Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014; 39 (2) ; 119–125
doi: 10.5505/tjb.2014.83997

Research Article [Araştırma Makalesi]

Yayın tarihi 30 Haziran, 2014 © TurkJBiochem.com

[Published online 30 June, 2014]

Toxicopathological evaluation of *Picralima nitida* seed aqueous extract in Wistar rats

[Wistar sıçanlarında *Picralima nitida* tohumu sıvı ekstresinin toksikopatolojik değerlendirilmesi]*

Taofik Olatunde Sunmonu[1],
Oyelola Bukoye Oloyede[1],
Tajudeen Alowonle Owolarafe[1],
Musa Toyin Yakubu[1],
Omotayo Olutola Dosumu[2]

Departments of [1]Biochemistry, [2]Chemistry, University of Ilorin, Ilorin, Nigeria

Yazışma Adresi
[Correspondence Address]

Taofik Olatunde Sunmonu

Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa.
Tel. +27 33 260 5136
E-mail. sunmonu@ukzn.ac.za

*** Translated by [Çeviri]** Dr. Özlem Dalmızrak

Registered: 22 April 2013; Accepted: 12 September 2013

[Kayıt Tarihi: 22 Nisan 2013; Kabul Tarihi: 12 Eylül 2013]

ABSTRACT

Objective: *Picralima nitida* is a widely used medicinal plant in West Africa for treating malaria, diarrhea and inflammation. The objective of this study is to evaluate the toxicological effect of aqueous seed extract of the plant in Wistar rats.

Methods: Twenty-four apparently healthy animals were randomized into 4 groups comprising 6 rats each and orally administered with aqueous extract of *P. nitida* seeds at doses of 100, 200 and 400 mg/kg body weight with distilled water as control for 14 days. Specific liver and kidney function indices were assayed alongside haematological and histopathological analyses to monitor toxicity according to standard methods.

Results: Phytochemical screening revealed the presence of alkaloids, glycosides, saponins, steroids and tannins. The extract had no significant effect on all kidney function indices assayed but caused a significant reduction ($P < 0.05$) in the activities of liver enzymes accompanied by significant decrease in liver to body weight ratio, serum total protein and globulin concentrations. No significant alteration was observed in the serum levels of albumin and conjugated bilirubin whereas the extract brought about significant increase ($P < 0.05$) in serum total bilirubin concentration. Haematological analysis revealed no significant effect on erythrocyte indices in contrast to white blood cell count and its differentials which were significantly elevated ($P < 0.05$) following extract administration. Histopathological studies further showed no distortion of cell structures in the studied organs.

Conclusion: The available evidences in this study suggest that aqueous extract of *P. nitida* seeds exhibits mild and selective toxicity with liver as the target organ. Therefore, the herb may not be completely 'safe' as an oral remedy; and long term administration should be avoided.

Key Words: *Picralima nitida*, phytochemical screening, toxicology, histopathology, haematology

Conflict of Interest: The authors declare no conflict of interest.

ÖZET

Amaç: *Picralima nitida* Batı Afrika'da sıtma, diyare ve inflamasyon tedavisinde sıklıkla kullanılan tıbbi bir bitkidir. Çalışmanın amacı Wistar sıçanlarında bu bitkinin sıvı tohum ekstrelerinin toksikolojik etkilerini incelemektir.

Metod: Sağlıklı 24 hayvan gelişigüzel olarak her bir grupta 6 sıçan olacak şekilde dört gruba ayrılmıştır. Sıvı *P. nitida* tohum ekstresi oral yolla, dozu 100, 200 ve 400 mg/kg vücut ağırlığı olacak şekilde uygulanmıştır. Kontrol grubuna 14 gün boyunca distile su verilmiştir. Hematolojik ve histopatolojik analizler ile birlikte karaciğer ve böbrek fonksiyon göstergeleri de incelenmiştir.

Bulgular: Fitokimyasal tarama ekstrenin alkaloid, glikozid, saponin, steroid ve tanninleri içerdiğini göstermektedir. Ekstrenin böbrek fonksiyonuna bir etkisi olmadığı saptanmıştır. Fakat karaciğer enzim aktivitelerinde belirgin azalma ($P < 0.05$) ile birlikte karaciğer : vücut ağırlığı oranında, serum total protein ve globulin derişimlerinde düşme gözlenmiştir. Serumda albumin ve konjuge bilirubin düzeyleri değişmezken serum total bilurubin derişimi belirgin bir şekilde ($P < 0.05$) artmaktadır. Hematolojik analizler ekstrenin eritrosit indeksine bir etkisinin olmadığını, buna karşılık beyaz kan hücresi sayısının önemli derecede ($P < 0.05$) yükseldiğini ortaya koymuştur. Histopatolojik incelemelerde çalışılan organların hücre yapılarında herhangi bir bozulmaya rastlanmamıştır.

Sonuç: Çalışma kapsamında elde edilen kanıtlar, *P. nitida* tohum ekstresinin karaciğer üzerinde hafif ve seçici toksisiteye neden olduğunu göstermektedir. Bu nedenle bu bitki tamamen 'güvenli' değildir. Uzun dönem kullanımlardan kaçınılmaldır.

Anahtar Kelimeler: *Picralima nitida*, fitokimyasal tarama, toksikoloji, histopatoloji, hematoloji

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Traditional medicines support well over 80% of the population in developing countries especially in the rural areas [1]. Available evidence suggests that even in urban areas which are well served by modern healthcare facilities, a good number of patients rely on traditional healers to meet some of their healthcare needs [2]. However, their general acceptability has been limited by lack of dose regimen and adequate toxicity data to evaluate their safety [3]. It is therefore imperative to provide information on the safety or toxicity risk associated with the cure use of these plants for the treatment of ailments.

Picralima nitida (Apocynaceae family) is one of such medicinal plants. It is a wildly grown tree and widely distributed in the tropical rain forests of Africa. When fully grown, it is about 20 m high with white flowers and large paired fruits. In Nigeria, it is popular referred to as *Osi-Igwe* by the *Igbos* and *Abere* by the *Yorubas*. Elsewhere in West Africa, the plant is called *Gbe-Fon dangné* (Benin Republic), *Adangme* (Ghana), *Abure ebissi* (Ivory Coast) and *Susu balunyi* (Sierra Leone) [4]. The tree has several medicinal uses. A decoction made from its stem bark is taken for the treatment of diarrhea, gonorrhea and intestinal worms [5]. Its bark exhibits properties that act against trypanosomiasis [6]. Crushed or powdered seed of the plant is taken to treat malaria, diarrhea and inflammation [7, 8]. Both its fruit rinds and bark are used for treating protozoan diseases while the stem bark, roots and seeds are used for the treatment of malaria [9].

Despite the widespread abundance and traditional use of *P. nitida* seeds, no systematic study has been done on the toxicological effects of this herb to the best of our knowledge. The present study was therefore designed to evaluate the safety/toxicity risk associated with the use of aqueous seed extract of *P. nitida* based on functional indices and histology of rat liver and kidney.

Materials and Methods

Assay kits and chemicals

Assay kits for electrolytes and enzymes were obtained from Randox Laboratories Ltd, United Kingdom. Albumin, globulin and total protein assay kits were supplied by Fortress Diagnostics Ltd, United Kingdom while bilirubin, creatinine, urea and uric acid assay kits were products of Agappe Diagnostics, India. All other chemicals and reagents used were of analytical grade.

Plant material and authentication

The seeds of *Picralima nitida* used in this study were obtained from a local herbal market in Ilorin, Nigeria and authenticated at the Herbarium Unit of the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria where a voucher specimen with number FHI 108794 was deposited.

Preparation of aqueous extract

The seeds were thoroughly rinsed under running tap and distilled water afterwards to remove dust and soil particles before oven drying at 50°C for 72 h. The dried seeds were ground into powder using a Milling machine after which 400 g of the powdery material was extracted in 1.5 liter of distilled water for 72 h at room temperature with intermittent shaking. The mixture was passed through Whatman No.1 filter paper and the resulting filtrate was freeze-dried to give a yield of 9.2 g. This was further reconstituted in distilled water to obtain the required extract doses of 100, 200 and 400 mg/kg body weight used for the experiment.

Phytochemical analysis of Picralima nitida seeds

Phytochemical analysis of the aqueous extract of *Picralima nitida* seeds was performed following standard procedures [10, 11].

Animals used

Male albino rats of Wistar strain with a mean weight of 200 ± 6.57 g were obtained from the Animal Holding Unit of the Department of Biochemistry, Faculty of Science, University of Ilorin, Nigeria. The animals were housed in clean metabolic cages placed in a well-ventilated house with optimum condition (temperature: 23±1°C; photoperiod: 12 h natural light and 12 h dark; humidity: 45-50%). They were acclimatized to animal house conditions and allowed free access to commercial pelleted rat pellets (Bendel Feeds and Flour Mill Ltd, Ewu, Nigeria) and water. The cleaning of the cages was done on a daily basis. This study was carried out following approval from the Ethical Committee on the use and care of animals of the University of Ilorin, Nigeria and an ethical clearance number (SUNMONU 2012/011) assigned for the project.

Experimental design

A total of 24 male rats were completely randomized into 4 groups comprising 6 animals each. Rats in Group 1 (Control) were orally administered with 0.5 ml distilled water (the vehicle) while those in Groups 2 to 4 were administered with the same volume of *P. nitida* seeds aqueous extract at 100, 200 and 400 mg/kg body weight/day, respectively and the treatment continued for 14 days.

Collection of blood sample and isolation of organs

After 14 days of treatment, the rats were sacrificed by ether anaesthetization and the neck area was quickly cleared of fur to expose the jugular vein which was slightly displaced and sharply cut with sterile surgical blade. An aliquot (2 ml) of the blood was collected into ethylene diamine tetra-acetic acid (EDTA) embedded sample bottles (BD Diagnostics, preanalytical systems, Midrand, USA) for haematological analysis. Another 5 ml

of the blood was collected and centrifuged at 2000 *g* x 5 min and the serum was carefully aspirated with a Pasteur pipette into sample bottles and used within 12 h for the biochemical assays. The rats were quickly dissected and the whole liver and two kidneys were excised, freed of fat, blotted with clean tissue paper and then weighed. The organ to body weight ratio was determined by comparing the weight of each organ with the final body weight of each rat. Known weights of the liver and kidney were cut, chopped into pieces and homogenized with ice cold 0.25 M sucrose solution (1 in 5 dilution) using precooled pestle and mortar in a bowl of ice- chips. The supernatant was carefully collected for enzyme assay.

Determination of biochemical parameters

The concentrations of creatinine, urea, uric acid, bilirubin, total protein, electrolytes, albumin and globulin were determined in the serum following standard procedures as described in the respective assay kits. Alkaline phosphatase (ALP) activity was assayed in the liver and kidney according to the method of Wright *et al*. [12] while the activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined in the liver following the method of Reitman and Frankel [13].

Determination of haematological parameters

Using the standard method of Alexander and Griffiths [14], the following haematological indices were determined namely red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cells (WBC) and white blood cell differential counts (Horiba ABX 80 Diagnostics, ABX Pentra Montpellier, France).

Histological examination of organs

Portions of the liver and kidney were fixed immediately on removal from the animals in 10% Buffered Neutral Formalin (BNF) for 72 h at room temperature for histological analysis using the method described by Krause [15].

Statistical analysis

Data were expressed as mean ± SD of six replicates and were subjected to one way analysis of variance (ANOVA) followed by Duncan multiple range test to determine significant differences in all the parameters. Values were considered statistically significant at $P < 0.05$.

Results

Phytochemical profile

Phytochemical screening of aqueous extract of *Picralima nitida* seeds revealed the presence of some phytochemicals which probably suggests its usage for medicinal purposes. Alkaloids and tannins are present in higher concentrations; saponins are moderately present while glycosides and steroids are present in relatively reduced concentrations.

Liver function indices

The effect of aqueous extract of *P. nitida* seeds on some liver function indices in the experimental rats is presented in Table 1. Compared to the control, the extract caused a significant reduction ($P < 0.05$) in the activities of ALP, ALT, AST as well as liver to body weight ratio at all doses investigated while the levels of albumin and conjugated bilirubin in the serum of the animals were not significantly altered following administration of the extract. In contrast, there was dose specific effect on the total protein, globulin and total bilirubin levels in the serum. For instance, the extract at all tested doses significantly reduced ($P < 0.05$) serum globulin concentration whereas total serum protein significantly dropped along with increase in extract doses administered when compared with the control.

Kidney function indices

Administration of aqueous extract of *P. nitida* seeds at all doses investigated in the study did not significantly affect kidney to body weight ratio and ALP activity. Also, the serum levels of sodium, potassium, chloride and bicarbonate ions; as well as urea, creatinine and uric acid concentrations compared favourably well with the control throughout the experimental period (Table 2).

Haematological and histopathological responses

Continuous administration of aqueous extract of *P. nitida* seeds at all tested doses did not produce any significant effect on the erythrocyte indices investigated (PCV, RBC and Hb counts). However, WBC counts and its differentials significantly increased ($P < 0.05$) in rats administered with 200 and 400 mg/kg body weight of the extract (Table 3).

Photomicrograph sections of the liver cells are shown in Figure 1. The section revealed preservation of the architecture, normal hepatocytes with appropriate nuclear to cytoplasmic ratio, normal central vein with no evidence of adhesion or inflammation, portal tracts with appropriate number of bile ducts and blood vessels. Generally, the features observed were essentially similar to the control. A similar situation of persevered glomerular structure was observed in kidney when compared with the control (Figure 2).

Discussion

One major problem associated with the use of herbs is the choice of dosage as most of them are administered without any standard dosage which may have serious toxicological implications on vital organs in the body. The present study has clearly demonstrated that aqueous extract of *P. nitida* seeds has some useful phytochemicals but long term administration at high doses may compromise normal functioning of the liver.

Phytochemical screening revealed that aqueous extract of *P. nitida* seeds is rich in alkaloids, saponins, tannins,

Table 1. Effect of aqueous extract of *P. nitida* seeds on some liver function indices of Wistar rats

		P. nitida seed extract (mg/kg body weight)		
Parameter	Control	100	200	400
Liver-body weight ratio (%)	3.43±0.08[a]	3.34±0.08[b]	3.09±0.07[c]	3.02±0.02[c]
Liver ALP activity (U/L)	33.52±0.25[a]	30.27±1.08[b]	18.37±1.36[c]	8.58±0.27[d]
Liver ALT activity (U/L)	70.90±0.77[a]	62.50±0.22[b]	39.60±0.60[c]	17.20±1.36[d]
Liver AST activity (U/L)	146.00±0.42[a]	139.70±1.36[b]	139.60±0.75[b]	123.00±1.55[c]
Total protein (g/L)	76.00±0.70[a]	73.17±0.91[b]	67.50±0.96[c]	55.83±0.73[d]
Albumin (g/L)	37.50±5.48[a]	37.40±3.28[a]	36.33±2.38[a]	35.67±2.95[a]
Globulin (g/L)	38.50±2.37[a]	28.50±1.95[b]	28.20±1.16[b]	24.17±2.76[b]
Total bilirubin (mmol/L)	1.10± 0.11[a]	1.42±0.08[ab]	1.70±0.06[bc]	1.88±0.08[c]
Conjugated bilirubin (mmol/L)	0.32±0.05[a]	0.32±0.04 [a]	0 .27±0.03[a]	0.28±0.03[a]

Data are means ± SD (n=6). Row values with different superscripts are statistically significantly different (Overall *P value* = 0.019).

Table 2. Effect of aqueous extract of *P. nitida* seeds on some kidney function indices of Wistar rats

		P. nitida seed extract (mg/kg body weight)		
Parameter	Control	100	200	400
Kidney-body weight ratio (%)	1.14±0.03[a]	1.13±0.04[a]	1.13±0.02[a]	1.12±0.04[a]
Kidney ALP activity (U/L)	33.52±2.25[a]	30.27±3.08[a]	28.67±2.36[a]	28.58±2.27[a]
Sodium (mmol/L)	136.50±1.63[a]	136.80±1.59[a]	140.83±1.54[a]	141.50±1.54[a]
Potassium (mmol/L)	8.00±1.08[a]	7.86±0.35[a]	7.75±0.19[a]	7.65±0.26[a]
Chloride (mmol/L)	78.20±3.93[a]	74.50±2.61[a]	72.50±3.14[a]	70.50±3.81[a]
Bicarbonate (mmol/L)	20.70±0.33[a]	20.20±0.37[a]	20.00±0.73[a]	19.33±0.62[a]
Urea (mmol/L)	3.52±0.27[a]	3.58±0.71[a]	4.03±0.14[a]	4.08±0.26[a]
Creatinine (*μ*mol/L)	33.00±5.18[a]	31.20±1.66[a]	30.83±3.10[a]	30.80±3.66[a]
Uric acid (*μ*mol/L)	0.12±0.01[a]	0.12±0.01[a]	0.12±0.01[a]	0.13±0.01[a]

Data are means ± SD (n=6). Row values with different superscripts are statistically significantly different (Overall *P value* = 0.061).

Table 3. Effect of aqueous extract of P. *nitida* seeds on some haematological indices of Wistar rats

	P. nitida seed extract (mg/kg body weight)			
Parameter	*Control*	*100*	*200*	*400*
PCV (%)	*28.40±0.75a*	*28.80±0.58a*	*29.20±0.74a*	*31.20±1.58a*
RBC (x 1012/L)	*1.77±0.07a*	*1.80±0.04a*	*1.83±0.04a*	*1.84±0.09a*
Hb (g/L)	*7.27±0.15a*	*7.66±0.19a*	*7.71±0.13a*	*7.92±0.23a*
WBC (x 109/L)	*10.28±0.21a*	*10.90±0.11ab*	*11.30±0.30b*	*11.60±0.23b*
Neutrophils (%)	*31.80±1.11a*	*31.80±3.73a*	*32.00±1.82a*	*39.20±1.86b*
Lymphocytes (%)	*57.60±1.21a*	*61.80±1.02ab*	*63.80±1.94b*	*69.20±0.74c*
Monocytes (%)	*1.00±0.02 a*	*2.00±0.03b*	*2.00±0.05b*	*2.33±0.01c*
Basophils (%)	*0.39±0.04a*	*0.39±0.03a*	*1.00±0.05b*	*1.83±0.07c*
Eosinophils (%)	*1.83±0.17a*	*2.00±0.10a*	*2.67±0.33b*	*2.83±0.17b*

Data are means ± SD (n=6). Row values with different superscripts are statistically significantly different (Overall P value = 0.041).

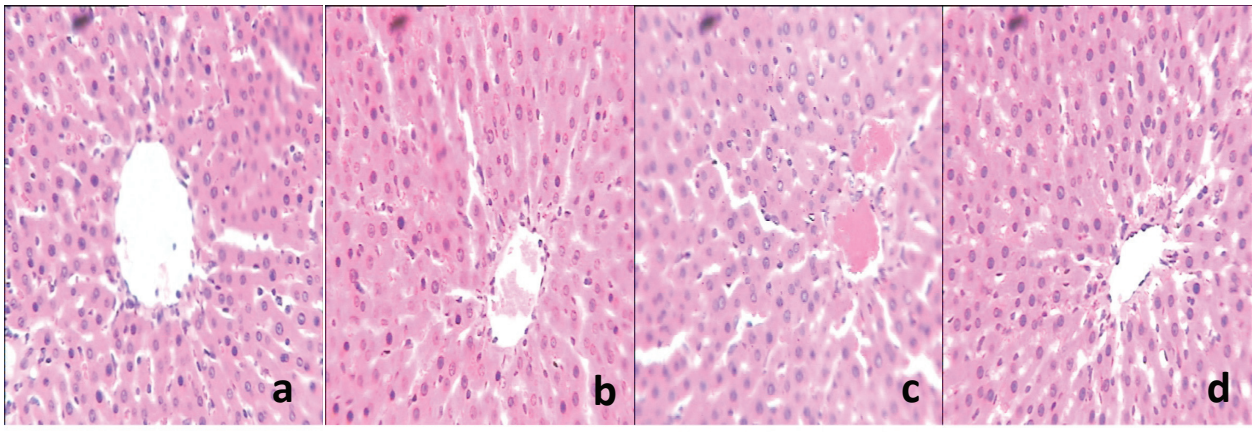

Figure 1. Photomicrograph sections showing intact rat hepatocytes (x400) after 14 days oral administration of (a) distilled water (Control) (b) 100 mg/kg body weight (c) 200 mg/kg body weight (d) 400 mg/kg body weight of *P. nitida* seeds aqueous extract.

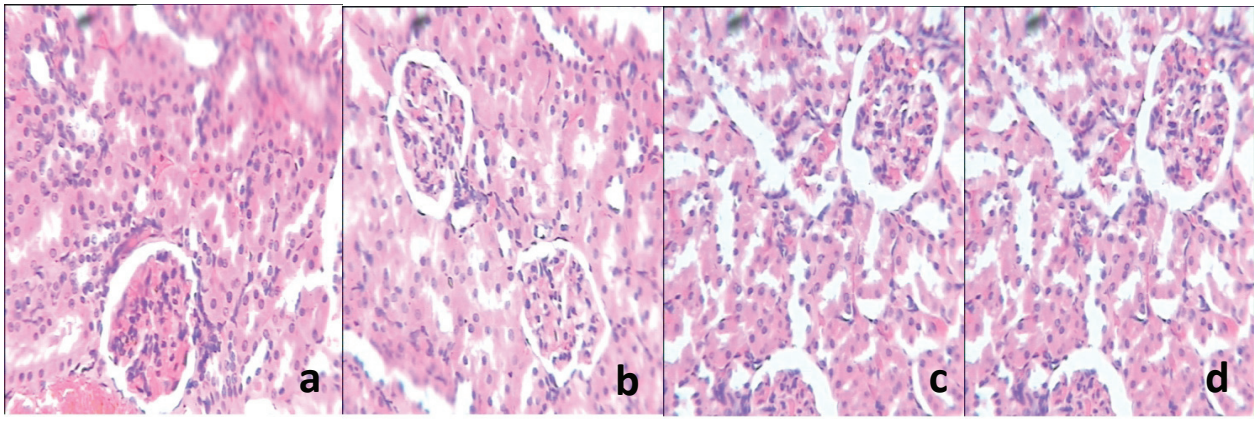

Figure 2. Photomicrograph sections showing intact rat glomeruli (x400) after 14 days oral administration of (a) distilled water (Control) (b) 100 mg/kg body weight (c) 200 mg/kg body weight (d) 400 mg/kg body weight of *P. nitida* seeds aqueous extract.

glycosides and steroids. This suggests that the seeds can be exploited for several medicinal purposes [10]. The presence of alkaloids suggests that the extract can be used as anti-inflammatory and anti-diabetic. This submission agrees with the findings of Inya-Agha [16]. The saponins and tannins contents of *P. nitida* seeds may also enhance its medicinal value. This is because these phytochemicals have been reported to prolong human life by preventing stress [17, 18].

Alteration in weight is an indication of impairment in the normal functioning of body organs. Organ to body weight ratio may indicate organ swelling, atrophy or hypertrophy [19]. The reduction in liver to body weight ratio following the administration of aqueous extract of *P. nitida* seeds in rats may be a result of atrophy. This submission is in agreement with earlier report by Ashafa *et al.* [20]. According to these authors, the significant reduction in organ-to-body weight observed in rats administered with *P. dioica* aqueous extract was attributed to atrophy. This may be an indication that the extract portends serious adverse effects on the growth potentials of the experimental rats. The reduction may also be attributed to abnormality in nutrient absorption by the liver since the animals fed quite well during the study [21].

Measurement of enzyme activity is a valuable tool in clinical diagnosis because it provides information on the effect and nature of pathological damage to tissues. Furthermore, damages to biological tissues can be assessed by changes in their enzyme activity, which indicate the catalytic influence of various factors such as inhibitors and activators, during pathological conditions. ALP is a marker enzyme often employed to assess the integrity of plasma membrane and endoplasmic reticulum [22]. Hence, damage to structural integrity of organs is reflected by decrease in the activity of this enzyme in the affected tissue. The transaminases (AST and ALT), on the other hand, are well known enzymes used as biomarkers to

predict possible toxicity to the liver [23]. Therefore, the reduction in ALP activity in the liver and kidney of rats following administration of aqueous extract of *P. nitida* seeds may in part be due to damage to the plasma membrane of the two organs leading to a compromise of membranal integrity. It could also be attributed to inhibition of the enzyme by the administered extract or inactivation of the enzyme molecules *in situ*. Reduction in liver activities of both transaminases as observed in the present study suggested damage to the liver cells and interference with protein metabolism or inhibition of the enzyme by the extract [24, 25]. Previous studies have also implicated plant extracts in the reduction of enzyme activity in tissues [26, 27]. Thus, our findings in this study agree with earlier submissions.

The concentrations of albumin, globulin, proteins and bilirubin in the serum can be used to assess the health status of the liver and can be used to ascertain different types of liver damage [21, 26]. The reduction in serum total protein concentration following treatment with aqueous extract of *P. nitida* seeds in rats could be attributed to a decrease in functional capacity of the liver possibly caused by some components of the extract. It is also possible that the herb brought about increase in protein catabolism leading to the observed decrease in serum protein concentration [28]. Reduction in serum globulin level also suggests damage to the liver [29]. Therefore, the reduced levels of these proteins as observed in this study are indications of diminished synthetic capacity of the liver. Elevated serum level of bilirubin at all tested doses may be as a result of reduced uptake arising from liver disease. All the data obtained with respect to the liver function indices indicate cellular toxicity of the aqueous extracts of *P. nitida* seeds on the liver of Wistar rats. The damage is however not total as the extract did not affect albumin and conjugated bilirubin concentrations in the serum.

The functional capacity of the kidney can be measured by the dye excretion tests, clearance test, and determination of serum concentrations of excretory and electrolyte constituents [30]. In the present study, the extract had no significant effect on all the kidney function indices investigated. This suggests that that the secretory ability and normal functioning of the kidney in relation to these parameters were unaffected. This may be an indication that the functions of the kidney were not compromised following administration of *P. nitida* seed aqueous extract for 14 days.

Administration of medicinal compounds or drugs can alter the normal range of hematological parameters positively or negatively [31, 32]. Assessment of these parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts in living systems [33]. The various haematological constituents investigated in this study are useful indices that can be employed to assess the toxic potentials of plant extracts/botanicals in living systems [34]. Such toxicity testing is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity, when data are translated from animal studies [35]. The non-significant effect of the aqueous extract of *P. nitida* seeds on RBC could mean that the balance between the rate of production and destruction of blood corpuscles (erythropoiesis) was not affected negatively. RBC, Hb and PCV are associated with the total population of red blood cells. Therefore, the absence of observable significant effect of the extract on these parameters may be an indication that neither the incorporation of haemoglobin into the red blood cells nor the morphology and osmotic fragility of the red blood cells was altered [36]. Hb is a major constituent of erythrocytes which function in oxygen transport and can be used as an index to evaluate physical condition of an animal [37]. The herb did not affect Hb concentration and this further suggests that the extract did not destroy red blood cells and therefore maintained the oxygen-carrying capacity of the blood at the dosages administered. However, selective immune modulatory effect and localized toxicity may occur as recorded in WBC and differentials counts of *P. nitida* seed extract-treated rats. This probably suggests mild effect on the heamatological status of experimental rats.

Histological analysis can be used to examine the morphological changes in organs to reflect possible effect of xenobiotics. These changes are a late manifestation of a chemical, physical, mechanical or inflammatory assault on the affected tissue and usually complement enzyme study [38]. The absence of any obvious histopathological effect in the studied tissues (liver and kidney) of the treated groups implies that there is no significant damage and adverse effects of the extract on the integrity of the tissues. The architectural organization of the tissues were neither disorganized nor distorted as hypochronic infiltration, which is a common features of damaged hepatocytes and glomerulus, was not observed.

In conclusion, the present study showed that aqueous extract *P. nitida* seeds has no serious effect on the kidney and haematology of rats for the duration of experiment. This probably explains the absence of any visible histopathological derangement in the studied organs. However, the alterations in some liver function indices following treatment with the herb are suggestive of adverse effects. Therefore, we conclude that the extract may not be completely safe in rats when repeatedly administered for 14 days at the investigated doses.

Acknowledgement

The authors would like to thank the Herbarium Unit of the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria for assistance in identifying the plant.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

[1] Klaassen CD, Eaton DL. Principles of toxicology. *In* (Eds. Amdur MO, Doull J, Klaassen CD) The basic science of poisons. McGraw Hill 1991; pp. 12-49, Willey, New York.

[2] Kilima PM, Ostermayer I, Shija M, Wolff MM, Evans PJ. Drug utilization, prescribing habits and patients in City Council Health Facilities, Darus Salaam, Tanzania 1993; Swiss Tropical Institute, Basel.

[3] Pousset JL. Medicinal plants used as a source of raw material for the pharmaceutical industry: Problems and prospects 1988; OAU, Ife-Nigeria.

[4] Burkill HM. The useful plants of West Tropical Africa (2nd edn) 1985; Royal Botanic Gardens, Kew, Richmond, United Kingdom.

[5] Fakeye TO, Itiola OA, Odelila HA. Evaluation of the antimicrobial property of stem bark of *Picralima nitida* (Apocynaceae). Phytother Res 2000; 14:386-390.

[6] Wosu LO, Ibe CC. Use of extracts of *Picralima nitida* bark in the treatment of experimental trypanosomiasis: A preliminary study. J Ethnopharmacol 1989; 25:263-268.

[7] Ezeamuzie IC, Ojinnake MC, Uzygna EO, Oji SE. Anti-inflammatory, antipyretic and antimalarial activity of a West African medicinal plant–*Picralima nitida*. Afr J Med Med Sci 1994; 23:85-89.

[8] Duwiejua M, Woode E, Obiri DD. Pseudo-akuammigine, an alkaloid from *Picralima nitida* seeds has anti-inflammatory and analgesic actions in rats. J Ethnopharmacol 2002; 8:73-79.

[9] Kapadia GJ, Angerhofer CK, Ansa-Asamoah R. Akuammine: An antimalarial indolemonoterpene alkaloid of *Picralima nitida* seeds. Planta Med 1993; 59:565-566.

[10] Sofowora A. Medicinal Plants and Traditional Medicines in Africa 1993; John Wiley & Sons, New York.

[11] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotech 2005; 4:685-688.

[12] Wright PJ, Leathwood AO, Plummer DT. Enzymes in rat urine: Alkaline Phosphatase. Enzymologia 1972; 42:317-327.

[13] Reitman S, Frankel S. A colometric method for the determination of serum GOT and GPT. Am J Clin Pathol 1957; 28:56-63.

[14] Alexander RR, Griffiths JM. Haematocrit: Basic Biochemical Methods, 2nd edn 1993; John Willey and Sons Inc, New York.

[15] Krause WJ. The art of examining and interpreting histologic preparations: A Student Handbook 2001; Partheton Publishing Group, UK.

[16] Inya-Agha SI, Ezea SC, Odukoya OA. Evaluation of *Picralima nitida:* Hypoglycaemic activity, toxicity and analytical standards. Int J Pharmacol 2006; 2:576-580.

[17] Duke JA. Handbook of biologically active phytochemicals and their activities 1992; CRC Press, Boca Raton, Florida.

[18] Shibata Y, Matsui K, Kajiwara T, Hatanaka A. Purification and properties of fatty acid hydroperoxide lyase from green bell pepper fruits. Plant Cell Physiol 1995; 36:147-156.

[19] Amresh GR, Singh PN, Rao VC. Toxicological screening of traditional medicine Laghupatha (*Cissampelos pareira*) in experimental animals. J Ethnopharmacol 2008; 116:454–460.

[20] Ashafa AOT, Sunmonu TO, Afolayan AJ. Effect of leaf and berry extracts of *Phytolacca dioica* L. on haematological and weight parameters of Wistar rats. Afr J Pharm Pharmacol 2011; 5:150–154.

[21] Sunmonu TO, Oloyede OB. Biochemical assessment of the effects of crude oil contaminated catfish (*Clarias gariepinus*) on the hepatocytes and performance of rat. Afr J Biochem Res 2007; 1:83 – 89.

[22] Akanji MA, Olagoke OA, Oloyede OB. Effect of chronic consumption of metabisulphite on the integrity of rat liver cellular system. Toxicol 1993; 81**:**173-179.

[23] Rahman MF, Siddiqui MK, Jamil K. Effects of vepacide (*Azadirachta indica*) on aspartate and alanine aminotransferase profiles in sub-chronic study with rats. J Hum Exp Toxicol 2001; 20:243-249.

[24] Wolf PL, Williams D, Tsudaka T, Acosta L (1972) Methods and Techniques in Clinical Chemistry 1972; John Wiley & Sons Inc, USA.

[25] Karmen A, Wroblewski F, La Due JS. Transaminase activity in human blood. J Clin Invest 1995; 34:126-130.

[26] Yakubu MT, Bilbis LS, Lawal M, Akanji MA. Evaluation of selected parameters of rat liver and kidney function following repeated administration of Yohimbine. Biochem 2003; 15:50-56.

[27] Ashafa AOT, Sunmonu TO, Afolayan AJ. Toxicological evaluation of aqueous leaf and berry extracts of *Phytolacca dioica* L. in male Wistar rats. Food Chem Toxicol 2010; 48:1886-1889.

[28] Rannels DE, Mckee EE, Morgan HE. Action of insulin. *In* (Ed. Litwack G) Biochemical Actions of Hormones 1977; pp 135-195, Academic Press, New York.

[29] Naganna B. Plasma proteins. *In* (Eds. Tawlar GP, Srivastava LM, Moudgils KD) Textbook of Biochemistry and Human Biology 1989; pp 172-181, Prentice-Hall, India.

[30] Panda NC. Kidney. *In* (Eds. Talwar GP, Srivastava LM, Moudgil KD) Textbook of Biochemistry and Human Biology, 2nd edn 1989; pp 276-292, Prentice-Hall, India.

[31] Adeneye AA. Haematopoetic effect of methanol seed extract of C*itrus paradisi* Macfad (grape fruit) in Wistar rats. Biomed Res 2008; 19:23-26.

[32] Ajagbonna OP, Onifade KI, Suleiman U. Haematological and Biochemical changes in rats given extract of *Calotropis procera*. Sokoto J Vet Sci 1999; 1:36-42.

[33] Ashafa AOT, Yakubu MT, Grierson DS, Afolayan AJ. Effects of aqueous extract from the leaves of *Chrysocoma ciliata* L. on some biochemical parameters of Wistar rats. Afr J Biotech 2009; 8**:**1425-1430.

[34] Sunmonu TO, Oloyede OB. Performance and haematological indices in rats exposed to monocrotophos contamination. Hum Exp Toxicol 2010; 29:845–850.

[35] Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Deun KV, Smith P, Berger B, Heller A. Concordance of toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 2000; 32:56-67.

[36] Adebayo JO, Adesokan AA, Olatunji LA, Buoro DO, Soladoye AO. Effect of ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. Biokem 2005; 17:45-50.

[37] Suchantabud A, Talubmook C, Chomko S, Narkkong N. Some hematological values and ultrastructure of blood cells in *Piper sarmentosum* Roxb. and *Tinospora crispa* Miers ex Hook. F & Thoms. treated diabetic rats. J Microsc Soc Thailand 2008; 22:65-70.

[38] Adesokan AA, Akanji MA. Effect of repeated administration of aqueous stem extract of *Enantia chlorantha* on the integrity of rat liver cellular system. Nig J Biochem Mol Biol 2007; 22**:**19-22.