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EFFECTS OF ETHANOLIC EXTRACT OF *EUPHORBIA HIRTA* LEAF ON THE BIOCHEMICAL PARAMETERS AND HISTOLOGY OF LIVER AND KIDNEY IN RATS

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ABSTRACT

Herbal medications are widely used in many parts of Nigeria and has become highly acceptable, accessible, cheap, readily available, potent, and relatively safe. This study evaluated the effects of ethanolic leaf extract of *Euphorbia hirta* (*ELEEH*) on the biochemical parameters and histology of the liver and kidney in rats. Twenty (20) adult male Wistar rats were randomly assigned to four groups of 5 animals each; a control group and treated groups vis 50mg/kg, 100mg/kg or 500mg/kg *ELEEH* for 14 days. Body weight was determined and the relative weights of the liver and kidney were evaluated and the tissues examined histologically. Serum biochemical analysis of the liver and kidney function were performed. Data were analysed using SPSS (version 23) and $p \leq 0.05$ was considered significant. Percentage body weight gain was non-significantly decreased while relative weight of the kidney was significantly decreased in the 50 and 100mg/kg treated groups compared with the control. There was no significant different in the relative weight of the liver, serum alkaline phosphatase and alanine aminotransferase and kidney histological architecture was comparable with control. However, serum aspartate aminotransferase, total protein, albumin, blood urea nitrogen and creatinine were significantly increased; especially in the 500mg/kg in the treated group, with signs of liver damage compared with the control. These findings suggest that *ELEEH* has the potentials of deleterious effects on the biochemical and physiological functions of the liver and kidney as well as liver histo-architecture in a rat model. Thus, caution should be exercised in the use of *Euphorbia hirta* as a medicinal plant.

***Keywords**: Biochemical parameters, Euphorbia hirta, Kidney, Liver, Histology.*

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1. INTRODUCTION

The use of plants' parts in health care delivery and for health and disease management especially in resource poor settings is increasing, with about eighty percent of the world's population depending on them for their primary health care purposes [1,2]. Though the use of these plants has shown

promising potential with a rising global demand, there are concerns about their safety [3]. Herbal products are regarded as safe or of low toxicity based on their long history of humans use [4], however, latest surveys have indicated that many of these products showed adverse effects [5]. Since safety continues to be a major concern with medicinal products, conducting toxicity studies on them is therefore important; especially in Nigeria where the use of traditional and herbal medicines/products is widely practiced [4,5].

Euphorbia hirta (*E. hirta*) is one of such plants used in Nigerian folkloric medicine. *E. hirta* belongs to the family of Euphorbiaceae, a herb common to tropical countries. The use of *E. hirta* as a traditional medicine for various diseases including asthma, diarrheal diseases, bronchitis, coughs, colds, emphysema, fever, heartburn, intestinal parasites, kidney stones, laryngeal spasms, menstrual problems, peptic ulcers, sterility, vomiting and venereal diseases is well documented [6]. The pharmaceutical application of this plant has shown promising evidence as an anti-bacterial, -fungal, -allergic, -inflammatory, -diarrheal, -oxidant, -tumor, -diabetic, -hypertensive, -malarial and as an immunomodulatory agent [6,7]. Preliminary phytochemical screening of ethanolic extract of *E. hirta* shows the presence of tannins, flavonoids, alkaloids, cardiac glycosides along with other anti-oxidants and the absence of saponin, cyanogenics and glycosides [8].

Target organ toxicity has only sometimes been reported in the literature, and there has not been much study done on how safe or otherwise the plant is. Adedapo *et al* [9] described an increase in serum biomarkers for liver and renal functions, as well as leucocytosis and uraemia in rats that were force-fed with *E. hirta* extracts. In many rural parts of the developing nations, medicinal plants are the most easily accessible and affordable health resources [10] because they are cost effective and generally assumed to be safe. However, researches have proven that not all medicinal plants are without adverse effects. Various deleterious effects have been reported with the administration of different herbs including systemic toxic effects of *Acacia ataxacantha* [11], *Caralluma dalzielii* [12] and developmental toxic effects of *Achyranthes aspera* [13]. The adverse effects can result from inherent toxic effect of the active principle, long-term use, overdosing or a combination of the aforementioned. Therefore, toxicological and pharmacological properties of medicinal herbs should be scientifically evaluated to warrant the outstanding quality and safety for patients' use [14]. Despite wide application of *E. hirta* in human health, the folkloric therapeutic efficacy and the safety profile of the leaf extract are yet to be scientifically validated. Therefore, the current study was designed to evaluate the toxicity profile of *E. hirta* by monitoring body weight gain, relative organ weight, biochemical and histological evaluation of the liver and kidney in rat following acute treatments. This is strongly in line with the World Health Organization set goals on determining the safety profile of any medicinal plants before being acceptable for human use. The finding of this study is expected to fill literature gaps on the short-term safety profile of this herbal plant and may also provide baseline data for further pre-clinical and clinical investigations.

2. MATERIALS AND METHODS

2.1. Collection and processing of plant material: *E. hirta* plants were collected from within the compound of College of Medicine, Ambrose Alli University, Ekpoma and the leaves were carefully picked from the plant and washed in clean tap water before air drying in the Histology Laboratory, Department of Anatomy, Ambrose Alli University, Ekpoma. The dried leaves were pulverized into fine powder using an electronic blender.

2.2. Plant extraction: Ethanolic extraction of the pulverized leaf was prepared following the method by Majekodunmi and Nubani [15]. 500grams of the powdered leaf was weighed into 2000ml conical flasks containing 1000ml of 98% ethanol. The mixtures were covered and sealed with foil paper with periodic shaking for 24hours at the end of which filtration was done sequentially via a cotton cloth mesh and through a Whatman filter paper No. 1. The resulting dark-green filtrates were then

concentrated at room temperature to dryness. The final products were sticky dark-green substances which were stored in universal bottles and refrigerated at 4^{o}C prior to testing.

2.3. Experimental animals: Apparently healthy male Wistar rats (N= 20; 120 to 160g) obtained from the Animal House of the College of Medicine, Ambrose Alli University, Ekpoma. They were moved to the Animal Holding Unit of the Department of Anatomy. The animals were allowed 2 weeks of acclimatization before commencement of the experiment. They were fed on standard rat chow diet and water *ad libitum* throughout the duration of the acclimatization period and experiments. All studies involving the experimental animals were conducted in compliance with general guidelines for methodologies on research and evaluation of traditional medicine as promulgated by WHO [16].

2.4. Experimental design: The rats were completely randomised into 4 groups as group I (control; administered 0.5 ml of distilled water) and groups II, III and IV were administered orally 50, 100 and 500mg/kg body weight of the extract respectively. The administration was done repeatedly on daily basis for two weeks using metal oropharyngeal cannula.

2.5. sample collection and Measurement of body and relative organ weights: On day 15, the rats were weighed and sacrificed by cervical dislocation. Blood samples were collected via cardiac puncture for biochemical analysis. The animals were weighed at the beginning and at the end of the experiment and the level of weight gain (%) was calculated using the relation;
Weight gain (%) = (Wf−Wi/Wi) x 100 {where Wf = final weight; Wi = initial weight}. After blood collection, the liver and kidneys were excised, weighed and assessed macroscopically. The offcuts of the organs were conserved for histopathological assessment. Relative organ weight of each harvested organ was determined using the relation; Relative organ weight (%) = (organ weight/body weight) x 100.

2.6. Measurement of serum biochemical parameters: Blood samples for biochemical analysis were gently placed in bottles to avoid haemolysis of the blood cells. Blood serum was obtained by centrifugation of the blood sample and was used for biochemical estimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP), total protein (TP), albumin (ALB), urea (BUN) and creatinine (CRE) using standard methods.

2.7. Histological processing: The fixed kidney and liver tissue were taken for histological processing following standard histological procedures that encompassed the following steps: fixation, dehydration, clearing, impregnation, embedding, sectioning, staining and microscopy [17]. Microscopic slides were examined under compound light microscope. Tissue sections from the treated groups were evaluated for evidence of histopathological changes as compared to those of the controls. Photomicrographs of selected slides of each of the organs under study were taken using digital Photo camera mounted on a binocular compound microscope (Axiostar MWIB, US).

2.8. Statistical analysis: The data obtained are expressed as mean ± standard error of the mean (SEM) and analysed for statistical significance using student t test and one-way ANOVA followed by the Least Significant Difference (LSD) test; in the Statistical Package for Social Sciences (SPSS version 21). $P < 0.05$ was considered significant. Histological findings were presented in plates of photomicrographs. The results were presented using suitable tables, chats and micrograph.

3. RESULTS

3.1. Effect of ELEEH on body weight and relative weight of the liver and kidney of rats.

Extract treated rats showed no observable toxicity sign compared to the control and they all survived throughout the 14 days of treatment. Figure 1 shows the effect of ethanolic leaf extract of *E. hirta*

(ELEEH) on percentage body weight gain and relative weight of the liver and kidney of rats after 14 days of administration. There was a dose-dependent non-significant increased (p>0.05) in percentage body weight gain in the extract treated groups and these were non-significantly decreased compared with the control. There was no significant difference (at p>0.05) in the relative weight of the liver in the extract treated groups compared to the control. The relative weight of the kidney was significantly lower in the 50 mg/kg (0.24±0.01 g/100g bwt) and 100mg/kg (0.30±0.00 g/100g bwt) treated groups compared to the control (0.65±0.02 g/100g bwt).

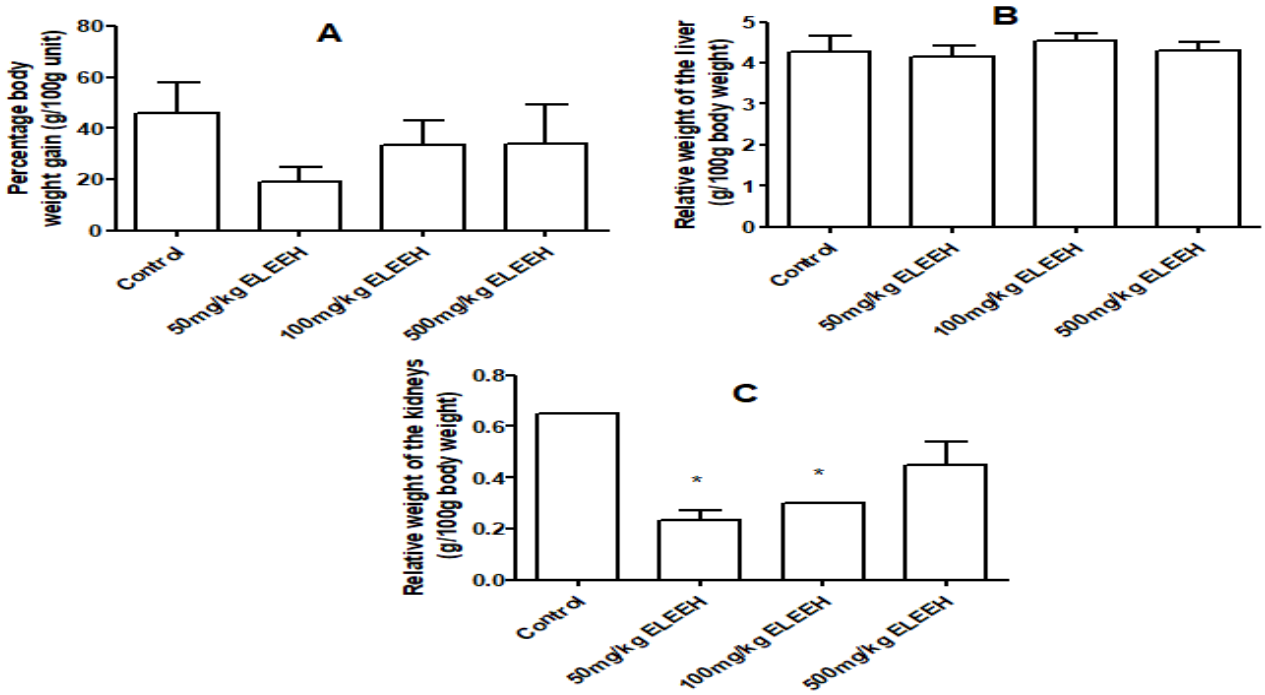


Figure 1. Effect of ethanolic extract of *E. hirta* leaf on (a) percentage body weight gain, relative weight of the (b) liver and (c) kidney of adult male Wistar rats after 14 days of oral administration.

3.2. Effect of ELEEH on biochemical parameters of the liver and kidneys of rats after 14 days of administration

The biochemical parameters analysed were liver and kidney function parameters. Compared to the control, serum alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were non-significantly increased in a dose dependent manner in the extract treated groups. On the other hand, serum aspartate aminotransferase (AST) and albumin (ALB) were significantly increased in the 50, 100 and 500mg/kg extract treated groups compared with the control. Blood urea nitrogen (BUN) was significantly increased in the 100 and 500mg/kg extract treated groups while serum total protein (TP) and creatinine (CRE) were only significantly increased in the 500mg/kg extract treated group compared with the control.

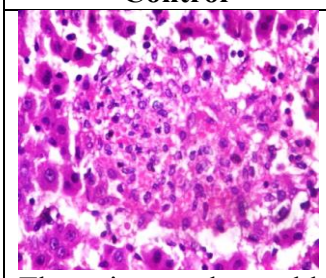
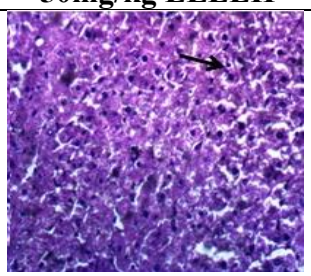
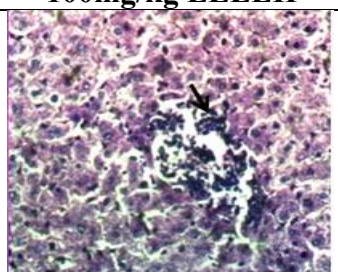
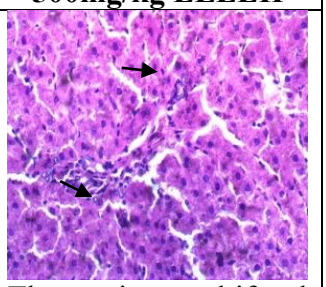
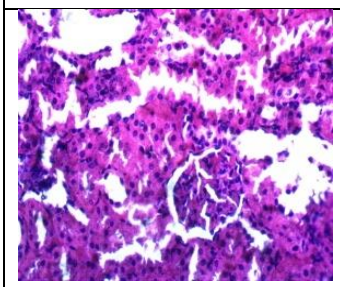
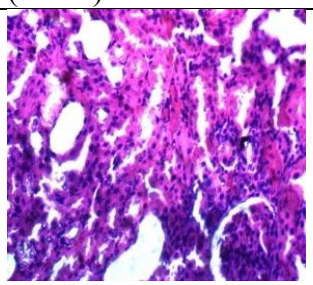
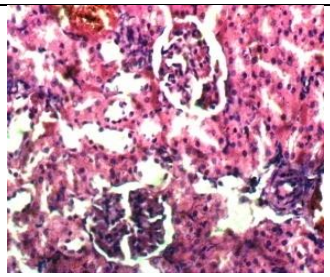
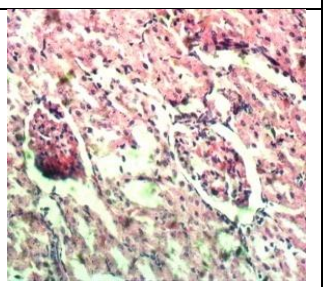
3.3. Effect of ELEEH on the histology of the liver and kidneys in rays after 14 days of oral administration

Plates 1 presents the photomicrographs on the effect of ELEEH on the histology of the liver and kidneys in rats after 14 days of oral administration. While there was normal liver histoarchitecture in the control, oral administrations of ELEEH caused multifocal hepatocellular degeneration, necrosis and inflammation in the 50mg/kg, 100mg/kg and 500mg/kg treated groups. On the other hand, there was no observable change in the histological features of the kidney in the extract treated groups compared with the control.

Table 1. Effect of ELEEH on liver and kidney biochemical parameters of rats after 14 days of administration

Parameter	Control	50mg/kg bwt	100mg/kg bwt	500mg/kg bwt
ALP (U/L)	70.33+6.11	75.67± 2.53	77.67+2.31	79.00+2.00
ALT (U/L)	27.33+1.53	32.00+3.61	32.67+3.51	35.00+3.61
AST (U/L)	37.00+1.73	42.33+2.52*	47.00+2.00*	47.67+3.06*
TP (g/dl)	6.40+0.27	6.90+0.46	7.43+0.43	8.00+0.17*
ALB (g/dl)	2.30±0.36	2.53±0.15*	2.67±0.21*	3.30±0.30*
BUN (mg/dl)	14.73+0.65	15.57+0.45	16.00+0.346*	16.23+0.47*
CRE (mg/dl)	0.50± 0.00	0.60± 0.00	0.63+0.12	0.67+0.06*

Values are the mean ± SD; n = 5 rats. * indicates a significant difference ($P < 0.05$) from controls. Keys: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total Protein (TP), Albumin (ALB), Blood Urea Nitrogen (BUN), Creatinine (CRE).

Control	50mg/kg ELEEH	100mg/kg ELEEH	500mg/kg ELEEH
 There is no observable lesion	 There is multifocal hepatocellular degeneration, necrosis and inflammation (arrow)	 There is multifocal necrotizing hepatitis (arrow)	 There is multifocal hepatocellular necrosis and inflammation (arrow)
 There is no observable lesion	 There is no observable lesion	 There is no observable lesion	 There is no observable lesion

Plates 1. Photomicrographs on the effect of ELEEH leaf on the histology of the liver and kidneys in rats after 14 days of oral administration (H and E X400).

4. DISCUSSION

The use of various traditional herbal medicines to treat several diseases and ailments is common in developing countries [18]. Although, many studies have been undertaken to investigate the

pharmacological potential of such remedies, rather little work has been done to assess the potential toxicities of such products. There is now growing evidence that many herbal medicines do cause serious toxicity [19] and therefore, more scientific attention is now being given than before to assess the potential toxicity of herbal medicines. The increased use of this plant has resulted in concerns over both the efficacy and safety of the product. Although various biological effects of *E. hirta* have been published to include possessing antioxidant, anxiolytic, antidiabetic, anticancer, sedative, anti-inflammatory, analgesic, and antipyretic potentials [20-22], there are no sufficient data on the toxicological profile of the plant and hence, this study.

Sub-acute toxicity testing is useful in assessing haematological, biochemical and target organ impacts of extracts/agents since these effects are usually not observable in acute toxicity testing [23]. Sub-acute toxicity in rodents provides valuable information on the possible undesirable effects of compounds or plant extracts administration. These toxicology data are important to fix the appropriate dosage for long-term studies [23]. The sub-acute toxicity profile of the EEEHL was evaluated in rats via evaluating body and relative organ weights, biochemical and histopathological parameters of the liver and kidney in this study. Mortality, severe clinical signs, loss of body weight and altered food intake are crucial indicators of the possible effects of various plant extracts or even drugs on test animals [24]. In this study, all animals were active and responded positively to stimuli. No deaths and no clinical signs of local or systemic toxic effects were observed to be different in the treated groups compared to the control. The behavior of the animals recorded daily were comparable to the control. Thus, the extract is assumed to be safe as there was no identifiable physical toxicity.

In general, an increase or decrease in the body weight of an animal has been used as indicator of an adverse effect of drugs and chemicals [24] and it will be significant if the body weight loss or gain occurred in excess of 10% from the initial body weight [25]. In the present study, the body weight of all treated rats did not differ significantly from those of the control groups. It indicates that the extract did not adversely affect food intake at all doses, and had no harmful effect on body growth patterns. Thus, oral administration of the extract appears safe and in relation to its folkloric practice in the administration of the herbs through oral route to treat patients. Therefore, *E. hirta* extract can be considered as non-toxic up to the maximum dose of 500mg/kg used in this study.

The kidneys and liver of rats are usually used to assess the effect of drugs or plant materials [26]. In toxicity studies, organ weight changes, effects on enzymes, physiologic disturbances and target organ injury are sensitive indicators of toxicity [27]. An increase in organ weight suggests the occurrence of hypertrophy while a decrease suggests necrosis [25]. While organ weights provide useful signals indicating test agent-related effects, organ weight data must be interpreted in an integrated fashion with gross pathology, clinical pathology, and histopathology findings [28]. The gross pathological examination of the liver and kidneys of the treated rats showed no change in color, shape, size, and texture compared to the control group. Also, there was no significant difference in the relative weights of the liver in the extract-treated rats compared with the control rats but there was in the kidneys where the 50 and 100mg/kg presented significant decrease in relative kidney weight. The non-significant change in relative weight of the kidneys in the highest dose (500mg/kg) treated rats question the observable significant decrease with the 50 and 100mg/kg treated groups. Thus, this decrease in relative weight of the kidney at 50 and 100mg/kg may be unrelated to the extract under study.

The decrease in kidney weight observed in the rats treated with extract was not dose dependent and therefore of no toxicological significance; moreover, it is at variance with the histopathological examination which showed normal kidney histo-architecture. Liver histopathological examination showed dilated multifocal hepatocellular degeneration, necrosis and inflammation. The serum clinical biochemistry analyses were done to evaluate the possible alterations in hepatic and renal functions as influenced by the extracts. The clinical significance of serum biochemical parameters; as

physiological indicators of stress in animals, is well established [29]. Measurement of serum biochemical parameters can be especially useful to help identify the target organs of toxic effects as well as the general health status of animals, and it is advocated to provide early warning of potentially deleterious changes in stressed organisms [30].

Biochemical evaluation of hepato-renal functional indices is important because kidney and liver toxicity has been reported following the use of phytotherapeutic products [31]. Hepatic and renal functions are crucial, with one being used for the metabolism of ingestion and the other for excretion of the waste product, respectively [32]. To evaluate the toxicity of any new compound, it is essential to know the state of these two vital organs, which can be verified by biochemical estimation [32]. In this study, biochemical parameters, which include: Urea, creatinine, albumin, total protein, ALP, ALT and AST were analysed and used to assess renal and hepatic functions.

There are many enzymes found in the serum that did not actually originate from the extracellular fluid. During tissue damage, some of these enzymes find their way into the serum, probably by leakage [33]. Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. Serum liver function tests provide insight into the state of the liver and include ALT, AST and ALP [34]. The aminotransferases (ALT and AST) describe its cellular integrity; alkaline phosphatase (ALP) describes its link with the biliary tract while albumin and total protein levels describe its functionality [35]. They are frequently used to diagnose or screen for hepatobiliary diseases, examine the progression of the disease as well as to monitor or detect the hepatotoxicity that may arise from the use of drugs or substances [36]. Enzymes are sensitive indices of cellular injury and are elevated above normal from tissue leakage before changes are noted with clinical and histological tests [37]. A significantly high level of liver enzymes is sign of hepatocellular disease or toxicity [38], while a decrease in the serum levels may indicate enzyme inhibition [39]. These enzymes are usually found in large quantities in the liver where they play an important role in the metabolism of amino acid [40] and may leak from the hepatocytes into the circulation where their levels become elevated due to damage or toxicity to the liver [41]. However, ALT is the most sensitive marker of liver damage or toxicity since AST is also found in abundance in kidneys, testes, cardiac and skeletal muscles, and ALP is also abundant in growing bone [42]. In this study, AST levels were increased significantly at all the doses of the extract when compared with control. This suggests that the extract may be toxic to the liver at the dose given to the rats for the period of 14 days. This, with the liver histopathological findings confirmed the possible hepatotoxic effect of this plant as reported in this study. These findings corroborated that of Whitehead *et al*., [40]. Apart from liver enzymes, the functionality of the liver can also be assessed by the serum protein, globulin and albumin levels since they are synthesized and metabolised by the liver [43]. A reduction in serum levels of proteins, globulin and albumin is a sign of reduced synthetic function, which occurs in liver disease or damage, but an increase may occur in cancerous conditions, or following high protein diet [44]. In this study, there were significant increase in the serum total protein and albumin levels in the extract treated groups compared to the control group. This suggests hepato-toxic nature of the extract.

The biochemical indices of the kidney such as electrolytes, creatinine, urea and bilirubin levels as well as the synthetic products of the liver like albumin and protein can be used as 'markers' for assessing the functional capacities of the organs [45]. The plasma concentration of urea and creatinine has been used as markers of the glomerular filtration rate or renal function [46]. Creatinine is the most used indicator of renal function, and a high value of urea is indicative of acute renal dysfunction whilst an increased level of creatinine is indicative of chronic renal dysfunction [47]. Several extra renal factors influence the circulating urea concentration limiting its value as a test of kidney function. For example, plasma urea concentration is increased by high protein diet, increased protein catabolism and dehydration. In these pre-renal situations, the plasma creatinine concentration is usually normal [48]. The raised plasma urea seen in the treated rats could be because of dehydration

or extract induced. In this study, there were increases in both urea and creatinine levels, these increases were statistically significant and thus suggest that the herb may be nephrotoxic especially at the highest dose.

The kidneys and liver have fundamental roles in the metabolism and excretion of drugs or plant products. During the biotransformation process of drugs and plant products, there is the generation of reactive metabolites, and the presence of secondary metabolites in the plant materials that may result in toxicity or cell and tissue damage on these two organs [49]. Histopathological investigation of these organs therefore may reveal changes caused by novel drugs that may not be easily revealed by hematological and biochemical markers. Some of the main histopathological changes observed under a microscope during hepatotoxicity include necrosis, fatty changes, congestion, lyses in the blood cells, WBC infiltration, and vascular lesions around the central vein or sinusoids of the liver [50,51]. The current study revealed similar changes in rats treated with the extract at all three doses such as multifocal hepatocellular degeneration, necrosis and inflammation. Therefore, the oral administration of the extract appears to have caused damage to the liver tissue and this corroborate with the elevation of the biochemical enzymes.

Histopathological changes observed in the microscopic examination of the kidney section include necrosis, urinary space obliteration, an increase in cellularity of the glomerulus, tubular degradation, hydropic changes, loss of microvilli, and inflammatory cellular infiltration [50]. In the present study, there were no such changes in the kidney of rats treated with the extract at all three doses. This indicates that oral administration of the extract has no marked effect on the kidney tissue of rats at the studied doses. This however does not agree with the findings from the biochemical parameters investigated in the current study. Thus, the observed changes in blood urea nitrogen and creatinine may have been induced by other factors rather than the extract.

5. Conclusion

Conclusively, the ethanolic leaf extract of *EH* altered the liver biochemical and histology architecture while selectively altering kidney biochemical parameter of male Wistar rats investigated in this study. This study therefore revealed that the extract has mild and dose specific hepato-and nephrotoxic effects and may not be completely safe as an oral remedy in male rats. thus, the need for further mechanistic studies.

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