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Graded doses of grape seed methanol extract attenuated hepato-toxicity following chronic carbamazepine treatment in male Wistar rats

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ABSTRACT

Aim: This study investigated the effects of co-administration of carbamazepine (CBZ) with grape (*Vitis vinifera*) seed methanolic extract (GSME) on liver toxicity.
Method: Thirty-five male rats (145−155 g) were randomized into 5 groups (n = 7) and administered with propylene glycol (PG 0.1 mL/day), CBZ (25 mg/kg), CBZ (25 mg/kg) + GSME (200 mg/kg), CBZ (25 mg/kg) + GSME (100 mg/kg), or CBZ (25 mg/kg) + GSME (50 mg/kg) orally for 28 days. Twenty-four hours after the last dose, changes in the body weights were determined. The rats were euthanized by cervical dislocation. The liver was weighed and later homogenized; while the supernatant was analyzed biochemically. The liver tissues were preserved in 10 % neutral-buffered formalin for the histomorphological investigation.
Result: There was significant ($p = 0.0001$) decrease in the body weight following carbamazepine treatment. The relative liver weight also decreased significantly ($p = 0.0004$) across the treatment group compared with control. The activities of the liver enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and glutathione activities), including the concentrations of malondialdehyde, increased significantly ($p \leq 0.0004$) following carbamazepine treatment. Various morphological alterations were observed, especially in the photomicrograph of the CBZ treated rats. However, these derangements were attenuated significantly in the CBZ - GSME co-treated group.
Conclusion: This study concludes that GSME treatment may serve as a potential therapeutic agent in carbamazepine-induced hepatotoxicity/ dysfunction.

1. Introduction

One of the chronic neurological disorders across the globe is epileptic seizures, with an estimated population of 50 new cases per year in every 100, 000 population [1]. This debilitating disease affects about 6 million people in Europe [2] and 10.1 per 1000 individuals residing in West Africa [3]. Seizures, and bipolar disorder, among other central nervous system dysfunctions, are often managed with standard antiepileptic drugs (AEDs) [4].

Carbamazepine (5 *H*-dibenzo[*b*,*f*]azepine-5-carboxamide), is an iminostilbene derivative, composed of cellulose compounds, dextrates, iron oxides, magnesium stearate, mannitol, polyethylene glycol, sodium lauryl sulfate, and titanium dioxide. It is structurally similar to tricyclic antidepressants, and it is ionized within the intracellular fluid [5]. As a result of its efficacy, availability, and affordability, CBZ remains one of the most prescribed AEDs in the treatment of partial and generalized tonic-clonic seizures [6]. However, this drug could only provide symptomatic relief without a complete cure. This necessitates its use for a very long period with attendant adverse effects [6]. Moreover, studies have shown the adverse effects of CBZ on various systemic functions. This stems from impairment of central nervous system [7]; alterations of hematological parameters [8]; testicular dysfunction/ hormonal deregulation [9], tubulointerstitial nephritis/ necrosis [10], cholestatic hepatitis [11], CBZ-epoxide-induced hepatic toxicity [12] to CBZ-induced teratogenicity [13]. Moreover, studies carried out in the United States of America have shown that AEDs, especially, carbamazepine, valproic acid, phenytoin, and phenobarbital contribute about 3% of all intoxications [14,15]

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The liver is the major metabolic organ whose disposition is often challenged by many xenobiotics and different oral medications such as carbamazepine, phenytoin, and several other conventional AEDs. Although both endobiotics and xenobiotics are metabolized across the liver cell plate and secreted into bile [16], this AED, CBZ undergoes extensive hepatic metabolism through the cytochrome P450 enzyme system and produces a pharmacologically active metabolite, carbamazepine-10,11-epoxide [17].

A recent study has implicated the product of hepatic drug-metabolizing enzymes (cytochrome P450 enzyme) as a potent reactive agent with endogenous proteins [17], while its activation is not without consequential idiosyncratic reactions [16,18]. There are two types of carbamazepine-initiated idiosyncratic liver injuries; hypersensitivity, and toxin-induced [19]. It is feasible that both are due to the accumulation of toxic metabolite(s), and arene oxides, damaging derivatives of carbamazepine metabolism [20].

The generation of reactive metabolites, followed by adduct formation with endogenous proteins, is critical for the development of CBZ-induced liver injury [21]. Owing to the high intracellular GSH content, the liver acts as a major detoxifying organ of the body. Therefore, alterations in the level of GSH contribute to several liver dysfunctions, and in some cases, it can be conditionally lethal [20]. Interestingly, findings from an *in vitro* study suggested that reduced glutathione (GSH) significantly suppressed the metabolism-dependent cytotoxicity of CBZ in human peripheral blood mononuclear leukocytes and the irreversible binding of [^{14}C]-CBZ to human liver microsomes (17).

Polyphenols in food plants are a versatile group of phytochemicals with many potentially beneficial activities in terms of disease prevention [22]. However, the seed of *Vitis vinifera* is reported to constitute the bulk proportion of proanthocyanidins [23,24]. Proanthocyanidins are a class of phenolic compounds that takes the form of oligomers or polymers of polyhydroxy flavan-3-ol units, such as (+)-catechin and (−)-epicatechin, all of which contain water-soluble molecules and several phenolic hydroxyls [25]. The polyphenolic nature of GSPE allows relatively easy release of protons, a leverage through which this agent exhibits its substantial antioxidant activity [26]; which is usually higher than vitamins C and E, the gold standards [27]. Studies have shown the inhibition of haloperidol-induced liver toxicity [27], and Gleevec-induced apoptosis, liver injury, and Ki67 alterations in rats [28] following the administration of grape seed proanthocyanidin. However, the untoward effects of carbamazepine on the liver in epilepsy patients, despite its efficacy, availability, and, affordability have been a serious concern over the years [29]. Therefore, there is a need to search for an adjuvant agent that can mitigate the hepatotoxic aftermath of carbamazepine chronic administration.

2. Materials and methods

2.1. Animals

Thirty-five male Wistar rats (145–155 g) were obtained from the Empire Farms, Osogbo, Osun State, Nigeria. The rats were maintained inside the plastic cages in the animal holdings of the College of Health Sciences, Osun State University, Osogbo, Nigeria. These animals were acclimatized for a week and permitted to feed on standard chow and drinking water *ad libitum*. All the experimental procedures were according to the approved schedule of animal care and treatment of the Health Research and Ethics Committee (HREC) of the College of Health Sciences, Osun State University, Osogbo, Nigeria.

2.2. Extraction of proanthocyanidin from grape seed

The extraction of the GSME followed the method of Zam et al. [30] as modified by Adefisayo et al. [31]. Briefly, the pulverized specimen of grape seed (1.0 kg) was soaked in 70 % methanol with vigorous shaking for 72 h with the aid of an electric shaker followed by filtration using Whatman no. 1 filter paper. The filtered extract was evaporated under reduced pressure using a rotary evaporator (Rotavapor R-210/215, India) at a temperature of 40 °C and then lyophilized with a freeze dryer (Labconco-7752020, the USA) to obtain the final product referred to as GSME

% Yield = Yield of GSME(g)/ Weight of pulverized seed X 100 %

Therefore, % yield of GSME = 78g/ 1000g X 100

= 7.8 %

2.3. Drugs administration and the study protocol

Carbamazepine [CBZ] (C4024) (Sigma Aldrich, USA) and grape seed methanolic extract (GSME) were dissolved in propylene glycol (PG) on days of experiments. The GSME was administered in doses of 200 mg/kg, 100 mg/kg and 50 mg/kg body weight. Thirty-five adult male Wistar rats were randomized into five groups (n = 7). Each group received propylene glycol (PG 0.1 mL/day), CBZ (25 mg/kg) [32], CBZ (25 mg/kg) with GSME (200 mg/kg), CBZ (25 mg/kg) with GSME (100 mg/kg) or CBZ (25 mg/kg) with GSME (50 mg/kg). The drugs and vehicle were administered orally for 28 consecutive days. Twenty-four hours after the last dose, the difference in body weights from day 1to day 29 was determined for each rat, and the percentage of body weight change was evaluated for each animal. The rats were euthanized *via* cervical dislocation, while the liver was weighed, homogenized, and the supernatant aspirated from the homogenate was preserved at −20 °C for the biochemical analysis. Also, representative liver tissue was preserved within 10 % neutral-buffered formalin for the histomorphological evaluation.

2.4. Biochemical analysis

The supernatant of the liver tissue homogenate was analyzed for the activities of aspartate aminotransferase (AST), alanine transferase (ALT), and alkaline phosphatase (ALP) according to the method of El-Awdan et al. [27].

2.5. Oxidative stress

Activities of the catalase, Superoxide Dismutase (SOD), reduced glutathione (GSH) and concentrations of the product of lipid peroxidation, malondialdehyde (MDA) in the liver were estimated spectrophotometrically [27]

2.6. Histological analysis

The fixed liver tissues were sectioned into 5-micron thickness and stained with Haematoxylin and Eosin (H&E) according to the method of Antai et al. [33], while the photomicrographs presented at 400 magnifications.

2.7. Statistical analysis

Data were subjected to descriptive and inferential statistics using GraphPad Prism software version 5.01. *T*-test, one-way analysis of variance (ANOVA) and Student-Newman-Keuls post hoc analysis were used where appropriate. The results were presented as mean ± standard error of the mean (SEM) in graphs or tables, while the level of significance was taken at $p < 0.05$.

3. Result

3.1. Effects of co-administration of carbamazepine with graded doses of GSME on the body weight, liver weight and relative weight of the liver to the total bodyweight

The final body weight (g) decreased significantly in the CBZ treated rat compared with the control ($p = 0.0001$), while GSME co-treatment with CBZ had no significant effect ($p = 0.2975$). There was significant decrease ($p = 0.0004$) in the liver weight (g) and relative liver weight respectively following CBZ, CBZ + GSME (100 mg/kg) and CBZ + GSME (50 mg/kg) treatment compared with the control (Table 1).

3.2. Effects of co-administration of carbamazepine with graded doses of GSME on the activities of liver enzymes in male Wistar rats

Chronic CBZ treatment induced a significant increase ($p = 0.0001$) in the activities of the AST, ALT, and ALP (IU/L) relative to the control. However, there was a significant ($p = 0.0036$) decrease in the activities of the AST, ALT, and ALP (IU/L) following CBZ + GSME (200 mg/kg and CBZ + GSME (100 mg/kg) treatments compared with the CBZ treated rats. Moreover, AST, ALT and ALP (IU/L) enzymatic activities increased significantly ($p = 0.005$) in the CBZ + GSME (50 mg/kg) treatment group relative to the CBZ + GSME (200 mg/kg and CBZ + GSME (100 mg/kg) treatment groups (Table 2).

3.3. Effects of co-administration of carbamazepine with GSME on the markers of oxidative stress in the liver of male Wistar rats

In this study, CBZ treatment had no significant effect ($p = 0.0588$) on the liver SOD activities (nmol/g tissue), while this was increased significantly ($p = 0.0019$) in the CBZ + GSME (200 mg/kg) and CBZ + GSME (100 mg/kg) compared with the control. Moreover, the SOD activities increased significantly in the CBZ-GSME co-treatment compared with the CBZ treated rats, but decreased in the CBZ + GSME (50 mg/kg) relative to the CBZ + GSME (200 mg/kg) treated rats.

The activities of catalase increased significantly ($p = 0.0001$) in the CBZ + GSME 200 mg/kg treated group but remain insignificant ($p = 0.0573$) in the CBZ, CBZ + GSME (100 mg/kg) and CBZ + GSME (50 mg/kg) compared with the control (Table 3).

Chronic CBZ treatment had no significant ($p = 0.5534$) effect on the liver GSH activities (nmol/g tissue), while it was increased significantly ($p = 0.0149$) following the CBZ + GSME co- treatment compared with the control. However, the liver GSH decreased significantly ($p = 0.0476$) in the CBZ + GSME (100 mg/kg), and CBZ + GSME (50 mg/kg) treated rats compared with the CBZ + GSME (200 mg/kg) treated (Table 3).

The product of lipid peroxidation, malondialdehyde concentration increased significantly ($p = 0.0005$) in the liver tissue following CBZ treatment. However, this was attenuated significantly ($p = 0.0001$) in the CBZ + GSME (200 mg/kg) treated group compared with the control. In addition, the concentration of MDA increased significantly ($p = 0.0297$) in the CBZ + GSME (100 mg.kg), and CBZ + GSME (50 mg/kg) compared with the CBZ + GSME (200 mg/kg) treated group (Table 3).

Table 1
Effects of co-administration of carbamazepine with graded doses of GSME on the body weight, liver weight and relative liver weight of male Wistar rats.

Treatment groups	Final body weight (g)	Liver weight (g)	% Relative liver weight (g/ 100 g body weight)
Control	168.7 ± 6.33	160.0 ± 2.08 $^{\beta}$	5.42 ± 0.09
CBZ	150.0 ± 1.16$^{\alpha}$	5.40 ± 0.20$^{\alpha}$	3.60 ± 0.10$^{\alpha}$
CBZ + GSME 200 mg/kg	167.7 ± 5.70 $^{\beta}$	7.91 ± 0.12 $^{\beta}$	5.27 ± 0.22$^{\beta}$
CBZ + GSME 100 mg/kg	158.0 ± 1.53 $^{\beta}$	7.00 ± 0.23 $^{\alpha\ \beta}$	4.66 ± 0.37 $^{\alpha\ \beta}$
CBZ + GSME 50 mg/kg	160.0 ± 2.08 $^{\beta}$	7.03 ± 0.23 $^{\alpha\ \beta}$	4.68 ± 0.18 $^{\alpha\ \beta}$

α: decrease compared with the control ($p = 0.0446$).
β: increase compared with CBZ ($p = 0.0446$).

Table 2
Effects of co-administration of carbamazepine with graded doses of GSME on the activities of liver enzymes in male Wistar rats.

Treatment groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	56.2 ± 3.20	21.5 ± 1.59	353 ± 55.70
CBZ	104 ± 1.55 $^{\beta}$	51.0 ± 2.04 $^{\beta}$	679 ± 37.1 $^{\beta}$
CBZ + GSP 200 mg/kg	91.3 ± 3.34 $^{\beta\ \alpha}$	35.7 ± 1.29 $^{\beta\ \alpha}$	357 ± 54.5 $^{\alpha}$
CBZ + GSP 100 mg/kg	91.1 ± 0.54 $^{\beta\ \alpha}$	39.4 ± 2.84 $^{\beta\ \alpha}$	455 ± 5.76 α
CBZ + GSP 50 mg/kg	102 ± 0.44 $^{\beta\ \delta\mu}$	45.5 ± 1.74 β δ μ	624 ± 27.9 β δμ

AST: Aspartate aminotransferase (ALT); Alanine aminotransferase (ALP); Alkaline phosphatase.
β: increase compared with control ($p = 0.0001$).
α: decrease compared with CBZ ($p = 0.0036$).
δ: increase compared with CBZ + GSME 200 mg/kg ($p = 0.005$).
μ: increase compared with CBZ + GSME 100 mg/kg ($p = 0.005$).

Table 3
Effects of co- administration of carbamazepine with graded doses of GSME on the markers of oxidative stress in liver tissue of male Wistar rats.

Treatment groups	SOD (nmol/g tissue)	CATALASE (nmol/g tissue)	GSH (nmol/g tissue)	MDA nmol/g tissue)
Control	2.36 ± 0.18	16.83 ± 0.40	2.01 ± 0.26	1.58 ± 0.50
CBZ	1.65 ± 0.21	16.84 ± 0.62	2.14 ± 0.13	4.68 ± 0.20 β
CBZ + GSME 200 mg/kg	4.36 ± 0.62$^{\beta\ \alpha}$	26.96 ± 0.48 $^{\beta}$	5.76 ± 0.95 βα	2.72 ± 0.50 β μ
CBZ + GSME 100 mg/kg	3.47 ± 0.33 $^{\beta\ \alpha}$	16.62 ± 0.47	3.28 ± 0.19 $^{\beta\alpha\delta}$	4.51 ± 0.22β ϒ
CBZ + GSME 50 mg/kg	2.58 ± 0.11 α δ	16.51 ± 0.59	2.95 ± 0.20 βαδ	4.69 ± 0.21$^{\beta\ \Upsilon}$

SOD: Superoxide dismutaseGSH: Reduced glutathione MDA: Malondialdehyde.
β: increase compared with control ($p = 0.0273$).
α: increase compared with CBZ ($p = 0.0159$).
δ: decrease compared with CBZ + GSME (200 mg/kg) ($p = 0.0476$).
μ: decrease compared with CBZ ($p = 0.0214$).
ϒ: increase compared with CBZ + GSME (200 mg/kg) ($p = 0.0297$)).

3.4. Effects of co-administration of carbamazepine with graded doses of GSME on the histomorphology of the liver

The liver tissue from the control group showed normal central venules and portal tracts without congestion (blue dart), the morphology of the hepatocytes appeared normal (blue dart), the sinusoids appeared moderately dilated and mildly infiltrated by inflammatory cells (slender black dart) (Fig. 1).

The histomorphology of the liver tissue from the CBZ treated rat showed normal central venules and portal veins with severe congestion (yellow dart), the morphology of the hepatocytes appeared relatively normal (blue dart) but with mild cytoplasmic fat infiltration in some hepatocytes, while the sinusoids appear normal and not infiltrated (slender dart) (Fig. 1).

The liver section of CBZ + GSME (200 mg/kg) and CBZ + GSME (100 mg/kg) showed moderate portal triditis; portal tracts with modest infiltration of inflammatory cells (blue dart), the morphology of the hepatocytes appear normal (blue dart), the sinusoids appear mildly dilated and infiltrated by inflammatory cells (slender dart) (Fig. 1).

The liver tissue of the CBZ + GSME (50 mg/kg) showed normal central venules with mild congestion (yellow dart), the morphology of the hepatocytes appear normal (blue dart), the sinusoids show scanty infiltration of inflammatory cells (slender dart) (Fig. 1).

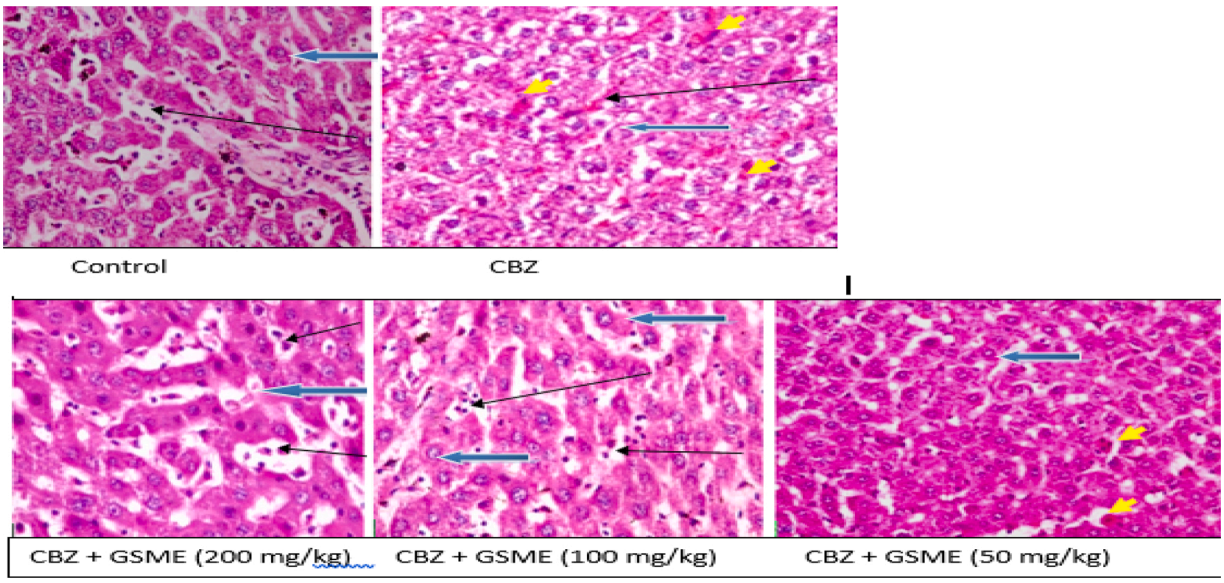

Fig. 1. Effects of co-administration of carbamazepine with graded doses of GSME on the histomorphology of the liver.
Magnification: 400; Stain hematoxylin and eosin

4. Discussion

The introduction of CBZ to the field of neuropharmacology is more than six decades. To date, it remains one of the widely prescribed medications in the field of neurology and psychiatry [12]. Clinical reports on the liver toxicity following the chronic treatment with psychotropic drugs [12,29] necessitate experimental studies on the co-administration of CBZ with a proven antioxidant agent. This is in a bid to mitigate the potential toxicity. In this study, a significant decrease in the body and liver weights following CBZ treatment is suggestive pieces of evidence of CBZ potential toxicity [34]. The mechanism of action of GSME in the prevention of CBZ-mediated liver weight loss in this study is unclear. However, being an agent with potent antioxidant effect, GSME is likely to have scavenged the free radicals generated by the CBZ. Significant reduction in the concentration of malondialdehyde and hepatic congestion following the CBZ-GSME co-treatment is an assertion that GSME lowers the CBZ potential hepatotoxicity. In this study, CBZ had no significant effects on the activities of liver SOD, catalase and GSH, while CBZ + GSME co-treatment increased the activities of SOD and catalase in a dose-dependent manner. A suggestive indication that GSME is a potential anti-oxidative agent and a shred of evidence that it could serve as a useful tool in the prevention of tissue damage related to oxidant production. Surprisingly, there was an increase in the GSH activities following CBZ treatment. This may be attributed to the automaticity of the biological system that must have been triggered by an abrupt rise in the accumulation of reactive oxygen species and MDA accumulation in the liver tissue.

The activities of alanine transferase reflect damage to hepatocytes, and is a highly sensitive and specific preclinical and clinical biomarker of hepatotoxicity [35,36]. In this study, a significant increase in the activities of ALT following CBZ chronic treatment suggests CBZ-induced hepato-toxicity. Findings from the study of Leo et al. [37] in their work implicated the unusually elevated levels of alkaline phosphatase as a red flag, suggesting a cholestatic picture, which might progress to acute fulminant hepatitis with fatal outcomes. However, treatment with GSME in a dose-dependent manner significantly decreased the ALP activities.

The enzyme CYP3A4 primarily metabolized carbamazepine to ring hydroxyl metabolites such as 2 and 3 hydroxyls CBZ 10, 11 CBZ-epoxide [12], while Higuchi et al. [38] reported accumulation of reactive metabolites with resultant hepatic injuries either by direct or immune-related mechanisms. In this study, depletion of the activities of liver glutathione following CBZ treatment suggests induced liver toxicity with resultant alteration in the histoarchitectural profile characterized by severe hepatic congestion and reduced sinusoids with infiltration of inflammatory cells. This finding is in agreement with the previous conclusion of the European Association for the Study of the Liver [19] that the pathogenesis of drug-induced liver injury usually involves the participation of a toxic drug or metabolite that either elicits an immune response or directly affects the biochemistry of the cell. In either case, the resultant cell death is the event that leads to the clinical manifestation of hepatitis.

In conclusion, the cause of hepatotoxicity following chronic CBZ treatment in this study is multifaceted. This ranges from body and organ weight loss, elevation of liver enzymes, lipid peroxidation, and disorganization of the histoarchitectural profile of the liver. However, co-administration of GSME with CBZ attenuated most of these disturbances in a dose-dependent manner. Therefore, GSME may serve as a potential therapeutic agent in the carbamazepine-induced hepatotoxicity/ dysfunction.

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CRediT authorship contribution statement

Opeyemi Samson Osuntokun: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Gbola Olayiwola:** Conceptualization, Validation, Writing - original draft, Writing - review & editing. **Tope Gafar Atere:** Investigation, Data curation, Writing - original draft. **Kabiru Isola Adedokun:** Validation, Funding acquisition. **Olayemi Olutobi Oladokun:** Investigation, Funding acquisition.

Declaration of Competing Interest

The authors report no conflict of interest.

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