INVESTIGATION OF THE HEPATOPROTECTIVE POTENTIAL OF FRACTIONS AND PHYTOSOMAL COMPLEXES OF *TELFAIRIA OCCIDENTALIS* HOOK F LEAF EXTRACTS ON ALCOHOL-INDUCED HEPATOTOXICITY IN RAT MODELS

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ABSTRACT

The aim of this work was to evaluate the hepatoprotective potential of fractions and phytosomal complexes of *Telfairia occidentalis* leaf extracts on ethanol-induced hepatotoxicity in rat models. Phytosomal complexes of ethanol extract fractions were prepared by the thin film hydration technique. Hepatoprotective activities were evaluated by biochemical assays and histopathological examination of liver sections. Intoxication of animals with pure ethanol significantly ($p < 0.05$) elevated their liver enzyme titers, whereas co-administration with various extracts and phytosomal complexes reversed the increases. Histopathological findings revealed liver tissue protective effects of the extracts with the phytosomal complexes exhibiting greater effects ($p < 0.05$). Significant differences at $p \leq 0.05$ were observed in the liver indices of animals treated with co-administered ethanol and extract fractions or phytosomes complexes versus the positive control group. *T. occidentalis* leaf extract formulated as phytosomes, therefore, has good potential of enhancing the hepatoprotective activities of the extract.

INTRODUCTION

Recent disease surveillance reports show that 2 million deaths per year equivalent to 4 % of all global deaths are linked to various types of chronic liver diseases (CLD) such as liver cirrhosis, hepatocellular carcinoma and acute hepatitis[1]. This reports also showed that liver cirrhosis is the 10th, 9th and 5th highest cause of deaths in Africa, South East Asia/Europe and the Eastern Mediterranean regions, respectively. A 2019 global health report also showed that 25 % of cirrhosis-caused deaths were attributed to alcohol consumption[2]. Alcohol consumption and associated liver diseases, therefore, constitute serious global health, social and economic burden borne by every nation, community and many individuals. Another 2019 cross sectional analysis showed that United States alone spent over 81.1 billion dollars on hospitalized patients with chronic liver diseases between 2012 and 2016[3]. Given this scenario, the search for new preventive approaches, and novel pharmacological treatments of alcohol induced liver disorders has remained very intense.

A lot of orthodox and alternative (herbal) remedies have been deployed in this effort, either to produce new effects or to improve the effectiveness of existing remedies/ medications. Recent advances in the management of CLDs has seen the use of non-selective beta blockers, gut-selective antibiotics such as, rifaximin, diuretics and even statins, all of which are associated with some serious side effects[4]. Herbal therapies have been accepted globally as effective and safe alternative and sometimes good replacement for synthetic chemical-based orthodox medicines. Virtually every part of the plant structure, including leaves, barks, roots and the sap is utilized as herbal and/or pharmaceutically formulated

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products for the treatment of one disease or the other, or as nutraceutical or as the so called, "immune booster". The advantages offered by herbal medicines are numerous.

Phytomedicines are of natural origin, cheap and easy to source from the immediate local environments. Herbal medications are believed to be free of the common side effects associated with synthetic and semi synthetic chemicals, and their processing techniques are environmentally friendly. Locally sourced therapies enjoy high patronage and trust among indigenous people. Green medicines have subsequently attracted high research interest in the recent decades. Many recent works have reviewed/investigated and reported the potential and actual therapeutic effectiveness of a lot of plant materials for the treatment of a variety of common ailments such as gastric ulcer[5], liver disorders[6], arterosclerosis[7], parkinsonism[8], obesity[9], cancer[10], toxic hepatitis[11] and many other diseases. Silymarin, a phytoextract of the milk thistle plant (*Silybum marianum*) which is commercially available as tablet and suspensions has proven to be one of the most effective hepatoprotective medicines currently in clinical use[12]. Recent interview with some indigenous herbal practitioners in various parts of South eastern Nigeria revealed the use of aqueous and ethanol leaf extracts of *Telfairia occidentalis* for the treatment of suspected liver disease symptoms such as jaundice, hepatomegaly, palpable stomach, constipation and sometimes combined with other herbs, in the preparation of remedies for treatment of venereal diseases. Unfortunately, more enquiries from both practitioners and users revealed that large daily doses of the extract (500-2000 mL) are usually required to produce significant therapeutic effects suggesting low therapeutic activity or poor systemic bioavailability of the active components. Large dose requirement is a common feature of crude medicinal plant extracts; a feature which researchers have attributed to the micro level composition of active material per unit portion of the extract[13]. Poor pharmacokinetic profiles of many phytochemicals have also been attributed to their large and complex molecular structure and high molecular weights[14]. These challenges have necessitated intense research into alternative systems for effective oral delivery of plant-based medicines. The results included the discovery of a number of novel phytochemical nanocarrier systems that not only exhibit capacity to improve the oral, topical and transdermal delivery of drugs of herbal origin, but also target bioactive materials to specific body sites[15]. Systems like liposomes, phytosomes, niosomes, ethosomes, nanoemulsions, among others have been studied and proven to deliver therapeutic phytochemicals to specific and desired site of action in an improved manner[16]. Among these carrier systems, phytosomes have attracted high research interest due to their simplicity, high loading capacity and relative stability.

A phytosome is a chemical complex formed through a hydrogen bond linkage between stiochiometric proportions of a phospholipid molecule (phosphatidylcholine) and a phytochemical which, upon hydration, undergoes self aggregation to form a versicular nanostructure. The principle of phytosome formation is linked to the bipolar structure of the phosphatidylcholine molecule, wherein the choline component is hydrophilic (polar), while the aliphatic tail portion of the compound is hydrophobic[17]. Phytochemical species in the extract possessing active hydrogen atoms (eg, –COOH, -OH, -NH$_2$ or NH) are able to bind to the choline (polar) portion through the hydrogen atom[18]. Upon hydration, the free hydrophobic tails of numerous complexes undergo self aggregation ostensibly to shield themselves from the aqueous environment, thereby leaving the drug bearing polar portion in the vicinity of water. Oppositely positioned tails produce a layer of hydrophobic region surrounded by two layers of equally oppositely oriented extract-complexed choline heads. This bipolar, bilayer structure is capable of entrapping both hydrophilic compounds like the choline/extract complexes and hydrophobic phytochemicals within the inner lipophilic tail-populated middle region (Fig. 1). Phytosomes improve the pharmacokinetic and pharmacodynamic profiles of their cargo phytomaterials by overcoming the common absorptive and distributive limitations of free phytochemicals. A lot of phytochemicals like phytophenols, glycosides, flavonoids aglycones and tannins are water soluble, but their absorption through the hydrophilic paracellular pathways of cell membranes is limited by their inherent complex structures[19,20]. Similarly, their passage through the lipidic transcellular pathways
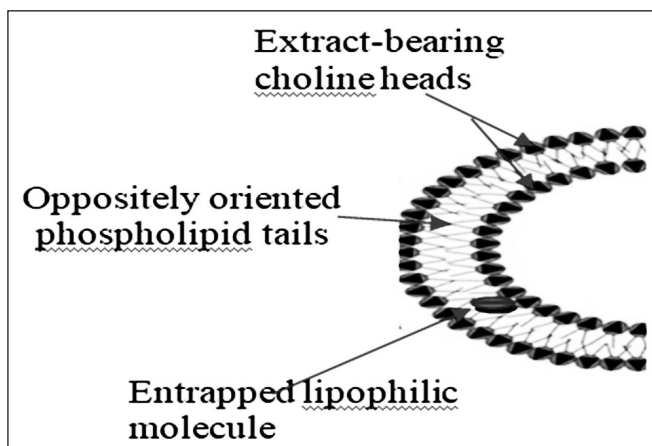

Fig. 1: Modified schematic diagram of section of a phytosome[26]

is challenged by their poor lipophilicity. Phytosomes are able to overcome these challenges because of their nano scale sizes, dual polarity, structural flexibility and cell like structural integrity[21, 22]. These features facilitate greater solubilization of phytomaterials, easy passage through the various cellular membrane pathways and unhindered integration into the intracellular spaces[23, 24, 25]. Fig. 1 is a schematic representation of a portion of a phytosome structure[26].

Phospholipids, which constitute a major component of phytosomes, are also the predominant natural component of many body cells and this makes phytosomes fully biocompatible with the cellular environment[24]. Phosphatidylcholine, which is the main phospholipid used for the current phytosome formation, is reported to possess inherent hepatoprotective activities, and as such has the potential to synergize with the extract[27].

T. occidentalis Hook F (*T. occidentalis)*, generally known as fluted pumpkin, belongs to the family Cucurbitaceae. It is widely cultivated and the leaves and seeds are copiously consumed as vegetable, mostly in the south Eastern communities of Nigeria and some other West African countries. It is a perennial creeping/climbing stem plant with dark greenish multifolate (3-6) leaves. The plant is called by many native names such as ugu, ugwu, ukong-ibong, eweroko, efurun and others by the Igbo, Hausaa, Efik/Ibibio, Yoruba and Ijaw tribes of Nigeria, respectively. The fruit has a characteristic flute shaped pod which bears many non-endospermic edible seeds[5]. Extracts of various parts of the plant are widely utilized as ethnomedicinal remedies. Traditional use of *T. occidentalis* in the management of jaundice, symptomatic hepatomegaly, anemia, diabetes mellitus and variety of other ailments as well as literature claims of the effectiveness of many fluted pumpkin parts in the treatment of alopecia, prostate enlargement, cancer, high cholesterol, urinary incontinence and many other disease conditions have been reported[28, 29]. There is, however, no available report on the formulation of the leaf extract in the form of phytosomes. The aim of the current study was to design phytosomal complexes of *T. occidentalis* leaf extract for enhanced hepatoprotective activities in rat models. The major objectives were to obtain aqueous and ethylacetate extract fractions of the *T. occidentalis* leaf, formulate phytosomal complexes of the extract and evaluate the hepatoprotective effects of the complexes against alcohol-induced liver toxicity in Wistar rat. Effective delivery of *T. occidentalis* extract through phytosome formulation has the potential of reducing its daily dose requirement, improving its efficacy and minimizing possible side effects through improved systemic bioavailability.

MATERIAL AND METHODS

Materials

Mature fresh leaves of *T. occitentalis* Hook F plant were harvested on 10th August 2022 from a rural farm in Uda village in Enugu Ezike, Igbo-Eze North Local Government Area of Enugu State, South East Nigeria. Wistar rats of both sexes weighing 160-200 g were obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. Diagnostic kits, including alkaline aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP) and bilirubin (TBL) kits were products of Diagnosticum Zrt Company, Budapest, Hungary, and were direct gifts from Adonai Medical Diagnostic Laboratories, Nsukka, Enugu State, Nigeria, Silymarin and phosphatidyl choline (phospholipid) were gifts from Bioresearch Laboratories, Obechara Junction, Nsukka, Enugu State, Nigeria. All chemicals and reagents used in the study were obtained from a licensed chemical vendor, Jeochem (Nig) Ltd., Nsukka, Nigeria and were used as supplied unless otherwise specified.

Methods

Plant material collection and extraction

The plant material was identified by Mr Samuel Ozioko (a Taxonomist) at the Bioresource Centre, Obuechara Junction, Nsukka, Enugu State, Nigeria with the sample identity number, BR/PTM/2022/45 and a voucher specimen retained in the Centre's herbarium. The leaves were washed three times under running tap water, rinsed with distilled water, drained. and then shade-dried for 10 days. The dry leaves were then coarsely milled using a mechanical grinder and 500g of the powder extracted with 450 mL of ethanol using a Soxhlet extraction machine (ST 243 Soxtec™ Denmark). The crude extract was filtered through a No. 1 Whatman's filter paper, and the filtrate reduced to $^1/_{10}$th of its volume by rotary evaporation. A dry mass was achieved by rotary evaporation using hot water bath at 45°C, and kept in a clean bottle at 4°C. A 50 g portion of the pasty extract were dissolved in 250 mL of deionized water, while another 50 g portion was dissolved in 250 mL of ethylacetate. The two solutions were concentrated to pasty masses using a rotary evaporator (HEIDOLPH), Germany: CR-2001), and then freeze dried in a high vacuum pressure-low temperature freeze drier (BIOEVOPEAK INC. LY060-IS, USA) to obtain dry water and ethylacetate extract fractions.

Oral acute toxicity studies of the extract fractions

The new two-phase Lorke's method for testing acute toxicity of materials in animal models reported by Chinedu et al[30]., was used in the current study. Briefly, in phase one, nine animals were divided into three groups, tagged as groups 1, 2 and 3 with the different groups receiving 10, 100 and 1000 mg kg^{-1} b.w. of the extract, respectively. The animals were subsequently observed for behavioral changes, morbidity and/or mortality for a period of 24 h following the receipt of the extract. In stage two, three groups of animals made up of one animal per group were administered with 1600, 2900 and 5000 mg kg^{-1} b.w. of the extract, and again observed for 24 h for mortality or morbidity. Equation 1 was used to calculate the LD_{50}.

$$LD_{50} = \sqrt{(D_0 \times D_{100})} \quad \text{------------- (Equation 1)}$$

where D_0 = highest dose of extract that caused no mortality and D_{100} = lowest dose that caused death of any animal

Preparation of phytosomes

A modified version of the solvent evaporation-thin film hydration technique described by Dhase & Saboo[31] was used to prepare both the aqueous fraction phytosomes (EqFPh) and the ethyl acetate fraction phytosomes (EaFPh) in two separate processes. For the preparation of the EaFPh, 60 mg weight of phosphatidylcholine was introduced into a round bottomed flask containing 45 mL volume of ethylacetate, into which 60 mg of the dry ethylacetate extract fraction (Eaf) had earlier been dissolved. The resulting mixture was sonicated (JP; Barcelon. Spain) for 10 min. Thereafter, the ethyl acetate was recovered in a rotary evaporator at 50 °C. The thin film of the mixture left at the bottom and sides of the flask was then hydrated with deionized water, and further sonicated for 20 min in the presence of ice pack to check heat dissipation. In a similar process, an equivalent quantity of aqueous extract fraction (in place of EaF) was used to produce the AqFPh. The resulting phytosomes of each of the fractions were separately placed in amber coloured bottles and stored at 6 °C until use.

Animal handling and experimental protocol

Thirty five (35) albino rats, Wistar strain of both sexes weighing 183 ± 0.22 - 201 ± 0.32 g were obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria Nsukka, Nigeria. The animals were maintained on standard pellet diet and tap water *ad libitum* and acclimatized for 14 days before use. The use and care of laboratory animals followed were in accordance with animal rights as per the Guide for the Care and Use of Laboratory Animals as approved by the Enugu State University of Science as Technology Animal Use Ethics Committee, which issued the approval certificate number ESUT/AEC/0205/AP129. The rats were randomly divided into seven groups of five animals each. The experimental protocols are shown in Table I.

Table I: Animal grouping and treatment

Group	Daily treatment for 14 days	Status
I	Distilled water	Control (-)
II	Ethanol (7 g kg^{-1} body weight (b.w.)	Control (+)
III	Silymarin (250 mg kg^{-1} b. w. + ethanol 7 g kg^{-1} b.w.)	Reference
IV	Aqueous fraction (250 mg kg^{-1} b. w. + ethanol 7 g kg^{-1} b.w.)	Experimental
V	Ethylacetate fraction (250 mg kg^{-1} b.w. + ethanol 7 g kg^{-1} b.w.)	Experimental
VI	Aqueous fraction phytosomes (250 mg kg^{-1} b.w. + ethanol 7 g kg^{-1} b.w.)	Experimental
VII	Ethylacetate fraction phytosomes (250 mg kg^{-1} b. w. + ethanol 7 g kg^{-1} b.w.)	Experimental
VIII	Aqueous fraction phytosomes (500 mg kg^{-1} b.w. + ethanol 7 g kg^{-1} b.w.)	Experimental

After the 14-day treatment, the animals were sacrificed under chloroform anesthesia and blood samples withdrawn by direct heart puncture and centrifuged at 3000 rpm to isolate the serum, while the liver was excised washed with phosphate buffer, and put in formalin solution for further investigations.

Determination of liver indices of animal groups

The average weight of each group of animals was determined prior to treatment. On completion of the various treatments, the animals were sacrificed and their livers were surgically incised. The average weight of the livers of each animal group was also determined. The liver index of each group was then calculated using equation 2[32].

$$\text{Liver index (LI)} = \frac{liver\ weight}{body\ weight} \times 100\% \text{ --------- (Equation 2)}$$

Determination of biochemical parameters of animal serum

The basic biochemical parameters of the separated serum, including ALT, AST, ALP and total bilirubin, were assayed using commercial rapid biochemical diagnostic kits (Diagnosticum Zrt Company Budapest, Hungary) which has been successfully used in previous works[11].

Histopathological examinations of liver sections

Paraffin embedded liver sections of the sacrificed rats were stained with haematoxylin and eosin (H&E), and examined under a microscope at 40X for histopathological manifestations.

Statistical analysis

Data were reported as means ± standard deviation with n = 5, while statistical analysis were carried out using the GraphPad Prism version 8 (California, USA), and then analysed using the one-way analysis of variance (ANOVA), and post hoc Tukey's test. Differences with p < 0.05 were considered as statistically significant.

RESULTS

LD$_{50}$ of extract

No death was recorded among the animals, including the one that received a dose of 5000 mg kg^{-1} b.w. However, for the animals in this group, there were some observable behavioral changes including soft stooling, weak response to needle stick injury and reduced feeding activities within the 5 - 13th h following administration of the extract. These changes had subsided at the 24th h of observation.

Organ index calculation

The results of the liver organ index tests for various animal groups that received the control, induction or experimental formulations are shown in Table II.

Formulations that had higher potential to induce hepatotoxicity exhibited higher LI. For instance, animals that received ethanol alone (a potent hepatotoxic beverage) had LI value of 1.98 %, while the group that received distilled water had LI value of 1.23 %. Group II animals which received ethanol co-administered with a standard hepatoprotective drug, silymarin, had LI value of 1.31 % comparable to groups VII and VIII animals that received ethanol co-administered with the synthesized phytosome formulations. Statistically significant differences were observed among the groups, as indicated in Table II.

Table II: Liver indices

Animal group	Treatment	Animal weight (g)	Organ weight (g)	Organ weight Index (%)
I	Distilled water	198 ± 0.45	2.45 ± 0.56	1.23
II	EtOH	194 ± 0.98	3.85 ± 0.77	1.98**
III	EtOH + Silymarin	199 ± 0.32	2.61 ± 0.87	1.31#
IV	EtOH + EqF	187 ± 0.77	2.69 ± 0.76	1.43#
V	EtOH + EaF	178 ± 0.14	2.97 ± 0.47	1.67
VI	EtOH + EaFPh	180 ± 0.51	2.41 ± 0.45	1.38##
VII	EtOH + EqFPh (250)	200 ± 1.06	2.65 ± 0.32	1.35#
VIII	EtOH + EqFPh (500)	195 ± 0.22	2. 58 ± 0.55	1.32#

*Results are presented as mean ± S.D., n=5.**: significantly different from the negative (distilled water) control group at p ≤ 0.01. #, and ##: significantly different from the positive control (EtOH) group at p ≤ 0.05, and 0.01, respectively.*

Assay for biochemical markers

Fig. 2 (A – D) are the bar charts showing the levels of tested liver toxicity biomarkers recorded for various treatment protocols. Distilled water served as the negative control while ethanol (70 %) administered at 7 g kg^{-1} b.w. given for 14 days was used as the induction (positive) control. The same dose was co-administered with aqueous fractions, ethylacetate fractions and phytosome formulations of the two extract fraction to various experimental animal groups. The results obtained are shown in Figs. 2 (A-D).

It was generally observed that the values of the four biochemical markers; AST, ALT, ALP and TLB increased significantly after the 2-week administration of ethanol vis-a-vis the negative control group and groups that received ethanol supplements with extract fractions and phytosome complexes. For instance, the AST level for animals that received EtOH (7 g kg^{-1} b.w.) for 14 days was 215.734 ± 0.42 iu L^{-1} (Fig. 2 C) while the values when ethanol was co-administered with silymarin (250 mg kg^{-1}
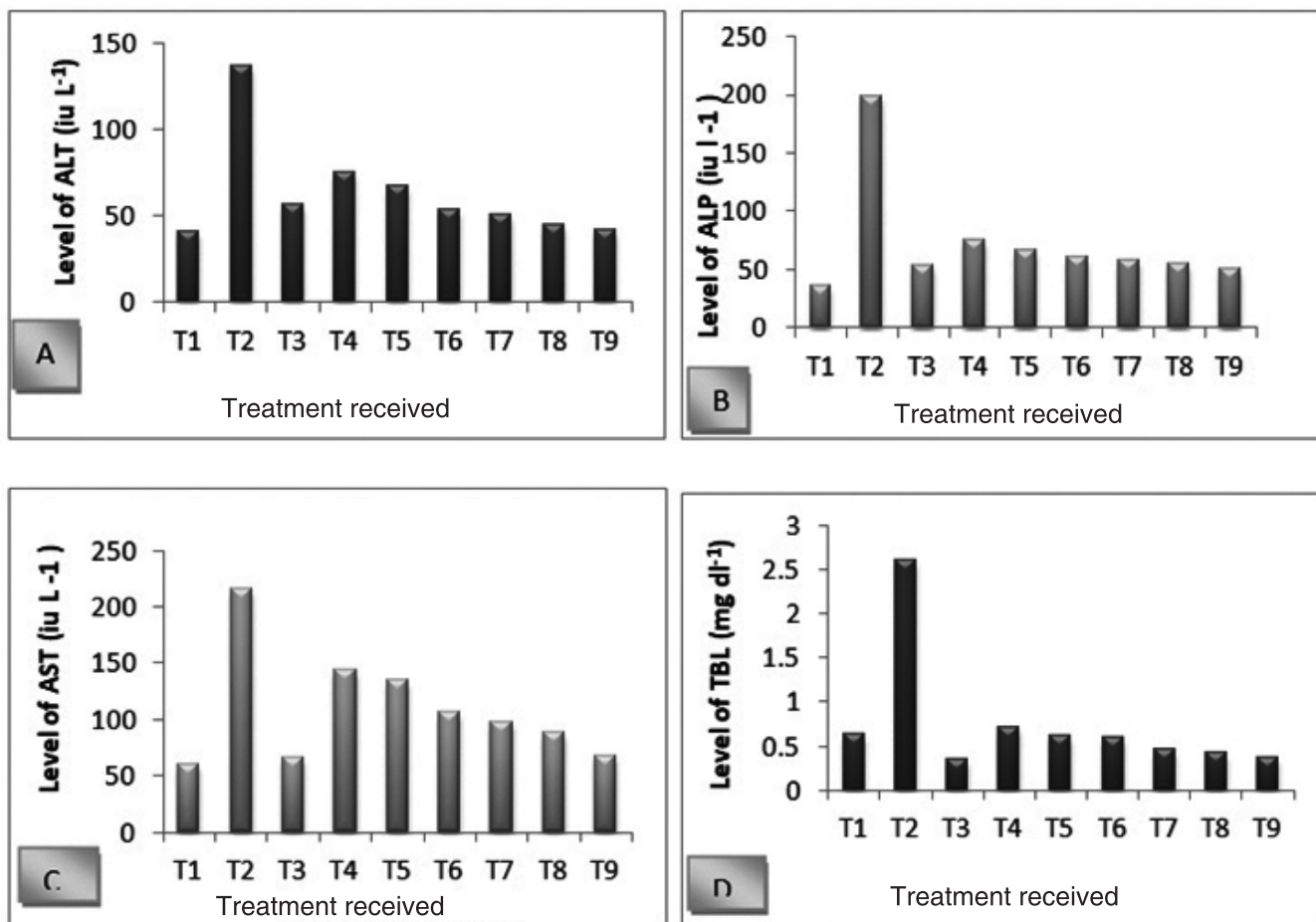
Key (Fig A – D):

T1 = Dist water (250 mg kg⁻¹ b.w.)

T2 = EtOH (250 mg kg⁻¹ b.w.)

T3 = Silymarin 250 mg kg⁻¹ b.w.+ EtOH (250 mg kg⁻¹ b.w.)

T4 = HF (250 mg kg⁻¹ b.w. + EtOH (250 mg kg⁻¹ b.w.)

T5 = EaF (250 mg kg⁻¹ b.w.+ EtOH (250 mg kg⁻¹ b.w.)

T6 = HFPh (250 mg kg⁻¹ b.w. + EtOH (250 mg kg⁻¹ b.w.)

T7 = HFPh (400 mg kg⁻¹ b.w. + EtOH (250 mg kg⁻¹ b.w.)

T8 = EaFPh (250 mg kg⁻¹ b.w. + EtOH (250 mg kg⁻¹ b.w.)

T9 = EaFPh (400 mg kg⁻¹ b.w. + EtOH (250 mg kg⁻¹ b.w.)

Fig. 2 (A-D): Post treatment biochemical assay

b.w.) + EtOH (250 mg kg⁻¹ b.w.), EqF (250 mg kg⁻¹ b.w. + EtOH (250 mg kg⁻¹ b.w.), EqFPh (250 mg kg⁻¹ b.w.) + EtOH (250 mg kg⁻¹ b.w.) and EaFPh (250 mg kg⁻¹ b.w.) + EtOH (250 mg kg⁻¹ b.w.) were, 60.987 ± 1.64 iu L⁻¹, 120.223 ± 0.17 iu L⁻¹, 88.872 ± 0.17 iu L⁻¹ and 93.432 ± 0.47 iu L⁻¹, respectively. The two phytosomes (EqFPh and EaFPh) at the dose of (250mg kg⁻¹ b.w.) + EtOH (250 mg kg⁻¹ b.w.) exerted higher hepatoprotective effects depicted by lower values of the biomarkers when compared to the effects of the uncomplexed fractions. For instance, the ALT value of the group that received EaF with ethanol was 113.654 but reduced to 78.43 when the fraction was complexed with phosphatidylcholine (phytosome). Similarly, while the TBL level after administration of EqF was 0.68, it

reduced to 0.51 in groups that received equivalent doses of EqFPh. The effects of the two phytosome formulations were observed to be dose-dependent. Animals that received 500 mg kg⁻¹ b.w. of the phytosomal complexes with ethanol experienced lower values of the biomarkers than the groups that received 250 mg kg⁻¹ b.w. of the same formulation. The effect of the 500 mg kg⁻¹ b.w. of the phytosome-loaded extract was comparable to that of silymarin (250mg kg⁻¹ b.w. + EtOH (250 mg kg⁻¹ b.w.).

Histopathological studies

The histopathological examination of the liver sections of the sacrificed experimental animals showed diverse histopathological changes, varying from degeneration
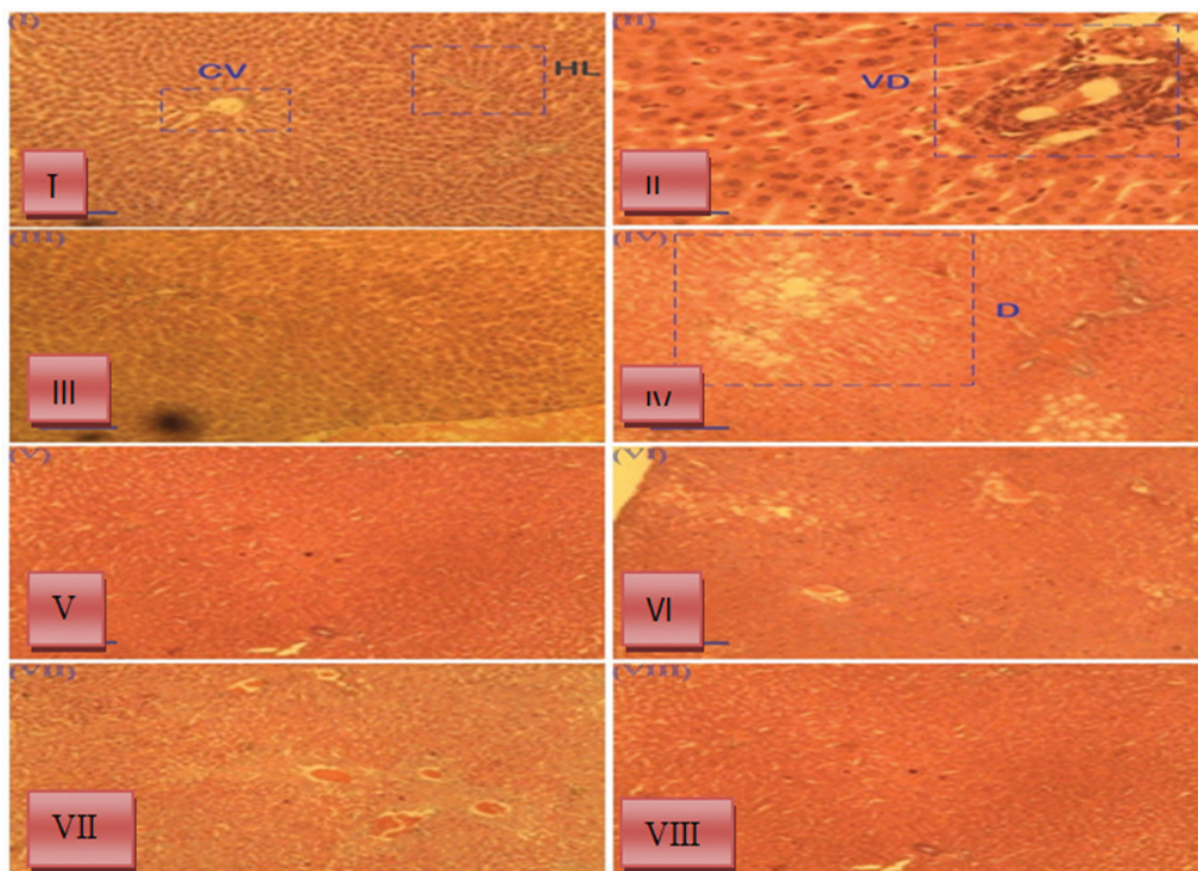
Fig. 3(I - VIII): Photomicrographs of liver section of rats treated with various extract formulations

to tissue necrosis and inflammatory cellular infiltrations, which represented the effects of various treatment regimens. The photomicrographs of the liver sections of various rat groups (post treatment) are shown as Fig. 3 (I – VIII).

Fig. 3(I) shows the photo micrographs of liver section of rats that received only food and water. The section exhibited normal histological architecture with prominent hepatic lobules (HL) and central veins (CV), surrounded by radiating hepatocytes arranged in chords. No evidence of steatosis or other inflammatory lesions was observed.

Photomicrographic view of the liver sections of the induction control animals (group II) that were intoxicated with ethanol (7g kg^{-1} b.w.) for 14 days showed severe vacuolar degeneration (VD) of the hepatocytes around the peripheral vein, severe lobular necrosis, inflammation and congestion around the portal veins (Fig. 2). Fig. 3 shows the microscopic view of the liver section of the animals treated with ethanol co-administered with silymarin, which served as reference hepatoprotective agent. Liver sections of animals that received aqueous

extract fraction co-administered with ethanol (group IV) equally exhibited reduced inflammatory responses including less vascular degeneration (D) of the hepatocytes and reduced presence of necrotic tissues (Fig. 2 IV). Group V rats which received ethanol supplemented with ethyl acetate fractions showed mild to moderate portal inflammation and observable reduction in the congestion of the portal tract (Fig. 2V). Group VI animals received the induction doses of ethanol co-administered with the aqueous fraction phytosomes (EqFPh), while group V rats received ethanol with ethyl acetate fraction phytosomes (EaFPh). Liver sections of both groups showed significant reduction in the occurrence of various histopathological changes compared to the sections of animals that received ethanol with aqueous-fraction or ethylacetate fraction of the extract (Figs. 2V & VII).

DISCUSSION

Ethanol is a known liver toxicant, and this was corroborated by the LI, biochemical assay and histopathological evaluation of the animal group that received only ethanol. Similar conclusion had been

reached in previous works[2, 3]. The LD_{50} results suggested that the extracts were generally non toxic and safe at doses used in these tests. The trend of response to dose of 5000 mg kg^{-1} b.w may be attributed to onset of side effects at the 5th h and reduction or cessation of these effects at later hours, probably due to extract excretion or systemic metabolism, both of which may have caused depletion or complete exhaustion of extract in the animal plasma. To ensure safety of animals, low dose of 250 mg kg^{-1} b.w. was used for the tests except for one animal group in which 500 mg of the test samples kg^{-1} b.w. was used. The lower values of LI observed in animals that received ethanol co-administered with either extract fractions or phytosomonal complexes vis-à-vis the value for animals intoxicated with ethanol alone clearly suggested that these formulations countered the liver inflammation process induced by ethanol. The results also showed that the phytosomal complexes exhibited hepatoprotective effects comparable to that of silymarin, and that both effects were superior to that of the non complexed extracts (EtOH+ EqF and EtOH+ EaF). This observation is in tandem with previous reports[23, 24, 25]. The higher LI values seen in formulations with higher tendency for liver toxicity is believed to be due to the liver, inflammatory response processes which involve increased presence of many enzymes and biochemical inflammatory mediators that cause the swelling of the liver and subsequent increase in their (liver) weights[33]. Increased post treatment liver weight arithmetically translates to higher LI. The relative effects of the various formulations equally agreed with the results obtained from the assay of post treatment liver toxicity biomarkers.

The biochemical assay results corroborated the analysis of the LI results, showing that ethanol actually induced hepatotoxicity (compared to control) in the animals. This effect was, however, significantly reduced ($p > 0.01$) by the co-administration of silymarin, extract fractions and fractions-phytosome complexes. The aqueous fractions and phytosomes caused more hepatotoxicity inhibition than the ethyl acetate fractions and phytosomes possibly due to the greater solubility of the relevant phyto compounds in water[27].

Observation from both the biochemical assay and the histopathological examination, again showed that the phytosomal complexes exhibited greater hepatoprotective effects than the extract fractions alone and that this effect was dose dependent. The phytosome acted as effective nanocarrier system that facilitated the delivery and enhanced the therapeutic efficacy of the extract. The enhancement of the hepatoprotective effect of some bioactive substances by phyotosome complexation has been reported by several researchers[24, 34, 35]. A previous report had attributed this to the ability of phytosomes to enhance the solubility, membrane permeability and intracellular compatibility of the bioactive chemicals[18]. The phospholipid component of the phytosome has also been reported to have intrinsic liver cell regeneration activity[17].

CONCLUSION

T. occidentalis leaf extracts exhibited good potential for protecting rat liver against alcohol-induced toxicity. Extracts conjugated with phosphatidylecholine in form of phytosomes showed higher hepatoprotective effects than the free extract fractions, but a little lower than the silymarin. Formulation of phytoextracts as phytosomes increases their therapeutic effectiveness, probably by enhancing the solubility, membrane permeability and overall bioavailability of the extract. The observed liver protection effects was corroborated by the reversal of the elevation of major liver enzymes earlier induced by the ethanol as well as by the relative values of the liver weight indices. Thus, the investigated extract may be a good alternative to many orthodox medicines currently in use for the treatment of both acute and chronic liver diseases, as it offers the advantages of affordability, higher safety profiles, biodegradability and biocompatibility. Poor yields of both crude extract and the phytosomes were major challenges to the work. Future works may explore other techniques for synthesizing the phytosomes and establish the effect of higher dosing of the phytosomal complexes above 500 mg kg^{-1} b.w. on its hepatoprotective effectiveness.

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