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Pharmacognostic Evaluation and Hepatoprotective Property of Crude and Fractions of *Triumfetta cordifolia* A. Rich. (Tiliaceae)

Chikeokwu Ikenna [a], Ene Erica Chiamaka [a], C. Mba Theodora [a*] and Uche Estella Odoh [b]

[a] Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu, Nigeria.
[b] Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: The role of phytochemicals in modern medicine is abundant with more evidence being published. *Triumfetta cordifolia* is a local plant known for its vast medicinal applications in traditional medicine with limited documented evidence. This study thus aims to evaluate the leaves of *Triumfetta cordifolia* to determine its Pharmacognostic parameters and hepatoprotective potential.

Methodology: The leaves were collected, identified, dried and subsequently pulverized before maceration in methanol. This was followed by fractionation using different solvent with increasing

**Corresponding author: E-mail: mba.theodora@esut.edu.ng;*

polarities (n-hexane, n-butanol, ethyl acetate and aqueous methanol). Physicochemical evaluations such as macroscopy, organoleptic tests, qualitative phytochemical analysis and total phenolic content was subsequently conducted on the extracts. Finally, an acute toxicity test and hepatoprotective evaluation was performed, the latter using carbon tetrachloride.

Results: The leaves *Triumfetta cordifolia* on macroscopic evaluation shown a simple, alternate, stipules triangular, densely stellate hairy leaves. The organoleptic evaluation indicated a pungent aromatic smell and a slightly bitter taste. Several phytochemicals such as tannins, saponins, glycosides, steroids and flavonoids were identified qualitatively.The extracts was safe at a dose less than 5,000mg/kg. The ethyl acetate fraction showed consistent hepatoprotective activity at a concentration of 400 mg/kg when compared to Gallic acid (positive control). This was possibly due to its high composition of phenolic compounds which was recorded during the study.

Conclusion: The ethyl acetate fraction of *Triumfetta cordifolia* leaves exhibited a hepatoprotective effect thus showcasing the potential benefits of the plant in mitigating liver damage. More research is however needed to identify specific compounds being extracted that may be responsible for the activity recorded in this study.

Keywords: Triumfetta cordifolia; hepatoprotective; phytochemical; ethyl acetate; pharmacognostic.

1. INTRODUCTION

Metabolism is a core function in all living organisms essential for the maintains of life. In humans, the liver plays major roles in different forms of metabolism; from modulating the concentration of glucose through reversible conversion to glycogen to making xenobiotics more water-soluble thus promoting their elimination [1]. In adults, the liver weighs about 1.4 kg comprising hepatocytes which generate a wide variety of metabolically active enzymes and are arranged in lobules within the liver [2]. However, the high rate of blood perfusion to this organ from the portal vein and hepatic artery positions it to maximally utilize its enzyme diversity and has been implicated in the first-pass metabolism of drugs.

With an increase in the consumption of xenobiotics, from orthodox drugs (such as codeine, warfarin, propranolol, etc) to herbal formulations, and processed foods, the detoxification role of the liver is ever more important to eliminate harmful compounds [3]. This detoxification process is split into phase I and II reactions, characterized by processes such as oxidation and reduction (facilitated by Cytochrome P450 enzymes) for the former and glucuronidation, sulphation and acetylation for the latter [4]. Some drug metabolites are however more active than the parent drug, for example, desmethyldiazepam, a metabolite of diazepam is more active than the parent drug. This generation of reactive metabolites during liver metabolism has been attributed to the development of acute and chronic drug-induced liver injury (hepatotoxicity) with an estimated annual incidence of 13.9 – 24.0 per 100,000 inhabitants [5,6].

Hepatotoxicity is divided into intrinsic and idiosyncratic which the former being more dose-dependent such as in cases of acetaminophen toxicity [7]. This occurs due to the accumulation of N-acetyl-p-benzoquinone imine (NAPQI), a metabolic intermediate of acetaminophen, which exceeds the capacity of the glutathione stores to conjugate and eliminate [8]. Thus, the concept of introducing external antioxidants, either to replenish internal stores or other compounds with similar activity is the basis of the research interest in plants. Several plants have been noted in the literature to possess hepatoprotective including *Ficus carica, Alangium salviifolium, Carissa opaca*, among others [9]. These activities have been attributed to the presence of phytochemicals which exert biological action when used in man.

Triumfetta cordifolia is a shrub which grows in most parts of Africa including Nigeria. It has various ethnobotanical uses, for example, its roots are used to treat burns and diarrhoea, its leaves for easing childbirth and mitigating sterility in women and its bark for managing muscular pains [10]. These variety of uses could imply an abundance of varying phytochemicals within different parts of the thus, thus necessitating the generation of scientific evidence on the activity of the plant. An example shown by Ngondi *et al.* showed that the plant promoted weight loss in *in-vivo* models used in the experiment thus suggesting its possible benefit in managing metabolic disorders [11]. However, there is an absence of scientific evidence on the possible protective benefit of this plant on the liver and thus, this work was designed to evaluate the hepatoprotective potential of the leaves of *Triumfetta cordifolia* using an *in-vivo* model.

2. METHODS

2.1 Plant Collection and Identification

Triumfetta cordifolia A. Rich. was collected from Nsukka Local Government Area of Enugu State, Nigeria on January, 2022. It was identified by a taxonomist, Mr. Ozioko from the International Center for Ethnobotanical Medicine and Drug Development.

2.2 Extraction and Fractionation

Leaves weighing 1000g were collected from the harvested plants and pulverized. The powdered leaves were subsequently soaked in 5.5liters of methanol and left for 72 hours at room temperature of 25° ± 2°C with agitation at regular intervals. The extract was first filtered using a fine-grade cloth, followed with several bouts of filteration using Whatman No. 1 filter papers. The extract was concentrated to dryness under reduced pressure (below 40°C) using a rotary evaporator to yield the crude methanol extract. The crude methanol extract of *Triumfetta cordifolia* (50 g) was fractionated using a fractionating column with the silica gel acting as the stationary phase [12]. Organic solvents of increasing polarity such as n-Hexane, ethyl acetate, n-butanol and aqueous methanol were used as the mobile phase, to obtain the different fractions. The percentage yield of the extract was then determined and transferred into an airtight container and stored at 4° to 2°C in a refrigerator.

2.3 Macroscopical Analysis

Macroscopical studies of the specimen which comprised of organoleptic characters (colour, odour, appearance, taste, shape, texture) of the fresh leaves of *Triumfetta cordifolia* were evaluated following standard procedures as described by Trease and Evans [13].

2.4 Physicochemical Evaluation

Analysis of the physicochemical constants of the powdered leaves of *Triumfetta cordifolia* was determined such as total ash, water-soluble ash and acid-insoluble ash values were calculated as per WHO guidelines [14]. Alcohol and water-soluble extractive values, as well as moisture content and pH were also determined.

2.5 Qualitative Phytochemical Analysis

The leaves of *Triumfetta cordifolia* were tested to identify the presence of alkaloids, glycosides, steroids, terpenoids, flavonoids, saponins, tannins and reducing sugars [13,15,16].

2.6 Total Phenolic Content of the Extract and Fractions by Folin Ciocalteu's Assay

The total phenolic content of the extract and fractions were determined using the method described by Kim et al. [17]. The absorbance of the tested samples was read at 760 nm using a UV-VIS spectrophotometer against blank (prepared with only 7.6% sodium bicarbonate and distilled water). The total phenolic content was estimated from the calibrated curve which was made by preparing a Gallic acid solution and expressed as milligrams of Gallic acid equivalent (GAE) per gram of the extracts.

2.7 Acute Toxicity Test for Determination of Fixed Dose

The median lethal dose (LD_{50}) was carried out using the method described by Lorke. Eighteen mice were selected for this study, all weighing 17 to 23g. The animals were starved for 18 hours prior to the study and were only allowed access to water. The animals were divided into six treatment groups labelled groups 'A to F'. All treatments were administered orally. Groups A to C had 3 animals each. Group A received 10mg/kg of extract while groups B and C received 100mg/kg and 1000mg/kg respectively of extract. Groups D, E and F, each containing just one mice, received 1600mg/kg, 2900mg/kg and 5000mg/kg of extract respectively. The animals were observed for signs and symptoms of toxicity including mortality for 24 h after treatment. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, that is, the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded [18].

2.8 Evaluation of Hepatoprotective Effect

Seven groups consisting of six mice were prepared for the different fractions (n-hexane, ethyl acetate, n-butanol, aqueous methanol), the crude extract, and the positive and negative controls. For the fractions and crude extract, two doses were used (200mg/kg and 400mg/kg) while for the positive control (Gallic acid), a standard dose of 100 mg/kg was used. Of the 6 animals in each group (for the fractions and extract), 3 were administered 200 mg/kg while the remaining three were administered 400 mg/kg. The negative control comprised of the vehicle which in this case was 5% tween 80.

Groups 7 were administered orally, with the fractions, and controls accordingly, for 10 days after which hepatotoxicity was induced with carbon tetrachloride [CCl_4]. Blood was collected through an ocular puncture and the blood was centrifuged to obtain the serum. The ALP, ASP and ALT tests (liver function tests) were conducted using the mice's serum with standards kits with detailed instructions.

2.9 Statistical Analysis

The values of the parameters used to ascertain the hepatoprotective property of *Triumffeta cordifolia* such as concentration of ALP, ATP and AST enzymes in the serum were analysed. The data was subjected to descriptive statistics using SPSS statistical software. The data was then analyzed by one-way analysis of variance (ANOVA) and this was followed by multiple post hoc tests for comparing mean separation and to assess the statistical significance of the difference between the study groups. The data analysis was set at 95% confidence level; differences were considered statistically significant when P was less than 0.05.

3. RESULTS

The extraction process employed in our study yielded a 0.63% yield (6.3g). The leaves of *Triumfetta cordifolia* on macroscopic evaluation has shown to be simple, alternate, stipules triangular, densely stellate hairy leaves. The organoleptic evaluation indicated a pungent aromatic smell and a slightly bitter taste. Qualitative phytochemical analysis of our sample tested positive for all phytochemicals tested except reducing sugars and hydrogen cyanide as seen in Table 1. Water and methanol ranked first and second respectively in terms of extractive value as seen in Table 2. While the n-butanol fraction had the highest phenolic content, ethyl acetate exhibited higher reproducibility (with a smaller standard deviation) in the total phenolic content being extracted by the solvent (Table 3). Figs. 1 to 3 shows the results of the hepatoprotective evaluation with at least one dose of the ethyl acetate fraction significantly reducing all three liver function enzymes. On the charts, an asterisk was used to indicate the concentrations of the different fractions that showed a statistically significant ($p < 0.05$) reduction in the liver function enzyme when compared to the positive control (Gallic acid). The acute toxicity results also showed that the extract were safe at a dose less than 5000mg/kg.

4. DISCUSSION

Triumfetta cordifolia has been used in traditional medicine for various purposes with little evidence. These purported claims have been suggested to be due to the variety of phytochemicals isolated from the plant. Our study shows that saponins, tannins, steroids, glycosides and flavonoids were identified. Our findings were similar to another study which also tested for phytochemicals from the leaves of *Triumfetta cordifolia* with the exception of terpenoids which were isolated in this study [19]. While the plant was obtained from the same geographical area, other factors such as time of harvest and storage factors may be responsible for the slight variation in the results obtained [20]. Another study utilizing the fruits of *Triumfetta cordifolia was* additionally able to isolate alkaloids thus supporting the assumption of the diversity of the phytochemical constituents of the plant under study [21]. This also supports the evidence being generated indicating its potential benefit for treating and managing ulcer, diarrhoea and diabetes but considering that there are a lot of potential compounds existing within these phytochemical classes, it is, therefore, important to understand their distribution within the plant's parts which would further aid in understanding which plant extracts will be most effective for specific ailments.

When exposed to carbon tetrachloride (CCl_4), the ethyl acetate fraction consistently exhibited a statistically significant reduction in the serum liver function values (Figs. 1-3) which is supported by the high total phenolic content recorded for the ethyl acetate fraction (Table 3). This is due to the fact there is an established body of evidence supporting the antioxidant characteristics of phenolic compounds, including polyphenols and flavonoids [22-24]. For example, a study showed that the flavonols quercitrin, rutin and rosmarinic acid were responsible for the antioxidant activity of *Flacourtia indica, Calotropis procera* and *Zygophyllum hamiense*. However, when compared to n-butanol which recorded a higher peak total phenolic content, it only showcased better activity than the positive control in reducing alkaline phosphatase (ALP) in the

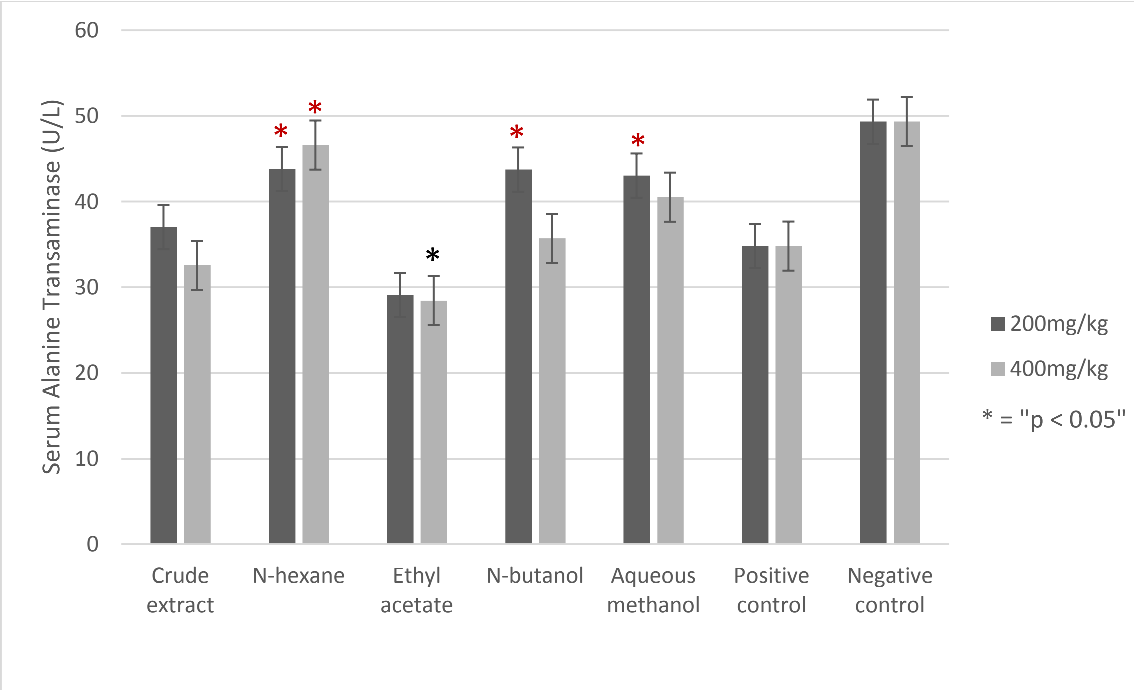

Fig. 1. Effect of extracts and fraction on serum ALT

*Key: * = The fraction shows a statistically significant reduction in ALT compared to the positive control;*
** = The positive control shows a statistically significant reduction in ALT compared to the fractions.*

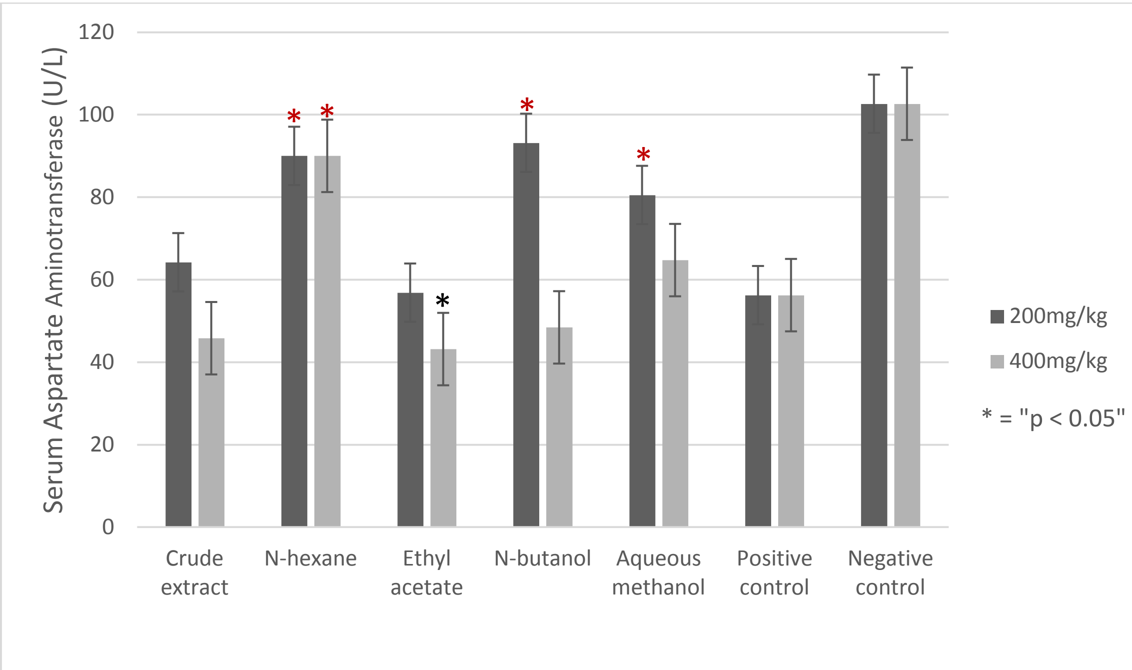

Fig. 2. Effect of extract and fraction on serum AST

*Key: * = The fraction shows a statistically significant reduction in AST compared to the positive control;*
** = The positive control shows a statistically significant reduction in AST compared to the fraction*

serum tested. This deviation was also observed in another study where the antioxidant activities of the different fractions did not follow the direction of the total phenolic content [25]. This suggests that it is not just important to ascertain the quantity of phenolic content present in an extract but also the type of compounds as this can determine their relative activities within the biological system. This is supported by Wen & Walle who indicated that methylated flavonoids were more metabolically stable than unmethylated flavonoids [26]. Thus, it may be possible that the type of compounds (methylated vs non-methylated) extracted by each vehicle would have played a major role in its activity which itself is a function of the polarity of the solvents used for the extraction process. It is therefore possible that if formulated to overcome its instability (due to first-pass metabolism after absorption) using nanotechnological formulation techniques, the n-butanol fraction may show better antioxidant activity. Additionally, n-butanol exhibited a larger standard deviation on the total phenolic content compared to the ethyl acetate thereby also suggesting an inconsistency in the concentration of the phenolic compounds being extracted which could impact its biological activity.

Finally, this study observed that the plant extract was safe up to 5,000mg/kg when the acute toxicity test was conducted. While this implies that the 200mg/kg and 400mg/kg would not be lethal at this point of the research, further concentrating these bioactive compounds could drastically lower both the LD_{50} and IC_{50}. For example, Noreen *et al* showed that the 5,7,4′-trihydroxy-3′-methoxy flavone exhibited 85.4% greater antioxidant activity than the positive control (Trolox, a branded formulation of Vitamin E) at a concentration of 50 µg/mL [25]. While the study does not calculate the LD_{50}, it is possible that the LD_{50} would not be as high as what was recorded in our study. This shows the underlying importance of conducting the acute toxicity test at each step of this research.

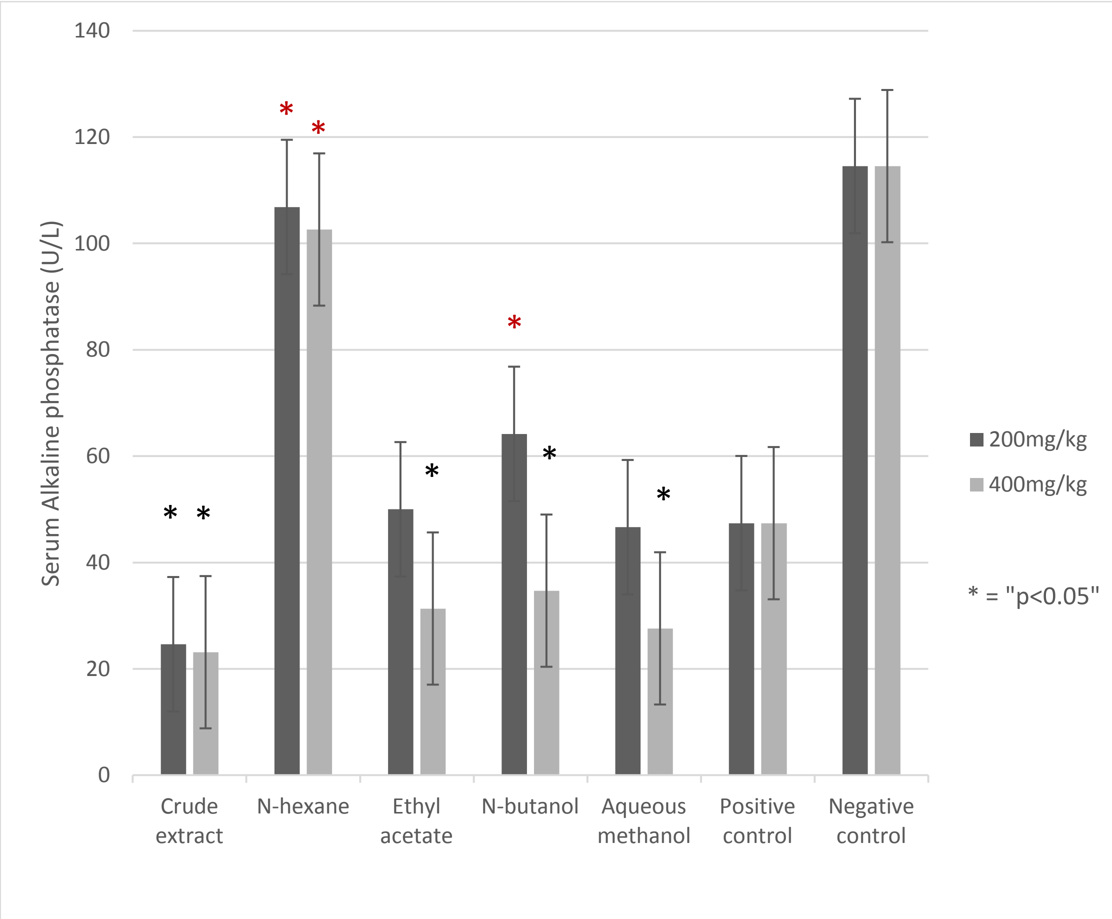

Fig. 3. Effect of extract and fraction on serum ALP

*Key: * = The fraction shows a statistically significant reduction in ALP compared to the positive control;*
** = The positive control shows a statistically significant reduction in ALP compared to the fraction*

Table 1. Results of the phytochemical analysis carried out on *T. cordifolia*

Phytochemical test	Saponins	Tannins	Alkaloids	Terpenoids	Steroids	Glycosides	Flavonoids	Hydrogen cyanide	Reducing sugars
Result	+	+	-	-	+	+	+	-	-

(+) = Present; (-) = Absent

Table 2. Results of analytical standards

Parameter	Numerical Constants/Standards	Numerical Constants/Standards
Extractive values	Water	19.25± 3.00
	Methanol	15.75 ± 2.75
	Chloroform	4.75 ± 0.25
	Ethylacetate	3.75 ± 0.25
	n-hexane	1.25 ± 0.75
Ash values	Total ash	10.17 ± 0.50
	Water soluble ash	4.00 ± 1.00
	Acid Insoluble ash	10.00 ± 0.00
Total ash		9.75 ± 0.75

Table 3. Results of total phenolic content presented as concentration ± standard deviation

Fractions	Total Phenolic Content(Mg GAE/G)
n-hexane	109.85 ± 106
n-butanol	198.65 ± 106
Ethyl acetate	129.99 ± 21
Crude extract	104.70± 21

Our inability to confirm the identity of the compounds that were extracted by the different solvents constitutes a limitation to our study as such information may have been crucial in understanding the hepatoprotective potential of our extracts. Additionally, while water was shown to have the highest extractive value, we utilized methanol for the crude extraction. This was done to mitigate the impact of microbial contamination in this study.

5. CONCLUSION

The ethyl acetate fraction of *Triumfetta cordifolia* exhibited consistent hepatoprotective activity at a dose of 400mg/kg as revealed by reductions in the level of serum ALP, ATP and AST when hepatotoxicity was induced using carbon tetrachloride. The acute toxicity study also shows that this concentration was also safe and therefore suggests the potential benefit of using this plant in the prevention of liver damage, although further studies are necessary to identify precise bioactive agents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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