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Research Article
Hepatoprotective Effects of Ethanol Extracts of *Gongronema latifolium* Leaves in a Carbon Tetrachloride (CCl_4)-Induced Murine Model of Hepatocellular Injury

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Abstract

Background and Objective: Liver diseases are some of the commonest causes of death worldwide. *Gongronema latifolium* leaves are used in treatment of hepatic disorders in traditional medicine. The aim of this study was to assess the effect of ethanol leaf extracts of *Gongronema latifolium* (EEGL) on carbon tetrachloride (CCl_4)-induced liver damage. **Materials and Methods:** Twenty five rats were divided into five groups of five rats each. Group I (vehicle control) received feed and water only. Groups II to V received a single dose of CCl_4 in olive oil (1 mL kg^{-1} b.wt.) on day 7; while group II (positive control) were treated with the standard drug, silymarin (50 mg kg^{-1} b.wt.), and group III (negative control) were left untreated. Groups IV and V (test groups) were pre-treated with 200 and 400 mg kg^{-1} b.wt. doses of EEGL for seven consecutive days prior to CCl_4 challenge. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatases (ALP) and the concentrations of malondialdehyde (MDA) were determined in serum. **Results:** Pre-treatment with varied doses of the extract for seven days prior to CCl_4 intoxication led to significant decrease ($p<0.05$) in activity of AST relative to negative control while there were non-significant ($p>0.05$) decreases in the activities of ALT and ALP. Malondialdehyde concentrations were decreased significantly ($p<0.05$) by 200 mg kg^{-1} b.wt. of EEGL when compared to the negative control. **Conclusion:** The results obtained from this study showed that EEGL might be useful for prevention of hepatotoxicity induced by CCl_4 through reduction in oxidative stress.

Key words: *Gongronema latifolium*, carbon tetrachloride, hepatoprotection, liver disease

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The liver plays a vital function in xenobiotic metabolism and it is involved in the regulation of several physiochemical processes in the body like oxidation, reduction, hydroxylation, hydrolysis, conjugation, sulfation, acetylation etc[1]. There is impairment in liver function when the hepatic tissues are destroyed or damaged[2]. Carbon tetrachloride (CCl_4) is a common toxicant for liver tissues employed in many experimental models[3]. The mechanisms of hepatotoxicity induced by CCl_4 are via the production of reactive intermediates like trichloromethyl radical ($CCl_3^{\bullet}$) and its derivative trichloromethyl peroxy radical ($CCl_3OO^{\bullet}$), which are produced by cytochrome P_{450} of liver microsomes. These free radicals are believed to cause lipid peroxidation via their reaction with membrane lipids[3]. The aftermaths of CCl_4-induced lipid peroxidation include damage of the liver cells membrane with resultant liberation of aminotranferases, alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and γ-glutamyltransferase (γ-GT) which are indicators of liver damage, centrilobular necrosis and steatosis[3]. The concentration of the reactive oxygen species within the cell is dependent on the rate of their production and removal by several enzymatic and non-enzymatic antioxidants produced endogenously[4, 5].

The choice of drugs for the treatment of hepatic ailment is still contentious because orthodox drugs used in the management of these diseases are inadequate and have some serious adverse effect[6]. The use of plants in the treatment of several diseases is as old as mankind because of its minimal side effects and safety[7]. Additionally, medicinal plants or herbal drugs are widely employed in disease treatment due to their low cost even when their active components are not fully explored[8]. It is on this premise that research attention is now focused on "green" compounds that can help in disease management.

Gongronema latifolium, commonly called 'utazi' and 'arokeke' in the South Eastern and South Western parts of Nigeria, respectively, is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine[9]. *Gongronema Latifolium* leaves are rich in phytochemicals and nutrients which are related to their therapeutic and nutritional potentials in various parts of Nigeria. A previous report indicates that *G. latifolium* leaves contain appreciable quantities of flavonoids, terpenes, tannins, saponins and alkaloids[10]. The leaves of *G. latifolium* have been utilized in the making of several herbal products which are administered orally for treatment of several ailments[11]. Some previous studies reported the ability of *G. latifolium* leaves-supplemented diets to protect diabetic oxidative stress and liver damage was probably due to its content of several antioxidants vitamins, minerals, cofactors and phytochemicals[12, 13].

Based on the above findings, and the folk-lore use of the plant, the present study was undertaken to explore the hepato-protective potentials of ethanol leaf extracts of *G. latifolium* in rats in which liver injury was induced using carbon tetrachloride. This objective was achieved by monitoring the concentrations of key enzyme markers of hepatocellular integrity [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP)] and a lipid peroxidation maker [malondialdehyde (MDA)], in the serum of the rats. The results will be presented and discussed subsequently.

MATERIALS AND METHODS

The study was carried out at the Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria, for a period of four months (February to May 2017).

Plant sample collection and preparation of the leaf extract: Fresh leaves of *Gongronema latifolium* were obtained from the Meat market Abakaliki, Ebonyi State, Nigeria in February, 2017 during dry season. The plants were authenticated by a taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The plant leaves were shade-dried for a period of two weeks at room temperature. The dried leaves were ground to powdered form using a portable mill. The extract was prepared as described by Ogbadoyi *et al.*[14]. Fifty grams of the powder was weighed out and extracted under reflux in 400 mL of ethanol. The extraction lasted for 2 h. The extract was filtered while hot using a muslin cloth and the filtrate evaporated and concentrated in a water bath. The crude extract obtained was transferred into a clean universal bottle and stored in the refrigerator prior to use. The two test doses were worked out from the stock.

Animals and treatment: Twenty five male Wistar albino rats obtained from the Animal House of Pharmaceutical Sciences, University of Nigeria, Nsukka that weighed between 85-180 g were used. They were safely transported to the Animal House, Alex Ekwueme Federal University Ndufu-Alike, housed in polypropylene cages and were acclimatized for 14 days. The

rats had *ad libitum* access to water and feed (Royal feeds, Enugu, Nigeria). The procedures used in this study conformed to the principles guiding research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the Care and Use of animals[15]. The rats were purposively randomized into five experimental groups of five rats each, as follows:

- Group I served as the vehicle control and received olive oil (1 mL kg^{-1} b.wt.)
- Group II (positive control) received a standard drug, silymarin (50 mg kg^{-1} b.wt.)+a single oral dose of CCl_4 in olive oil (1 mL kg^{-1} b.wt.) on day 7
- Group III (negative control) received only an oral administration of CCl_4 (1 mL kg^{-1} b.wt.) in olive oil on day 7
- Groups IV received 200 mg kg^{-1} b.wt. EEGL for 7 days+a single oral dose of CCl_4 in olive oil (1 mL kg^{-1} b.wt.) on day 7
- Groups V was pretreated with 400 mg kg^{-1} b.wt. EEGL for seven days+a single oral dose of CCl4 in olive oil (1 mL kg^{-1} b.wt.) on day 7

Three days after oral administration of CCl_4 (on day 10), the animals were sacrificed by cardiac puncture and blood was collected into plain bottles, allowed to clot and centrifuged at 1000 rmp for 30 min. The sera were carefully collected and stored at -4°C prior to analyses.

Biochemical analyses

Determination of liver function enzymes: The activities of serum ALT and AST were determined enzymatically and colorimetrically using test kits procured from Randox. The principles were based on those described by Reitman and Frankel[16]. Serum ALP activities were determined using a similar method as described by Rec[17].

Malondialdehyde (MDA) determination: The level of lipid peroxidation in the experimental rats was quantified using the MDA assay, otherwise known as thiobarbituric acid reaction method as described by Witte *et al.*[18].

Statistical analysis: All data were arranged and analyzed using the IBM-SPSS Statistics version 20.0 (IBM Corp., Armonk, NY). Results were expressed as Mean±standard deviation (SD). Comparative analysis between groups was done using one way analysis of variance (ANOVA) using least significant difference (LSD). A significance threshold of ($p<0.05$) was adopted for all statistical analyses.

RESULTS

Liver function parameters: The results showed a significant ($p<0.05$) decrease in serum AST activity in groups pre-treated with the extracts relative to negative control group (Fig. 1). However, there was no significant difference between the AST values of the vehicle control, positive control and the pre-treated groups. The results in Fig. 2 showed non-significant decrease in ALT activities of pre-treated groups ($p>0.05$) when compared to the negative control group. From Fig. 3 it is seen that there was a non-significant decrease in the activity of the group pre-treated with 200 mg kg^{-1} of extract when compared with the negative control group. There was

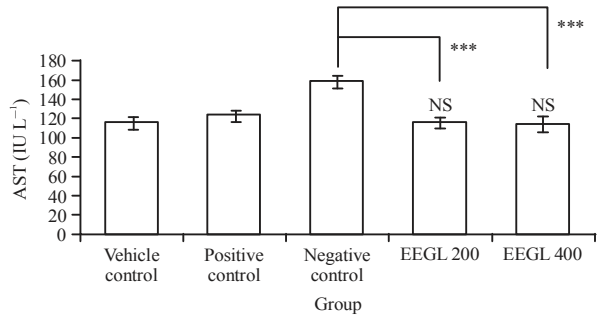

Fig. 1: Mean aspartate aminotransferase (AST) activity of the different groups, EEGL: Ethanol extract of *Gongronema latifolium* leaf. Comparisons to the positive control are indicated on the error bars, otherwise, the comparisons are to the negative control. ***Indicates significant differences at $p<0.001$, NS: Not significant

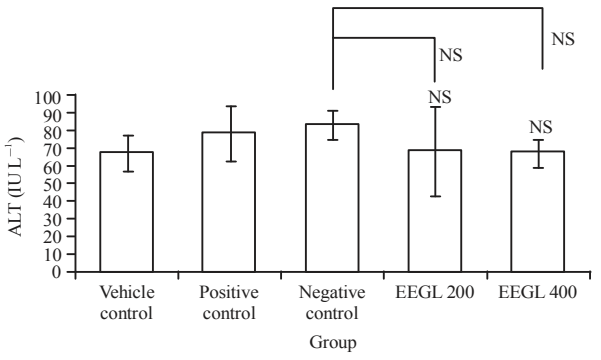

Fig. 2: Mean alanine aminotransferase (ALT) activity of the different treatment groups, EEGL: Ethanol extract of *Gongronema latifolium* leaf. Comparisons to the positive control are indicated on the error bars, otherwise, the comparisons are to the negative control, NS: Not significant

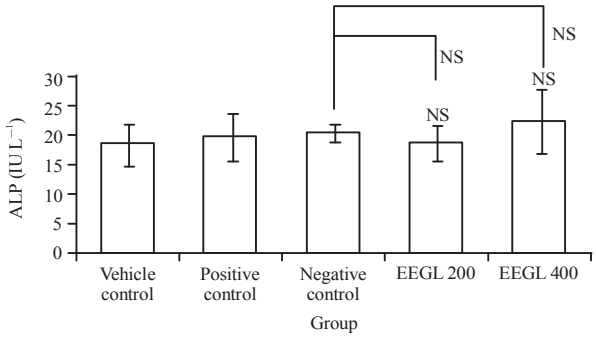

Fig. 3: Mean alkaline phosphatase (ALP) activity of the different treatment groups, EEGL: Ethanol extract of *Gongronema latifolium* leaf. Comparisons to the positive control are indicated on the error bars, otherwise, the comparisons are to the negative control, NS: Not significant

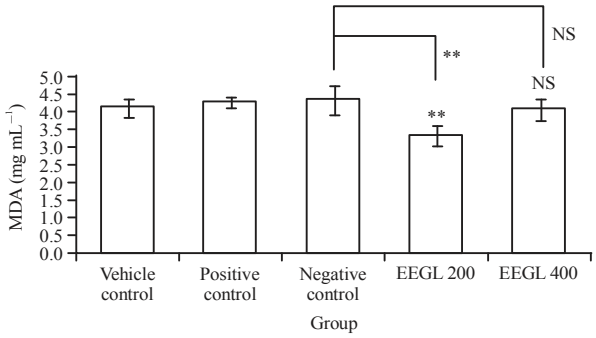

Fig. 4: Mean Malondialdehyde (MDA) values of the different treated groups, EEGL: Ethanol extract of *Gongronema latifolium* leaf. Comparisons to the positive control are indicated on the error bars, otherwise, the comparisons are to the negative control. **Indicates significant differences at p<0.01, NS: Not significant

no significant difference in the ALP values of all the test groups (p>0.05) relative to the negative control group.

Lipid peroxidation marker: The results of mean MDA shown in Fig. 4 revealed significantly (p<0.05) lower concentration of MDA in the EEGL 200 mg kg^{-1} group relative to all other groups while no significant difference (p>0.05) was seen between the vehicle control, positive control, negative control and EEGL 400 mg kg^{-1} group.

DISCUSSION

The results from this study indicate that pre-treatment of rats with 200 and 400 mg kg^{-1} EEGL for seven days prior to intoxication with CCl_4 resulted in a considerable lowering of the serum concentrations of AST and MDA relative to the negative control. Implicit in this is that the extracts were able to prepare the liver cells to either resist, or quickly recover from, the insults emanating from CCl_4 exposure. This, it may have achieved by quenching the radicals produced by CCl_4 or by promoting accelerated regeneration of parenchymal cells, hence preventing the fragility of the cell membrane and leakage of liver marker enzymes into circulation. It is worthy of note, that the test doses in all cases either matched the activity recorded by the standard drug or even surpassed it (as is seen in the data for serum MDA concentrations). To get this kind of activity in a crude extract is promising as purification of the constituents to liberate the extracts from unwanted (possibly interfering compounds) will very likely heighten its activity. The observation of a reduced activity at the 400 mg kg^{-1} b. wt. does appear to support this line of thought. This is however without prejudice to possible synergistic accentuation of effects that often happens with phytochemicals[12].

The changes in the level of activity of the liver enzymes system have been used clinically in assessing the toxicity of any substance foreign to the body (a xenobiotic). This is due to the fact that any derangement of biochemical processes in experimental animals due to the presence of a xenobiotic would be reflected as an increase or a decrease in the activity of such enzymes including AST and ALT [19]. The potency of any liver prophylactic drug is majorly dependent on its ability in either reducing the deleterious effect or in maintaining the normal liver physiological function which has been changed by the hepatotoxin[20].

In this study, there was elevation in the serum levels of AST, and insignificant elevations in the activities of ALT and ALP, in the animals intoxicated by CCl_4, which is an indication of cellular leakage and compromise in activities of cell membrane in liver[21]. These results are consistent with previous findings which revealed that intoxication of rats with CCl_4 increases levels of ALT, AST and ALP in serum which indicates liver damage[22,23,24]. Specifically, an elevated level of ALT is indicative of hepatocyte damage[25,26]. The findings in this study indicated that pre-treatment of the rats for seven days before administration of CCl_4 caused a significant (p<0.05) decrease in AST and non significant decrease (p>0.05) in ALT activity relative to negative control. This result is in agreement with previous finding of significant decrease of AST, ALT and ALP activity in rats on pre-treatment with diet formulated with the flour of *Garcinia kola* for 21 days prior to $CC1_4$ administration[27]. This showed that ethanol extract of leaves of *Gongronema latifolium* may have protective effect on CCl_4 induced liver damage in Wistar albino rats. In fact, some earlier studies had

pointed to this[28,29]. The studies however either used a different solvent and adopted co-treatment[28] or the same solvent and treatment method but a different method to induce hepatotoxicity[29]. The data presented here therefore enriches the literature on the usefulness of *Gongronema latifolium* in hepato-protection irrespective of nature of extracts, toxicant or treatment.

CCl_4 induces hepatotoxicity through generation of reactive oxygen species or free radical and depletion of glutathione culminating in oxidative stress[30]. Intoxication of CCl_4 provokes free trichloromethyl radical ($CCl_3^{\bullet}$) which induces liver damage as a result of activation of the NADPH-Cyt P_{450} system of liver endoplasmic reticulum[31] which culminates in production of more reactive radical, trichloromethyl peroxy ($CCl_3OO^{\bullet}$) that induces peroxidation of lipids, alteration of homeostasis of Ca^{2+} and apoptosis[32]. These alterations in the function and morphology of the cellular membrane and death of the cells result in leakage of liver enzymes while the free radicals cause fatty acid oxidation which releases lipid peroxides[31]. MDA is generated from the oxidation of poly-unsaturated fatty acids as a secondary product[33] and serves as a main indicator to quantify the levels of lipid peroxidation[34]. Besides, membrane damage is a function of rate of peroxidation of lipid and this lipid peroxidation depicts changes in the morphology and function of cellular membrane[35]. The significant ($p<0.05$) elevation in MDA levels in the negative control group, compared to the other groups, may be as a result of the enhanced membrane lipid peroxidation by free radicals generated and failure of antioxidant defence mechanisms to prevent formation of excessive free radicals[36]. There was a significant ($p<0.05$) decrease in MDA levels in the group pretreated with 200 mg kg^{-1} of extract when compared to the negative and positive control groups which suggests that the leaf extract may possess anti-oxidant properties which is consistent with reports of Nwanjo *et al.*[37]. The observation that the extract did not lower the MDA concentration significantly at 400 mg kg^{-1} b.wt. suggests that at high concentrations, the extract may not be as efficient in preventing lipid peroxidation. This may be due to antagonistic reactions between phytochemicals, or the presence of pro-oxidants which may become problematic at high doses.

This study is however limited by financial constraints for which further experiments directed at identifying more robustly, the possible mechanisms action of the extract could not be done. Again, the study may have benefitted from a longer pre-treatment period, say up to two to three weeks, as this would have been better for evaluating the efficacy of the extract. Finally, it would be important to identify the exact active phytochemical(s) responsible for prophylactic activity of EEGL.

CONCLUSION

In conclusion, this study investigated the ability of ethanol extracts of the leaves of *Gongronema latifolium* (given as a pre-treatment to rats) to protect liver cells from CCl_4-induced damaged. The extracts significantly reduced damage to hepatocytes and reduced the lipid peroxidation of their membranes. Given that the extracts were more effective at the 200 mg kg^{-1} b.wt. dose, caution is warranted in the possible application of these extracts as antagonisms may exist at high doses.

SIGNIFICANCE STATEMENT

This research showed that methanol extracts of the leaves of *Gongronema latifolium* are capable of reducing hepatocellular injury caused by CCl_4, in rats that are pre-treated with it. This is beneficial to the scientific society as it searches for novel "green" compounds that can be used in managing the rising cases of liver diseases. With these results, researchers can better focus on possible candidates for drug development in this area. Finally, a new theory on the prevention of hepatic injury by phytochemicals can be arrived at.

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