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Hepatotoxic effects of Euphol-rich fractions from *Euphorbia bivonae*—Relevance to cytotoxic and anti-tumor activities

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ABSTRACT

These studies were designed to evaluate the preliminary oral toxicity profile of the crude ethanolic aerial part extract of *E. bivonae* in the Male albino Wistar rats and its active chemical constituents. The 24-h LD_{50} was determined using probit analysis method. The single dose $LD_{50}$was found to be 2568.64 mg/kg bw when administrated orally in mice. Additionally, the Wistar rats were used to evaluate the subchronic toxicity of *E. bivonae* ethanolic extract. The serum biomarkers, lipid peroxidation and antioxidants status in liver and histopathological analysis were investigated in normal and treated groups. Subchronic toxicity studies in rats with oral doses of 50, 150, 350 and 500 mg/kg body weight showed significant increase in alanine aminotransferase, aspartate aminotransferase and total bilirubin levels. In addition, the administration of this extract significantly ($p < 0.05$) decreased superoxide dismutase, catalase and glutathione peroxidase and an increment in lipid peroxidation and protein carbonyls. Finally, we suggest that the three compounds of *E. bivonae* extract (sitosterol, euphol and lupeol) are the mainly responsible of this toxicity.

1. Introduction

The study of the toxicities of medicinal plants is important in predicting their safety. Most of the methods used in the evaluation of acute and subacute toxicities of chemicals are based on the routes of administration/exposure to humans: oral; dermal; intrapéritonéale [1], inhalation; and the eye irritation assays [1]. Toxic plants compound can affect several organ and systems. Additionally, pharmacological and toxicological studies of medicinal plants are essential for drug safety and development [2–4]. According to the World Health Organization (WHO), more than 4 billion people worldwide (or 80% of the global population) use medicinal plants in the primary health care [5]. However, evidences suggest that although the adverse effects of using medicinal plants are less frequent than those from conventional drugs, scientific studies confirm that they exist [6]. This fact was corroborated with the 18,579 cases record of plant poisoning in Brazil, between 1999 and 2009 (National System of Toxico-Pharmacological Information - Sinitox, 2011). The genus *Euphorbia* (Euphorbiaceae) is composed of more than 8000 species, which are distributed all over the world especially in Africa and Central and South America. The most species of this family are used in traditional medicines, on the treatment of tuberculosis, tumour, and chronic tracheitis [7,8] and are scientifically reported for its antiviral, antimicrobial, anticancer, cytotoxic, and antitumor activities [9]. In Tunisia, *Euphorbia* has been used folk medicine for rheumatism, swelling, and especially as a wart remover. However, this family has different compounds responsible for their toxic properties such as the lectins and esters of certain diterpene alcohols, phorbol derivatives, tigliane, daphnane, and ingenane diterpene ester toxins [10]. Thus, this medicinal plant has been found to be potentially toxic, mutagenic, carcinogenic and teratogenic. However, the potential toxicity of herbs has not been recognized by the general public or by traditional healers. This plant has been reported to induce severe alterations in liver and kidney tissues [11]. Although some poisoning from medicinal plants are related to misidentification of medicinal plants used for extract preparation, inadequate preparation, and inappropriate administration [12], other cases are related to the presence of toxic substances in the medicinal plants. Several medicinal plants from Africa have been implicated in the etiology of liver diseases, and hepatotoxicity related to medicinal plants may be higher than

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actual expectations. This raises concern about the potential toxic effects resulting from the short-term and long-term use of such medicinal plants. Therefore, evaluating the toxicity effects of any medicinal plants extracts intended. In these tests a living tissue, organism, or group of organisms is used as a test organism for the determination of the potency of any physiologically active substance of unknown activity [1]. For example, the toxicity effects of many members of the Euphorbiaceae family have been shown on molluscicidal activity [13].

This investigation was designed to study the toxic effect of *E. bivonae* ethanolic extract on liver in the Male albino Wistar rats and its impact on antioxidant enzymes activities (SOD, CAT, and GPx), oxidative stress markers levels and histopathological section of the rats liver.

2. Materials and methods

2.1. Plant materials

Aerial part from *Euphorbia bivonae* Steudel were collected in Mai 2014 in Djebel Bou Ramli (34°31′8″ N and 8°32′48″ E), Gafsa (South Tunisian).. A herbarium voucher specimen was deposited in the herbarium of the Facultat de Farmàcia, Universitat de Barcelona (BCF) (BCF 38,639).

2.2. Extraction procedure

The aerial part of this plant (500 g) was powdered and extracted for 24 h with 1000 ml of ethanol (80%) at room temperature, followed by rapid paper filtration through Whatman 0.45 mm filter paper. The resulting solutions were evaporated under vacuum at 60 °C by a rotary evaporator (Büchi Rotavapor R-200, Büchi Heating Bath B-490). This fraction was then stored at −20 °C until use.

2.3. GC/MS analysis of ethanolic extract

The GC–MS analysis was performed in the conditions previously optimized. The chromatographic system used was a DB-5MS (5%-phenylmethylpolysiloxane) fused silica capillary column of dimensions 30 m × 0.25 mm i.d. × 0.25 μm film thikness supplied by Agilent. Data acquisition was performed with Agilent Enhanced MDS Productivity Chem Station software (version E.02.02). The optimized chromatographic conditioned were: oven temperature program 250–300 °C/min (hold 15 min); injector temperature 280 °C; carrier gas helium at a constant flow rate of 1 ml/min; injection volume 2 μl with a split ratio of 20:1. The EI spectra were recorded at 70 eV from *m/z* 40 to 600 in a scan mode. The MS source was set to 230 °C, the quad rupole temperature was 150 °C, and the transfer line temperature was set to 280 °C. Wiley and NIST library (version 2.0) was used to assist in compound identification. Further identification was based on the relative retention times compared with the reference standards and the literature values. The fraction (1 mg) were accurately weighed and diluted of ethanol using calibrated micropipettes.

2.4. Experimental animals

Male albinos Wistar rats (140–200 g) and Swiss albino mice (25–30 g) used in the present study were obtained from the Central Pharmacy (SIPHAT, Tunis, Tunisia). They were maintained under standard laboratory conditions (temperature 22 ± 2 °C; 12 h light–dark cycle). The animals had free access to water and commercial standard pellet diet and acclimatized for 1 week before the experiment design. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the Ethical Committee of the Sfax Faculty of Sciences.

2.5. Toxicological bioassay

2.5.1. Acute toxicity study in mice

The mice (25–30 g), fasted for 12 h, were weighed and divided into 12 groups of 10 in each. The ethanolic extract was administrated at increasing doses of 5 mg/kg bw (5–7000 mg/kg) by oral route (Hodge and Sterner Scale). After administration of the extract, the animals were observed continuously for 24 h. Changes in the normal activity of mice due to acute toxicity were monitored. The lethal dose that killed 50% of the mice was estimated after 24 h, applying the method of Finney [14]. The experimental protocols were conducted in accordance with the guide for the care and use of laboratory animals issued by the University of Sfax, Tunisia, and approved by the Committee of Animal Ethics of Sfax.

2.5.2. Subchronic toxicity studies in rats

A total of 25 male albino Wistar rats (150–200 g) were divided into four groups and used for subchronic toxicity studies. The rats were housed in polypropylene cages under identical animal house condition and provided with standard pellet diet and water ad libitum. These groups were control (group 1). Groups 2–4 at daily treatment doses of 50 is equivalent to 1.94% of the oral LD_{50}), 125 (is equivalent to 4.86% of the oral LD_{50}), 350 (is equivalent to 13.62% of the oral LD_{50}) and 500 mg/kg bw (is equivalent to 19.47% of the oral LD_{50} from the acute toxicity study in mice), respectively.

All groups received daily orally administered with respect to each treatment, by using gavage technique, through five consecutive weeks. During experimental period, all animals were checked daily for behavior and general health conditions. They were caged individually and kept under standard laboratory conditions (temperature 25 ± 1 °C, natural light-dark cycle). The rats had free access to drinking water and commercial standard laboratory diet. At the end of the experimental period, all animals were anesthetized by ketamine–xylazine (KX) and scarified by decapitation for blood and tissue collections.

Blood was collected from the jugular vein into plain centrifuge tubes and was allowed to stand for 1 h. Serum was prepared by centrifugation at 3000 rpm for 15 min. The supernatant was used for the estimation of biochemical indices. Subsequently, the abdomen of each rat was surgically opened and liver was quickly removed and washed in ice-cold 1.15% Kcl solution to remove blood stain, dried and weighted. Part of these tissues were fixed with 10% of phosphate buffered neutral formalin, dehydrated in graded (50–100%) alcohol, embedded in paraffin and used for histology. The remaining tissues were homogenized and centrifuged at 3500 rpm for 15 min. the supernatant were used immediately for the biochemical tissue analysis.

2.6. Biochemical assays

2.6.1. Estimation of plasma markers of hepatic damage

The activities of serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP) and serum total bilirubin (TB) were estimated according the procedures using commercially kits (Biomaghreb, Ariana, Tunisia. Moreover, Red blood cell (RBC), white blood cell (WBC), differential leucocyte counts, packed cell volume (PCV), haemoglobin concentration (Hb), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were determined in an automatic hematological assay analyzer (Beckman Coulter, USA).

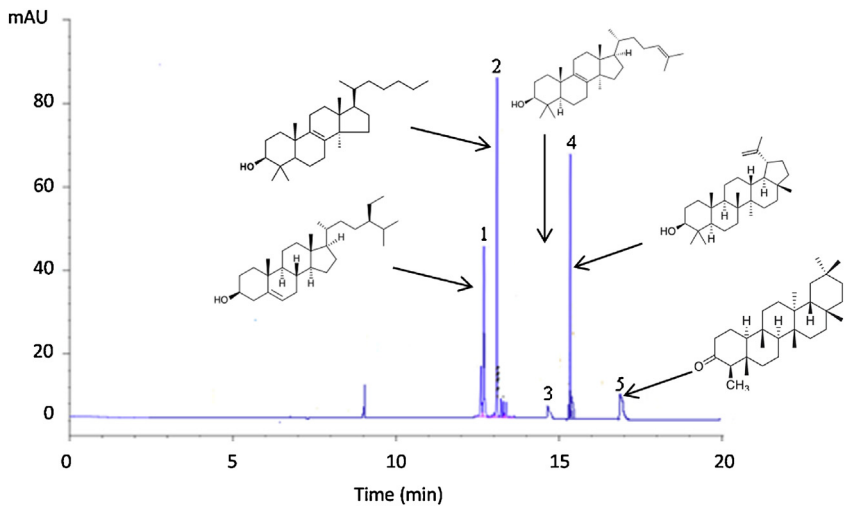

Fig. 1. Compounds analysis of *Euphorbia bivonae* crud extract. Peak assignment: 1. sitosterol; 2. euphol; 3. lanosterol; 4. lupeol; 5. friedelin.

2.6.2. Oxidative stress markers in liver tissues

The malondialdehyde (MDA) level in liver tissues was measured using the protocol described by Niehaus and Samuelsson [15]. Briefly, 0.5 ml of liver supernatant was mixed with 1 ml of trichloroacetic acid solution and centrifuged at 2500 × g for 10 min. Then, 1 ml of thiobarbituric acid (TBA) solution (0.67%) and 0.5 ml of supernatant were incubated for 15 min at 90 °C. Finally, the absorbance of TBA–MDA complex was measured at 532 nm.

Total protein carbonyl level in liver supernatant of different groups was determined according the method developed by Levine et al. [16]. The results were expressed as nmol/mg protein.

2.6.3. Protein content

Proteins were quantified by Lowry et al. [17] method, using a bovine serum albumin (BSA) calibration curve. Absorbance was measured at 700 nm.

2.6.4. Enzymatic antioxidant status

Homogenates of liver was used for the determination of superoxide dismutase activity was estimated by the method developed by Beauchamp and Fridovich [18]. The reaction mixture concentration 50 mM of liver tissue homogenates in potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 2μM riboflavin and 75 mM nitro blue tetrazolium (NBT). The absorbance was detected at 560 nm and the results are expressed as units (U) of SOD activity/mg protein. The catalase activity (CAT) was determined by the protocol described by Aebi [19]. Glutathione peroxidase (GPx) activity was determined according to protocol described by Flohe and Gunzler [20]. The enzyme activity was expressed as units of GPx activity/min/mg protein.

2.7. Histology of liver

For histological studies, the liver tissues were fixed with 10% phosphate buffered neutral formalin, dehydrated in graded (50–100%) alcohol and embedded in paraffin. Thin sections (5 μm) were cut and stained with routine hematoxylin and eosin (H & E) stain for photo microscopic assessment.

2.8. Statistical analysis

Mean values of various treatments were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics 20.0 Package Program. Significance level was determined ($p < 0.05$) and significant difference was separated using Duncan's Multiple Range Test (DMRT). The LD_{50} values were calculated by probit analysis with a reliability interval of 95%.

Table 1
Average estimated LC values and confidence limits.

Exposure		95% confidence limits	
Point	Concentration (mg/kg)	Lower	Upper
LD_1	18.51	11.45	22.12
LD_5	78.54	63.45	84.75
LD_{10}	169.67	142.71	201.32
LD_{15}	285.32	201.46	307.45
LD_{50}	2568.64	2415.47	3214.12
LD_{85}	8124.36	1213.62	5321.61
LD_{90}	12884.17	3321.37	16789.87
LD_{95}	17006.66	8954.46	21456.08
LD_{99}	26324.37	18123.11	87307.42

3. Results

3.1. GC/MS analysis

The compositions of *Euphorbia bivonae* extract were determined and the result was given in Fig. 1. In our investigation five compounds were determined in this plant species. In this study euphol was the dominant compounds. Sitosterol; euphol; lanosterol; lupeol; friedlin found in *E. bivonae* extract. Minor compound including lanosterol and friedelin.

3.2. Mice lethality assay

After 24 h exposure, the polar extract (ethanolic fraction) of *E. bivonae* exhibited the highest toxicity on the mice. The LD_{10}, LD_{50} and LD_{90} values and their corresponding LD_{99} values obtained through probit analysis of the bioassay data of these samples are presented in Table 1. The oral administration of this extract was produced mortality in mice from to 18.5 mg/kg bw. The single dose LD_{50} was found to be 2568.64 mg/kg bw (Fig. 2).

3.3. Subchronic toxicity

3.3.1. Biochemical and hematological analyses

Results shown in Table 2 clearly revealed the serum hepatic marker enzyme levels and total bilirubin of normal and

Table 2
Serum ALT, AST, ALP and total bilirubin of the studied groups.

Treatment	Dose (mg/kg)	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	Bilirubin (mg/dl)
Group I	Control	93.51 ± 5.46^{a}	92.63 ± 3.41^{a}	271.31 ± 8.07^{a}	0.63 ± 0.07^{a}
Group II	50	107.31 ± 4.12^{b}	101.21 ± 2.84^{b}	294.45 ± 6.51^{b}	1.12 ± 0.11^{b}
Group III	125	112.63 ± 7.51^{c}	111.08 ± 5.87^{c}	308.15 ± 4.64^{c}	1.32 ± 0.09^{c}
Group IV	350	127.22 ± 3.62^{d}	116.31 ± 1.74^{d}	318.71 ± 3.42^{d}	1.39 ± 0.12^{d}
Group V	500	141.19 ± 8.71^{e}	121.13 ± 4.31^{e}	332.66 ± 2.85^{e}	1.51 ± 0.05^{e}

Resultsare expressed as mean of three experiments ± SD. Values bearing the same letters showed no significant difference($P < 0.05$). The results are sorted in crescent order: a < b<c < d<e.

Table 3
Effect of *Euphorbia bivonae* ethanolic extract on hematological changes after subchronic treatment in rat.

Treatment and parameters	Hb (g/dl)	RBC ($\times 10^6$ mm^3)	PCV (%)	MCV (m^3)	MCHC (%)	WBC ($\times 10^3$ mm^3)	Neutrs (%)	Lymphs (%)
Control	12.78 ± 2.31^{e}	7.08 ± 1.22^{e}	41.05 ± 2.31^{e}	61.32 ± 2.45^{a}	31.43 ± 2.65^{e}	6.75 ± 0.52^{e}	55.62 ± 1.56^{e}	46.23 ± 2.31^{e}
50 mg/kg	11.07 ± 1.56^{d}	6.32 ± 0.82^{d}	39.63 ± 2.45^{d}	64.37 ± 1.45^{b}	30.12 ± 2.16^{d}	6.41 ± 0.25^{d}	52.42 ± 2.45^{d}	43.56 ± 1.45^{d}
125 mg/kg	10.32 ± 2.05^{c}	5.72 ± 0.23^{c}	38.34 ± 1.67^{c}	66.08 ± 3.56^{c}	27.57 ± 2.53^{c}	5.08 ± 0.35^{c}	47.63 ± 1.72^{c}	40.12 ± 2.42^{c}
350 mg/kg	9.56 ± 0.78^{b}	4.56 ± 0.41^{b}	36.74 ± 3.07^{b}	72.45 ± 3.14^{d}	21.65 ± 1.74^{b}	4.82 ± 0.42^{b}	42.73 ± 1.87^{b}	36.75 ± 1.37^{b}
500 mg/kg	8.04 ± 1.08^{a}	4.12 ± 0.17^{a}	30.25 ± 2.41^{a}	73.68 ± 4.45^{e}	18.72 ± 2.32^{a}	4.26 ± 0.53^{a}	40.12 ± 1.64^{a}	33.56 ± 0.87^{a}

Results are expressed as mean of three experiments ± SD. Values bearing the same letters showed no significant difference ($p < 0.05$). The results are sorted in crescent order: a < b<c < d<e.

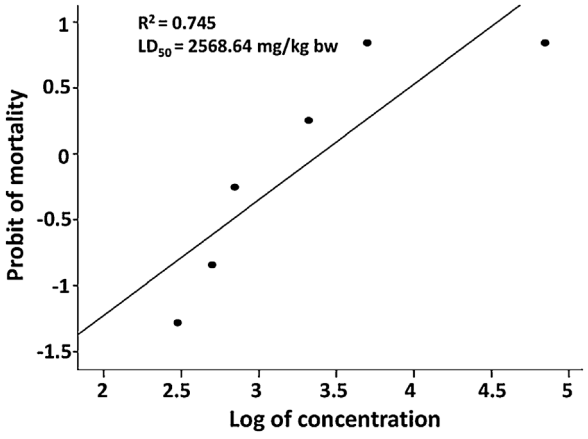

Fig. 2. Toxic effects of the *E. bivonae* ethanolic extract after 24 h using mice lethality assay.

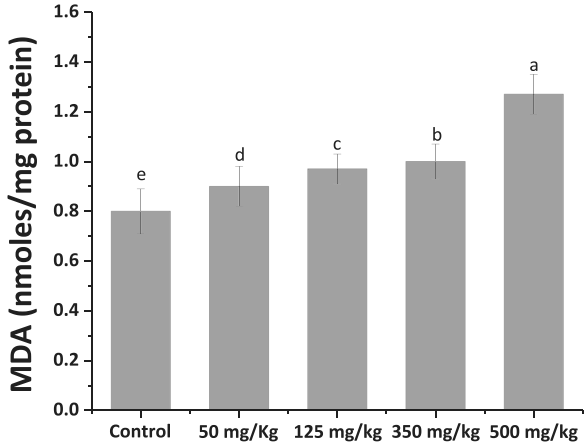

Fig. 3. Effect of *E. bivonae* ethanolic extract on lipid peroxidation in rats. Values are mean of six determinations (n = 6); ± standard deviation. Bars having different letters are significantly different ($p < 0.05$). The results are sorted in crescent order: a < b<c < d<e.

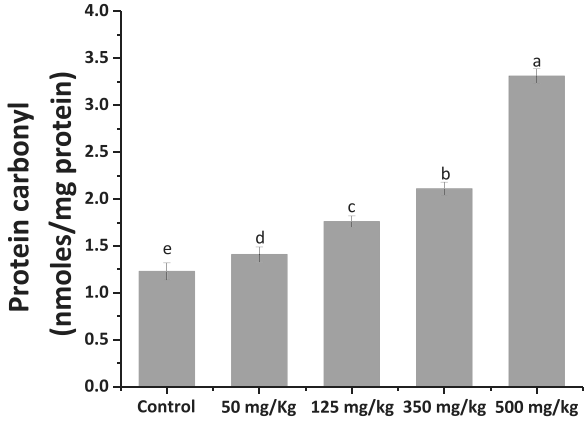

Fig. 4. Effect of *E. bivonae* ethanolic extract on protein carbonylation in rats. Bars having different letters are significantly different ($p < 0.05$). Values are mean of six independent determinations (n = 6); ± standard deviation. The results are sorted in crescent order: a < b<c < d<e.

treated rats. Oral administration of ethanolic fraction induce significantly ($p < 0.05$) increased in hepatic serum markers. The aspartate transaminase, alanine transaminase and the total bilirubin (TB) levels were significantly increased ($p < 0.05$) in *E. bivonae*- intoxicated rats, to compare with control group. The hematological parameters including Hb, RBC, PCV, MCV, MCHC, total WBC, neutrophils and lymphocytes in the rats treated with the ethanolic extract of *E. bivonae* up to 500 mg/kg/day differ significantly ($p < 0.05$) from that of the control group (Table 3).

3.3.2. Lipid peroxidation and protein oxidation indices

The hepatic lipid peroxidation and protein carbonyl contents of hepatic supernatant in control and treated rats are given in Figs. 3 and 4, respectively. The levels of lipid peroxidation (LPO) and protein carbonyl content (PCC) were significantly enhanced ($p < 0.05$) in ethanolic extract-treated rats when compared with normal rats.

3.3.3. Enzymatic antioxidant status in liver

The change levels of antioxidant enzymes SOD, CAT and GPx in the hepatic supernatant of the experimental rats have been shown in Fig. 5. Our results showed that the *E. bivonae* ethanolic extract administration significantly ($p < 0.05$) decreased the activities of the antioxidant enzymes compared to the control rats. Accordingly, ethanolic extract (500 mg/kg) treatment show the maximum decrease in CAT, SOD and GPx by (-25%), (-42.3%) and (-29.76%), respectively, compared to those of control group.

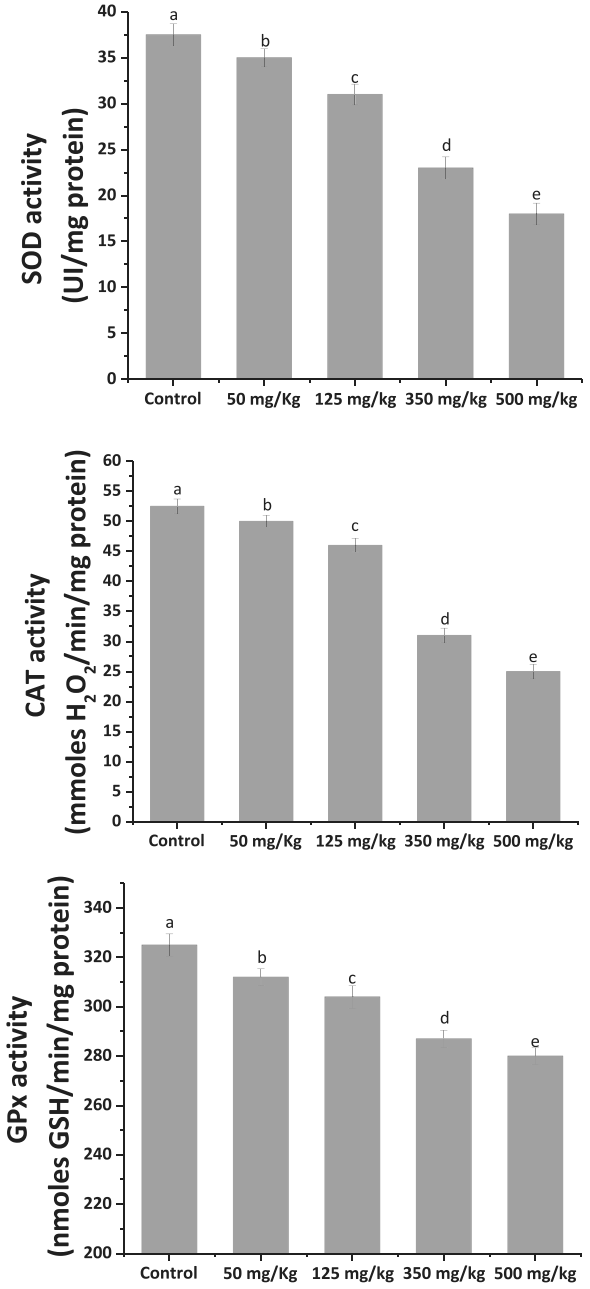

Fig. 5. Antioxidant enzymes activities in liver tissues of the studied groups. A, SOD; B, CAT and C, GPx. Values followed by different superscript in each column are significantly different ($p < 0.05$). The results are sorted in crescent order: a<b<c<d<e.

3.3.4. Histological observations

Fig. 6 represents the histological section of the hepatic tissue of the control and treated groups. Control liver sections showed a normal hepatic architecture. However, ethanolic extract exposure induced severe pathological alterations in hepatic tissues, these lesions in the form of the parenchyma dilation. Extensive degeneration of hepatocyte with focal dilatation in the liver was also detected.

4. Discussion

Many indigenous plants are used without the actual understanding of their potential for toxicity [21]. The safety of herbal medicines used in third world and economically disadvantage countries is a major concern although not frequently addressed in the literature. However, the biotransformation process bioactivities some chemicals to render them more hepatotoxic. Although some poisoning from medicinal plants are related to misidentification of medicinal plants used for extract(s) preparation, inadequate preparation, and inappropriate administration [22]. However, because toxic plant extracts necessarily contain physiologically active constituents, further research should concentrate on the isolation of those compounds responsible for the antitumor activity. Previously, several medicinal plants have demonstrated combinatory antioxidant and anti-proliferative effects [23]. This cytotoxic effect of *E. bivonae* may be explained by the presence of some steroids molecules such as sitsoterol and lanosterol and highest euphol concentration. These results of our study are consistent with the observation by Awad et al. [24], who showed that the cytotoxic effect attributed to steroid molecules. Gupta et al. [25] also found that the steroids molecules have some compatibility with nuclear receptor; and can act enzyme inhibitors, cytotoxic molecules. Nascimento et al. [26] reported that the *Euphorbia* species it was shown that these cytotoxic activities were associated with terpenoids. Moreover, Luz et al. [27] indicated that the euphol compounds have a cytotoxic effect. The terpenoids euphol is one of the distinctive and active in this herb, with cytotoxicity. In addition, phenolic compounds of *Euphorbia lagascae*, *Euphorbia tuckeyana*, and *Pycnanthus* were mentored antiproliferative activity [27]. Luz et al. [27] also revealed that *Euphorbia umbellate* extract induced apoptosis process in Jurkat cancer cell line. Moreover, Kwan et al. [28] found that *Euphorbia hirta* extracts stimulated growth inhibition of MCF-7 cells. This plant shows a very significant cytotoxicity activity. But, toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules.

The results of the mice lethality test indicated different indices of toxicity. In the present finding, the aerial part extract of *Euphorbia bivonae* could be characterized slightly toxic. The main compounds responsible for the toxic properties of Euphorbiaceae are the lectins and esters of certain diterpene alcohols. In addition to phorbol derivatives, tigliane, daphnane, and ingenane diterpene estertoxins are found in *Euphorbia* species [29]. The toxicity of this plant was suggested to be due to the presence of indole diterpenoids such as euphol [30]. Similar to this result, Li et al. [31] revealed that euphol compound acted on the intestinal smooth muscle with propulsion of feces involving the irritation of the intestines with acute inflammatory reactions. In the present study, the oral administrations of the plant extract induced elevation on biochemical hepatic parmeters. These findings were supported by elevated activities of AST, ALT and ALP and concentration of total bilirubin in the serum. This extract affects cellular membrane integrity, resulting in pores in the cell membranes. AST, ALT, ALP and bilirubin are generally present in the hepatic cells, when hepatic injuries occur these enzymes leak out from the cells into the blood stream leading to elevated levels in serum [32].Therefore, the elevation of ALT, AST, ALP and bilirubin levels in serum could be explain by severe hepatic alterations [33]. Our results showed a significant increases in serum AST, ALT, ALP and total bilirubin levels in the groups receiving *E. bivonae* extract compared to those of the control groups. Accordingly Dzoyem et al. [34] reported that *Diospyros canaliculata*m ethanolic extract significantly ($p < 0.05$) enhanced AST, ALP and AST activities in serum at 2 g/kg bw (rats). Atsafack et al. [35] also found that the *Pteleopsis hylodendron* extract significantly increased in liver enzymes (ALT and AST). Similarly, the oral administration of the ethanolic extract at 50, 125, 350 and 500 mg/kg bw/day for 30 days induced changes in hematological parameters. The decreases in PCV, Hb and RBC indicated anaemia. Our result is in consistent with Al-Yahya et al. [36], who reported that the decrease in PCV, Hb and RBC is a response of the organ to the toxic extract. Additionally, Maphosa et al. [37] have also reported that the of *Leonotis leonu-*

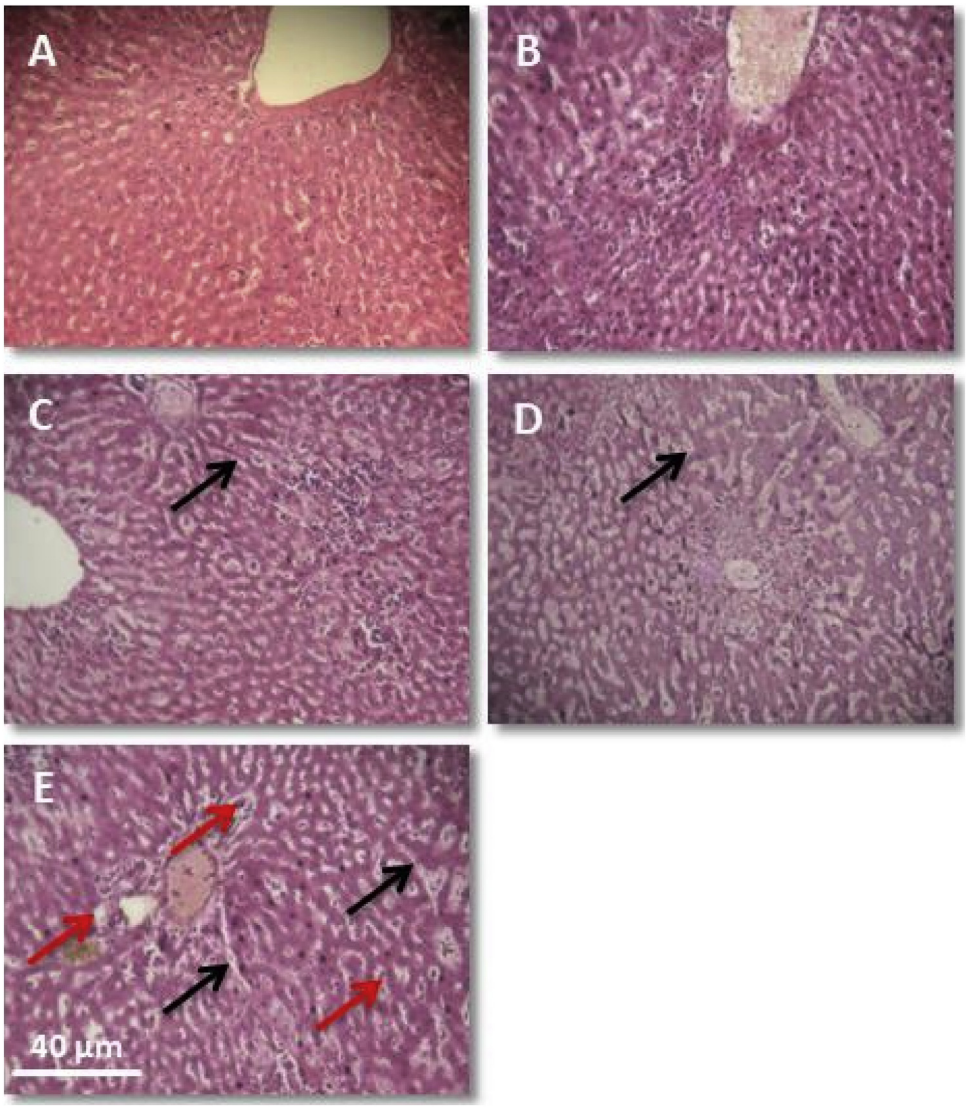

Fig. 6. Paraffin section photograph(s) of rat liver controls and experimental group. (A) Control group. (B) Group treated with ethanolic extract (50 mg/kg) showing near normal liver tissues. (C) Group treated with ethanolic extract (125 mg/kg). (D) Ethanolic extract (350 mg/kg)-treated rat liver. (E) Ethanolic extract (500 mg/kg)-treated rat liver. All sections were stained with Hematoxylin/eosin; 200 × for all panels. Black arrows indicate extensive parenchyma dilation of hepatocytes. Red arrows indicate extensive hepatocyte degenerative. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rus extract induced a significant changes in RBC, PCV, hemoglobin concentration, mean corpuscular volume, platelets and WBC.A significant increase in body temperature and a significant decrease in the RBC count, Hb count, and hematocrit values was observed with of *Ficus exasperate* ethanolic leaf extract [38].

Similar to biochemical markers, the MDA level significantly increased in the groups treated with *E. bivonae* extract. This elevation in MDA concentration indicated that the *E. bivonae* extract is capable of inducing oxidative stress. Direct free radical damage to membrane proteins may occur as a result of lipid peroxidation leading to their activation [39]. In the present study, hepatic protein carbonyl contents significantly increased *E. bivonae* extract treated groups. The antioxidant enzymes such as SOD, CAT and GPx present the first line of defense against free-radical damage [40]. In the present finding, the antioxidants status; SOD, CAT, and GPx were significantly ($p < 0.05$)reduced in hepatic tissues of plant-treated rats in comparison to the control group, which indicated that this plant was able to induce oxidative stress. The inhibitory action of ethanolic extract on SOD may be due to competition between biomolecules of this extract and Zn or Cu that is required for this enzyme. Catalase is a hemeprotein, which metabolizes excess hydrogen peroxide to water and oxygen. The decrease in catalase activity by ethanolic extract may be attributed to the decreased absorption of iron, an essential trace element required for the activity of catalase.

Furthermore, these results of hepatic biochemical markers are confirmed by a histopathological study. This plants altered several histological parameters in experimental animals. *E. bivonae* extract caused extensive degeneration of hepatocytes with focal necrosis, vacuolation and inflammatory cell infiltrations in portal region in the liver were also observed. In addition, several studies indicate that the Euphorbiaceae family induced liver alterations [41].Our results are consistent with the observation by Okoye et al. [42], who indicated that of *Annonas enegalensis* aqueous extract showed degeneration and necrosis of the hepatocyte cells.

5. Conclusion

E. bivonae is a medicinal plant with wide range of medicinal properties. Despite its potential activity still now no work has been

done to evaluate the medicinal properties of the plant. On the basis of pharmacological findings (antioxidant, antimicrobial, antitumor) of *E. bivonae* extracts, it was selected to assess toxic activity. In this study, the toxicity of *E. bivonae* ethanolic extract was noticed to be dose-dependent. Furthermore, ethanolic extract of *E. bivonae* rich in toxic compounds induced hepatotoxicity by decreasing antioxidant enzyme activities, stimulating lipid peroxidation and increasing the levels of hepatic marker. In future, work will be done to isolate bioactive constituents of fresh *E. bivonae* extract to locate potential pharmacological agents.

Competing interests

The authors declare that they have no competing interests and non-financial competing interests.

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