Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.56, 2016
www.iiste.org

Acute and Subchronic Toxicity Study of Methanol Seed Extract of Passion Fruit (Passiflora edulis var. flavicarpa) in Albino Rats

Amedu AM[1] *Wasagu RSU[1] Lawal M[1] Sabir AA[2] Sahabi SM[3] Zaharadeen A[1]
1. Department of Biochemistry, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria
2. Department of Medicine, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria
3. Department of Histopathology, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria

Abstract
The present study investigates the toxicological properties of the methanol seed extract of *Passiflora edulis var. flavicarpa*. For the acute toxicity study, a limit test dose of 5000mg/kg was used while in the sub-chronic toxicity study, the treatment groups received a daily oral dose of the extract at 1000, 2000 and 3000 mg/kg for 28 days. The acute toxicity study revealed that the extract was safe up to 5000mg/kg. Results of sub-chronic toxicity study revealed significant ($p<0.05$) increase in body weight at the dose of 3000mg/kg. Neutrophils in all the treatment groups were significantly increased ($p<0.05$) while all the other haematological parameters tested showed no significant difference with the control group. The extract also showed mild to moderate deleterious effects on some biochemical parameters. ALT, ALP. AST and GGT levels were significantly increased ($p<0.05$) with mild distortion of hepatic architecture in the liver of the group that received the highest dose (3000mg/kg). No pathological changes in the kidneys were observed at the various doses. This suggests that the extract should be used with caution with increasing dose.
Keywords: *Passiflora edulis var. flavicarpa,* acute toxicity study, sub-chronic toxicity study, haematological parameters, biochemical parameters.

Introduction

It is documented by the World Health Organization (WHO) that 80% of the World's population relies solely on traditional medicine, particularly plant-based remedies for their primary healthcare [20]. The primary benefit of using plant derived-medicine is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and affordable treatment [26]. The WHO also notes, though, that "inappropriate use of traditional medicines or practices can have negative or dangerous effects" and that "further research is needed to ascertain the efficacy and safety" of several of the practices and medicinal plants used by traditional medicine systems [29]. It is thus important to evaluate the toxicological properties of these plants.

Passiflora edulis (Passion fruit) belongs to the family *Passifloraceae* which contains 12 genera with about 500 species [3]. The species of this genus are distributed in the warm temperate and tropical regions of the world, but they are much rarer in Asia, Australia, and tropical Africa [5]. Two types of *P. edulis* are grown commercially, the purple form (*P. edulis* Sims) and a yellow form (*P. edulis* var. *flavicarpa*) [5, 19]. The yellow passion fruit accounts for 95% of the passion fruit-cultivating area, owing to the quality of its fruits, vigor, yielding and juiciness [17]. Passion fruit is traditionally reported to possess anticonvulsant, antidepressant, anstringent, cardiotonic (tones balances, strengthens the heart, disinfectant, nervine (balances / calms nerves), neurasthenic (reduces nerve pain), tranquilizer and vermifuge (expels) worm activities [14]. All parts of the plant have been ascribed to have medicinal properties.

Materials and Methods

Plant Materials: Matured fresh *Passiflora edulis var flavicarpa* fruits were collected from Vandeikya Local Government Area of Benue State, Nigeria. The fruits were identified by a Taxonomist in the Botany unit, Biological Science Department, Usmanu Danfodiyo University, Sokoto and a voucher specimen was deposited at the Herbarium of the same department with a voucher specific number UDUH/ANS/0059. The seeds were removed from the pulp of the fruits and air dried for two weeks.

Extraction: The seeds were pulverized into fine powder and 200g of the powder was extracted with 2 litres of methanol at room temperature for 72hours. It was filtered through Whatman No. 1 filter paper. The filtrate was concentrated to dryness using rotary evaporator and the yield of the extract (23.5% w/w) calculated.

Experimental Animals

Albino rats of both sexes weighing 115-130g were purchased from Nigerian Institute for Trypanosomiasis Research, Kaduna state. They were kept in a well-ventilated room under supervision in the animal house with free access to feeds and tap water *ad libitum*. The rats were allowed to acclimatize in the environment for two (2) weeks before the commencement of the experiment.

Acute Toxicity Study

Acute oral toxicity study was carried out according to Organization for Economic and Cultural Development method [21]. Five (5) randomly selected animals were used for the experiment. The extract was administered at

5000mg/kg body weight (bw) in a single dose. Each animal was dosed and observed one after the other. Observation time for the first 8hrs, 14hrs, 24hrs, 48hrs for any signs of toxicity like tremors, itching, depression, weakness, food and water refusal, salivation and death if any. The animals were observed for a further 14 days for any signs for delayed toxicity. If three (3) or more animals died within 48 hrs, the LD_{50} is less than 5000mg/kg and if one, two or none died within 48hrs, the LD_{50} is greater than 5000mg/kg.

Subchronic Toxicity Study

The albino rats were randomly divided into four (4) equal treatment groups containing five (5) animals each. The methanol extract of *Passiflora edulis var. flavicarpa* was administered to the rats at the dose of 0 (control), 1000, 2000 and 3000mg/kg daily for 28 days. Body weight of each animal was determined before treatment and weekly. On the 29th day of the experiment, after an overnight fast, the animals were anaesthesized under light chlorofoam anesthesia. The blood of the animals were collected for haematological and biochemical analysis and the liver and kidneys of each animal were dissected out and weighed in grams. The relative organ weight of each animal was then calculated as follows.

$$\text{Relative Organ Weight} = \frac{\text{Absolute Organ weight (g)}}{\text{Body weight of rat on sacrificed day (g)}} X100$$

Haematological Parameters

Packed Cell Volume (PCV), Haemoglobin (Hb) and Red blood cells count (RBC) were determined using the method described by Jain [12] while White blood cells count (WBC), Platelets, Neutophils and Lymphocytes were determined using Leishman's stain method [6].

Biochemical Parameters

Serum alkaline phosphatase (ALP) activity was determined using the method of Sood [25], serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were determined by the method of Reitman and Frankel [23], serum gamma glutamyl transferase activity was determined using the method of Szasz and Bergmeyer [27], serum total protein level was estimated by using biuret method as described by Gomall *et al*., [10]; serum albumin level was estimated by bromocresolgreen method as modified by Doumas *et al.,* [7]; serum bilirubin level was estimated using method described by Jendrassik and Groff [13]; serum creatinine level was estimated using Jaffe's method [4] while serum urea level was measured using Berthelot colorimetric method [30].

Histopathological Studies

Kidney and liver of the sacrificed rats were identified and harvested. They were fixed in 10% buffered formalin for 72 h. The tissues were then dehydrated in alcohol of graded concentrations and embedded in paraffin. Embedded tissues were cut into sections of 5 µm thick and these were stained with hematoxyline and eosine for photomicroscopic assessment. Photomicrographs of the samples were then taken [8].

Data Analysis

The data obtained was represented in mean standard error of the mean. Result was analysed statistically by One way ANOVA followed by Duncan's, multiple comparison test using the statistical package – SPSS version 20. Values were considered statistically significant at $p<0.05$.

Results

Acute Toxicity Study

The oral administration of methanol seed extract of *Passiflora edulis var. flavicarpa* at a single dose of 5000mg/Kg body weight did not cause rat mortality during the 48 h and 14 day observation and there was no indication of toxicity, behavioural or physiological changes.

Sub-Chronic Toxicity Test

Table 1.0 shows the effect of different doses of methanol seed extract of *Passiflora edulis var. flavicarpa* on the body weight (g) of rats. All the rats had significant increase in their body weight compared to their starting weight. There was a significant difference ($p< 0.05$) between the treatment group at the dose of 3000mg/kg and the control group. All the other groups showed no significant difference.

Table 1.0: Changes in body weight (g) of rats during treatment with different doses of methanol seed extract of *Passiflora edulis var flavicarpa*

Group	Week 0	Week 1	Week 2	Week 3	Week 4
Normal	123.67±13.57[a]	136.33±15.30 [a]	146.33±14.62 [a]	150.67±14.19 [a]	155.33±9.13 [a]
1000mg/kg	110.00±5.77 [a]	120.33±1.86 [a]	129.00±17.50 [a]	137.33±16.86 [a]	144.33±16.76 [a]
2000mg/kg	123.00±0.577 [a]	125.67±4.26 [a]	133.67±10.73 [a]	140.33±11.83 [a]	152.67±12.43 [a]
3000mg/kg	129.67±8.95 [a]	142.00±11.71 [a]	159.33±11.61 [a]	167.00±14.42 [a]	176.33±11.41 [b]

Values are expressed as mean ± Standard Error of Mean of five replicates. Mean Value Having Different Superscript Letters in rows are significantly different **($p<0.05$) (One way ANOVA followed by Duncan's, multiple comparison test)**.

Table 1.1 presents the effect of different doses of methanol seed extract of *Passiflora edulis var flavicarpa* on the relative organ weight of rats. There was no significant difference ($p< 0.05$) between the

treatment groups and control groups.

Table 1.1: Effect of sub-chronic oral treatment with methanol seed extract of *Passiflora edulis var flavicarpa* on organ weights (per 100 g body weight) of rats

Group	Liver	Kidney
Normal	3.62±0.11[a]	0.71±0.05[a]
1000mg/kg	3.97±0.50[a]	0.70±0.01[a]
2000mg/kg	3.82±0.32[a]	0.67±0.05[a]
3000mg/kg	3.73±0.16[a]	0.62±0.04[a]

Values are expressed as mean ± Standard Error of Mean of five replicates. Mean Value Having Different Superscript Letters in rows are significantly different **(p<0.05) (One way ANOVA followed by Duncan's, multiple comparison test)**.

Table 1.2 presents the effect of different doses of methanol seed extract of *Passiflora edulis var flavicarpa* on haematological parameters of rats. The result shows that there was a significant increase (p<0.05) in neutrophils at all the dose levels when compared to the control group. All the other haematological parameters investigated showed no significant difference between the different dose levels and control group.

Table 1.2: Effect of sub-chronic oral treatment with methanol seed extract of *Passiflora edulis var flavicarpa* on haematological parameters of rats.

Parameters	Treatment (mg/kg)			
	Normal	1000	2000	3000
PCV (%)	35.00±1.15[a]	32.67±3.33[a]	35.33±1.20[a]	38.33±1.76[a]
RBC (10^6/µl)	6.59±0.09[a]	6.84±0.31[a]	6.36±0.32[a]	6.69±0.41[a]
Hb (mg/dl)	12.03±0.26[a]	12.21±0.55[a]	11.98±0.24[a]	12.99±0.30[a]
WBC (10^3/µl)	6.30±0.64[a]	5.17±0.82[a]	4.90±0.91[a]	6.00±2.11[a]
Neutrophils (%)	12.33±3.84[a]	19.33±1.20[b]	21.67±1.45[b]	20.33±0.88[b]
Lymphocytes (%)	87.67±3.84[a]	82.67±0.88[a]	82.67±2.03[a]	78.00±5.54[a]
Platelets (10^3/µl)	133.00±1.04[a]	130.33±0.96[a]	129.67±0.33[a]	130.00±0.81[a]

Values are expressed as mean ± Standard Error of Mean of five replicates. Mean Value Having Different Superscript Letters in rows are significantly different **(p<0.05) (One way ANOVA followed by Duncan's, multiple comparison test)**.

Legend: **PCV** = Packed Cells Volume; **RBC** = Red Blood Cells; **WBC** = White Blood Cells; **Hb** =Hemoglobin.

Table 1.3 presents the effect of different doses of methanol seed extract of *Passiflora edulis var flavicarpa* on some biochemical parameters of rats. The result showed that the extract had effect on some biochemical parameters. ALT, ALP, AST and GGT serum levels were significantly increased (p>0.05) at the dose of 3000mg/Kg bwt when compared with the normal control while the other dose levels (1000mg/Kg and 2000mg/Kg bwt) had no significant difference. All the other biochemical parameters investigated had no significant difference at the different dose levels compared with the control group.

Table 1.3: Effect of sub-chronic oral treatment with Methanol Seed Extract of *Passiflora edulis var flavicarpa* on some biochemical parameters of rats.

Parameters	Treatment (mg/kg)			
	Normal	1000	2000	3000
Urea (mmol/l)	6.89±0.57[a]	5.95±0.25[a]	5.69±0.25[a]	5.34±0.69[a]
Creatinine (mg/dl)	1.05±0.06[a]	0.96±0.04[a]	0.95±0.03[a]	0.92±0.04[a]
Total Protein (g/dl)	6.55±0.11[a]	6.18±1.18[a]	4.49±0.67[a]	4.85±0.44[a]
Albumin (g/dl)	1.78±0.18[a]	1.92±0.34[a]	1.68±0.28[a]	2.28±0.08[a]
Total Bilirubin (mg/dl)	1.04±0.22[a]	0.70±0.32[a]	0.61±0.26[a]	0.50±0.27[a]
Direct Bilirubin (mg/dl)	0.11±0.05[a]	0.16±0.02[a]	0.14±0.03[a]	0.20±0.03[a]
ALP (U/L)	263.41±3.34[a]	265.66±0.35[a]	266.49±0.46[a]	282.63±1.15[b]
ALT (U/L)	66.94±0.81[a]	69.48±0.73[a]	69.73±0.46[a]	100.82±1.58[b]
AST (U/L)	115.32±0.86[a]	117.01±1.01[a]	117.10±1.02[a]	140.32±2.32[b]
GGT (U/L)	12.73±0.08[a]	12.94±0.06[a]	12.92±0.08[a]	17.70±0.14[b]

Values are expressed as mean ± Standard Error of Mean of five replicates. Mean Value Having Different Superscript Letters in rows are significantly different **(p<0.05) (One way ANOVA followed by Duncan's, multiple comparison test)**.

Legend: **ALT**= Alanine Amino Transferase; **AST**= Aspartate Amino Transferase; **ALP** = Alkaline Phosphatase **GGT** =Gamma Glutamyl Transferase.

Histopathological Evaluation of the Liver and Kidney of animals administered with Methanol Seed Extract of *Passiflora edulis var flavicarpa*.

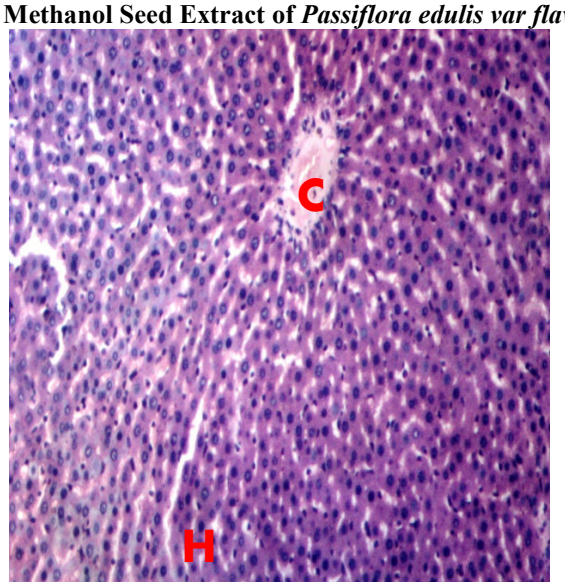

Plate 1a: Photomicrograph of liver section of normal rat (control group) showing the central vein (C) and normal hepatocytes (H) with no histopathological lesion. Hematoxylin and Eosin (H&E) X 100.

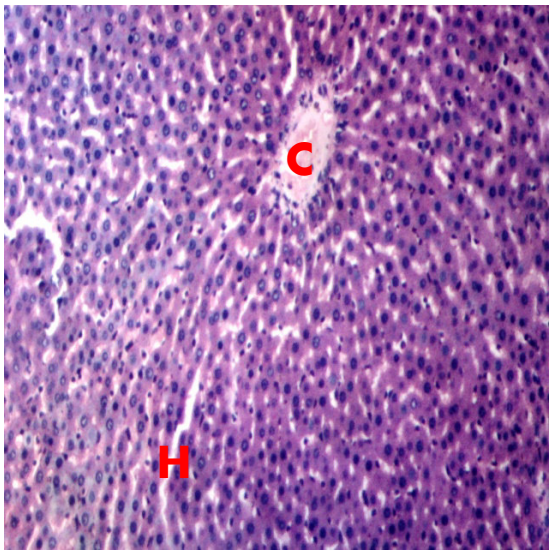

Plate 1b: Photomicrograph of liver section of rat administered with 1000 mg/kg of the seed extract for 28 days showing no pathological changes. Hematoxylin and Eosin (H&E) X 100.

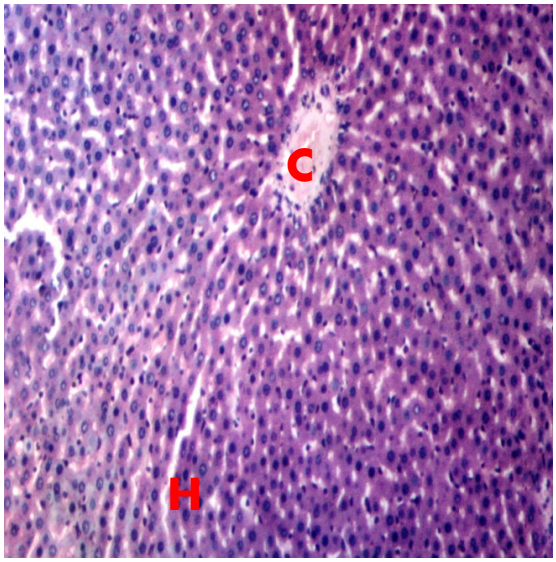

Plate 1c: Photomicrograph of liver section of rat administered with 2000 mg/kg of the seed extract for 28days showing no pathological changes. Hematoxylin and Eosin (H&E) X 100.

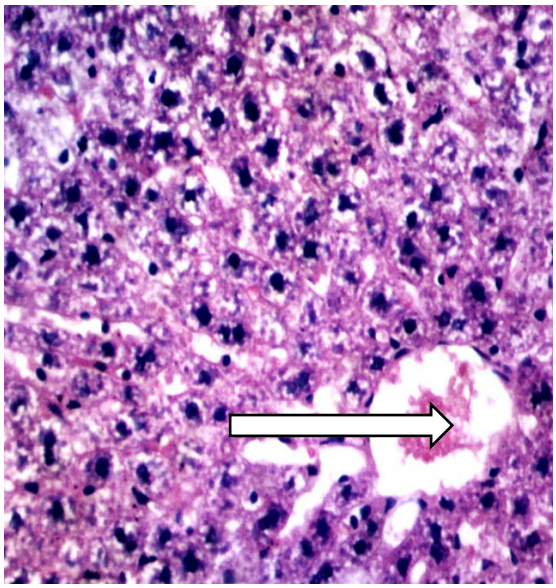

Plate 1d: Photomicrograph of liver section of rat administered with 3000 mg/kg of the extract for 28 days showing mild distortion of hepatic architecture. Hematoxylin and Eosin (H&E) X 200.

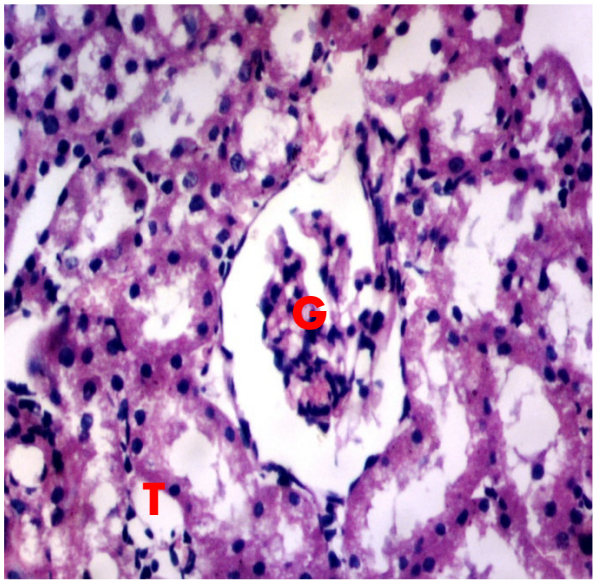

Plate 2a: Photomicrograph of the kidney of normal rat (control group) showing normal histological structures of the kidney: Glomerulus (G) and Renal tubule (T). Hematoxylin and Eosin (H&E) X 200.

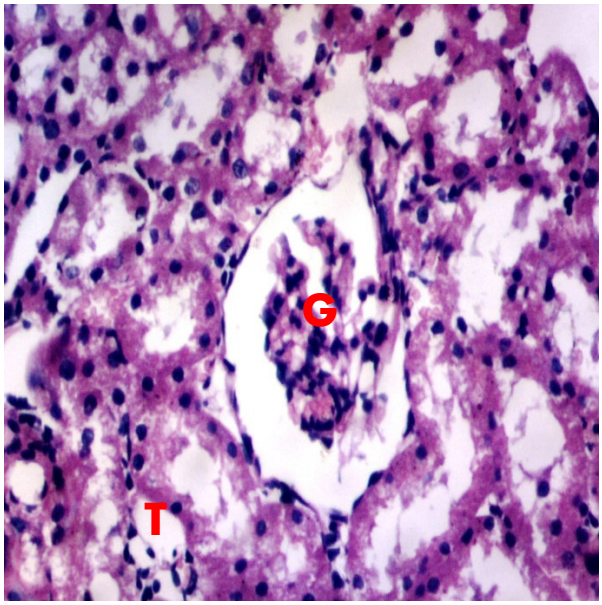

Plate 2b: Photomicrograph of kidney of rat administered with 1000 mg/kg of the seed extract for 28days showing no pathological changes. Hematoxylin and Eosin (H&E) X 200.

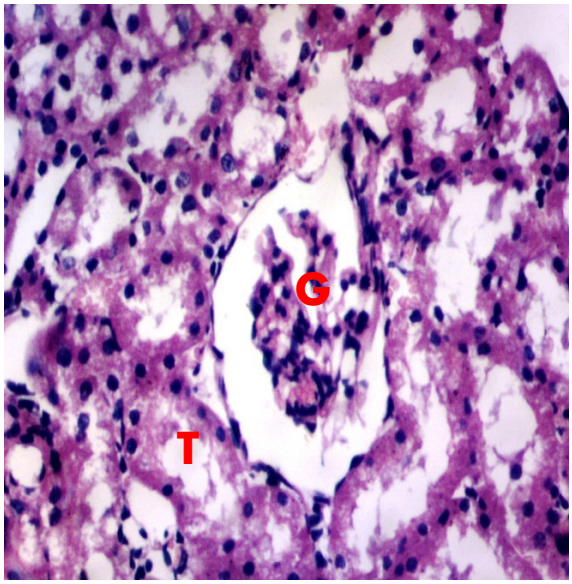

Plate 2c: Photomicrograph of kidney of rat administered with 2000 mg/kg of the seed extract for 28days showing no pathological changes. Hematoxylin and Eosin (H&E) X 200.

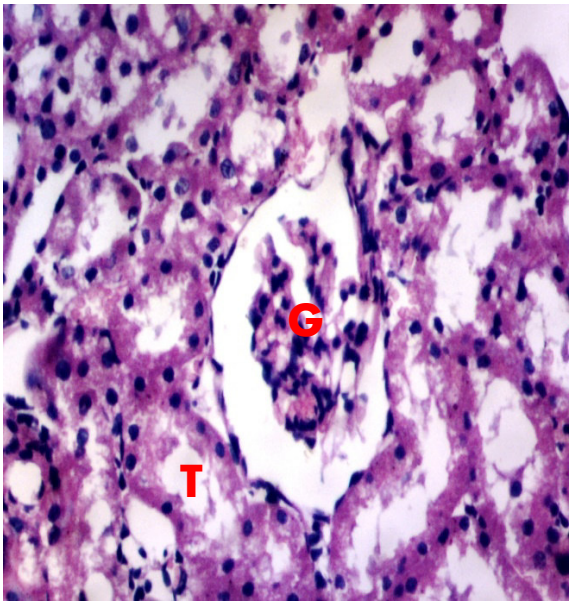

Plate 2d: Photomicrograph of kidney of rat administered with 3000 mg/kg of the seed extract for 28days showing no pathological changes. Hematoxylin and Eosin (H&E) X 200.

Discussion

It is important to evaluate the toxic effects of plants in order to assess their safety for both human and animal use. In the acute toxicity study of the methanol seed extract, no mortality or adverse effects were observed at a single dose of 5000mg/kg indicating that the LD_{50} of the extract is greater than 5000mg/kg. This result shows that acute exposure to high doses of the methanol extract of *passiflora edulis var. flavicarpa* seeds is safe.

Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals [11]. In the sub-chronic study, there was substantial increase in the mean weights of the treatment groups and the control**.** There was a significant difference ($p<0.05$) between the treatment group at the dose of 3000mg/kg at week 4 and the control, all the other groups showed no significant difference. This indicates that the extract might have deleterious effect at increasing dose and over a long period of time.

In addition to bodyweight, relative organ weight has also been used as another basic indicator to determine whether the rats have been exposed to harmful agents [18, 28]. In the current study, the relative organ weights of the liver and kidneys in all the treatment groups were not significantly different ($p<0.05$) from those of the control groups.

The haematopoietic system is very sensitive to toxic compounds and serves as an important index of the

physiological and pathological status in both animals and humans [2]. The effect of the methanol seed extract on haematologic parameters of the treatment groups showed a significant increase ($p<0.05$) in neutrophils in all the doses when compared to the control. This may be as a result of the body's immune system perceiving the extract as an external agent. Neutrophils defend the body against infection and antigens. All the other haematological parameters tested showed no significant difference from that of the control groups.

The results of biochemical study indicate that the administration of methanol seed extract of *Passiflora edulis var. flavicarpa* for a period of 28 days has mild to moderate deleterious effects on some biochemical parameters. It has been well established that elevated levels of AST, ALT, ALP and GGT are indicative of cellular leakage and loss of functional integrity of hepatic cell membranes implying hepatocellular damage [1].

Alanine aminotransferase (ALT), Aspartate aminotransferases (AST) and Alkaline Phosphatase (ALP) are biomarkers used to predict hepatic function [24]. Although ALT is a more specific marker because it is more sensitive to liver damage. The serum levels of ALT, AST and ALP are usually elevated in conditions associated with injuries or diseases affecting the liver which leads to the release of these hepatocellular enzymes into the bloodstream [21]. The activity of serum γ - glutamyl transferase (GGT) is generally elevated as a result of liver disease, since γ - glutamyl transferase is a hepatic microsomal enzyme [15]. GGT is also more specific than ALP in detecting hepatic diseases. Also, it is more responsive to biliary obstruction than are AST and ALT [16]. The ALP, GGT, AST and ALT serum concentration were significantly higher ($p<0.05$) in the treatment groups at the dose of 3000mg/kg compared to the control group while at the dose of 1000mg/kg and 2000mg/kg, there was no significant difference. The increase in serum concentration could be due to the different active compounds present in the extract which are acting differently at the doses tested. This result indicates that the extract might have toxic potential on liver with increasing dose. There was no significant change in the serum concentration of both total and direct bilirubin of the treated groups compared with the control group indicating that that the conjugating ability of the liver was not affected. Also, the non-significant effect of the extract on the serum total protein and albumin concentration indicates that the secretory capacity of the liver was not affected as well.

Urea and creatinine are both markers of kidney function. The results of the serum levels of both creatinine and urea of the treatment groups showed no significant difference ($p<0.05$) compared to the control. This indicates that the kidney was not affected by the extract.

In toxicological studies, histopathological examination provides supportive evidence for biochemical and haematological observations [9]. The photomicrograph of the liver revealed that there was no visible effect of the extract on the liver cells of group II (plate 1b) and group III (plate 1c) which received 1000mg/Kg bwt and 2000mg/Kg bwt of the extract respectively which is similar to that of the control group (plate 1a) showing a normal central vein and hepatocytes radiating from the central vein while the photomicrograph of the liver cells of group IV (plate 1d) which received 3000mg/Kg revealed that there was mild distortion of hepatic architecture showing a normal central vein with cytoplasmic vacuolations of hepatocytes.

The photomicrograph of the kidney revealed that the histological structures of the kidneys (glomeruli and renal tubules) were normal in all the treatment groups just as the control group.

Our result revealed that the methanol seed extract of *Passiflora edulis var. flavicarpa* could have hepatotoxic effect at high doses and over a long period of time and as such, it should be used cautiously at high doses over a long period of time because it might cause damage to the liver.

Conflicts of interest

I Rabiu Umar Aliyu Wasagu declare that they have no conflict of interest

References

1. Abd-Algader, N.N., El-Kamali, H.H., Ramadan, M.M., Ghanem K.Z. and Farrag A.H. (2013). *Xylopia aethiopica* Volatile Compounds Protect Against Panadol-Induced Hepatic and Renal Toxicity in Male Rats. *World Applied Sciences Journal*; 27(1): 10-22.
2. Adeneye, A.A., Ajagbonna, O.P., Adeleke, T.I. and Bello, S.O. (2006). Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. *J. Ethnopharmacol.*, 105: 374-379.
3. Armstrong, G.M. and Armstrong, J.K. (2013). Reflection on the wilt Fusaria. *Annual Review of Phytopathogy* 13:95-103.
4. Bartels, H. and Bohmer, M. (1971). Micro determination of Creatinine. *Clin. Chin. Acta*; 32: 81-85.
5. Beninca, J.P., Montanher, A.B., Zucolotto, S.M., Schenkel, E.P. and Frode, T.S. (2007). *Food Chemistry,* 104:1097–1105.
6. Coles, E.H. (1989). *Veterinary Clinical Pathology*. 4th Edn. W.B.Saunders Co. USA pp. 130-148.
7. Doumas, B.T., Watson, W. and Briggs, H.G., (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chem. Acda*; 3: 187-196.

8. Drury, R.A., Wallington, E.A. and Cameron, R. (1967). *Carletons histological technique*. 4th ed. Oxford University press.New York. Pp48-66.
9. Eroschencho, V.P. (2000). *Atlas of histology with functional correlations*, 9th edn, Williams and Wilkins Lippincott, USA; p 12.
10. Gomall, A.G., Bardawill, C.S. and David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem*; 177(2): 751-766.
11. Hilaly, J.E., Israili, Z.H. and Lyouss, B. (2004). Acute and chronic toxicological studies of AjuvaIva in experimental animals, *J. Ethnopharmacol*, 91(1): 43–50.
12. Jain, N.C. (1986). Schalm's veterinay haematology 4th Ed. Lea and Febiger Philadelphia, USA.
13. Jendrassik, L. and Grof, P. (1938).Simplified photometric methods for the determination of bilirubin. *Biochem.Zchr*. 297: 81-89.
14. Joy, P.P. (2010). Passion fruit (*Passiflora edulis sims*): *Passifloraceae*. Pineapple Research Station (Kerala Agricultural University), Vazhakulam-686 670, Muvattupuzha, Ernakulam District, Kerala, India. Pp 1-8.
15. Kanchana, N. and Sadiq, M.A. (2011). Hepatoprotective Effect of Plumbago Zeylanica on Paracetamol Induced Liver Toxicity In Rats. *Int. J. Pharm. Pharm. Sci*; 3: 151-154.
16. Lum, G. and Gambino, S.R. (1972). Serum Gamma-Glutamyl Transpeptidase activity as an indicator of disease of liver, pancreas, or bone. *Clin Chem;* 18: 358 - 62.
17. Meletti, L.M. and Brückner, C.H. (2000). Melhoramento Genético. In: Brückner, C.H.; Picanço, M.C. Maracujá: tecnologia de produção, pós-colheita, agroindústria e mercado. Porto Alegre: Cinco Continentes. pp 345-385.
18. Mohamed, E.A, Lim,C.P, Ebrika,O.S., Asmawi, M.Z., Sadikun, A. and Yam, M.F. (2011). "Toxicity evaluation of a standardised 50% ethanol of Orthosiphon stamineus," *J. Ethnopharmacol*; 133(2): 358–363.
19. Montanher, A.B., Zucolotto, S.M., Schenkel, E.P. and Frode, T.S. (2007). *J. Ethnopharmacol*; 109: 281–288.
20. Njagi, J.M., Piero, M.N., Kibiti, C.M. Ngeranwa, J.N., Njagi, A.N., Njue ,W.M. and Gathumbi, P.K. (2012). Herbal management of diabetes mellitus: a rapidly expanding research avenue *Int J Curr Pharm Res*, 4(2):1-4.
21. OECD, (2001). Guideline for testing chemicals. Acute toxicity – up and down procedure. No. 425, pp: 1-26.
22. Pagana, K.D. and Pagana, T.J (2002). *Mosby's Manual of Diagnostic and Laboratory Tests*. 2nd Edn.,Mosby, St Louis, Missouri USA, pp: 1-44.
23. Reitman, S., and Frankel, S. (1957). A calorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic tranaminases. *.Amer.J. clin.Pathol*; 28: 56-63.
24. Shaaraway, S.M., Tohamy, A.A., Elgendy, S.M., Elmageed, Z.Y., Bahnasy, A., Mohamed, M.S., Kandil, E. and Matrougui, K. (2009). Protective effects of garlic and silymarin on NDEA induced rats hepatotoxicity. *Int J Biol Sci.,* 5(6):549-557.
25. Sood, R. (1999). *Medical Laboratory Technology. Method and Interpretation.*Sed.Jaypee Brothers Medical Publishers Ltd. Pp 488-490.
26. Suffnes, M. and Dowos J. (1982). Current stating of the NCI Plant and Animal product program, *J. Natural prod*.; 45:1-14.
27. Szasz, G. and Bergmeyer, H.U. (1974). *Method of enzymatic analysis*. Weinheim Veriag Chemie.
28. Teo, S., Stirling, D., Thomas, S., Hoberman, A., Kiorpes, A. and Khetani, V. (2002). "A 90-day oral gavage toxicity study of D-methylphenidate and D,L-methylphenidate in Sprague-Dawley rats," *Toxicology*; 179(3): 183–196.
29. World Health Organization (2008). Traditional Medicine. Fact sheet no. 134
30. Young, D.S. (1997). Effects of Drugs on Clinical Laboratory Test. *Ann. Clin. Biochem.* 34: 579-581.