Science World Journal Vol. 20(No 4) 2025 https://dx.doi.org/10.4314/swj.v20i4.25
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

SERUM BIOCHEMICAL AND HISTOLOGICAL ANALYSIS FOLLOWING SUB-ACUTE DOSING OF ETHYLACETATE FRACTION OF *NELSONIA CAMPESTRIS* IN RATS

Hadiza Mohammed[1*], Hadiza L. Muhammad[2], Fatima Gogo Mayaki[1], Gideon Philip Azaki[3], Mary Abu Ladidi[1], Aliyu Olalekan Amuzat[1] and Usman Sa'adat[1]

[1]Department of Biochemistry and Biotechnology, IBB University Lapai, Niger State, Nigeria
[2]Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria
[3]Department of Chemistry, Federal University of Technology, Minna, Niger State, Nigeria

*Corresponding Author Email Address: mhadiza@ibbu.edu.ng Phone: +2348069179298

ABSTRACT
The present study assessed the effect of sub-acute administration of ethyl acetate fraction of *Nelsonia campestris* on biochemical and liver histopathology of male rats. The sub-acute toxicity study was conducted by oral administration of the extracts at daily doses of 150, 300 and 600 mg/kg body weight to rats for 28 days. The extract caused a significant ($p< 0.05$) increase in alkaline phosphatase (ALP), aspartate aminotransferase (AST). There was a slight increase in total protein at both 150 and 300 mg/kg in the parameters with ethyl acetate fraction. The serum alanine transaminase (ALT), bilirubin compared favorably ($p> 0.05$) with the control. The histopathology examination showed no remarkable changes in liver morphology of the rats treated with extract at 100 & 300 mg/kg body weight. However, the liver of animals treated with higher doses of ethyl acetate fraction (600 mg/kg) showed noticeable cellular alterations. In conclusion, ethyl acetate fraction shows significant alteration in some biochemical parameters of liver integrity.

Keywords: *Nelsonia campestris*, Biochemical parameters; Histopathology; toxicological profile.

1.0 INTRODUCTION

As free gift, nature has provided man with a store of remedies to cater to all of their ailments. Plants are valuable resources with their curative values laying in some of their synthesized compounds such as alkaloids, flavonoids, tannins accumulated in different parts of the plant. Plants are alternatively used for treatment in place of conventional drugs due to their presumed acceptability, effectiveness, affordability, safety, and low cost (Oguejiofor *et al.,* 2021). Although plants have been extensively used, specific evaluation on toxicity has not been fully documented and could lead to deleterious conditions (Yakubu *et al.,* 2013). Less than 10% of herbal products in the world market are standardized to known active components (Saidu *et al.,* 2017). World health organization has therefore recommended that safety should be the overriding criteria for the selection of medicinal plants for therapeutic purposes (Hong *et al.,* 2016). One such plant with several therapeutic application is *Nelsonia campestris.*

Nelsonia campestris (lam.) Sprung with the common name blue pussy leaf is of the family *Acanthaceae* with about 221 genera and 4000 species widespread around the world tropics (Dikko *et al.,* 2019). It is a creeping plant with villous stems and known to grow on roadsides, grasslands, river banks, and arid regions (Yoshida *et al.,* 2020). Since time memorial, this plant has been used in African traditional medicine to reduce fever and as an analgesic in varied conditions as colds, flu, and viral infections (Adebisi *et al.,* 2021). In Nigeria, it grows in the semi-arid region during the dry season. This plant is known by the traditional healers of Niger state, Nigeria as Isa bakaqqi. The root, fruit, and leaves are used for different medicinal purposes (Musa and Muhammad, 2022) It is used to treat measles alongside other opportunistic infections including pneumonia, conjunctivitis, diarrhea, and otitis media (Musa and Muhammad, 2022).

The unavailability of scientific literature on the toxicological profile of the solvent fractions of this plant is what prompted the present study. Therefore, the present study aims to evaluate the acute and subacute toxicity of ethyl acetate fractions of *N. campestris* leaves extract on some hepatic and renal indices in Wistar rats.

2.0 MATERIAL AND METHODS

2.1 Collection, Identification and authentication of Plant sample

Fresh samples of *Nelsonia campestris* were obtained from the premises of Federal University of Technology, Minna, Bosso Campus, Niger State Nigeria. The plant was identified and authenticated by the herbarium unit in the Department of Plant Biology at the Federal University of Technology in Minna with batch number 00046.

2.2. Sample preparation and extraction

The collected fresh samples of *Nelsonia campestris* were washed with clean running tap water, dried at room temperature and finally grounded into powder using a grinder mill. Extraction of plant materials was performed by weighing 50 g of the powdered plant sample and extracted by soxhlet extraction using 200 ml of methanol. The resulting extract was concentrated in a water bath and then fractionated with ethylacetate to give ethylacetate fraction.

2.3. Experimental Animals

Healthy albino rats of average weight weighing between 120-150 g were purchased from the biochemistry department, Federal University of Technology, Minna, Niger State Nigeria. The rats were kept in clean plastic cages and allowed unrestricted access to rat pellets (grower's mash) and tap water. They were allowed to acclimatize to the environment (temperature of 29 ^{0}C and natural illumination of 12/12 h daylight/dark cycles) for two weeks before commencement of the experiment

Science World Journal Vol. 20(No 4) 2025 https://dx.doi.org/10.4314/swj.v20i4.25
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

2.4 Experimental Design of Sub-acute toxicity study

This study was carried out according to the updated version of the Organisation for Economic Cooperation and Development (OECD) guidelines (OECD, 2023). Twenty (20) Wistar rats were selected for the sub-acute toxicity study. They were divided into four groups (A, B, C, D,) of five rats each. The three groups (Groups A, B, C) were treated with the ethylacetate fraction of *Nelsonia campestris* at doses of 150, 300, and 600 mg/kg body weight respectively. The animals in Group D were used for normal control and administered distilled water. The rats were weighed before the commencement of treatment thereafter; they were weighed weekly throughout the duration of the study.

2.5 Termination of the experiment

On the 29th day, the animals were put under light ether anesthesia (Orisakwe *et al.,* 2016). Blood was collected by cardiac puncture into heparinized and EDTA bottles, followed by centrifugation at 3000 rpm for plasma preparation. The plasma collected were kept in a bio-freezer (-20°C) until they were analysed for the biochemical indices of toxicity. The blood samples collected into EDTA bottles were analysed immediately for hematological indices. The kidney, liver, heart and spleen were also collected from the animals and washed in normal saline, weighed and stored in 10 % formalin in plastic bottles. The biochemical, hematological and histopathological parameters of organ toxicity were evaluated in the treated animals and compared with controls. The relative organ weights were also calculated and recorded.

2.6 Biochemical Analyses

All biochemical analyses were conducted using Randox Diagonistic kit (Randox Laboratories Ltd, Crumlin, UK). Alanine transminase (ALT) was analysed on the principle of catalytic action of ALT on alanine and α – oxoglutarate to form pyruvate and glutamate. Aspartate transaminase (AST) was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4 – dinitrophenylhydrazine (Gaber *et al.,* 2020). Serum total protein concentration was estimated based on the principle that cupric ions in an alkaline medium interact with protein peptide bonds resulting in the formation of a coloured complex which absorbed maximally at 546 nm (Elita *et al.,*2021). The bilirubin assay was based on the reaction between bilirubine and diazotized sulphanilic acid in alkaline medium to form a blue-coloured complex which absorbed at 578 nm (Rec 1972). Albumin concentration was assayed based on its quantitative binding to the indicator 3, 3', 5, 5'-tetrabromo-cresol sulphonephthalein (bromocresol green, BCG) (Hadiza *et al.,* 2023). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance is directly proportional to the concentration of albumin in the sample. For creatinine assay, creatinine in alkaline solution reacts with picric acid to form a colored complex (Haruna and Elinge, 2019).

2.7 Histopathological analysis

The histopathological analysis of the kidney, the liver, the spleen and the heart excised from the experimental animals were carried out in the histopathology laboratory Abuja, Nigeria. The various organs were cut and placed in embedded cassettes. Thereafter, they were fixed with 10% formalin for 1 hour and afterwards processed for histological analysis.

2.8 Data Analysis

Values were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± SE of the mean. Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $P < 0.05$ (Hong *et al.,* 2016).

3.0 RESULTS

3.1 Effect of sub-acute administration of ethylacetate fractions of *Nelsonia campestris* on serum biochemical parameters of male wister rats

The administration of ethyl acetate fraction at 150 and 600mg/kg caused a significant ($p< 0.05$) increase in alanine transaminase (ALT) activities. However, ALT activities at 300mg/kg ethyl acetate fraction compared favorably ($p> 0.05$) with the control **(Figure 1).** Ethyl acetate fractions caused a significant ($p< 0.05$) increase in aspartate aminotransferase (AST) and alkaline phosphatase (ALP) at all doses when compared with the control **(Figure 2 and 3).** But there was a significant ($p< 0.05$) increase in total protein concentration in the parameters with all doses of ethyl acetate fraction **(Figure 4).** Bilirubin compared favorably ($p> 0.05$) with the control at all doses with a slight decrease at 300mg/kg body weight ethylacetate fraction (Figure 5).

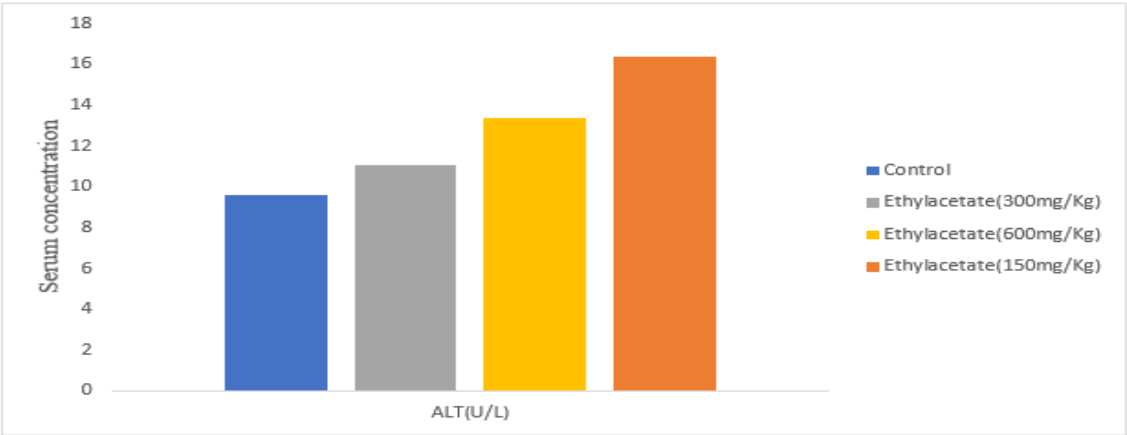

Fig 1: The effect of sub-acute administration of ethylacetate fractions of *Nelsonia campestris* on activity of serum liver enzyme (ALT). Values are mean ± SEM of 3 determinations

Science World Journal Vol. 20(No 4) 2025 https://dx.doi.org/10.4314/swj.v20i4.25
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

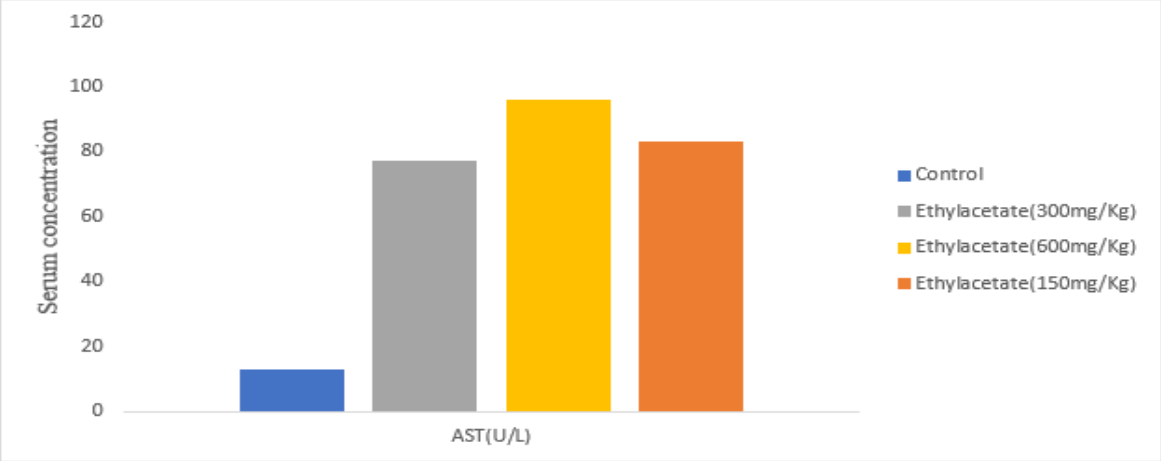

Fig 2: The effect of sub-acute administration of ethylacetate fractions of *Nelsonia campestris* on activity of serum liver enzyme (AST) in rats. Values are mean ± SEM of 3 determinations

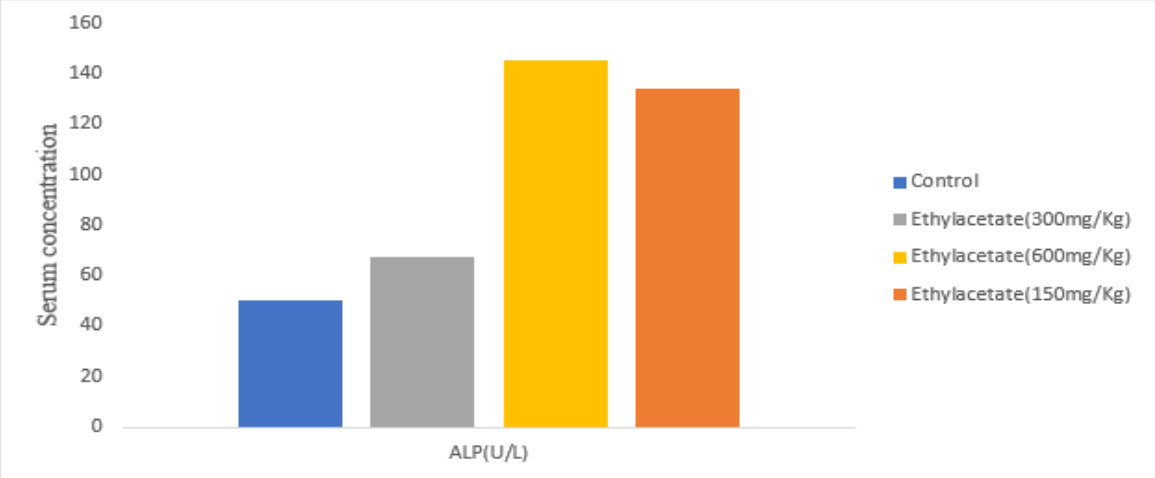

Figure 3: The effect of sub-acute administration of ethylacetate fractions of *Nelsonia campestris* on activity of serum liver enzyme (ALP) in rats. Values are mean ± SEM of 3 determinations

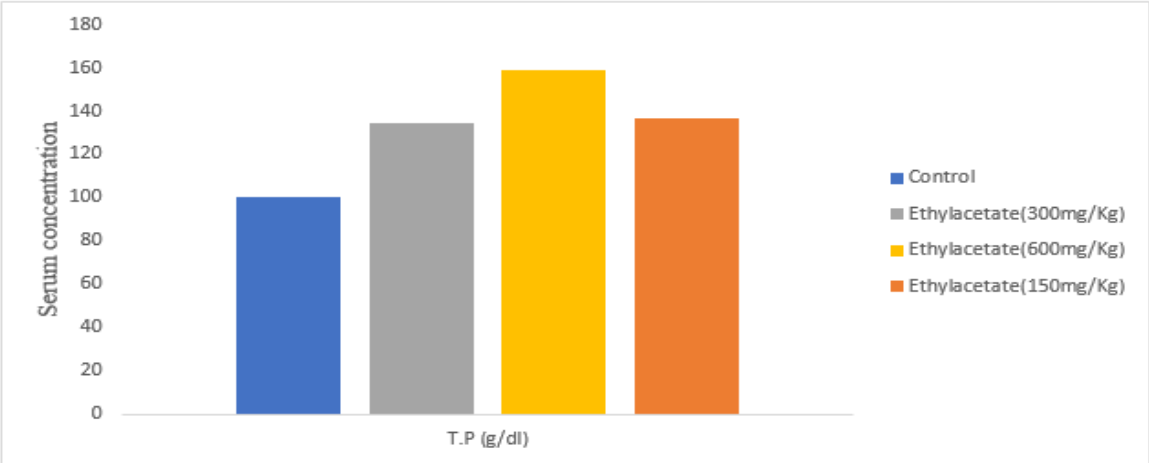

Figure 4: Effect of sub-acute administration of ethylacetate fractions of *Nelsonia campestris* on activity of serum liver enzyme (total protein) in rats. Values are mean ± SEM of 3 determinations

Science World Journal Vol. 20(No 4) 2025 https://dx.doi.org/10.4314/swj.v20i4.25
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

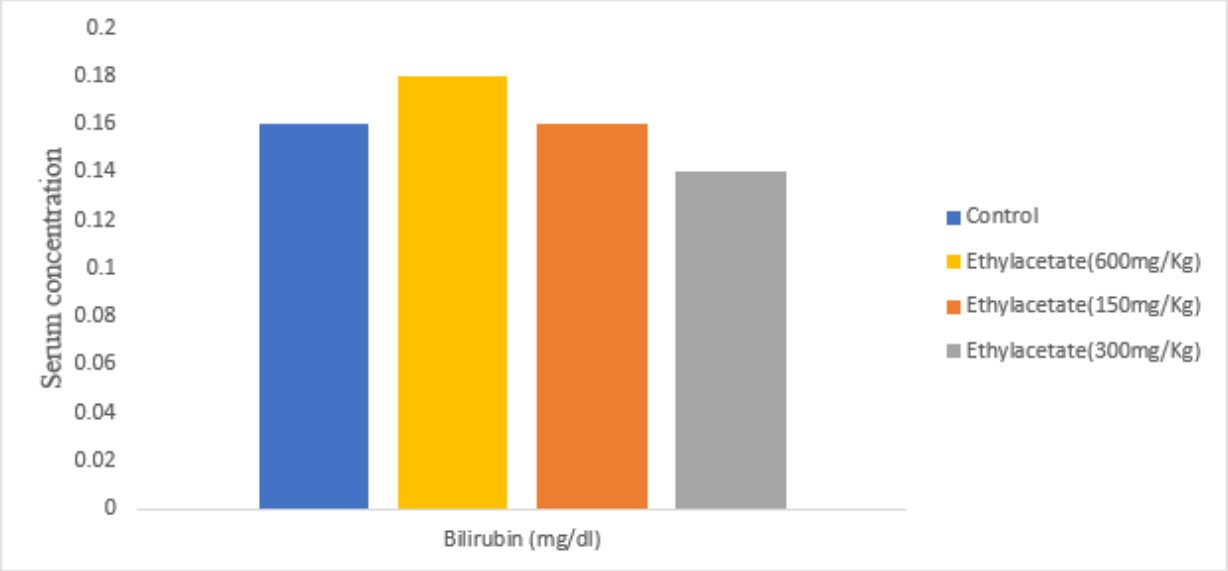

Figure 5: the effect of sub-acute administration of ethylacetate fractions of *Nelsonia campestris* on bilirubin. Values are mean ± SEM of 3 determinations

3.2 Effect of sub-acute administration of ethylacetate fractions of *Nelsonia campestris* on liver histopathology of male rats

The normal microscopic architecture of the liver is composed of hexagonal lobules and acini. Hexagonal lobules are centered on the central vein (CV) and have a portal triad containing branches of the portal vein (PV), hepatic artery (HA), and bile duct (BD). Compared with the control (figure 6), no remarkable changes in the morphology of organs of the experimental rats treated with both ethyl acetate extracts at 150 & 300 mg/kg body weight were noticed on gross examinations. The histologic section of the liver showed a preserved hepatic architecture, with hepatocytes arranged in plates with no vascular congestion; there were no areas of necrosis or hemorrhage, fatty change, or fibrosis in rats treated with ethyl acetate 150 mg/kg body weight (figure 6B) & 300 mg/kg body weight (figure 6C). However, the livers of rats treated with a higher dose of ethyl acetate fraction (600 mg/kg body weight) showed noticeable cellular alterations such as bridging inflammation of the central vein to portal tract with mildly congested vessels (shown in short arrows) & necrotic cells (as shown in long arrow) figure 6D.

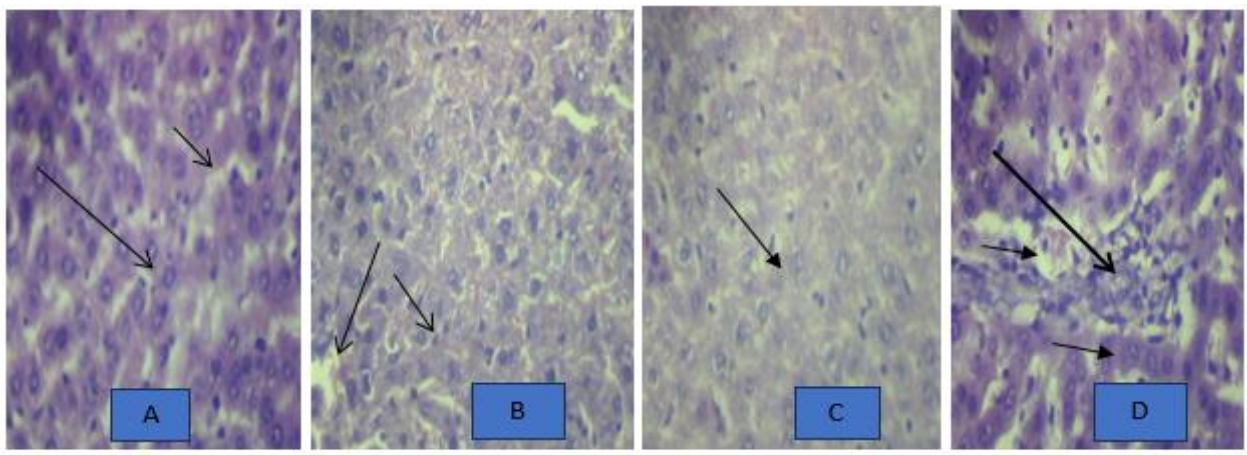

Figure 6: photomicrograph of the liver section of rat administered normal saline and ethylacetate of *N. campestris* for 28 days.

Key:
A: normal saline treated liver section showing unremarkable hepatocytes (long arrow) and portal tract (short arrow); B: liver section of rat administered 150mg/kg of ethylacetate fraction of *N. campestris* plant showing intact hepatocytes (long arrow) and portal tracts with congested blood vessles; C: liver section of rat administered 300mg/kgbw of ethylacetate fraction of *N. campestris* plant showing well preserved hepatic lobular architecture; D: liver section of rat administered 600mg/kgbw of ethylacetate fraction of *N. campestris* extract showing mild distortion of lobular hepatic architecture, bridging inflamation of the central veins and portal tract with mild congested vessels (short arrows) and necrotic cells (long arrow). H&E staining, magnification × 40.

Science World Journal Vol. 20(No 4) 2025 https://dx.doi.org/10.4314/swj.v20i4.25
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Table 1.0: Effect of sub-acute administration of ethylacetate fractions of *Nelsonia campestris* on hematological parameters in rats

GROUPS	HB	PCV	MCV	MCH	MCHC	RBC	PLT
Control	9.30±0.91[a]	27.66±2.72[a]	103.33±4.37[ab]	59.00±3.05[a]	54.00±2.00[a]	1.56±0.16[ab]	360.00±30.98[ab]
Ethylacetate (150mg/kg)	9.36±1.75[a]	27.66±5.23[a]	112.67±0.66[b]	82.00±3.46[d]	67.33±1.20[a]	1.36±0.33[a]	473.30±10.72[c]
Ethylacetate (300mg/kg)	10.03±0.84[a]	29.66±2.60[a]	83.66±14.81[a]	74.66±4.91[cd]	53.00±9.64[a]	1.60±0.17[ab]	427.67±28.01[bc]
Ethylacetate (600 mg/kg)	8.26±0.66[a]	28.00±0.000[a]	104.67±8.33[b]	72.33±2.66[bcd]	62.33±3.66[a]	1.40±0.00[a]	321.67±9.66[a]

Key; Mean corpuscular hemoglobin (MCH), mean cell volume (MCV), platelate count (PLT), red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), total white blood cell (WBC) and differential counts. Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).

Table 2.0: Effect of sub chronic administration of ethylacetate fractions of *Nelsonia campestris* on organs body weight ratios in rats

Groups	Kidney	Liver	Heart	Spleen
Control	0.0062±0.00[a]	0.0032±0.00[a]	0.0038±0.00[b]	0.0034±0.00[ab]
Ethylacetate (150mg/kg)	0.0052±0.00[a]	0.033±0.00[a]	0.0038±0.00[b]	0.0054±0.00[ab]
Ethylacetate (300mg/kg)	0.0066±0.00[a]	0.034±0.00[a]	0.0040±0.00[b]	0.0044±0.00[ab]
Ethylacetate (600 mg/kg)	0.0066±0.00[a]	0.032±0.00[a]	0.0021±0.00a	0.0039±0.00[ab]

Values are mean ± SEM of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).

4.0 DISCUSSION

Evaluation of serum biochemical indices in animals has become one of the most valuable tools for assessing the integrity and functionality of organs as well as risk assessment, pathological condition and general health status of the body (Aastha *et al.,* 2019). AST and ALT are biomarkers of hepatic integrity and to a certain level can be used to assess the extent of hepatocellular damage, the ALT activities however, give more valuable information relevant to the integrity of the hepatocyte than AST (Belmekki *et al.,* 2021). Consequently, in the present work, serum AST activities was significantly raised in rats dosed 150 and 300mg/kg of ethylacetate fractions of *N. campestris* for 4 weeks when compared with the control. The constituents of the extracts may have altered the enzyme activity or increased the amounts of important molecules needed for the optimum activities of the enzyme (Edith *et al.,* 2021). Such increase in AST activities will however, adversely affect the metabolism of amino acid and carbohydrate with consequent effect on ATP generation (Builders, 2019). It appears that the extract might have selectively affected the transaminases since ALT activities in the serum of the animals were not altered. This may be connected to the earlier mentioned, selective toxicity of natural products especially by the plant extracts (Emmanuel *et al.,* 2024).

Alkaline phosphatases (ALP) are often used to assess the integrity of plasma membrane and endoplasmic reticulum (Fongang *et al.,* 2020). The significant increase in serum ALP activities following administration of ethylacetate fractions of *N. campestris* for 4 weeks suggested that the integrity and functionality of endoplasmic reticulum and plasma membrane has been comprised (Hope *et al.,* 2021). It may also indicate that the extract activates, the activities of the enzyme molecule in situ (Mohaddesi *et al.,* 2015). This may also be as a result of the fact that the enzyme activity may be selectively increased by some molecules present in the plant extract due to the enzyme being most active in an alkaline medium. The levels of bilirubin, total protein, electrolytes creatinine and urea play important roles in determining the synthetic and excretory roles of the kidney and liver (Khetani *et al.,* 2017). The observed increase in the total protein content in rats dosed 150 and 300 mg/kg ethylacetate fraction suggests that the extract might have interfered with the equilibrium in the rate of synthesis or destruction of total protein from the system of the animals. Such increase could, however, lead to dehydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals (Adebisi *et al.,* 2021).

Organ body weight ratios are normally investigated to determine whether the size of the organ has changed in relation to the weight of the whole animal (Dikko *et al.,* 2019). Also, to access alterations that may be caused by organ injury. The kidney, liver and heart body weight ratios were not altered by all the doses of the extracts except a decrease in heart-body weight ratio by the 600 mg/kg ethylacetate fraction. The absence of an effect on the computed liver and heart /body weight ratios suggest that the extract did not cause any form of swelling, atrophy and hypertrophy on the organs as shown in Table 2.0 (Gaber *et al.,* 2020). This finding agrees with the studies by Hadiza *et al*., (2023), who reported that administration of crude aqueous extract of *N. campestris* at doses of 100, 300 and 600 mg/kg for 28-days did not elicit any deleterious effect on the weight of liver and heart.

According to Edith, (2021) functional studies in toxicology should be coupled with the appropriate histological studies because appropriate morphological studies are useful, especially during the anatomical localization of the action of a toxin. Histopathology is an important part of clinical work-up related with liver diseases. For example, an inflammation is often present within the liver and has multiple causes. Based on this, a histological study of the effect of ethylacetate fractions of *Nelsonia campestris* was conducted. It was observed that the extract (at doses of 150 & 300 mg/kg body weight) and the control groups caused no histopathological alterations in the cellular architectures of the liver. However, at higher dose (600 mg/kg body weight), there were noticeable histopathological alterations in the cellular architectures of the liver. The above histopathological damages at the high extract dose are

probably responsible for the alterations in the biochemical and hematological markers of the liver functions. This finding corroborates with the finding of Edith *et al.,* (2021) who observed mild to moderate hepatic and cortical necrosis of liver of animals treated with 150 and 600 mg/kg body weight of aqueous and methanolic extracts of *Nelsonia campestris.*

Table 1.0 revealed the hematological studies of the ethylacetate fraction of Nelsonia *campestris* extract. Among the erythrocytic indices evaluated in this study, MCH and MCV were significantly raised by the extract while HCT, HGB and MCH-C compared well with the control rats at all doses tested, although, with a significant ($p< 0.05$) decrease in red blood cells (RBC). This is an indication that ethyl acetate fraction of *N. campestris* has no beneficial effects in stimulating the erythropoietin release in the kidney, which is the humoral regulator of RBC production (Hadiza *et al.,* 2023).

5.0 Conclusion

This study has shown that the ethyl acetate fraction of *N. campestris* shown significant alteration in the biochemical parameter and functional integrity of the liver.

REFERENCES

Aastha, C., Ziad, S., David, C., & Hellen H. (2019). Global contributors to antibiotic resistance. *Journal of global infectious disesese*, 11(1), 36.

Adebisi, A., Fagade, S. & Fafioye, O. (2021). Toxicity of *Parkia biglobosa* and *Raphia vinifera* on Albino wistar rats. *African Journal of Biotechnology*, 3,11, 632-630.

Belmekki, F Himri, I., Bellahcen, S., Souna, F., Aziz, M., Bnouham, M., Zoheir, J., Berkia, Z., Mekhfi, H., & Saalaoui, E. (2021). A 90-day oral toxicity study of tartrazine, a synthetic food dye, in wistar rats. *International Journal of Pharmacy and Pharmaceutical Science*, 3, 160-169.

Builders, M. I., Isichie C. O., & Aguiyi, J. C. (2019). *Parkia biglobosa* stem bark in rats. *Modern Advances in Pharmaceutical,*1,5-8.

Dikko A. A., Goji, A. D., Tende, J. A. & Ezekiel, I. (2019). Effect of ethanolic leaves extract of *Moringa oleifera* on blood glucose levels of streptozocin-induced diabetics and normoglycemic Wistar rats. *British Journal of Pharmacology and Toxicology*, 2 (1), 1-4.

Edith, O. A. (2021). Antibacterial Properties of *Parkia biglobosa* and *Parkia bicolor* leaf extracts. *African Journal of Biomedical Research*. (5), 125.

Elita, S., Aragão, D., Lyvia, G., Juliana, L., Juliana., C. & Raúl, G. (2021). Hypoglycemic effects of *Cecropia pachystachya* in normal and alloxan-induced diabetic rats. *Journal of Ethnopharmacology,* 128 (3), 629-633.

Emmanuel O. A., Patrick A., Paul P. S. O., Eric A. F., Isaac K. A., William G. A., Samuel A., & Tracy N (2024). An acute and sub-acute toxicological assessment of *Reissantia indica* plant extract in male Sprague-Dawley rats: Hematological, serum biochemical and histopathology. *Scientific African,* 23; 1-11

Fongang, F. Y. S., Bankeu, K. J. J., Gaber, E. B., Iftikhar, A. & Lenta, N. B. (2020). Extraction of Bioactive Compounds from Medicinal Plants and Herbs. *Medicinal plants from nature,* DOI: 10.5772/intechopen.98602.

Gaber, E. B., Luay, M. A., Lamiaa, G. W., Amany, M. B., Eman, H. N. & Eman, K. R. (2020). *Syzygium aromaticum L.* (*Myrtaceae*): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. *Biomolecules.* 10,2,202. doi: 10.3390/biom10020202

Hadiza Lami Muhammad, Rahinat Garba, Abubakar Siddique Abdullahi, Hussaini Anthony Makun, Musa Bola Busari, Hadiza Kudu Muhammad, Funmilola Sherifat Adefolalu (2023). Subchronic Toxicological Studies of Methanol and n-Hexane Extracts of *Leptadenia hastata (pers) Decne* Leaves Used as Antihypertensive Agent. *Journal of Chemical Health Risks*, 13(3), 565-575

Haruna, Y. I., & Elinge, C. M. (2019). Median Lethal Dose and Phytochemical Studies of Aqueous Leaves Extract of Blue Pussy Leaf, *Nelsonia canescens (Lam.) Spreng*. Proteomics Bioinformatics, 1(2): 38-42

Henry, R. R. & Edward C. C. (2016). SGLT2 inhibition—a novel strategy for diabetes treatment. *Nature reviews drug discovery*, 9,7, 551-559.

Hong-Yu, X., Jun-En, S., Zhen-Ming, L., Xiao-Mei, Z., Wen-Fang, D. & Zhen-Hong X. (2016). *Natural Product Research* 24 (6), 542-547.

Hope M., Nchekwube I., Ifeoma J. O., Charity C. E., Cyril O. O., Chinenye H. N., & Felix A. O (2021). Pharmacognostic profile and evaluation of the antidiarrheal activity of *nelsonia canescens* plant (lam.) *acatheceae* in mice model. *International Journal of Modern Pharmaceutical Research*; 5(4), 04-15

Khetani, V. Teo, S., Stirling, D., Thomas, S., Hoberman, A. & Kiorpes, A. (2017). A 90- day oral gavage toxicity study of D-methyl penidate and DL methyl penidate Sprague – Dawley rats. *Toxicology*, 179, 183.

Mohaddesi, B., Ashok, B. K., Rabinarayan, A. & Vinay, J. S. (2015). Antiinflammatory activity of *Nelsonia ccanescens(Lam)Spreng*. Root in albino rats. *The journal of Phytopharmacology*, (4), 68-72

Musa, I. & Muhammad, B. N (2022). Assessment of Property Rating Administration and Management in Minna Between 2009 – 2019. *Journal of Biodiversity and Environmental Research*, 26(4) 2211-8735.

Oguejiofor I. J., Ifebi H. M. N., Ezea C. C., Ogbue C. O., Nedum C. H., & Iredu S. C (2021). Evaluation of anti-helmintics effect of *nelsonia canescens* plant lam. (*Acatheceae*) *European Journal of Biomedical AND Pharmaceutical sciences,* 8(7), 15-19.

Orisakwe, O. E., Chude, A. M., Onyenmechi, J. A., Ejeatuluchukwu, O. & Chudi, E. D. (2016). Sub-chronic toxicity studies of the aqueous extract of *Boerhavia diffusa* leaves. *Journal of health science*, 49 (6), 444-447.

Saidu, Y., Bilbis, L.S., Lawal, M., Isezuo, S.A., Hassan, S.W. & Abbas, A.Y. (2017). Acute and Sub-chronic Toxicity Studies of Crude Aqueous Extract of *Albizzia chevalieri Harms (Legumino(sae). Asian Journal of Biochemistry*. 2(4), 224-236.

Yoshida, J., Kodera, Y. & Ichikawa, M. (2020). "Pharmacokinetic study of allixin, a phytoalexin produced by garlic," *Chemical and Pharmaceutical Bulletin*, 50,3, 354–36.

Organisation for Economic Cooperation and Development (2023). Guidance document on histopathology for inhalation toxicity studies, supporting TG 412 (Sub-acute inhalation toxicity: 28-day study) and TG 413(Sub-chronic inhalation toxicity: 90-day study). National institute of health http://ntp.niehs.nihs.gov.ng