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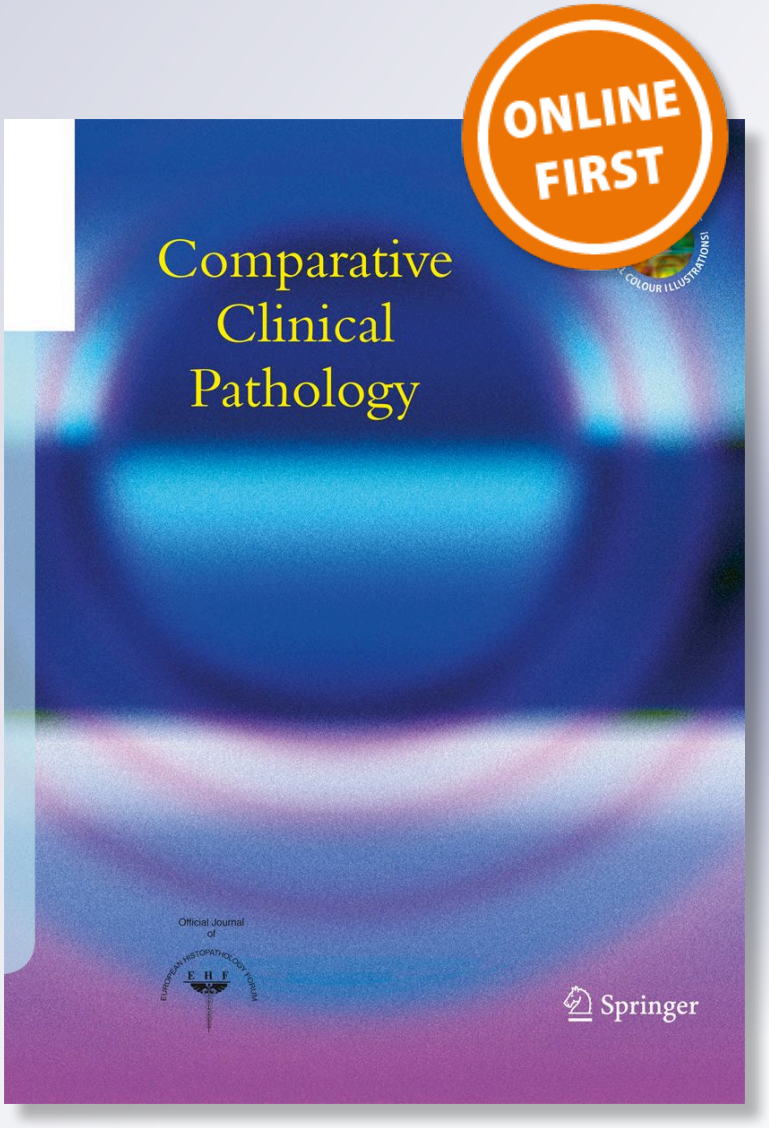

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ORIGINAL ARTICLE

Toxicity of aqueous root extract of *Cochlospermum planchonii* (an anti-malarial herb) in selected tissues of mice

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Abstract Aqueous root extract of *Cochlospermum planchonii*, an acclaimed anti-malarial herb was investigated for toxicity in selected tissues of mice at the doses of 50, 100, and 250 mg/kg body weight. Twenty Swiss albino mice were completely randomized into four groups: A–D consisting of five animals each. Animals in group A (control) were orally administered with 0.5 ml of distilled water on daily basis for 7 days while those in groups B, C, and D were treated like the control except that they received the same volume of the extract containing 50, 100, and 250 mg/kg body weight. The extract significantly ($p<0.05$) reduced the activities of alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH) in the liver, ALP and ACP of the kidney, LDH in the small intestine, plasma γ-glutamyl transferase (γ-GT), urea, creatinine, and albumin content of the animals while there was no significant effect on the liver γ-GT activity of the mice. The reduction in the LDH manifested only in the kidney at 100 and 250 mg/kg body weight of the extract. There was no significant change on the plasma bilirubin content, activities of ACP of small intestine, LDH, ALP, and ACP of the plasma. The extract also produced inflammations along the portal tract in the liver, extensive inflammations and focal necrosis of the tubular cells with infiltration of the interstitial cells in the kidney, and total destruction of the villi accompanied with complete loss of mucosal glands in the small intestine. Overall, the results from the present study suggest functional and structural dysfunction of the organs. Therefore, the aqueous root extract of *C. planchonii* when consumed on daily basis for 7 days at 50–250 mg/kg body weight (3,500–17,500 mg; doses adjusted to a 70 kg man) is not safe as an oral remedy.

Keywords *Cochlospermum planchonii* · Cochlospermeacea · Functional dysfunction · Structural dysfunction · Toxicity

Introduction

In recent times, the use of herbs and its by-products in the management of diseases is increasing worldwide. According to Phillipson and Wright (1991), approximately 80 % of the world populations still depend on traditional medicine for the management of diseases. With the steady rise in the use of herbs, the number of deaths and other toxic reactions has also increased (Sekar et al. 2007). Therefore, plants with acclaimed medicinal properties should be screened extensively for safety or otherwise. One of such plants with acclaimed anti-malarial property in the folklore medicine of Nigeria is *Cochlospermum planchonii.*

C. planchonii Hook Fx. planch (family Cochlospermacea), commonly called *Gbehutu* or *Feru* (Yoruba, Nigeria), *Yasubiyar*, *Bálágándéé* (Hausa, Nigeria), and *Avongongon* (Jukun, Nigeria), is a West African species which grow from Guinean region to Cameroon. It is a shrub of 2–4 m tall, with reddish yellow scabrid tri-lobed leaves and small brownish stem (Blench 2007). The flowers have five yellow petals which produced globose fruits. *C. planchonii* has been acclaimed to be used in the management of malaria, hepatitis, diabetes, infertility, trypanosomiasis, stomach disorder, typhoid fever, urinary tract infections, schistosomiasis, jaundice, fever, back pains, intestinal worms, and bilharziasis (Kone et al. 2002; Anthony et al. 2005; Pousset 2006; Togola et al. 2008; Blench 2007).

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Various parts of the plant have been substantiated with scientific evidence to exhibit diverse activities such as fungicidal, anti-plasmodial, trypanocidal, analgesic, anti-inflammatory, and anti-hyperglycemic (Atawodi 2005; Benoit-Vical et al. 2003; Nduagu et al. 2008; Anaga and Oparah 2009; Yakubu et al. 2010). Aqueous root extract of the plant have been reported to contain alkaloids, saponins, tannins, phenolics, steroids, anthraquinones, sodium (3.08×10^4 mg/kg), potassium (8.25×10^4 mg/kg), iron (91.4 mg/kg), calcium (26.80 mg/kg), magnesium (32.80 mg/kg), and minute amount of lead (1.00 mg/kg) and cadmium (0.20 mg/kg) (Nafiu et al. 2011a). Nafiu et al. (2011b) have also reported on the adverse effects of the aqueous root extract of *C. planchonii* at the dose of 50 mg/kg body weight in Wistar rats.

Although previous study by Nafiu et al. (2011b) reported on the adverse effect of aqueous root extract of *C. planchonii* at the dose of 50 mg/kg body weight, administered on daily basis for 15 on the some biochemical indices of liver and kidney damage of Wistar rats, there has not been any other report in the open scientific literature on the safety evaluation of the extract at the doses of 50, 100, and 250 mg/kg body weight in mice.

Therefore, the present study was aimed at assessing the toxicity of aqueous root extract of *C. planchonii* by using some biochemical parameters of organ function as well as the histological changes in the selected organs of mice. The animal model was chosen because of its susceptibility to *Plasmodium* species, hence the use of mice to evaluate the toxicity of this anti-malarial herb. The liver, kidney, and small intestine were selected because of their respective roles in the detoxification, elimination, and absorption of the extract of the plant when administered to the animals.

Materials and methods

Plant material and authentication

The plant samples which were obtained from traditional herb sellers at one of the markets (Oja-Oba) in Ilorin, Nigeria were authenticated at the Forestry Research Institute of Nigeria Ibadan, Nigeria. A voucher specimen (FHI 99093) was deposited at the herbarium of the institute.

Experimental animal

Swiss albino mice with age range of 8–11 weeks and average weight of 23.90±2.00 g were obtained from the Small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were kept in plastic cages with free access to rat pellets (Bendel Feeds and Flour Mills Ltd, Ewu, Nigeria) and contaminant-free tap water. The cages were placed in well-ventilated house conditions: (temperature 23±1 °C; photoperiod 12-h natural light and 12-h dark; humidity 45–50 %).

Assay kits and reagents

Assay kits for lactate dehydrogenase (LDH) and gamma glutamyl transferase (γ-GT) were products of Randox Laboratories Ltd., Ardmore, Co. Antrim, UK. Para-nitrophenylorthophosphate was a product of Sigma Chemical Company, St. Louis, USA. Other reagents used which were of analytical grades were prepared in distilled water and kept in air-tight reagent bottles.

Methods

Animal grouping

A total of 20 albino mice were grouped into four (A–D) consisting of five animals each. The animals in group A (control) were given 0.5 ml of distilled water (the vehicle), while those in groups B–D were administered with same volume of the extract corresponding to 50, 100, and 250 mg/kg body weight. Both the distilled water and extract were administered orally, once daily using oropharyngeal cannula for a period of 7 days. The animals were allowed to acclimatize for 2 weeks before the commencement of the experiment. The animals were handled humanely in accordance with the guidelines of European convention for the protection

Table 1 Liver enzyme activities of mice administered with aqueous root extract of *C. planchoni*

Enzymes[a]	Control	Extract (mg/kg body weight)		
		50	100	250
LDH	16.68±1.36c	6.66±0.62b	4.76±0.33ab	3.62±0.47a
ALP	1.49±0.40b	0.78±0.11a	0.84±0.04ab	0.49±0.27c
ACP	7.51±0.84b	2.57±0.51c	1.61±0.33a	1.02±0.12a
γ-GT	2.31±0.48a	2.55±0.24a	1.99±0.38a	1.71±0.03a

Values are mean of five determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different ($p<0.05$)

Table 2 Kidney enzyme activities of mice administered with aqueous root extract of *C. planchonii*

Enzymes[a]	Control	Extract (mg/kg body weight)		
		50	100	250
LDH	43.31±8.95b	46.78±3.51b	14.32±1.67a	5.99±0.58c
ALP	365.84±25.88c	243.85±48.79b	83.74±4.78d	151.06±18.13a
ACP	26.32±6.40b	7.93±0.92a	4.15±0.40c	19.10±1.00b

Values are mean of five determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different ($p<0.05$)

[a] Enzyme activities were measured in micromoles/minute/milligram protein

of vertebrate animals and other scientific purposes ETS-123 (European Treaty Series 2005).

Preparation of extract

The roots were washed in distilled water, air-dried at room temperature before being cut into thin pieces. The dried pieces were then pulverized. A portion (800 g) of the powder was extracted in 1.6 L of distilled water followed by thorough shaking for 24 h with magnetic stirrer. The mixture was then filtered and freeze-dried (Ilshin Freeze-Dryer, Model no. FD5518, Ilshan Laboratory Company Ltd., Seoul, Korea) to give a yield of 22.75 g which corresponds to a percentage yield of 2.84 % of the starting material. A calculated amount of the residue was weighed and reconstituted in distilled water to give the required doses of 50, 100, and 250 mg/kg body weight.

Preparation of plasma and tissue homogenates

All the animals in the various experimental groups were sacrificed 24 h after the completion of their daily doses (day 8). Under ether anesthesia, the neck of mice was quickly cleared of fur and skin to expose the jugular vein. These animals were made to bleed through their cut jugular vein. Blood was collected into clean, dry centrifuge tubes containing citrate phosphate dextrose adenine (anticoagulant) and was mixed thoroughly before being centrifuged at 224 × *g* for 15 min using a Uniscope Laboratory centrifuge (Model SM800B, Surgifriend Medicals, Essex, England). The plasma was thereafter aspirated into clean, dry, sample bottles using Pasteur pipettes and kept frozen before it was used for assay. The animals were thereafter quickly dissected and the liver, kidney, and small intestine, removed. The kidney was decapsulated, while the liver was cleaned of blood. Metabolic waste in the small intestine was also removed. The organs were homogenized in 0.25-M sucrose solution (1:5 *w/v*) as described by Akanji and Yakubu (2000). The homogenates were transferred into specimen bottles and kept frozen for 24 h before analyses.

Determination of biochemical indices

The biochemical parameters were determined using standard methods described for alkaline phosphatase (Wright et al. 1972a), acid phosphatase (Wright et al. 1972b), gamma glutamyl transferase (Szasz 1969), lactate dehydrogenase (Wroblewski and La Due 1955), urea (Fawcett and Scott 1960), creatinine (Bartels and Bohmer 1972), albumin (Grant and Kachmar 1987), and bilirubin (Jendrassik and Gof 1938). The organs were prepared for histopathological analysis according to the procedure described by Krause (2001) and stained with hematoxylin and eosin (H & E). The photomicrographs were captured at ×400 with software, Presto! Image Folio package.

Table 3 Small intestine enzyme activities of mice administered with aqueous root extract of *C. planchonii*

Enzymes[a]	Control	Extract (mg/kg body weight)		
		50	100	250
LDH	22.40±4.28c	2.26±0.45a	3.87±0.48ab	9.49±0.64b
ALP	107.45±8.42b	123.51±16.47b	124.45±3.14b	74.39±7.09a
ACP	3.65±0.49a	7.81±0.99b	4.35±0.21a	5.18±0.44a

Values are mean of five determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different ($p<0.05$)

[a] Enzyme activities were measured in micromoles/minute/milligram protein

Table 4 Plasma enzyme activities of mice administered with aqueous root extract of *C. planchonii*

Enzymes[a]	Control	Extract (mg/kg body weight)		
		50	100	250
LDH	54.88±0.11b	8.08±2.08a	80.37±0.32c	4.10±0.13a
ALP	11.53±0.45a	21.67±13.79a	110.54±1.88b	7.31±0.27e
ACP	12.27±0.51a	8.56±4.34a	56.41±0.61b	7.06±0.23c
γ-GT	9.71±0.25a	1.51±0.65b	3.45±0.12c	0.29±0.11d

Values are mean of five determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different ($p<0.05$)

[a] Enzyme activities were measured in micromoles/minute/milligram protein

Statistical analysis

Data were presented as mean of five determinations ± SEM. Statistical analysis were carried out using one way analysis of variance. Differences were considered statistically significant at $p<0.05$.

Results

The extract at the doses of 50, 100, and 250 mg/kg body weight significantly ($p<0.05$) reduced the activities of ALP, ACP, and LDH in the liver of the animals, while there was no significant effect on the liver γ-GT activity of the mice (Table 1). Furthermore, while there were decreases in the activities of ALP and ACP in the kidney of mice at all the doses investigated, the reduction in the LDH manifested only in the kidney of mice treated with 100 and 250 mg/kg body weight of the extract. The LDH activity in the kidney of mice administered with 50 mg/kg body weight of the extract was not significantly different from that of the control (Table 2).

While all the doses of the extract reduced the activity of LDH in the small intestine of mice, the pattern of effect on the organ's ALP activity was not definite (Table 3). Furthermore, the 50 mg/kg body weight of the extract increased the activity of ACP in the small intestine, whereas the higher doses of 100 and 250 mg/kg body weight produced enzyme activity that compared favorably with the control (Table 3).

Except for the plasma γ-GT activity that was reduced by all the doses of the extract, the activities of LDH, ALP, and ACP fluctuated at all the doses investigated (Table 4).

All the doses of the extract significantly reduced the plasma concentrations of urea, creatinine, and albumin in a dose-dependent manner, whereas the effect of the extract on bilirubin fluctuated between an increase at 100 mg/kg body weight dose and values that compared favorably with that of the control at both the doses of 50 and 250 mg/kg body weight (Table 5).

The extract produced histopathological changes ranging from inflammations along the portal tract in the liver to extensive inflammations and focal necrosis of the tubular cells with infiltration of the interstitial cells in the kidney (Figs. 1a–d and 2a–d). There was total destruction of the villi accompanied with complete loss of mucosal glands and subsequent replacement with inflammatory cells in the small intestine of mice administered with both the 100 and 250 mg/kg body weight. The 50 mg/kg body weight of the extract produced only inflammations at the base of the villus (Fig. 3a–d).

Table 5 Some liver and kidney function parameters of mice administered with aqueous root extract of *C. planchonii*

Parameters	Control	Extract (mg/kg body weight)		
		50	100	250
Urea (mmol/l)	6.54±0.42a	4.80±0.71ab	4.28±0.93bc	2.40±0.46c
Creatinine (mmol/l)	101.40±4.78a	74.60±6.83b	55.80±0.73c	43.40±4.48c
Albumin (g/l)	34.20±1.36a	19.00±2.95b	19.80±1.71b	14.00±0.89b
Bilirubin (mmol/l)	10.40±0.24a	11.80±1.59a	16.00±0.71b	12.60±1.12a

Values are mean of five determinations ± SEM. Test values carrying lowercase letters different from that of the control are significantly different ($p<0.05$)

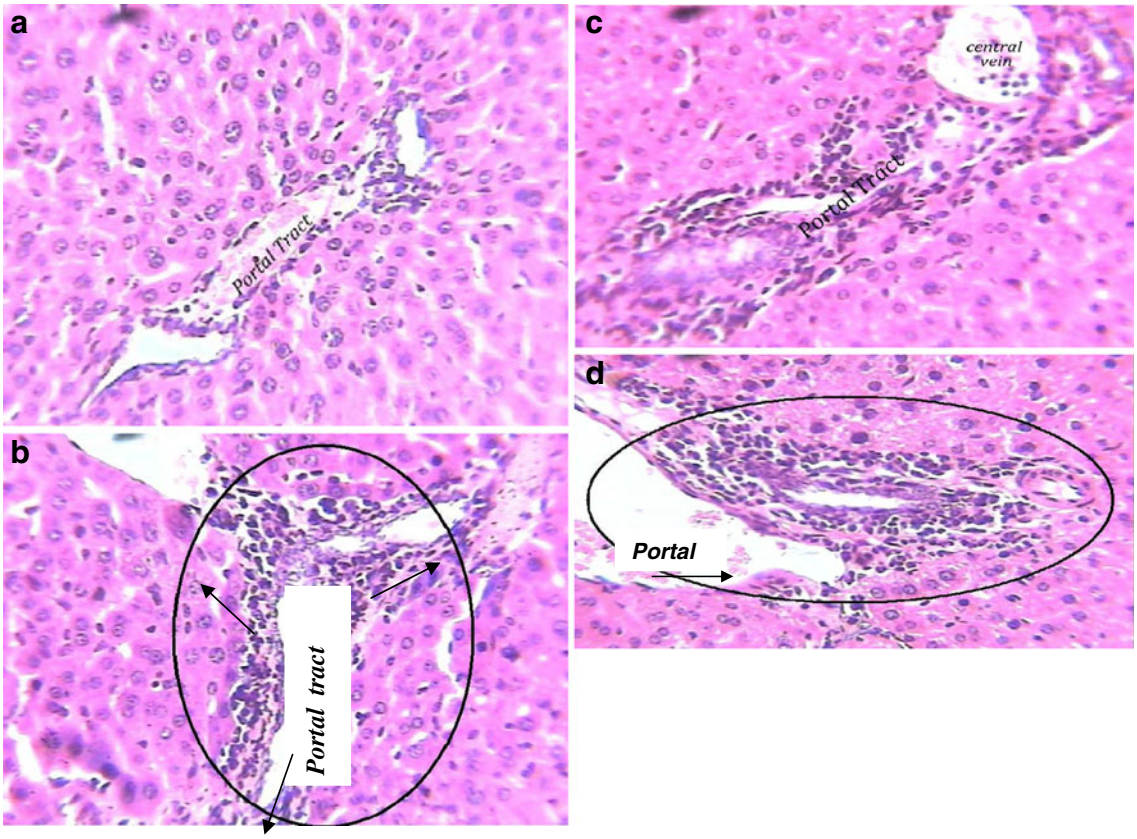

Fig. 1 **a** Photomicrograph of the liver of mouse administered with distilled water for 7 days. The liver is normal with no pathological changes. **b** Photomicrograph of the liver of mouse administered with 50 mg/kg body weight of aqueous root extract of *C. planchonii* for 7 days. There are inflammations along the *portal tract*. **c** Photomicrograph of the liver of mouse administered with 100 mg/kg body weight of aqueous root extract of *C. planchonii* for 7 days. There are inflammations along the *portal tract* and *central vein* with lobular inflammation. There is an extension of the hepatic lobe, and hepatocyte showed degenerative changes. **d** Photomicrograph of the liver of mouse administered with 250 mg/kg body weight aqueous root extract of *C. planchonii* for 7 days. The effect is similar to the animals treated with 100 mg/kg (Fig. 3)

Discussion

Toxicity studies in animals are commonly used to assess potential health risk in humans, caused by intrinsic adverse effects of chemical compounds or plant extracts (Klassen and Eaton 1991; Afolayan and Yakubu 2009). These adverse effects may manifest significant changes in the levels of biomolecules such as enzymes and metabolic, synthetic, and secretory products of some organs like the liver, kidney, testes, etc. (Yakubu et al. 2008; Afolayan and Yakubu 2009). When tissue damage occurs, these biomolecules leak into the extracellular fluid and such may be corroborated by histological changes which, in most cases, are often delayed before manifesting.

ALP, γ-GT, and ACP are ectoenzymes of the plasma membrane and lysosomal membranes, respectively, where they are used to assess the integrity of cell membranes (Akanji et al. 1993). LDH on the other hand is a "marker" of cytosolic disturbance. Therefore, the reductions in the activities of ALP and ACP in the liver and kidney of mice in the present study could be attributed to any of loss of membrane component including the enzymes itself, into the extracellular fluid (Malbica and Hart 1971), inhibition of enzyme activity by the chemical compound(s) in the extract, or inactivation of the activity of the enzyme in situ (Umezawa and Hooper 1982). Against all expectation, such decrease in the tissue phosphatases was not reflected in the plasma as the activity of the enzyme fluctuated throughout the period of exposure. Therefore, the reduction in the phosphatases may not be due to damage to the plasma membrane. Furthermore, it is also possible that some organs that were not investigated in the present study might have contributed to the pattern of enzyme activity recorded in the plasma. Again, the loss of LDH in the liver suggests disturbance in the cytosolic content of the hepatocytes. Such disturbance only manifested at higher doses of the extract in the nephrons. All these will consequentially affect the normal functioning of the enzymes. The findings in the present study with respect to the liver ALP are consistent with that

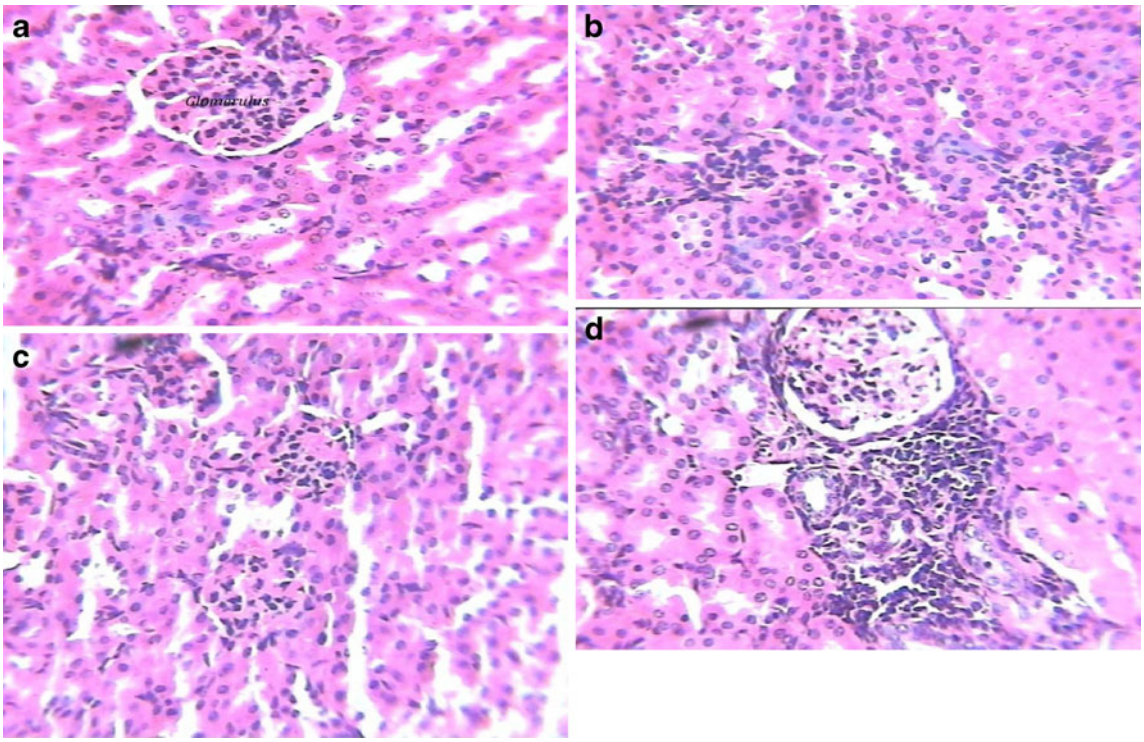

Fig. 2 **a** Photomicrograph of the kidney of mouse administered with distilled water for 7 days. The kidney is normal with no glomerular and tubular injury. **b** Photomicrograph of the kidney of mouse administered with 50 mg/kg body weight aqueous root extract of *C. planchonii* for 7 days. There are extensive inflammations and focal necrosis of the tubular epithelia cells with infiltration of the interstitial cells. **c** Photomicrograph of the kidney of mouse administered with 100 mg/kg body weight of aqueous root extract of *C. planchonii* for 7 days. There are inflammations and focal necrosis of the tubular epithelia cells more pronounced in the mouse administered 50 mg/kg of the extract with infiltration of the interstitial cells. **d** Photomicrograph of the kidney of mouse administered with 250 mg/kg aqueous root extract of *C. planchonii* for 7 days. There are extensive inflammations and focal necrosis of the tubular epithelia cells much more pronounced in the animals administered 50 and 100 mg/kg of the extract with infiltration of the interstitial cells. Some tubules have been destroyed. H & E, ×400

reported earlier by Nafiu et al. (2011b) following the administration of 50 mg/kg body weight of the aqueous extract of *C. planchonii* rhizome to rats. Again, the ACP activity in the liver, ALP and ACP activities in the kidney as well as the plasma phosphatase activities in the present study contrast the previous report by Nafiu et al. (2011b). The non-definite pattern of effect on the enzymes of the small intestine in the present study may suggest recovery attempt by the animals to counteract the effect of the extract. The absence of alteration in the liver γ-GT activity suggests selective effect on the enzymes of the liver by the extract since the plasma enzymes were also reduced.

Albumin and globulin are useful indicators of synthetic function of the liver, whereas bilirubin could be used to assess excretory function of the organ (Kaplan et al. 1988; Yakubu et al. 2003). The drop in the plasma albumin concentration of the groups treated with the extract compared with that of the control may not be unconnected with toxic effect of the extract on the liver, and this may consequently impair its excretory functions. Similarly, the reduction in both the urea and creatinine could be suggestive of either down-regulation in the synthesis of urea and creatinine or their enhanced excretion through urine, perhaps in response to possible extract-induced toxic assault on the kidney and consequent impairment of its functional capacity (Egbuonu et al. 2010). Such reductions in both the urea and creatinine levels in the plasma of the animals may have consequential effects on the synthetic functions of the liver with respect to these biomolecules. Furthermore, the decreased serum creatinine concentration as obtained in the present study may be predictive of glomerular hyper-filtration associated with increased metabolic risk (Tomaszewski et al. 2007). This is corroborated by the findings on the morphology changes in the nephrons of the kidney following the administration of the plant extract. The pattern of albumin in the present study agrees with that previously reported by Nafiu et al. (2011b), whereas that of bilirubin contrasts each other.

It has been reported that cells died as a result of necrosis or apoptosis when they are challenged with toxins, noxious agents, or injuries (Eroschencho 2000). Therefore, the various pathological changes such as extension of hepatic lobes, degeneration of hepatocytes, lobular inflammation by inflammatory cells, and periportal inflammation of the liver, focal necrosis, extensive inflammation, and infiltration

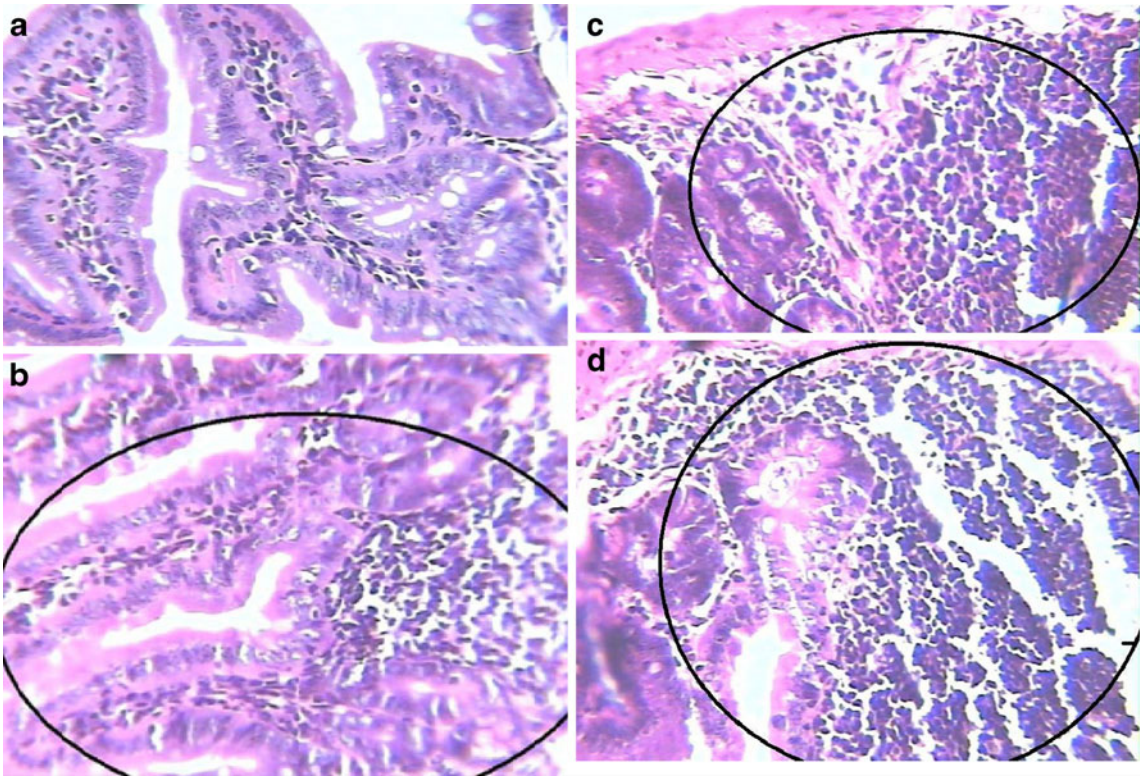

Fig. 3 **a** Photomicrograph of the small intestine of mouse administered with distilled water for 7 days. The small intestine is normal with no degenerative cells and necrosis. The lining of the epithelium and villi are normal. **b** Photomicrograph of the small intestine of mouse administered with 50 mg/kg body weight aqueous root extract of *C. planchonii* for 7 days. There are inflammations at the base of the villi. **c** Photomicrograph of small intestine of mouse administered with 100 mg/kg body weight aqueous root extract of *C. planchonii* for 7 days. There was a total destruction of the villi accompanied with complete loss of mucosal gland and replacement with inflammatory cells. **d** Photomicrograph of small intestine of mouse administered with 250 mg/kg body weight of aqueous root extract of *C. planchonii* for 7 days. There was a total destruction of the villi, complete loss of mucosal gland, and replacement with inflammatory cells. The effect is more pronounced than in the animal given 100 mg/kg body weight of *C. planchonii*. H & E, ×400

of the interstitial cells of the kidney and total loss of the villi, complete loss of mucosal gland, and replacement with inflammatory cells corroborate toxicity of the extract and may adversely affect the normal structural functioning of the organs.

A previous report had shown that the phytoconstituents of the plant is diverse (Nafiu et al. 2011a). Some of these compounds and mineral elements could be responsible for the toxicity profile of the root extract in the present study. For example, saponins have been implicated in a wide range of effects such as disrupting the biological membranes, generating free radicals that cause lipid peroxidation (Francis et al. 2002). Saponins make the lipid bilayer permeable to macromolecules by inducing pore-like structures which subsequently increase membrane fluidity leading to saponin-induced toxicity (Baumann et al. 2000; Francis et al. 2002). Consequently, changes in the levels of some biochemical parameters on both sides of the membrane (electrolytes, enzyme, substrates, and enzymes) may emerge as signs of saponin-induced biological effects (Nandi et al. 2004). Furthermore, tannins have been linked to cause bowel irritation, kidney irritation, liver damage, irritation of the stomach, and gastrointestinal pain (Elvin-Lewis and Lewis 1977). In addition, a variety of metals, such as cadmium and lead, have long been known to be associated with kidney disease, often causing proximal tubular dysfunction and glomerular damage (Delaney et al. 2008). Therefore, the observed alterations in the biochemical parameter of organ damage/dysfunction in the present study may not be unconnected with the presence of these chemical compounds. However, the specific phytochemical that was responsible for the observed effects awaits further investigation.

Overall, the alterations in the biochemical parameters and the histopathological changes in the organs investigated in the present study following the administration of aqueous root extract of *C. planchonii* at the doses of 50, 100, and 250 mg/kg body weight suggests that the extract has caused both functional and structural toxicities in the mice. Therefore, the use of the aqueous root extract of *C. planchonii* in the management of disease conditions in folklore medicine at these doses should be done with caution.

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