

## Full Length Research Paper

## Toxicological evaluation of extract of *Olox subsorpioidea* on albino Wistar rats

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This research work was undertaken in order to evaluate the toxicity effects of the leaf extract of *Olox subsorpioidea* on albino Wistar rats using the biochemical, haematological and histopathological indices. Five groups (A to E) of eight rats per group were used for this study. Animals in group A was administered with distilled water while the rats in groups B, C, D, and E were administered with 250, 500, 750 and 1000 mg/kg body weight of the extract of *O. subsorpioidea* via oral intubation for 28 days. Animals were subsequently anaesthetized in diethyl ether respectively and blood samples were collected for some biochemical and haematological assays, while the liver and kidney organs were isolated and processed for histopathological studies. Biochemical analysis revealed a significant decrease ( $p < 0.05$ ) in the levels of total bilirubin and alanine aminotransferase in the groups treated with 250 and 500 mg/kg body weight, while significant elevation ( $p < 0.05$ ) was observed in alkaline phosphatase, and albumin levels. Furthermore, haematological studies showed a significant reduction ( $p < 0.05$ ) in white blood cell count and haemoglobin level in the treated groups. Moreover, the group treated with 1000 mg/kg body weight of the extract exhibited a reduced ( $p < 0.05$ ) percentage mean cell haemoglobin and lymphocyte, while the percentage neutrophil was significantly increased ( $p < 0.05$ ). Histopathological studies conducted revealed that there was no significant damage on the liver and kidney tissues. The results suggest that extract of the leaf of *O. subsorpioidea* could alter the haematopoietic elements as well as some biochemical parameters and may not cause any adverse effect on the liver and kidney tissues.

**Key words:** *Olox subsorpioidea*, biochemical parameters, haematological parameters, histopathological studies.

### INTRODUCTION

The use of medicinal plants in the treatment of various illnesses is due to their phytochemical constituents and can be traced back to antiquity (Yakubu et al., 2007). Due

to the advancements in medicine, a lot of medicinal plants have been globally ignored. However, Africa and some parts in Asia utilize traditional medicine as an

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alternative for treatment. With the increasing discoveries of multifunctional herbs in these regions, herbal rebirth is thus taking over the world (Hoareu and DaSilva, 1999). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (Doughari et al., 2008). *Olox subscorpioidea* belongs to the family of Olacaceae and can either be a shrub or tree; it is about 10 m in height and widely distributed in Nigeria, Zaire and Senegal (Ayandele and Adebisi, 2010). It is known as “Mtungapwezi” in Swahili. It is called several names in Nigeria; for instance, it is called “Ukpakon” in Edo, “Ifon” in Southern Western Nigeria and “Aziza” in Nsukka (Ukwe et al., 2010). The plant is believed to possess medicinal properties; it is also reported to contain many bioactive compounds which are responsible for its diverse biological activities. Among them are tannins, alkaloids, saponin, flavonoids, glycosides, and steroids (Ayandele and Adebisi, 2010). Like some other African plants, religious and superstitious beliefs are attached to it. The plant is often used as genital stimulants, pain killers, treatment of venereal diseases, rheumatoid arthritis, tooth aches, etc (Borokin and Omotayo, 2012). Investigation on the saline extract of the plant showed that it possesses membrane stabilizing property, whereas the sodium hydroxide extract possesses anti-protease activity (Oyedapo and Famurewa, 1995). Similarly, the antimicrobial activity of the ethanolic stem extract has also been reported (Ayandele and Adebisi, 2007). Moreover, the plant root has been explored for the treatment of asthma and constipation (Okoli et al., 2007).

Since majority of the medicinal plants in Africa are believed to be multi-potent, they are used without knowledge of the potential toxic effects that may arise, thus, this study was aimed at evaluating the toxic effect of the ethanolic leaf extract of *O. subscorpioidea* by employing biochemical, haematological and histological indices in albino Wistar rats.

## MATERIALS AND METHODS

### Plant

The leaves of *O. subscorpioidea* were obtained from the campus of Covenant University, Canaan land, Ota, Ogun State, Nigeria in December 2012. The authentication was done by a botanist, Dr. A. C. Omonhinmin from the Department of Biological Sciences, Covenant University and a voucher specimen was also kept in the herbarium.

### Reagent sources

Absolute ethanol used for the plant extraction was obtained from Sigma, Aldrich, USA, whereas all the kits used for biochemical assays were obtained from Randox Laboratories London, UK.

### Plant extraction

The leaves were collected and air-dried for about three weeks and

then smoothly homogenized using a domestic blender and was subsequently prepared for extraction. Five hundred grams (500 g) of the powdered leaves of *O. subscorpioidea* were extracted in 95% ethanol using a Soxhlet apparatus. It was then concentrated at 50°C in a rotary evaporator to afford 95 g (19% yields) of the ethanolic extract (Ayandele and Adebisi, 2010).

### Experimental animals

Forty male Wistar rats, specific pathogen free, aged 2 to 8 weeks old were purchased from the University of Ibadan, Ibadan, Nigeria and were kept under standard environmental conditions (25 ± 21°C; 12/12 h light/dark cycle). Ten animals were kept in each cage and fed with standard diet (obtained from Graceline Feeds Ota, Ogun state) and clean water was given *ad libitum*. The animals were allowed to acclimatize for six weeks prior to the experiment. For experimentation, the animals were fasted overnight (Adebayo et al., 2010). The experimental animals were handled and used in accordance with the international guide for the care and use of laboratory animals (National Institute of Health, 1985).

### Experimental design

The animals were divided into 5 groups (A to E) of 8 rats per group. Group A was the control group whose animals were treated with distilled water, while animal subjects in groups B, C, D and E (Test group) were administered 250, 500, 750 and 1000 mg/kg body weight of ethanolic leaf extract of *O. subscorpioidea* (dissolved in distilled water), respectively, all groups were fed and treated for 28 days.

### Blood sample and organ collection

Animals were sedated with diethylether, blood samples were collected via cardiac puncture into two sets of lithium heparinized bottles. Plasma was obtained in one set by centrifuging the blood at 10,000 revolutions/min for 15 min and stored at -20°C in Eppendorff bottles until required for biochemical assays, while the whole blood was used for haematological studies. The organs (liver and kidney) were collected and placed in 10% formalin (Aliyu et al., 2007; Charity et al., 2012).

### Biochemical assay

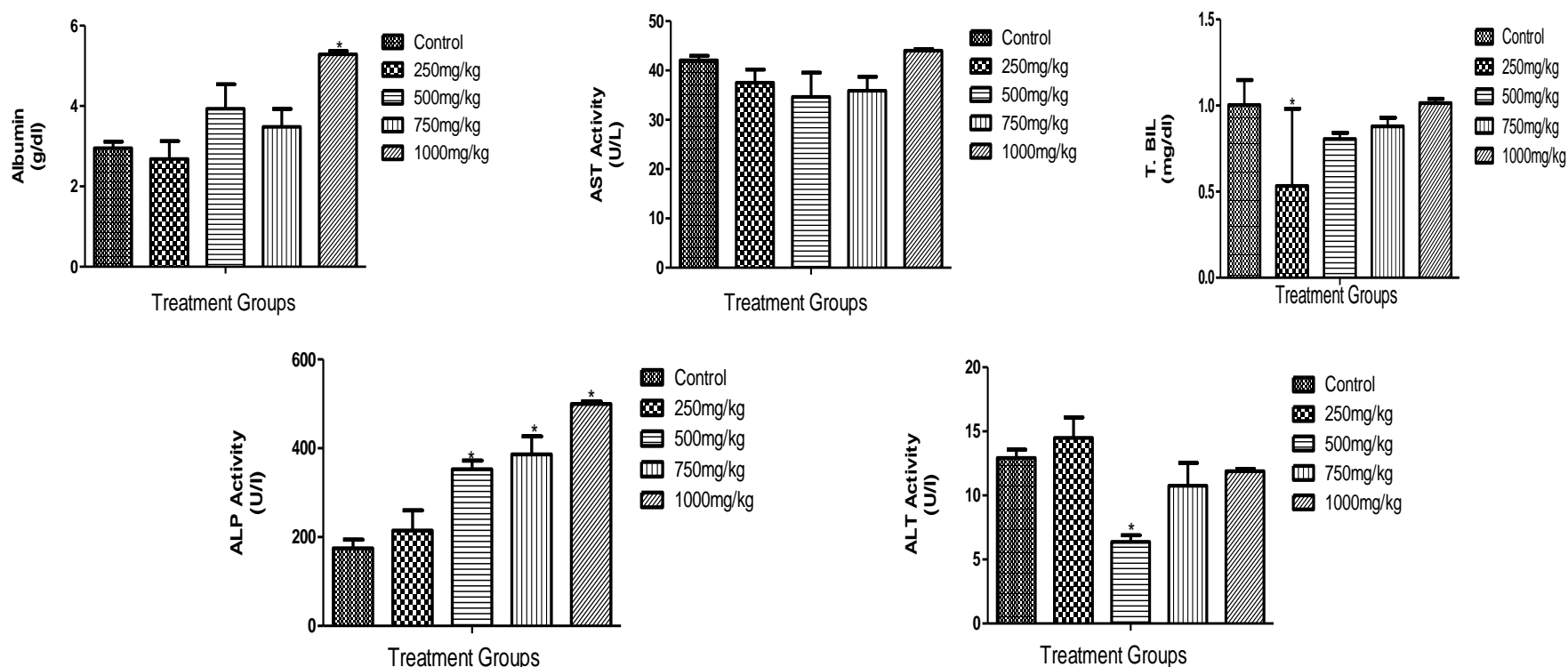
An auto-analyzer (Archem BM240, Turkey) was used to assay for biochemical parameters which include: aspartate amino transferase (AST) (Bergmeyer et al., 1986A), alanine aminotransferase (ALT) (Bergmeyer et al., 1986b), alkaline phosphatase (ALP) (Tietz et al., 1983), total bilirubin (Doumas et al., 1973), albumin (Doumas et al., 1971), and urea (Krieg et al., 1986).

### Haematological assays

An automated haematology system analyzer (Archem BM240, Turkey) was used to assay for white blood cell count (WBC), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), mean cell haemoglobin (MCH), percentage lymphocyte (% LYM), neutrophil (NEU) (Baker et al., 1998).

### Histopathological studies

The livers and kidneys tissues were fixed in normal saline for 72 h



**Figure 1.** Effect of leaf extract of *Olax subscorpioidea* on liver functions of albino Wistar rats.

AST, aspartate aminotransferase; T.BIL, total bilirubin; ALP, alkaline phosphatase; ALT, alanine amino transferase. Values represent mean  $\pm$  SEM of 8 replicates; \* $p < 0.05$ .

and sliced into a thickness of 2.1 mm. The tissues were dehydrated with alcohol of graded concentrations. They were further treated with paraffin wax and cast into blocks; sections of the tissues were then cut on a microtome to 5  $\mu$ m. these were later attached to a slide and allowed to dry. The sample slides were subsequently stained with haematoxylin-eosin and examined under a light microscope; photomicrographs of the samples were recorded (Aliyu et al., 2007; Charity et al., 2012).

#### Statistical analyses

All data were expressed as mean  $\pm$  standard error of mean (SEM), a probability of  $p < 0.05$  being considered significant.

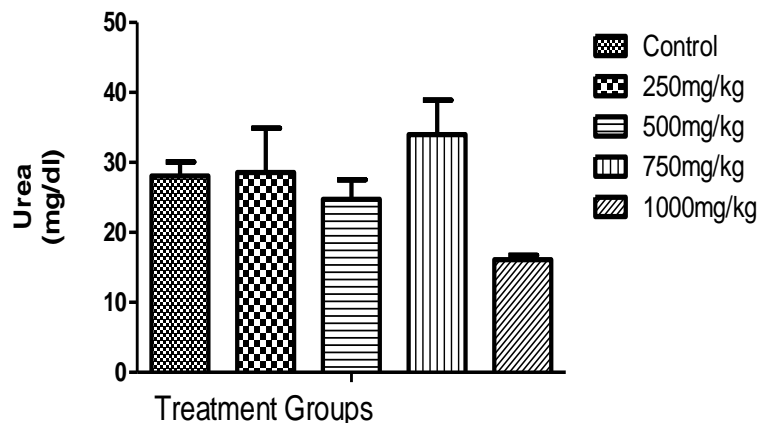
Group comparisons were done using the analysis of variance (ANOVA) test. Tukey's post hoc test was carried out to analyze significance of difference between different groups.

## RESULTS

### Biochemical analysis

The effects of the extract on liver functions in blood of rats were assessed (Figure 1). ALT, AST and total bilirubin were not significantly elevated

( $p > 0.05$ ) when compared with the control group although there was a significant reduction ( $p < 0.05$ ) in ALT and total bilirubin in the group treated with 500 and 250 mg/kg body weight of extract, respectively. A significant increase ( $p < 0.05$ ) in ALP was observed in the groups treated with 500, 750 and 1000 mg/kg body weight of extract, while the level of albumin in the group treated with 1000 mg/kg body weight of extract was also significantly increased ( $p < 0.05$ ). Analysis of the urea concentration revealed that there was no significant change observed in blood urea nitrogen (Figure 2).



**Figure 2.** Effect of leaf extract of *Otax subscorpioidea* on urea level of albino Wistar rats. Values represent mean  $\pm$  SEM of 8 replicates;  $p > 0.05$ .

(Figure 2).

### Haematological analysis

Haematological parameters such as: red blood cell count (RBC), mean cell volume (MCV), platelet count (PLT) and mean corpuscular haemoglobin concentration (MCHC) were not significantly affected ( $p > 0.05$ ), but there was significant decrease ( $p < 0.05$ ) in WBC count of all treated groups. Similarly, there was a significant reduction ( $p < 0.05$ ) in haemoglobin level in groups treated with 250, 500 and 1000 mg/kg body weight of extract. Moreover, MCH and percentage lymphocyte significantly dropped ( $p < 0.05$ ) in the group treated with 1000 mg/kg body weight of extract, while percentage neutrophil was significantly elevated ( $p < 0.05$ ) in the group treated with 1000 mg/kg body weight of extract (Figure 3).

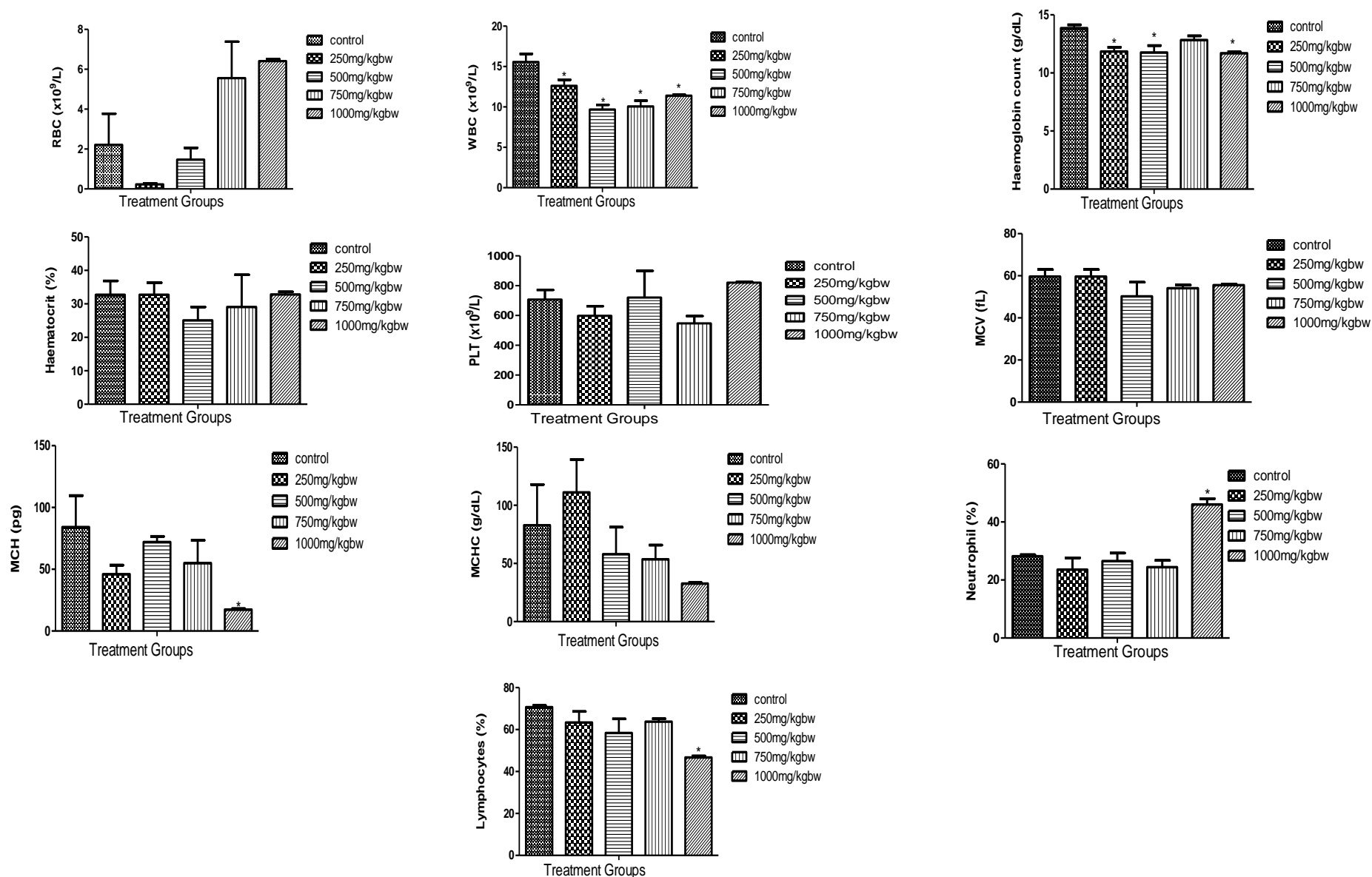
### Histopathological studies

No significant damage was observed in rats' liver and kidney tissues in groups treated with ethanolic leaf extract of *O. subscorpioidea* when compared with the control groups (Figures 4 and 5).

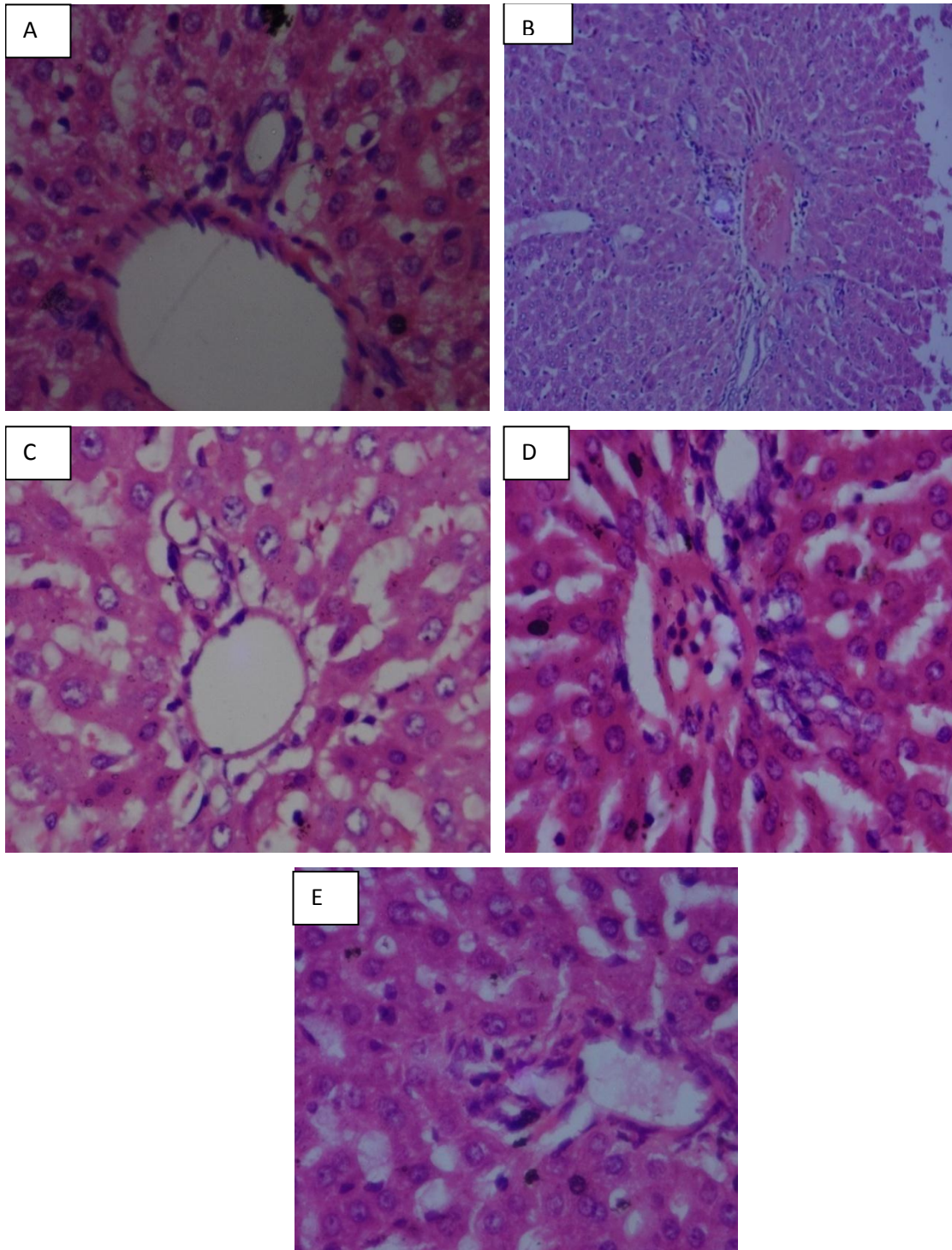
### DISCUSSION

Toxicological assessment of potential drugs, herbs and extracts are necessary to establishing the safety limit of these substances in animals. They are commonly used to assess the possible health risk in humans, caused by intrinsic adverse effects of compounds or plant extracts (Klassen and Eaton, 1991). Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are specific markers of hepatic injury and hepatocellular necrosis (Blackwood, 2001). The

insignificant elevation in the activities of ALT and AST reveals that the ethanolic extract may not be harmful to the liver, because increase in plasma ALT and AST activity are markers for hepatic damage (Rej, 1989), though the cause of the reduction of ALT activity in the group treated with 750 mg/kg body weight of the extract is unknown. This result however agrees with the study conducted on leaf of *Chrysophyllum albidum* G which also decreased ALT level in Wistar rats (Adebayo et al., 2010). The decrease observed may be attributed to the ability of the extract to alter the hepatocytes and down regulate its production which could be beneficial. ALT is localized in the cytosol of the hepatocytes and more sensitive marker of hepatocellular damage as opposed to AST from the mitochondrial which can also be produced from other tissues like heart, kidney and pancreas other than the liver (Mabeku et al., 2007). The contrasting dose dependent significant increase of alkaline phosphatase activity in the group that received the higher doses as shown in Figure 1 was also observed by Adedapo et al. (2009). ALP is a metalloenzyme whose activity change in the blood is xenobiotic-sensitive, because it is a glycosylphosphatidylinositol (GPI) anchored protein of the microsomal and cellular membrane of hepatocyte, this makes it susceptible to cleavage by phospholipase D, proteases and bile acids (Rej and Bretaudiere, 1980). Flavonoids present in the leaf extract of *O. subscorpioidea* have been implicated to induce the activity of ALP. Since ALP is also synthesized by other tissues of the body, a dose related rise in ALP activity may not be due to liver damage (Ayandele and Adebisi, 2007; Hsu et al., 2009). This result is supported from the histological assessment of the liver tissue where no obstructive jaundice and intrahepatic cholestasis as a consequence of high level of ALP was observed. The high concentration of albumin observed in group treated with 1000 mg/kg body weight of extract poses the risk of high oncotic blood pressure at higher doses of the plant



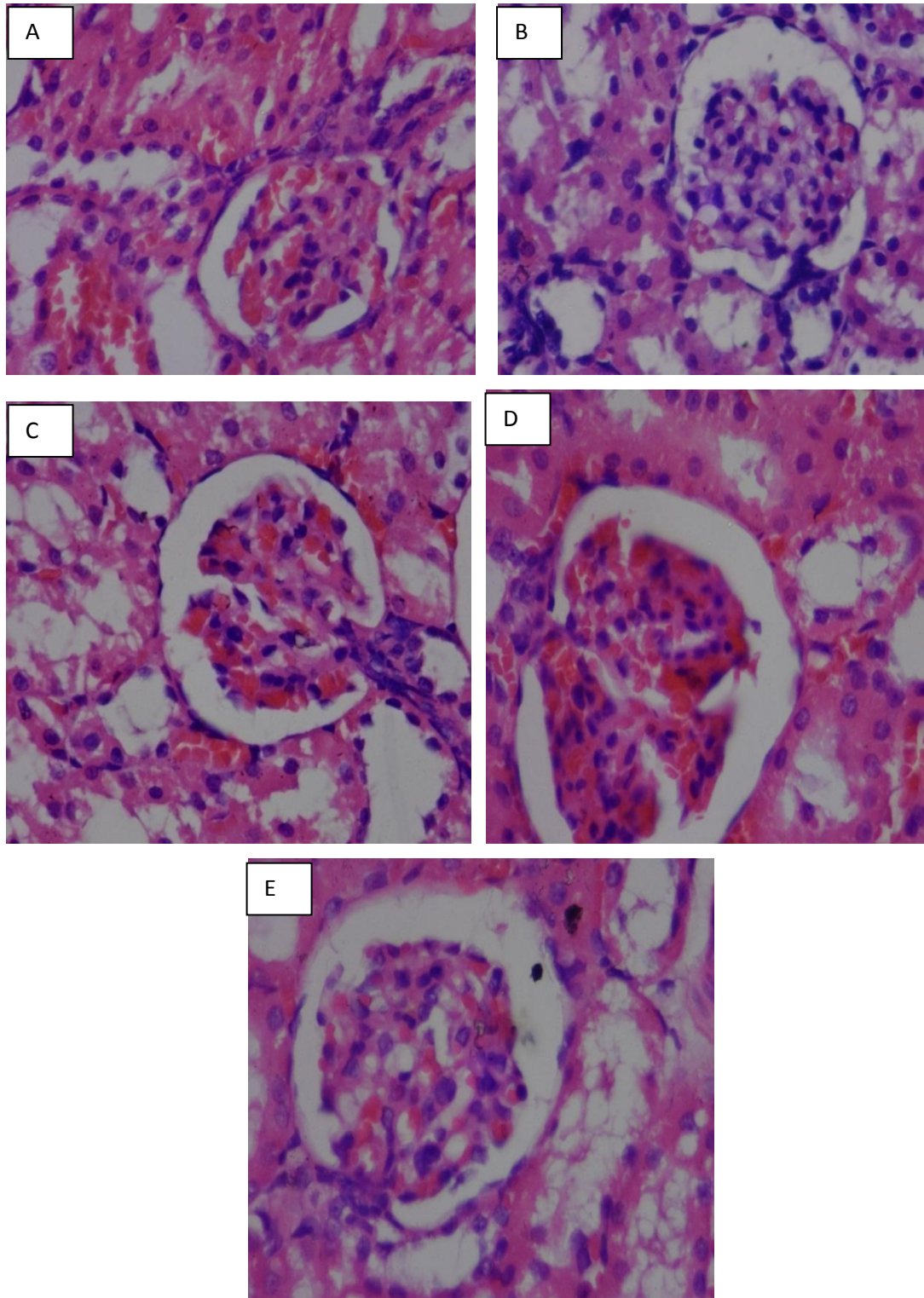
**Figure 3.** Effect of the leaf extract *O. subscorpiodea* on the haematological parameters of albino Wistar rat. Values represent mean  $\pm$  standard error of mean (SEM) of 8 replicates; \* $p < 0.05$ . RBC, red blood cell count; WBC, white blood cell count; PLT, platelet count; MCV, mean platelet volume mean corpuscular hemoglobin, MCH; MCHC, mean corpuscular hemoglobin concentration; LYM%, percentage lymphocyte.



**Figure 4.** Photomicrograph of the liver tissues (H&E x160) of albino Wistar rats treated with extract of *O. subscorpioidea*. Figures A to E show the tissues of rats treated with 0, 250, 500, 750 and 1000 mg/kg body weight respectively).

plant extract. Albumin apart from being a useful indicator of the integrity of glomerular membrane is also important in determining the severity of the disease (Adedapo et al., 2005). Increased albumin may be primarily due to high

synthetic ability of the liver. The elevated level observed in this group suggests an enhancement in the synthetic function of the liver (Kaplan et al., 1988). The non significant increase in urea during the treatment duration may be



**Figure 5.** Photomicrograph of the kidney tissues (H&E x160) of albino Wistar rats treated with extract of *O. subscorpioidea*. Figures A to E show the tissues of rats treated with 0, 250, 500, 750 and 1000 mg/kg body weight respectively).

may be attributed to the impairment of the urea cycle leading to the reduced production of urea suggesting that the extract may not possess any phyto-constituent

obstructing the urea cycle (Adebayo et al., 2003). The result corroborates the histopathological findings of the kidney tissues where no significant damage was observed.

The decrease in WBC count and lymphocyte makes the animal vulnerable to infections caused by pathogens (Anofi and Olugbenga, 2011). The decrease in the values of WBC in the treated rats indicates that the rats were fighting against some forms of infection probably induced as a result of administration of this plant extract. In a similar study conducted by Iweala and Obidoa (2009), they observed that the presence of phytosterols and flavonoids in the leaf extract of *Gompholobium latifolium* might possibly interfere with the process of WBC synthesis. The presence of these phytochemicals may however play synergistic roles in mediating this activity. The decrease in free haemoglobin level of groups B, C and E and MCH in group E reveals that the plant extracts may contain some harmful phytochemicals, posing the risk of anaemia although the insignificant change observed in the quantity of red blood cell is paradoxical. Saponins have been implicated to be highly toxic when injected into the blood stream and cause haemolysis of the red blood cells and eventually destroying the cells (Itodo et al., 2011). This therefore suggests that saponins from the plant extract might be responsible for the significant reduction of the haemoglobin count. Percentage neutrophil upsurge as seen in group E is a signal for immune-attack and anaemia (Harshida et al., 2013). Thus, further increase in the dose of the extract will compromise the immune system and the free blood haemoglobin status.

In conclusion, this study has demonstrated that the ethanolic leaf extract of *O. subscorpioidea* may not cause deleterious effects on the liver and kidney tissues, but could alter the haematopoietic elements as well as some biochemical parameters. Further investigation is needed to establish the bioactive compounds of the extract involved in mediating these biological effects.

### Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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