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Effect of *Cnidoscolus Aconitifolius* on Some Biochemical Parameters in Wistar Rats Orally Exposed to Bonny Light Crude Oil

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Abstract

Purpose: Crude oil is used in folklore medicine to treat various forms of diseases and in most cases in combination with medicinal plants. Studies have reported that *Cnidoscolus aconitifolius* possess medicinal properties. The present study investigated the effect of different concentration of Bonny light crude oil and *Cnidoscolus aconitifolius* on some biochemical parameters of Wistar rats. The rats were orally exposed to different concentrations of Bonny light crude oil and *Cnidoscolus aconitifolius* leaf extract individually and in combination.

Methodology: After an exposure period of 21days, the results revealed that Bonny light crude oil and the leaf extract at concentrations of 250 and 500mgkg⁻¹b.wt can induce damage to the liver and kidney within 21days of consecutive administration. This is shown in results of histological analysis gotten from the liver and kidney tissues.

Findings: This study revealed that the induction of the leaf extract of *Cnidoscolus aconitifolius* and Bonny light crude oil at concentrations of 250 and 500mgkg⁻¹b.wt for 21days may affect the liver and kidney.

Contributions to theory, policy and practice:Therefore, it is recommended that the use of this leaf extract and Bonny light crude oil at concentrations similar to the ones used in this study should be discontinued.

Keywords: *Crude oil, Wistar rats, hematological, folklore medicine, Cnidoscolus aconitifolius*

Introduction

Crude oil exploration leads to the pollution of our environment including Streams, Rivers and this constitutes a potential hazard to both aquatic and terrestrial species (Simon and Jean 2019). The presence of crude oil through oil spillages causes a large impact on the riverine ecosystem and it is a likely determinant factor of poor water quality in oil producing communities (Ayman 2014). When crude oil enters the environment through spillage or other anthropogenic sources, it undergoes rapid changes due to chemical reactions, photo-induced reactions, biochemical transformation and microbial degradation. Products from these reactions are sometimes more toxic than they original crude oil-based sources. Also, metabolites of Benzene found in crude oil are transformed by the cytochrome P450 linked polyunsaturated monooxygenase system, found in animals, to highly reactive and carcinogenic compounds (Barton 2014). Thus, both humans and animals in such environment who are regularly exposed to sub lethal doses of these xenobiotics may become vulnerable to carcinogenesis and other toxicological effects. Several studies have documented the application of plants and their products in managing crude oil-induced toxicity and they include *Andrographis paniculate*, *Chromolaona odorata*, and *Cnidocolus aconitifolius*. *C. aconitifolius*, which is also known as Chaya, tree spinach or spinach tree is a leafy perennial shrub that is believed to have originated in the Yucatan Peninsula of Mexico (Grubben and Denton 2004). This plant is commonly referred to as “Efo Iyana Ipaja” or “Efo Jerusalem” in south-West Nigeria and “Hospital too far” in Niger-Delta areas of Nigeria. In folklore medicine, *C. aconitifolius* leaves has been used for the treatment of malaria, intestinal worm, Jaundice etc. Other studies have reported that the plant is associated with health benefits such as its role in maintaining blood sugar level, anti-inflammatory, anti-anemic, anti-microbial, antioxidant effect (Pillai *et al.*, 2012), (Penghal *et al.*, 2018), (Babalola and Opeyemi 2015), (Amil *et al.*, 2021). While studies on the adverse effects of crude oil exposed to rats and the search for protective agents against its toxicity are still ongoing, no research has been carried out to investigate the ability of *C. aconitifolius* to mitigate the adverse effects of crude oil on exposed animals. This study helps in determining the possible effects of *C. aconitifolius* against crude oil-induced toxicity in experimental Wistar rats.

Materials and Methods

Study area: This research was carried out in the animal house of the department of Pharmacology and the research laboratory of the department of Biochemistry, University of Port Harcourt. In this study, twenty-eight (28) Wistar rats were obtained from the animal house of the department of Pharmacology, in University of Port Harcourt, Nigeria. The animals were allowed to acclimatize for one week under laboratory conditions at the animal house in the department of Biochemistry and they were given rat chow and water *ad libitum*.
Chemicals and Reagents: All chemicals and reagents used throughout the study were of analytical grade. Laboratory analysis were carried out in the research laboratory of the department of Biochemistry, University of Port Harcourt.

Crude oil sample: The test sample of Bonny light crude oil was obtained from the Port Harcourt Refining company (PHRC) limited, Alesa Eleme, Port Harcourt, Rivers State, Nigeria.

Plant collection and extraction: leaves of *C. aconitifolius* were collected from Abia State in Nigeria. The leaves were washed thoroughly with clean running water. *C. aconitifolius* were authenticated by the department of plant science and Biotechnology, University of Port Harcourt and assigned the herbarium number of UPH/P/264. Preparation of the extract was done using water and ethanol as solvent extractors. The leaves were air dried in the open air at room temperatures for 3 days and further dried in an oven at 45⁰c for 48hrs to obtained a constant weight. The dried leaves were pulverized to affine powder using an electric blender (Achuba and Offor 2020). After blending, the ethanol extraction was carried out using the method described by Achuba with slight modification. Here 131g of the powdered *C. aconitifolius* was soaked in 1182mL of 80%(V/V) ethanol and allowed to stand for 24hrs. The extraction mixture was filtered with cheesecloth and the filtrate was concentrated using a rotary evaporator at 45⁰c. It was further dried using a water bath. From the crude extracts, 1 and 2g of it were dissolved separately in 4ml of distilled water to bring the concentrations to 250 and 500 mgml⁻¹ respectively.

Experimental design: Animals weighing between 100 and 150g were divided into seven groups of four male rats each. Group1, the negative control, received neither the Bonny light crude oil (BCLO) nor the leaf extract. Group 2 and 3 received 250 and 500mgkg⁻¹ b.wt of BLCO, respectively while groups 4 and 5 received 250and 500mgkg⁻¹b.wt of *C. aconitifolius* leaf extract. These doses were based on what was used by local population in folklore medicine and administered daily (Singha *et al.*, 2007). At the end of the 21days exposure period, the animals were weighed and sacrificed under chloroform anesthesia. The liver and kidney were excised weighed and fixed in Bouin`s fluid for at least 48hrs. They were processed in an automatic processor and embedded in paraffin wax. Section 5µm thick were examined and photographed using Lietz light microscope.

Blood samples obtained from the Jugular vein and placed in EDTA container were used for Hematological and heavy metal analysis. Hematological parameters (PCV, Hb, WBC, RBC, Platelet, and N, L, E and M) were carried out using the automated method with the automatic analyzer, " Hematology auto- analyzer Sysmex KV- 2IN". Blood samples gotten from the jugular veins placed in a plane bottle were used for the measurement of biochemical parameters. The kidney parameters (Urea, Creatinine, Ca, Na and K) and Liver parameters (AST, ALT, Albumin, total protein, total and direct bilirubin) were determined using the spectrum kit manufactured by the Egyptian company for Biotechnology, Cairo, Egypt.

Statistical analysis: The values were reported as mean ±SEM. The least significance difference was used to test for differences between individual treatment groups and the difference in the body weight of the rats over the treatment period using statistical package for social sciences (SPSS) version 22.0

Results

The results obtained as shown in Table 1 shows the hematological profile which includes Packed cell volume (PCV), Hemoglobin (HB), Red blood cell (RBC), White blood cell (WBC), Platelet, Neutrophils (N), lymphocytes (L), eosinophil (E) and monocytes (M) of the Wistar rats orally exposed to BLCO and CA leaf extract. The PCV of the control group (37.67 ±

0.33%) showed a significantly ($p < 0.05$) higher value when compared with 500mgkg⁻¹b.wt CA ($32.67 \pm 1.45\%$), 250mgkg⁻¹ b.wt each of BLCO + CA ($34.00 \pm 0.58\%$) and 500mgkg⁻¹ b.wt, BLCO+ CA($34.67 \pm 1.45\%$). On the other hand, the PCV of the 250mgkg⁻¹b.wt BLCO (39.00 ± 0.33) and 500mgkg⁻¹b.wt BLCO (38.67 ± 0.33) groups showed significantly ($p < 0.05$) higher value when compared to the 250mgkg⁻¹ b.wt of BLCO+CA (34.00 ± 0.58) and 500mgkg⁻¹ b.wt, BLCO+ CA(34.67 ± 1.45). Similarly, 250mgkg⁻¹b.wt CA was significantly ($p < 0.05$) higher when compared with 500mgkg⁻¹b.wt CA. However, no significant difference ($p < 0.05$) exist between 250mgkg⁻¹b.wt CA, 250mgkg⁻¹b.wt each of BLCO+ CA ($34.00 \pm 0.58\%$), and 500mgkg⁻¹b.wt BLCO + CA ($34.67 \pm 1.45\%$), and between 500mgkg⁻¹b.wt CA, 250mgkg⁻¹ b.wt each of BLCO+CA ($34.00 \pm 0.58\%$) and 500mgkg⁻¹ b.wt, BLCO+ CA($34.67 \pm 1.45\%$). The HB concentration of the control group ($12.50 \pm 0.12\%$) has significantly ($p < 0.05$) higher value when compared with 500mgkg⁻¹b.wt CA ($10.87 \pm 0.49\%$), 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA ($11.37 \pm 0.20\%$), 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA ($11.50 \pm 0.46\%$). Also, 250mgkg⁻¹b.wt BLCO (13.00 ± 0.17), and 500mgkg⁻¹b.wt BLCO HB (12.87 ± 0.09) groups showed significantly ($p < 0.05$) higher value when compared to the 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA and 500 mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA respectively. However the 250mgkg⁻¹ b.wt CA (11.87 ± 0.49) is significantly ($p < 0.05$) higher when compared with 500mgkg⁻¹b.wt CA (10.87 ± 0.49). There is no significant ($p < 0.05$) between 250mgkg⁻¹b.wt CA and 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA, 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA ($11.50 \pm 0.46\%$). Also, there was no significant ($p < 0.05$) difference between 500mgkg⁻¹b.wt CA ($10.87 \pm 0.49\%$) and 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA ($11.50 \pm 0.46\%$). The RBC had a control ($5.37 \pm 0.09\%$) value that was significantly ($p < 0.05$) higher when compared to 500mgkg⁻¹b.wt CA ($4.70 \pm 0.23\%$), and 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA ($4.60 \pm 0.06\%$). Also, 250mgkg⁻¹b.wt BLCO and 500mgkg⁻¹b.wt BLCO were significantly ($p < 0.05$) higher when compared to 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA and 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA. Additionally, 250mgkg⁻¹b.wt BLCO had a higher value when compared to 500mgkg⁻¹b.wt BLCO with no significant ($p < 0.05$) difference. Also, the 250mgkg⁻¹ b.wt, CA (5.27 ± 0.26) was significantly ($p < 0.05$) higher when compared to each of 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA and 500mgkg⁻¹ b.wt BLCO+ 500mgkg⁻¹b.wt CA (4.77 ± 0.26).

Liver: The liver parameters, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), albumin (ALB), total bilirubin (TB), direct bilirubin (DB), and total protein (TP) of rats orally exposed to Bonny light Crude oil (BLCO) and *Cnidioscolus aconitifolius* (CA) leaf extract are presented in Table 2. The alanine aminotransferase concentration of the control group ($23.19 \pm 2.13\%$) was significantly ($p < 0.05$) higher when compared to 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA ($15.45 \pm 2.66\%$). Also 500mgkg⁻¹b.wt BLCO (26.44 ± 1.78) had a significantly ($p < 0.05$) higher value when compared to 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA. However, 500mgkg⁻¹b.wt CA (21.11 ± 0.23) was significantly ($p < 0.05$) higher when compared to 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA. Nevertheless, no significant ($p < 0.05$) difference exists between 250mgkg⁻¹b.wt BLCO ($23.35 \pm 1.76\%$), 250mgkg⁻¹b.wt CA and 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA ($25.11 \pm 2.07\%$), 500mgkg⁻¹b.wt CA. The TB of the control group ($1.58 \pm 0.27\%$) was significantly ($p < 0.05$) lower when compared to

500mgkg⁻¹b.wt BLCO (2.04 ± 0.43 %), 250mgkg⁻¹b.wt CA (2.03 ± 0.07 %) and 500mgkg⁻¹b.wt CA (2.66 ± 0.38 %). Similarly, the direct bilirubin value of the control (1.42 ± 0.94 %) group was significantly (p<0.05) higher when compared to 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA (0.29 ± 0.09 %). Also, the 250mgkg⁻¹b.wt BLCO (0.98 ± 0.17) had a significantly (p<0.05) higher value when compared to 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA. Also, 250mgkg⁻¹b.wt CA (1.32 ± 0.12) was significantly (p<0.05) higher value when compared to 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA. However, no significant (p<0.05) difference exists between 500mgkg⁻¹b.wt BLCO (0.83 ± 0.18 %) and 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA (0.79 ± 0.41 %). Also, no significant (p<0.05) difference exists between 250mgkg⁻¹b.wt CA and 500mgkg⁻¹b.wt CA (1.61 ± 0.14 %). In addition the TP level of the control group (6.64 ± 0.25 %) was significantly (p<0.05) lower when compared to 500mgkg⁻¹b.wt BLCO (16.43 ± 1.49 %) and 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA (10.93 ± 2.59 %). The 500mgkg⁻¹b.wt BLCO (16.43 ± 1.49) was significantly (p<0.05) higher when compared to 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA. However, no significant (p<0.05) difference exists between 250mgkg⁻¹b.wt BLCO (10.36 ± 0.91 %), 250mgkg⁻¹b.wt CA (9.57 ± 1.80 %) and 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA (10.07 ± 0.97 %). Also, no significant (p<0.05) difference exists between 250mgkg⁻¹b.wt CA and 500mgkg⁻¹b.wt CA (9.71 ± 1.00 %).

Kidney: The kidney parameters, Urea (U), Creatinine (C), Sodium (Na), Potassium(K) and calcium (Ca) of the rats orally treated with BLCO and CA leaf extract are shown in Table 3. The 500mgkg⁻¹b.wt BLCO (0.32 ± 0.10) was significantly (p<0.05) higher for C when compared to 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA (0.10 ± 0.03). However, no significant (p<0.05) difference exists between 250mgkg⁻¹b.wt BLCO (0.21 ± 0.04 %), 250mgkg⁻¹b.wt CA (0.21 ± 0.08 %) and 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA (0.16 ± 0.05 %). Also, no significant (p<0.05) exists between 250mgkg⁻¹b.wt CA and 500mgkg⁻¹b.wt CA (0.13 ± 0.05 %). However, the Na concentration of 500mgkg⁻¹b.wt BLCO (206.25 ± 23.66) was significantly (p<0.05) lower when compared to 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA (245.83 ± 16.27). However, no significant (p<0.05) difference exists between 250mgkg⁻¹b.wt BLCO (122.92 ± 39.75 %), 250mgkg⁻¹b.wt CA (106.25 ± 3.61 %) and 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA (183.33 ± 23.48 %). Also, no significant difference exists between 250mgkg⁻¹b.wt CA and 500mgkg⁻¹b.wt CA (181.25 ± 7.22 %). For K, the control (2.38 ± 0.31 %) was significantly (p<0.05) higher when compared to 500mgkg⁻¹b.wt BLCO (1.30 ± 0.10 %), 250mgkg⁻¹b.wt CA (1.27 ± 0.17 %), 500mgkg⁻¹b.wt CA (1.61 ± 0.27 %). However, no significant difference exists between 250mgkg⁻¹b.wt each of BLCO (1.90 ± 0.10%), 500mgkg⁻¹b.wt BLCO and 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA (1.73 ± 0.16 %), 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA (1.78 ± 0.37 %). Also, no significant (p<0.05) difference exists between 250mgkg⁻¹b.wt CA and 500mgkg⁻¹b.wt CA. For Ca, the control (3.66 ± 0.19 %) was significantly (p<0.05) higher when compared to 250mgkg⁻¹b.wt CA (2.75 ± 0.22 %), 500mgkg⁻¹b.wt CA (2.25 ± 0.10 %), 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA (2.42 ± 0.34 %), 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA (2.22 ± 0.42 %). Also, 250mgkg⁻¹b.wt each of BLCO (4.26 ± 0.12) and the 500mgkg⁻¹b.wt BLCO (3.53 ± 0.14) were significantly (p<0.05) higher when compared to 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA and 500mgkg⁻¹b.wt

BLCO + 500mgkg⁻¹b.wt CA. There was no significant ($p < 0.05$) between 250mgkg⁻¹b.wt CA and 500mgkg⁻¹b.wt CA.

The results of the histological investigations of the rats exposed to BLCO and CA were presented in Fig. 1-14. Fig. 1 showed the photomicrograph of the liver from the control group. It revealed a histologically normal liver with intact hepatocytes and sinusoids containing Kupffer cells. Fig. 2 showed the photomicrograph of the liver of rats administered 250mgkg⁻¹b.wt BLCO. There was evidence of histologically normal liver showing intact hepatocytes and sinusoids containing Kupffer cells. Fig. 3 showed the photomicrograph of the group administered 500mgkg⁻¹b.wt BLCO. Also, they have histologically normal liver, sinusoids containing Kupffer cells but with congested central vein. In fig. 4 the photomicrograph of the liver from Wistar rats administered 250mgkg⁻¹b.wt CA showed histologically intact hepatocytes, sinusoids containing Kupffer cells and potent central vein. The photomicrograph of the liver from rats administered 500mgkg⁻¹b.wt CA was shown in Fig. 5. They showed normal liver with intact hepatocytes, congested central vein and sinusoids containing Kupffer cells. The photomicrograph of liver of Wistar rats administered 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA was shown in Fig. 6. They showed histologically normal liver with potent central vein, intact hepatocytes and sinusoids containing Kupffer cells. In Fig. 7, the photomicrograph of the group administered 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA was shown. They showed histologically normal liver with intact hepatocytes, potent central vein and sinusoids containing Kupffer cells. Fig. 8 shows the photomicrograph of the kidney of Wistar rats from the control group. They have histologically normal kidney with intact glomeruli containing glomerular mesangial cells and patent Bowman's capsular space. Fig. 9 showed the photomicrograph of the kidney from the group administered 250mgkg⁻¹b.wt BLCO. They have distorted kidney showing enlargement of glomerular tuft with decrease of Bowman's capsular space and intact renal tubules. In Fig. 10, shows the photomicrograph of the group administered 500mgkg⁻¹b.wt BLCO. They have histologically distorted kidney showing enlargement of glomerular tuft with marked occlusion of Bowman's capsular space and intact renal tubules. The photomicrograph of the kidney from the group administered 250mgkg⁻¹b.wt CA was shown in Fig. 11. They have histologically distorted kidney showing infiltration of inflammatory cell into the interstitial tissue and hypercellularity of glomerular tuft. Fig. 12 shows the photomicrograph of kidney from Wistar rats administered 500mgkg⁻¹b.wt CA. also, they have histologically distorted kidney with hypercellularity of glomerular tuft, infiltration of inflammatory cells, intact renal tubule and patent Bowman's capsule. Fig. 13 shows the photomicrograph of the group administered 250mgkg⁻¹b.wt BLCO + 250mgkg⁻¹b.wt CA. They have histologically normal kidney with intact glomerular tuft, patent Bowman's capsule and intact renal tubule. In Fig. 14, we have the photomicrograph of the kidney from the group administered 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA. They have histologically distorted kidney showing enlarged glomerular tuft with occluded Bowman's capsular space and intact renal tubules.

Discussion

The results obtained from this study shows the biochemical implications of Wistar rats exposed to bonny light crude oil and *C. aconitifolius*. The determination of hematological indices

provides physiological information on the general picture of the blood including the reticuloendothelial system. The results of this study shows that consumption of 250mgkg⁻¹b.wt BLCO and 500mgkg⁻¹b.wt BLCO led to marked increase in PCV, HB and RBC in the experimental rats when compared with its combination with the extract. This is contrary to observations from similar studies (Nafagha *et al.*, 2018), (Zheleu *et al.*, 2016). The difference maybe as a result of the concentrations administered and mode of exposure of the crude oil to the rats. The liver maintains homeostasis in living systems. It is involved in biochemical pathways necessary for growth and fights diseases. This study shows that there was an increase in ALT, TB and TP at 500mgkg⁻¹b.wt BLCO when compared with its combination with the extract. A similar observation was made by (Chibuike *et al.*, 2012) and (Adedara *et al.*, 2012). The concentration of TP and bilirubin indicates the state of the liver and the type of damage (Chukwudoruo *et al.*, 2021) and (Imafidon and Okunrobo 2012). The toxic metabolites at high concentration of BLCO maybe responsible for the significantly high value of bilirubin, TP and ALT. Zhang *et al.*, (2015) reported that an increase in bilirubin concentration maybe as a result of metabolic disturbance in the liver arising from a defective conjugation and/or excretion of bilirubin. Also the photomicrographs at 500mgkg⁻¹b.wt BLCO indicates that there was a congestion of the central vein. According to Hilscher and Sanchez (2016), the congestion of the central vein could lead to ischemia, atrophy of hepatocytes and distinction of sinusoids and this may lead to hepatomegaly. This agrees with the photomicrograph shown in fig. 3. The administration of both BLCO and extract reversed this condition as seen in both table 1 and fig. 7.

The kidney maintains a constant extracellular environment through its role in the excretion of metabolites like Urea, Creatinine and Uric acid. Also, it aids in the regulation of water and electrolyte balance. Abnormal levels of these metabolites and some electrolyte indicate renal impairment. These impairments could be due to exposure to nephrotoxic substances (Pazhayattil and Shirali 2014). The present study showed no abnormality in kidney markers analyzed. Nevertheless, histological findings showed that 250mgkg⁻¹b.wt BLCO (Fig. 9) and 500mgkg⁻¹b.wt (Fig. 10) showed histologically distorted kidney showing enlargement of glomerulus tuft with marked decrease of Bowman`s capsular space. Also, 250mgkg⁻¹b.wt CA (Fig. 11) and 500mgkg⁻¹b.wt CA (Fig. 12) revealed histologically distorted kidney showing infiltration of inflammatory cell into the interstitial tissue and hypercellularity of glomerulus tuft while 500mgkg⁻¹b.wt BLCO + 500mgkg⁻¹b.wt CA (Fig.14) treated group revealed histologically distorted kidney showing enlarged glomerulus tuft with occluded Bowman`s capsular space. This indicates that the doses of BLCO and CA leaf extract used for the study has the tendency of causing renal damage. Additionally, the observed trend in potassium and calcium concentrations in the group treated with 500mgkg⁻¹b.wt BLCO, 250mgkg⁻¹b.wt BLCO and 500mgkg⁻¹b.wt BLCO are similar to the observations on rats orally exposed to high concentrations of crude oil. These changes in electrolyte level is related to the development of hypertension (Ojo *et al.*, 2015).

Conclusion

The present study revealed that the leaf extract of *Cnidioscolus aconitifolius* and BLCO induction at concentrations of 250 and 500mgkg⁻¹b.wt consecutively for 21days maybe

hazardous to the liver and kidney. This is evident from the result of the histological analysis that was carried out on the tissue extracts. Therefore, it is recommended that the use of *Cnidocolus aconitifolius* in folklore medicine at the above concentrations and duration should be discontinued.

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Statement of competing interest: The authors have no competing interests.

Table 1: Haematological profile of Wistar rats orally exposed to Bonny light crude oil and *C.aconitifolius* leaf extract

GRO UPS	PVC (%)	HB (g/dl)	RBC ($\times 10^{12}$)	WBC ($\times 10^9$)	PLATE LET ($\times 10^9$)				M (%)
						N (%)	L (%)	E (%)	
Contr ol	37.67 \pm 0.33 ^{a,b}	12.50 \pm 0.12 ^{a,b}	5.37 \pm 0 .09 ^{a,b}	8.97 \pm 0.15 ^a	232.67 \pm 7.22 ^a	32.67 \pm 1.45 ^a	56.67 \pm 0.88 ^a	3.67 \pm 0.33 ^{a,b}	7.67 \pm 0.33 ^a
BLC O (250 mg/k g bw)	39.00 \pm 0.58 ^a	13.00 \pm 0.17 ^b	5.70 \pm 0 .23 ^a	9.60 \pm 1.10 ^a	251.00 \pm 2.31 ^{a,b}	31.00 \pm 0.58 ^{a,b}	58.67 \pm 0.88 ^{a,b}	3.00 \pm 0.00 ^a	7.67 \pm 1.45 ^a
BLC O (500 mg/k g bw)	38.67 \pm 0.33 ^a	12.87 \pm 0.09 ^b	5.70 \pm 0 .12 ^a	9.87 \pm 0.38 ^a	239.67 \pm 12.99 ^{a,c}	25.67 \pm 0.88 ^c	62.67 \pm 1.45 ^b	5.00 \pm 0.00 ^c	7.00 \pm 0.58 ^a
CA (250 mg/k g bw)	35.67 \pm 1.45 ^{b,c}	11.87 \pm 0.49 ^{a,c}	5.27 \pm 0 .26 ^{a,c}	10.07 \pm 0.66 ^a	237.67 \pm 6.06 ^{a,c}	28.00 \pm 1.15 ^{b,c}	62.67 \pm 1.45 ^b	3.00 \pm 0.58 ^a	6.67 \pm 0.88 ^a
CA (500 mg/k g bw)	32.67 \pm 1.45 ^{d,e}	10.87 \pm 0.49 ^{d,e}	4.70 \pm 0 .23 ^{c,d}	8.17 \pm 0.20 ^a	256.00 \pm 5.20 ^{b,c}	28.67 \pm 2.03 ^{a,c}	62.67 \pm 1.45 ^b	3.00 \pm 0.00 ^a	6.00 \pm 0.58 ^a
BLC O (250 mg/k g bw)	34.00 \pm 0.58 ^{c,e}	11.37 \pm 0.20 ^{c,e}	4.60 \pm 0 .06 ^{d,e}	12.87 \pm 0.66 ^b	270.67 \pm 12.41 ^{b,d}	32.67 \pm 1.45 ^a	56.67 \pm 2.03 ^a	4.00 \pm 0.58 ^{a,c}	7.00 \pm 0.00 ^a

+									
(CA									
250									
mg/k									
g bw)									
BLC									
O									
(500									
mg/k									
g bw)	34.67±	11.50±	4.77±0	12.77	281.00±	30.67±	57.67±	4.67±	7.67±
+ CA	1.45 ^{c,e}	0.46 ^c	.26 ^{b,c,e}	±1.30 ^b	2.31 ^d	1.45 ^{a,c}	1.45 ^a	0.33 ^{b,c}	0.33 ^a
(500									
mg/k									
g bw)									

Values are reported as mean ± SEM of triplicate determination. Values with different superscript alphabets are significantly different at $p < 0.05$. The Least Significant Difference (LSD) was used to test for the difference between individual treatments groups using Statistical Package for Social Sciences (SPSS), version 22.0. BLCO = Bonny light crude oil; CA = *Cnidioscolus aconitifolius*; mg/kg bw = milligram per kilogram body weight.

Table 2: Liver profile of Wistar rats orally exposed to Bonny light crude oil and *C. aconitifolius* leaf extract

Groups	Aspartate Aminotransferase (U/L)	Alanine Aminotransferase (U/L)	Albumin (g/dL)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Total Protein (g/dL)
Control	44.57±5.96 ^a	23.19±2.13 ^{a,b}	8.83±1.99 ^a	1.58±0.27 ^{a,b}	1.42±0.94 ^a	6.64±0.25 ^a
BLCO (250 mg/kg bw)	36.50±8.67 ^a	23.35±1.76 ^{a,b}	11.50±1.88 ^a	1.36±0.17 ^a	0.98±0.17 ^a	10.36±0.91 ^{a,b}
BLCO (500 mg/kg bw)	39.50±4.01 ^{a,b}	26.44±1.78 ^{a,b}	10.50±2.00 ^a	2.04±0.43 ^{a,b,c}	0.83±0.18 ^a	16.43±1.49 ^c

CA (250 mg/kg bw)	53.73±11.81 ^a	24.95±0.28 ^{a,b}	8.83±0.71 ^a	2.03±0.07 ^{a,c}	1.32±0.12 ^a	9.57±1.80 ^{a,b}
CA (500 mg/kg bw)	50.67±4.31 ^a	21.11±0.23 ^b	8.33±0.71 ^a	2.66±0.38 ^{b,c,d}	1.61±0.14 ^a	9.71±1.00 ^{a,b}
BLCO (250 mg/kg bw) + (CA 250 mg/kg bw)	49.80±6.05 ^a	25.11±2.07 ^{a,b}	5.83±0.01 ^a	2.50±0.33 ^{b,c,d,e}	0.29±0.09 ^{b,c}	10.07±0.97 ^{a,b}
BLCO(500 mg/kg bw) + CA (500mg/ kgbw)	55.80±3.15 ^a	15.45±2.66 ^c	6.67±0.79 ^a	1.72±0.20 ^{a,e}	0.79±0.41 ^{a,c}	10.93±2.59 ^b

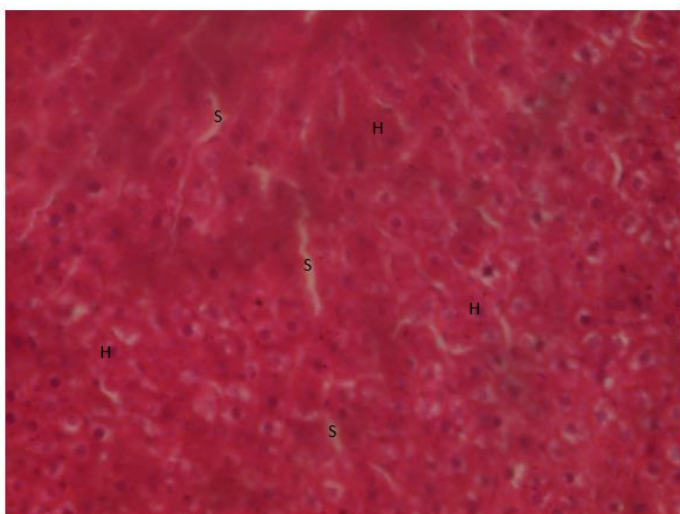
Values are reported as mean ± SEM of triplicate determination. Values with different superscript alphabets are significantly different at $p < 0.05$. The Least Significant Difference (LSD) was used to test for the difference between individual treatments groups using Statistical Package for Social Sciences (SPSS), version 22.0. BLCO = Bonny Light Crude Oil; CA = *Cnidioscolus aconitifolius*; mg/kg bw = milligram per kilogram body weight.

Table 3: Kidney profile of Wistar rats orally exposed to Bonny light crude oil and *C.aconitifolius* leaf extract

GROUPS	UREA (mmol/L)	CREATININE (mmol/L)	SODIUM (mmol/L)	POTASSIUM (mmol/l)	CALCIUM (Mmol/L)
Control	3.36±1.29 ^a	0.22±0.10 ^{a,b}	156.25±33.07 ^{a,b}	2.38±0.31 ^{a,b}	3.66±0.19 ^a
BLCO (250 mg/kg bw)	1.42±0.88 ^a	0.21±0.04 ^{a,c}	122.92±39.75 ^{a,b}	1.90±0.10 ^{a,c}	4.26±0.12 ^a

BLCO (500 mg/kg bw)	2.92±0.22 ^a	0.32±0.10 ^a	206.25±23.66 ^{a,c}	1.30±0.10 ^c	3.53±0.14 ^a
CA (250 mg/kg bw)	3.94±0.81 ^a	0.21±0.08 ^{a,b}	106.25±3.61 ^{b,e}	1.27±0.17 ^c	2.75±0.22 ^b
CA (500 mg/kg bw)	3.23±0.79 ^a	0.13±0.05 ^{b,c}	181.25±7.22 ^{a,e}	1.61±0.27 ^c	2.25±0.10 ^b
BLCO (250 mg/kg bw) + (CA 250 mg/kg bw)	2.56±1.27 ^a	0.16±0.05 ^{a,c}	183.33±23.48 ^{a,e,c}	1.73±0.16 ^{a,c}	2.42±0.34 ^b
BLCO (500 mg/kg bw) + CA (500 mg/kg bw)	3.72±0.42 ^a	0.10±0.03 ^{b,c}	245.83±16.27 ^{c,d}	1.78±0.37 ^{a,c}	2.22±0.42 ^b

Values are reported as mean ± SEM of triplicate determination. Values with different superscript alphabets are significantly different at $p < 0.05$. The Least Significant Difference (LSD) was used to test for the difference between individual treatments groups using Statistical Package for Social Sciences (SPSS), version 22.0. BLCO = Bonny light crude oil; CA = *Cnidioscolus aconitifolius*; mg/kg b.wt = milligram per kilogram body weight.

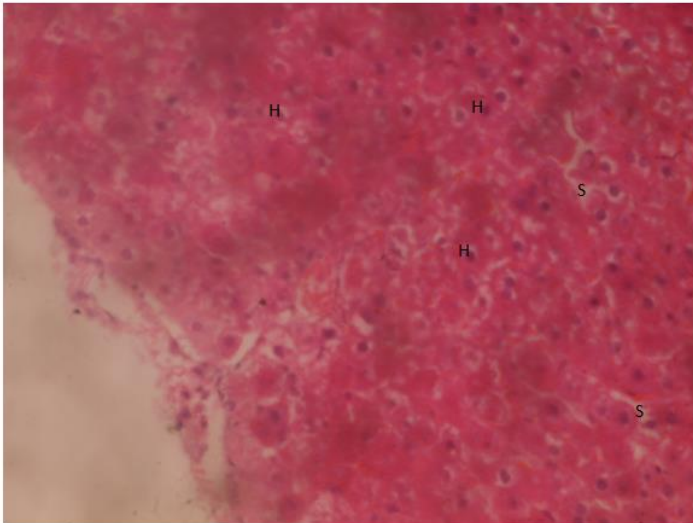


Liver 1

Magnification= X400 H&E

Fig. 1: Photomicrograph of liver from the control group

Histologically normal liver showing hepatocytes (H) intact, sinusoids (S) contain Kupffer cells

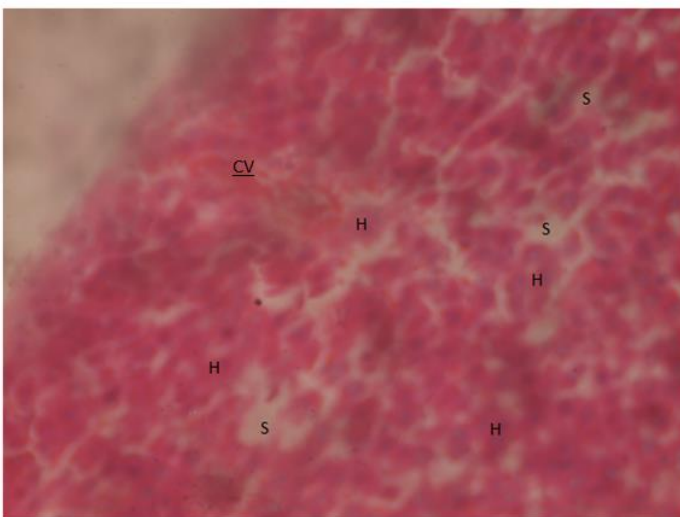


2

Magnification=X400 H&E

Fig. 2: Photomicrograph of the group administered 250mgkg⁻¹b.wt, of BLCO

Histologically normal liver showing hepatocytes (H) intact, sinusoids (S) contain Kupffer cells

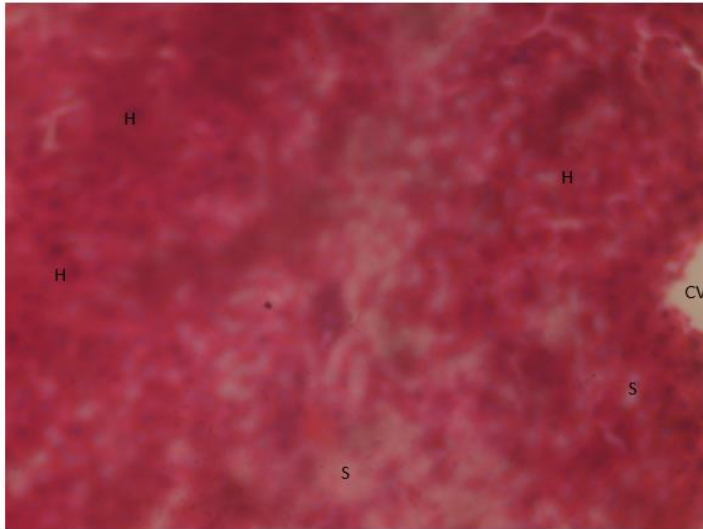


3

Magnification =X400 H&E

Fig. 3: Photomicrograph of liver from the group given 500mgkg⁻¹b.wt of BLCO

Histologically normal liver with intact hepatocytes(H), sinusoids(S) contain kupffer cells, congested central vein (CV)

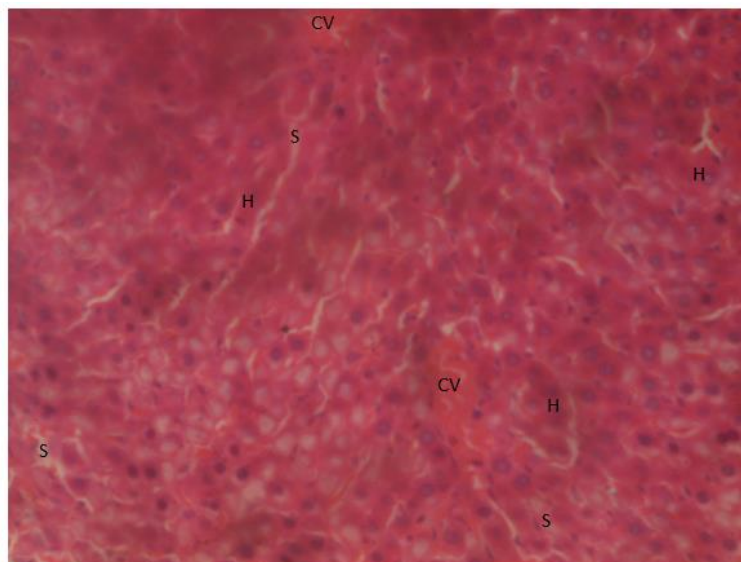


Liver 6

Magnification=X400 H&E

Fig. 4: Photomicrograph of liver from the group administered $250\text{mgkg}^{-1}\text{b.wt}$ of CA

Histologically normal intact hepatocytes (H), sinusoids (S) contain Kupffer cells, potent central vein (cv)

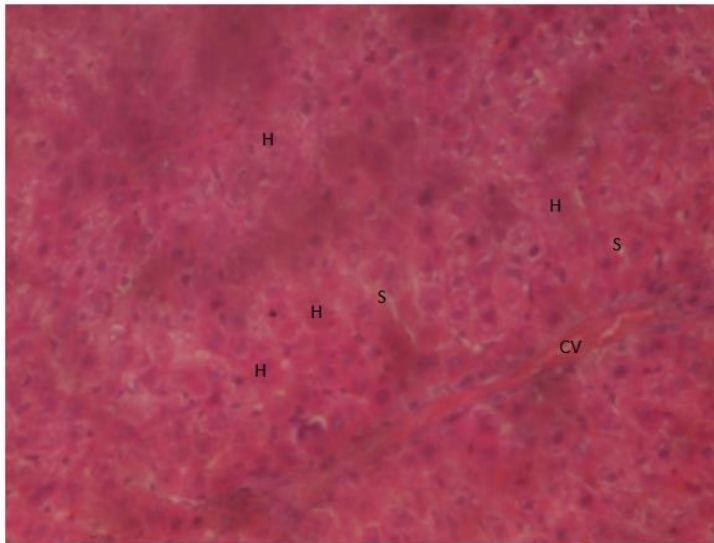


Liver 7

Magnification= X400 H&E

Fig. 5: Photomicrograph of liver from group administered $500\text{mgkg}^{-1}\text{b.wt}$ CA

Histologically normal liver with intact hepatocytes (H), congested central vein (CV), sinusoids (S) contain Kupffer cells



Liver 10

Magnification= X400 H&E

Fig. 6: photomicrograph of liver from group administered $250\text{mgkg}^{-1}\text{b.wt}$ of CA + $250\text{mgkg}^{-1}\text{b.wt}$ of BLCO

Histologically normal liver with potent central vein (CV), intact hepatocytes (H), sinusoids (S) contain Kupffer cells

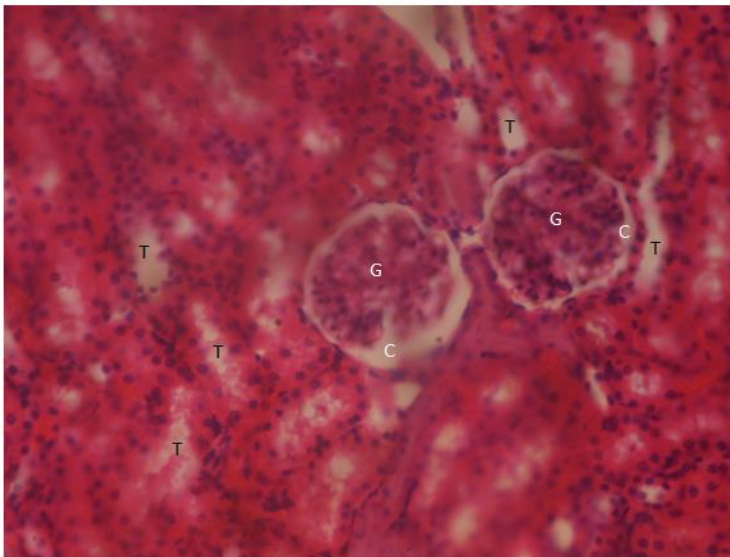


Liver 11

Magnification= X400 H&E

Fig. 7: Photomicrograph of group administered $500\text{mgkg}^{-1}\text{b.wt}$ CA + $500\text{mgkg}^{-1}\text{b.wt}$ BLCO

Histologically normal liver with intact hepatocytes (H), potent central vein (CV), sinusoids (S) contain Kupffer cells

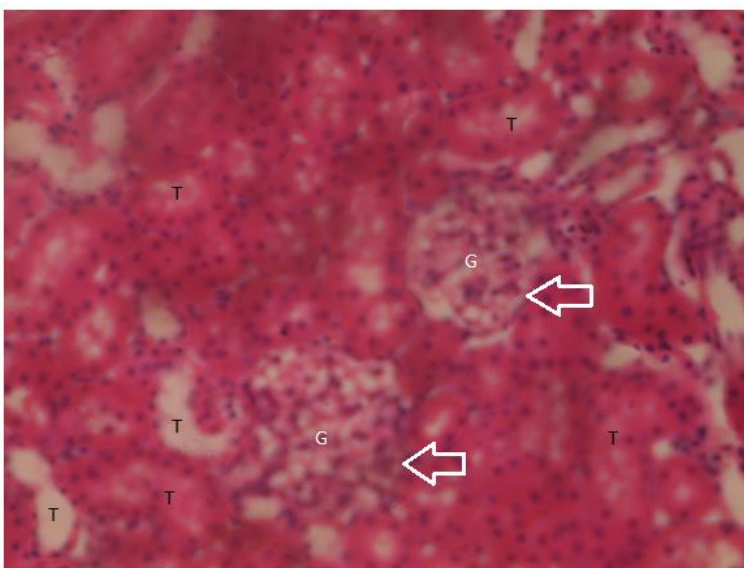


Kidney 0

Magnification=X400 H&E

Fig. 8: Photomicrograph of kidney from the control group

Histologically normal kidney showing intact glomeruli (G) containing glomerular mesangial cells, glomerular matrix and capillaries, patent Bowman`s capsular space(C), renal tubules (T).

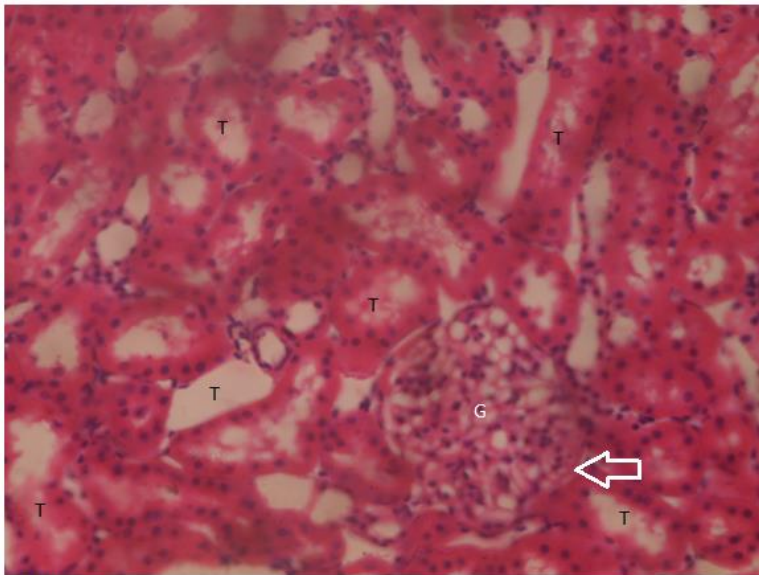


Kidney 1

Magnification= X400 H&E

Fig. 9: Photomicrograph of kidney from the group administered 250mgkg⁻¹b.wt BLCO

Histologically distorted kidney showing enlargement of glomerular tuft (G), with marked decrease of Bowman`s capsular space arrowed, intact renal tubules (T)

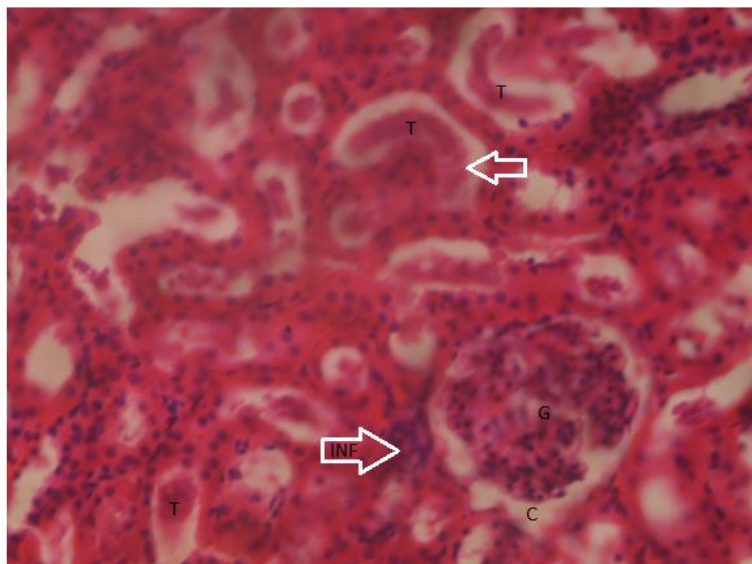


Kidney 2

Magnification= X400 H&E

Fig. 10: Photomicrograph of kidney from group administered $500\text{mgkg}^{-1}\text{b.wt}$ BLCO

Histologically distorted kidney showing enlargement of glomerular tuft(G) with marked occlusion of Bowman`s capsular space arrowed and intact renal tubules (T).

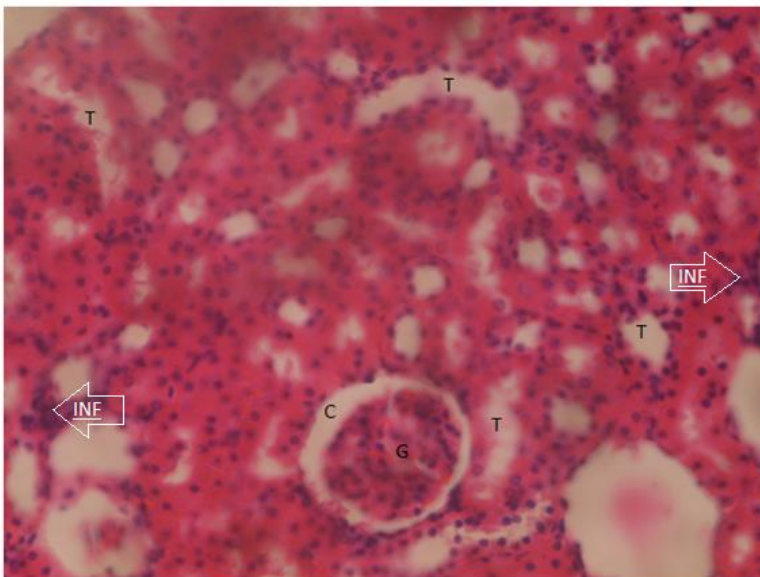


Kidney 5

Magnification= X400 H&E

Fig. 11: Photomicrograph of kidney from group marked $250\text{mgkg}^{-1}\text{b.wt}$ CA

Histologically distorted kidney showing infiltration of inflammatory cell (INF) into the interstitial tissue, hypercellularity of glomerular tuft (G)

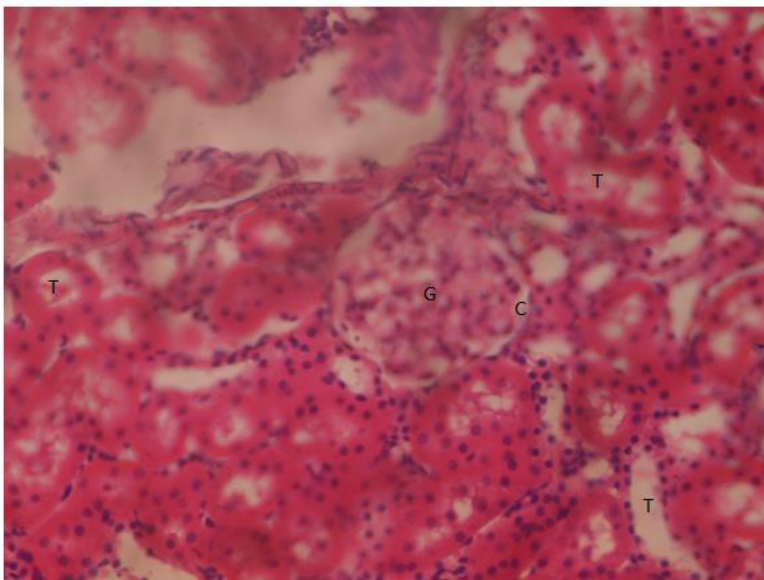


Kidney 6

Magnification= X400 H&E

Fig. 12: Photomicrograph of kidney from the group administered $500\text{mgkg}^{-1}\text{b.wt CA}$

Histologically distorted kidney with hypercellularity of glomerular tuft (G), infiltration of inflammatory cells (INF), intact renal tubule (T), patent Bowman`s capsule (C)

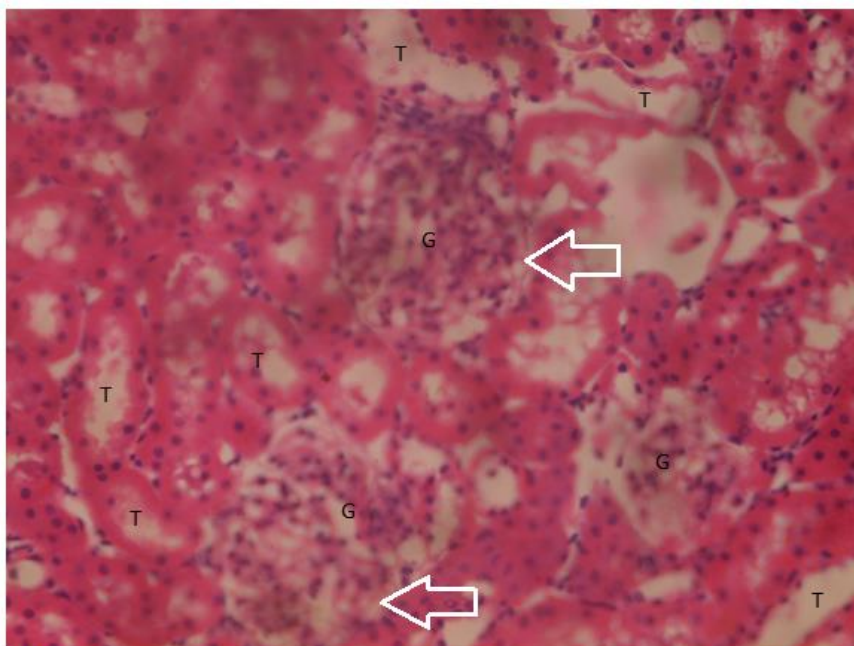


Kidney 9

Magnification= X400 H&E

Fig. 13: Photomicrograph of kidney showing group administered $250\text{mgkg}^{-1}\text{b.wt BLCO} + 250\text{mgkg}^{-1}\text{b.wt CA}$

Histologically normal kidney showing intact glomerular tuft (G), patent Bowman`s capsule (C)and intact renal tubule (T)



Kidney 10

Magnification= X400 H&E

Fig. 14: Photomicrograph of kidney from the group administered $500\text{mgkg}^{-1}\text{b.wt}$ BLCO + $500\text{mgkg}^{-1}\text{b.wt}$ CA

Histologically distorted kidney showing enlarged glomerular tuft (G) with occluded Bowman's capsular space (C) arrowed and intact renal tubules (T)

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