leonurus cardiaca* extract is indicative of non-toxic effect of the extract on heart tissues (Plates 1-10).



- Plate 1. Photomicrograph of positive control showing normal myocytes (white arrows)**  
**Plate 2. Photomicrograph of normal wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days for acute toxicity testing, showing, prevascular inflammation and normal myocytes (blue arrows)**  
**Plate 3. Photomicrograph of normal wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days for acute toxicity testing, showing, mild perivascular inflammation and normal myocytes (black arrows)**  
**Plate 4. Photomicrograph of normal wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days for acute toxicity testing, showing, mild perivascular inflammation and normal myocytes (white arrows)**

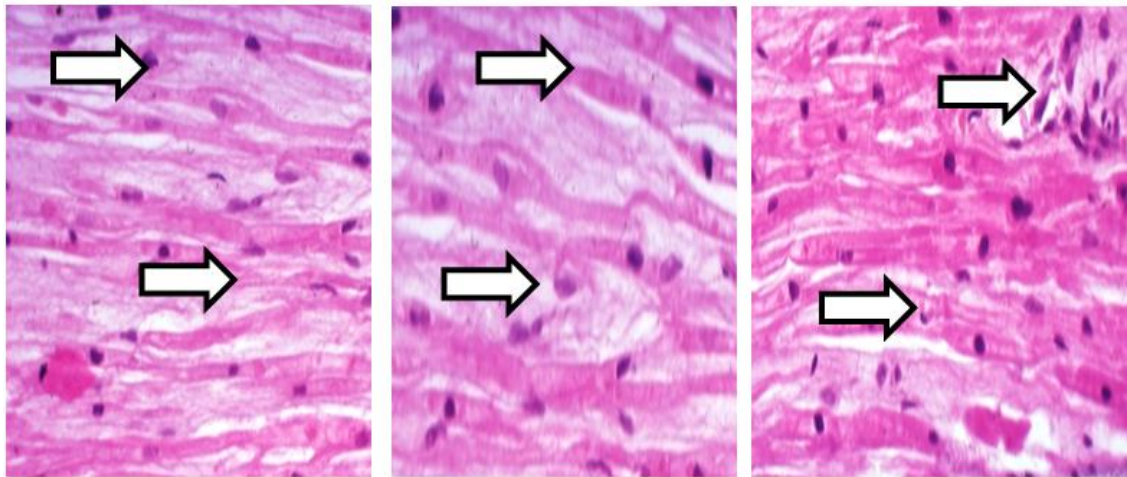


Plate 5

Plate 6

Plate 7

**Plate 5.** Photomicrograph of normal wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days for acute toxicity testing, showing, mild perivascular inflammation and intracellular fatty accumulation.

**Plate 6.** Photomicrograph of normal wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days for acute toxicity testing, showing, mild perivascular inflammation and intracellular fatty accumulation.

**Plate 7.** Photomicrograph of normal wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 21 days for acute toxicity testing, showing, mild perivascular inflammation and intracellular fatty accumulation.

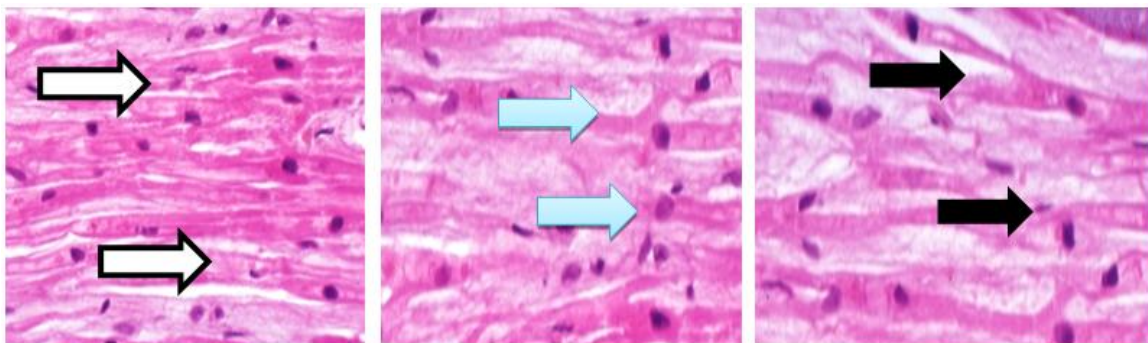


Plate 8

Plate 9

Plate 10

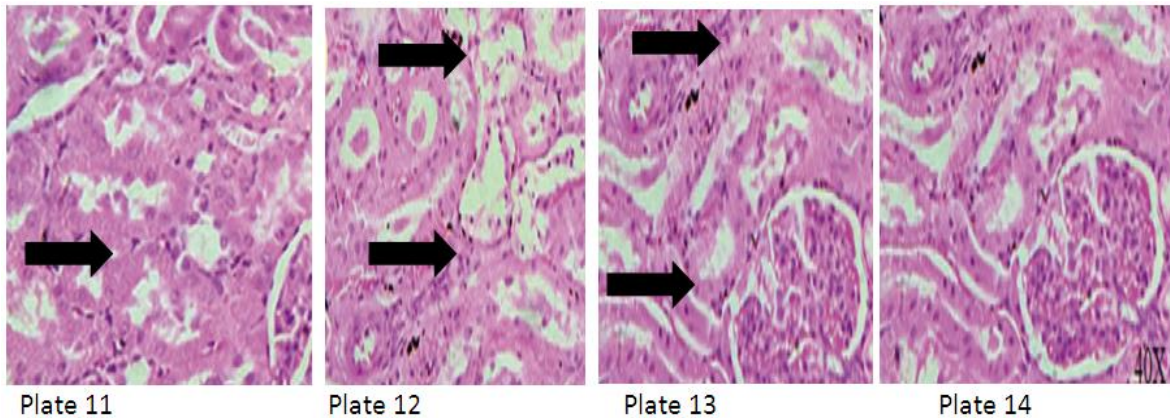
**Plate 8.** Photomicrograph of normal wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days for acute toxicity testing, showing, mild perivascular inflammation and normal myocytes

**Plate 9.** Photomicrograph of normal wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days for sub-acute toxicity testing, showing, normal perivascular inflammation and normal myocytes

**Plate 10.** Photomicrograph of normal wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 21 days for acute toxicity testing, showing, improved perivascular inflammation and normal myocytes

However, treatment with aqueous extract of the aerial parts of *Leonurus cardiaca* resulted in improvement on the kidney tissues when compared to the control (Plates 11-20). The presentation of normal kidney tissues after

treatment with the extract for 7, 14, and 21 days is suggestive of the non-nephrotoxic effect of aqueous extract of the aerial parts of *Leonurus cardiaca* (Plates 11-20)

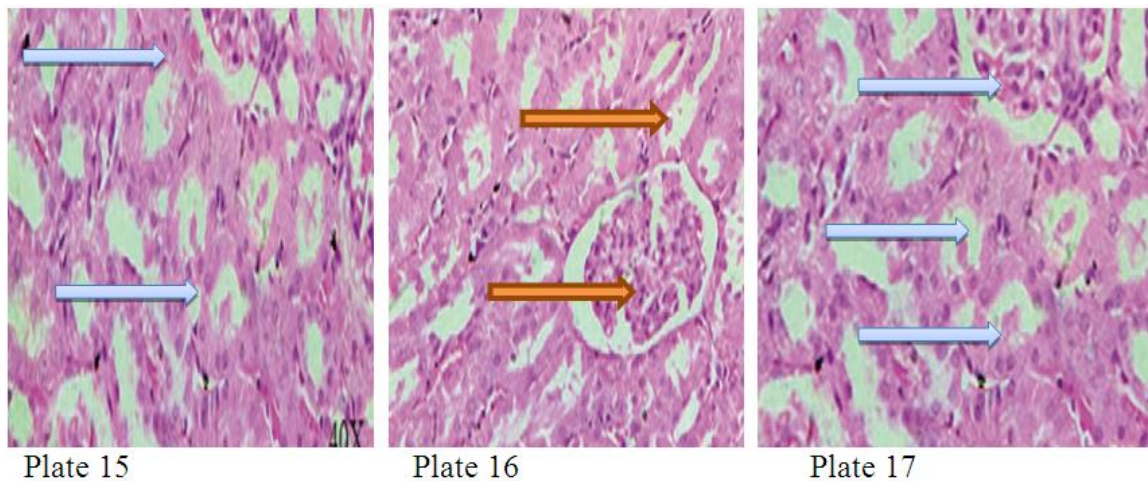


**Plate 11. Control Plate 12: 166 mg 7 days Plate 13: 166 mg 14 Plate 14: 166 mg 21 days, showing normal kidney architecture**

**Plate 12. Photomicrograph of normal in wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 7 days, for toxicity study of the extracts, showing normal kidney tissues**

**Plate 13. Photomicrograph of normal in wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 14 days, for acute toxicity study of the extract, showing normal kidney tissues**

**Plate 14. Photomicrograph of the kidney of normal in wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 21 days for chronic toxicity study of the extract, showing normal kidney tissues**



**Plate 15. Photomicrograph of the kidney of normal in wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 7 days, for acute toxicity study of the extract, showing normal kidney tissues**

**Plate 16. Photomicrograph of the kidney of normal in wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 14 days, for sub-acute toxicity study of the extract, showing normal kidney tissues**

**Plate 17. Photomicrograph of the kidney of normal in wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 21 days, for chronic toxicity of the extract, showing normal kidney tissues**

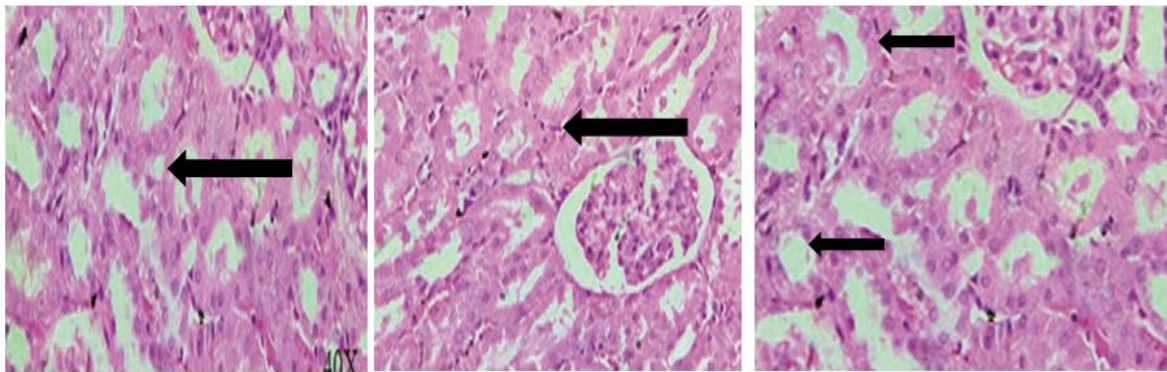


Plate 18

Plate 19

Plate 20

**Plate 18. Photomicrograph of the kidney of normal in wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 7 days, for acute toxicity testing of the extract, showing normal kidney tissues**

**Plate 19. Photomicrograph of normal in wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 14 days for sub-acute toxicity study of the extract, showing normal kidney tissues**

**Plate 20. Photomicrograph of the kidney of normal in wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 21 days chronic toxicity study of the extract, showing normal kidney tissues**

Plate 20: Photomicrograph of normal in wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 14 days for sub-acute toxicity study of the extract, showing normal kidney tissues and similar improvement was observed on the liver tissues as shown in plates 21-30.

Plates 21-24: Photomicrograph of the liver tissue of normal rats, treated with 166 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7, 14, and 21 days respectively (H&E) staining) x 400.

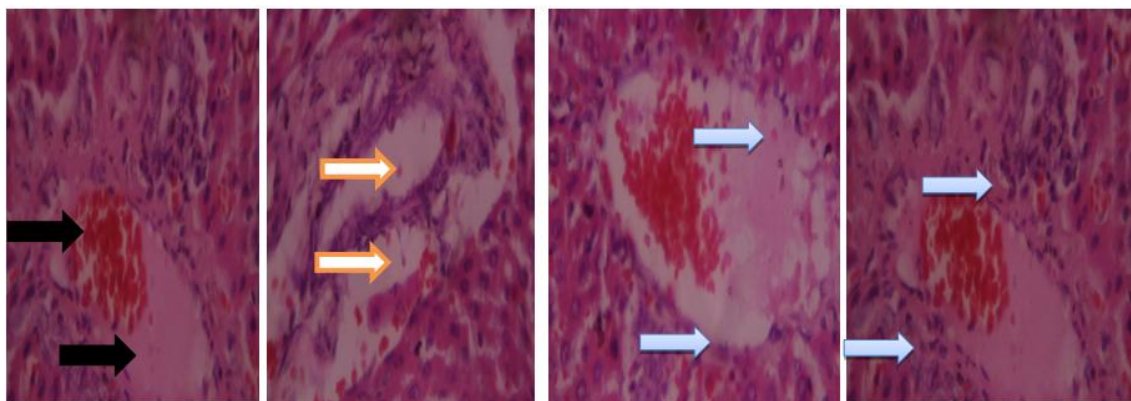


Plate 21

Plate 22

Plate 23

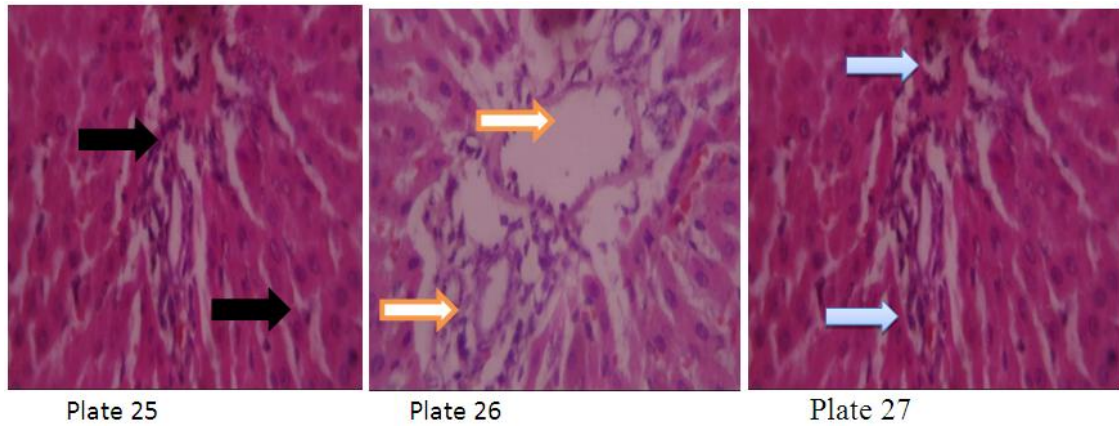
Plate 24

**Plate 21. Photomicrograph of liver tissue of normal control showing normal liver histology**

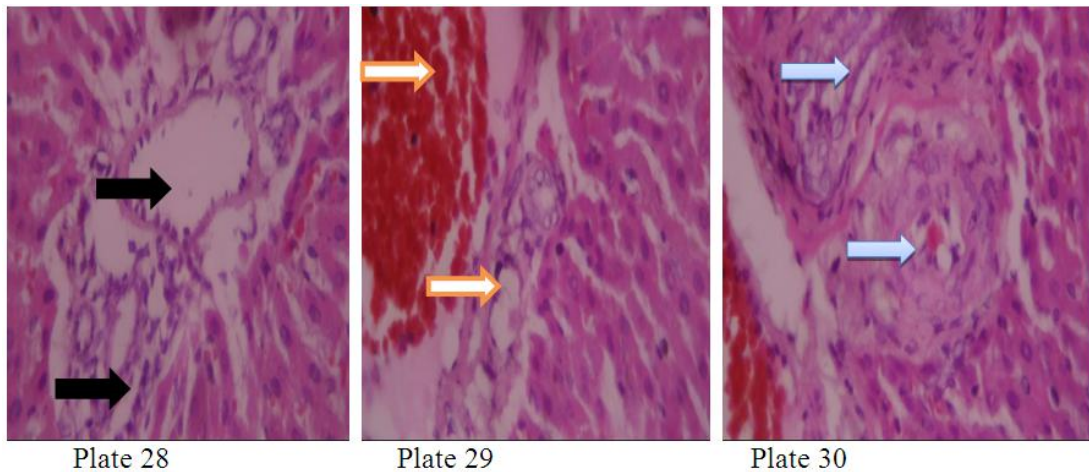
**Plate 22. Photomicrograph of liver tissue of normal rats, treated with 166 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days, showing inflammation and normal liver histology**

**Plate 23. Photomicrograph of liver tissue of normal rats, treated with 166 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days, showing improved liver histology**

**Plate 24. Photomicrograph of liver tissue of normal rats, treated with 166 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 21 days, normal liver histology**



**Plate 25.** Photomicrograph of liver tissue of normal rats, treated with 250 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days, showing improved liver histology  
**Plate 26** Photomicrograph of liver of normal rats treated with 250 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days, showing mild inflammation and improved liver architecture  
**Plate 27.** Photomicrograph of liver of normal rats treated with 250 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 21 days, showing improved liver histology



**Plate 28.** Photomicrograph of liver of normal rats, treated with 500 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days showing normal liver histology  
**Plate 29.** Photomicrograph of liver of normal rats treated with 500 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days showing normal liver histology  
**Plate 30.** Photomicrograph of liver of normal rats treated with 500 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 21 days, showing almost normal liver histology

Plates 25-27: Photomicrograph of the liver tissue of normal rats, treated with 250 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7, 14, and 21 days respectively (H&E) staining) x 400.

Plates 28-30: Photomicrograph of the liver tissue of normal rats, treated with 500 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7, 14, and 21 days respectively (H&E) staining) x 400.

#### 4. CONCLUSION

In the light of finding of acute, sub-acute and chronic toxicity studies of aqueous extract of *Leonurus cardiaca*, as per 425 indicated improvement on all assayed biomarkers, haematological profile and heart, kidney and liver tissues. The significantly improved effect observed from this study is suggestive that aqueous extract of the aerial parts of *Leonurus*

*cardiaca* elicited non-toxic effects and the plant extract is safe at 166, 250, and 500 mg/kg.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## NOTE

This study highlighted the effectiveness of “traditional medicine” which is an ancient tradition practiced in some parts of India. This ancient concept should be carefully investigated in the light of modern clinical science and can be adopted partially if considered appropriate.

## ETHICAL APPROVAL

All authors hereby declared that the principles of laboratory animal care were followed as well as scientific national laws where applicable. All experiments and procedures were thoroughly examined and approved by the ethical committee on human and animal research University of Port Harcourt.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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