

Antihepatotoxicity Studies of Crude Extract of *Zingiber officinale* on CCl₄ Induced Toxicity and Comparison of the Extract's Fraction D Hepatoprotective Capacity

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Abstract: The antihepatotoxic effect of ethanolic extract of ginger (*Zingiber officinale*) against CCl₄ (10 mL kg⁻¹ body weight) were investigated. Total 7 groups of rats were used in the investigation with alternative methods of administration of ginger extract and CCl₄ both at 24 h intervals as well as simultaneous administrations. All the administration methods involved injection of the substances intraperitoneally. Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT) decreased significantly ($p < 0.05$) when ginger ethanolic extract was administered first (1000 mg kg⁻¹ body weight) followed by CCl₄ 24 h later. Injection of CCl₄ followed by ethanolic ginger extract 24 h later gave a reduction in the serum enzyme but not as much as when ginger extract was first administered. The same result above was also obtained for lipid peroxidation production. Protein synthesis was not affected by the various groups although, CCl₄ and ethanolic extract of ginger caused increase in serum protein which did not show any significant increase ($p > 0.05$). Inorganic phosphate was increased by both CCl₄ and ethanolic extract administration. Fraction D was shown to have more hepatoprotective effect than even the ethanolic extract itself. Administration of ginger extract and CCl₄ simultaneously had the least hepatoprotective effect. Thus, preventive intraperitoneal administration of ginger ethanolic extract before liver injury had the highest efficacy against hepatotoxic induction using CCl₄.

Key words: Hepatoprotective, intraperitoneal, ginger, SGPT, SGOT, CCl₄, Nigeria

INTRODUCTION

Plants have various uses, particularly as primary source of food they could also be subjected to other uses. They are known to possess the active components used in drug production as well as raw materials for industrial products. Some traditional medicine, particularly those used as part of African medicine has been extensively used in various herbal extract forms for treatment of diverse ailments. Combination of garlic and ginger had been researched (Ahmed and Sharma, 1997) and shown to be effective in reducing blood glucose and serum lipids. Toxins are introduced into the body through food, water or air (including drugs, food additives, industrial chemicals and pesticides).

Even in healthy individuals, the toxins that abound in the modern environment can overload or inhibit liver function, possibly resulting in a steady, insidious accumulation of harmful substance (Elegbede *et al.*, 1993). The clearest toxicological evidence for CCl₄ arises from its effects on the liver. According to the findings by the

International Programme on Chemical Safety (IPCS 1999), CCl₄ was shown to be an outstanding and potent hepatotoxicant. Human exposure by any route including inhalation has been associated with various signs of liver damage including elevated hepatic enzyme and bilirubin levels in the serum jaundice, altered serum protein levels and a swollen and tender liver.

When enzymatically active cells are lysed or destroyed (Zimmerman and Henry, 1984), certain enzymes are released into the serum. These enzymes are measured to assess which tissue is damaged. Only active cells release high quantities of enzymes in the serum. The more acute and extensive tissue injury is the greater the rise in enzymes released from that tissue. A number of animals and *in vitro* trials have shown that many herbs can protect liver cells from damages against a variety of chemicals or immunological agents (Wagner, 1981). According to Leung and Foster (1996), the pungent principles of ginger helps to counter liver toxicity by increased bile secretion and its acetone and methanolic extract is a stomachic as well as a strong inhibitor of

gastric ulceration. In this study, it is examined the various hepatoprotective effects of *Zingiber officinale* crude extract and a particular fraction of the crude extract (Fraction D) on various liver toxic parameters such as enzymes, protein, inorganic phosphate and lipid peroxidation. CCl_4 was administered intraperitoneally. Crude ginger extract and Fraction D of the extract were administered intraperitoneally at protective as well as curative dosages.

MATERIALS AND METHODS

Equipments and instruments: The equipment and instruments used include the following: hotbox oven-Gallenkamp England, spectrophotometer-NOVASPEC Germany, sensitive electric balance-Ohaus Compact Scale, flask shaker, bench centrifuge-MSE, refrigerator-Thermocool F200, thermometer-MKV, pH meter-PYE MODEL 290, test tubes-PYREX AND BROSIL, cotton wool, aluminum foil, cuvette, liquid dispenser, beakers-PYREX, micropipettes, petri dish, spatula, measuring cylinder, sample bottles, hand gloves, filter papers, water bath-Grant instrument, hand grinding machine, chromatographic tank, Soxhlet extractor, fractionating column, plates, electrically operated laboratory type homogenizer.

Chemicals and biochemical reagents: All the chemicals and biochemicals used were reagent grade, some of them includes: glacial acetic acid (BDH, poole, U.K), sodium chloride (may and baker), concentrated sulphuric acid, thiobarbituric acid, chloroform, carbon tetrachloride (CCl_4) (Merck, darmstadt, F.R.G.), Ethanol absolute (Vicker, West York, U.K.), petroleum ether, silica gel.

Plant materials used: The fresh succulent rhizome of the plant (*Zingiber officinale* Rosc) were bought from the Oba market, suburb of Nsukka, Nigeria. They were identified by Mr. P.O. Ugwuozor, the Chief Consultant, Herbarium section of the Department of Botany, University of Nigeria, Nsukka. The rhizomes were washed to remove sand and other impurities before cutting into small pieces and subsequently air dried for 10 days. Milling of the dried ginger was done using a sterilized manual hand grinder.

Animals used: About 40 healthy inbred Wister albino rats of either sex between 4-6 weeks old weighing (46-80 g) were bought from the National Veterinary Research Institute Vom (NVRI), Plateau State, Nigeria. Additional 10 adult mice of both sexes were also bought from the animal house of the Faculty of Veterinary Medicine, University

of Nigeria Nsukka, Nigeria. They were quartered in stainless steel cages at room temperature of 28-32°C and a light period of 15-17 h daily for 3 weeks. The animals were fed with standard commercial chaw (Vital feeds brand produced by Grand Cereals and Oil Mills Ltd., Jos, Nigeria and obtained from Ogige Market Nsukka) and tap water administered to them *ad libitum*.

Extraction of plant material/components: About 170 g of the powdered rhizome of *Zingiber officinale* was packed into a Soxhlet extractor. Petroleum ether (2.4 L) was used to defat the material. Extraction was carried out using 2300 mL of 70% ethanol. The extraction process ran for 48 h. Concentration of the extract was carried out in a water bath at 40°C in a fume chamber for 72 h. The concentrated extract was then weighed and stored in a refrigerator below 5°C for further use.

Preliminary test for fraction by the Thin Layer Chromatographic (TLC) method: Thin Layer Chromatography (TLC) was carried out on the crude extract obtained from the rhizome to determine the best solvent system for separating its various fractions and the best ratio of solvent mixture needed.

TLC plates: About 15 glass plates each with a dimension of approximately 5×20 cm were used. The plates were washed and dried in the oven. About 200 g of silica gel were dissolved in 400 mL of distilled water to form slurry which was used to coat the plates with the aid of a spreader. The plates coated were air dried and subsequently activated in oven at 120°C for about 1 h.

Solvent system: Before the best solvent system for the separation was known, different solvents were tried, they include Ethanol: Chloroform in the ratio of 2.5: 7.5, Acetone: Chloroform: Acetic acid (2.5: 7.5: 2.5). Butanol: Ethyl Acetate: Methanol (2: 7: 1), Hexane: Methanol: Ethyl Acetate (2: 2: 2), Ethyl Acetate: Chloroform: Acetone: Toluene: Acetic Acid (3: 3: 2: 1: 1), Ethyl Acetate: Toluene: Ethanol (8: 0.5: 1.5), Ethyl Acetate: Toluene (5: 5), Toluene: Ethyl Acetate (9.3: 0.7). Each of these solvent mixture were tried by pouring into a clean chromatographic tank with the cover sealed with Vaseline Jelly to keep air tight and allowed to equilibrate for 20 min before introducing the spotted plates to be developed for a period of 1 h. Finally the system containing Ethyl Acetate: Toluene (9.3: 0.7) was used and the extract was separated into 4 bands after developing in an iodine tank and sprayed with Dragendorff's reagent, respectively.

Fractionation of the plant extract: The fractionation was carried out using a quick fit column fractionator whose dimensions are 4×30 cm. The column was gradually packed with adsorbent chromatographic silica gel. This was packed to about 80% of the length of the column; the extract was then introduced through the apex of the column fractionator.

With the tap gradually opened, more of the solvent was introduced and the eluent collected into test tubes at intervals of 30 min. All the test tubes had their contents spotted on TLC plates and developed in the chromatographic tank to know similar components. The similar components were then pooled as 1 fraction. The crude extract when separated gave 4 fractions namely: fraction A-D.

Experimental arrangement of animal specimen: All administration of substances was through injection (intraperitoneally), blood was collected 34 h after the administration of the 1st substance except for group G and arrangements of the various groups are as indicated:

Group A: This group was administered with the vehicle (10% tween 20) 10 mL kg⁻¹ body weight of rats. The rats in this group were 2 males weighing 243.0 and 201.8 g and 2 females weighing 206.0 and 158.5 g, respectively.

Group B: This group was administered with 10 mL kg⁻¹ body weight carbon tetrachloride only once a day for 2 days and served as the negative control. The group had 2 male and 2 female rats. The body weights of the male were 218.2 and 251.5 g and the females were 171.0 and 170.5 g.

Group C: This group was administered with the plant extract of 1000 mg kg⁻¹ body weight intraperitoneally for a period of 2 days. This group contained 2 male rats weighing 187.0 and 255.0 g and 2 females weighing 180.5 and 170.0 g.

Group D: This group was administered CCl₄ intraperitoneally and then the extract 24 h later for 2 days consecutively. This group had 2 male rats weighing 208.0 and 183.5 g, the group also consisted of 2 females weighing 177.0 and 170.0 g.

Group E: This group was treated with the extract 1000 mg kg⁻¹ body weight intraperitoneally then followed by CCl₄ 10 mL kg⁻¹ body weight 24 h later for 2 days. The rats were 2 male rats weighing 169.5 and 195.0 g; it also had 2 females weighing 164 and 163 g.

Group F: This group was administered ginger ethanolic extract and CCl₄ intraperitoneally at the same time for 2 days. The group was made up of 2 male rats weighing 210.0 and 190.0 g and 2 females 191.0 and 179.0 g.

Group G: This group was treated with fraction D intraperitoneally twice daily for 4 days; the group contained 2 males weighing 220.5 and 194.5 g and a female weighing 184.0 g.

Acute toxicity test (LD₅₀ determination): Widely differing doses were administered to the animals in acute toxicity test. About 10 adult mice of both sexes were used and 2 mice per group of 5 groups given various doses of the crude extract dissolved in 10% tween 20 solution. This was injected at concentrations of 10, 100, 1000, 2000 and 3000 mg kg⁻¹. Injection was carried out intraperitoneally.

Isolation of blood serum: Blood was collected from the ocular region of the live rats by use of capillary tubes (non-heparinized) and centrifuged at 3000 g for 10 min to obtain the serum.

Preparation of liver homogenate: About 4 g of each liver harvested and properly labeled was rinsed in normal saline and 5 mL of normal saline added to them in 25 mL beakers. They were then homogenized using a homogenizer (Electric Homogenizing Machine).

Biochemical tests: The blood and harvested liver were used for the various biochemical tests such as SGOT, SGPT, Protein, Inorganic Phosphate and Lipid Peroxidation tests Serum Glutamate Oxaloacetate Transaminase (SGOT) (Reitman and Frankel, 1957; Bergmeyer, 1974). In this calorimetric assay, 0.5 mL of substrate (sodium pyruvate) was warmed in a water bath at 37°C for 3 min. About 0.1 mL of serum was added, mixed gently and incubated for 60 min. The tubes were removed from the bath and immediately; 0.5 mL of Denitrophenol (DNPH) was added and mixed well. DNPH was allowed to react in all the tubes for 20 min at room temperature and then 5 mL of 0.4 N sodium hydroxide solution was added, mixed well and left for a further 10 min. The Optical Density (OD) was read at 540 nm.

Serum Glutamate Pyruvate Transaminase (SGPT) test: The methodology for determination of SGPT is similar to that of SGOT. Only difference being that SGPT substrate is used instead and the incubation time reduced to 30 min.

Lipid peroxidation test (Wallin *et al.*, 1993): About 1 mL of liver homogenate of all the animal groups were measured followed by addition of 0.1 mL of 10%

triton×100, 3 mL of Tricarboxylic acid and 1 mL of 0.6% Thiobarbituric Acid (TBA). The tubes were shaken vigorously and filtered. To 3 mL of the clear filtrates were added 2 mL of 0.6% TBA acid. The mixtures were incubated in boiling water for 15 min, cooled and 2 mL of chloroform added, centrifuged and OD read at 532 nm. The result was corresponded to that of the standard curve and concentration determined.

Protein determination was according to the Biuret's method: Egg albumin 1 g was dissolved in 100 mL of distilled water; serial dilution was made and incubated with biuret's reagent for 20 min at room temperature. The absorbance was read at 540 nm against a dionized water blank containing biuret reagent using a spectrophotomer. This was used for the standard curve for protein concentration determination. Egg albumin was replaced with 1 mL of blood serum from the various animal groups. These were diluted 20 folds and the absorbance read and the concentration of protein determined using the standard curve.

Inorganic phosphate: Modified Gomeri (1942), serial dilutions of stock solution were incubated for 20 min at room temperature and extinction read against the blank at 660 nm. The serum solution was treated similarly and extinction values (inorganic phosphate) extrapolated using the standard curve produced from the stock solution.

Statistical analysis: The statistical analysis was carried out by 1 way Analysis of Variance (ANOVA). Data were expressed as mean±SD (n = 4). The p-values<0.05 were considered significant.

RESULTS AND DISCUSSION

There was significant (p<0.05) reduction on the serum enzymes (SGOT and SGPT) on administration of ethanolic extract of *Zingiber officinal* Rosc alone and its fraction D. This is a clear indication that ginger has hepatoprotective properties. The acute toxicity tests showed that when ginger extract was administered to the mice at dosages of between 1000-2000 mg kg⁻¹ body weight there were no mortality recorded however there was 80% mortality at 3000 mg kg⁻¹ body weight and above. Thus, the administered extract dosage used through out the experiment were non lethal. The CCl₄ used on the rats was at a dosage of 10 mL kg⁻¹ body weight which caused hepatic toxicity as seen by the high level of SGOT, SGPT and lipid peroxidation represented by the group B as shown in Fig. 1-3.

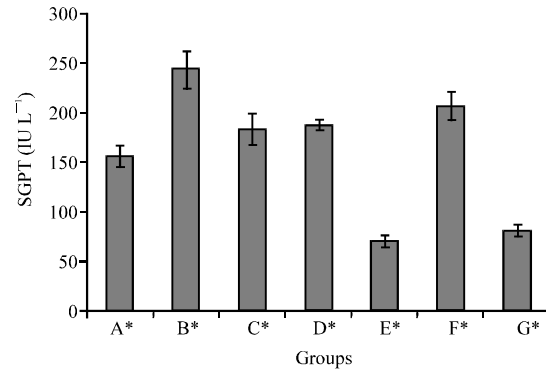


Fig. 1: Influence of ginger extract and its fraction D on serum SGPT level in CCl₄ induced hepatotoxicity

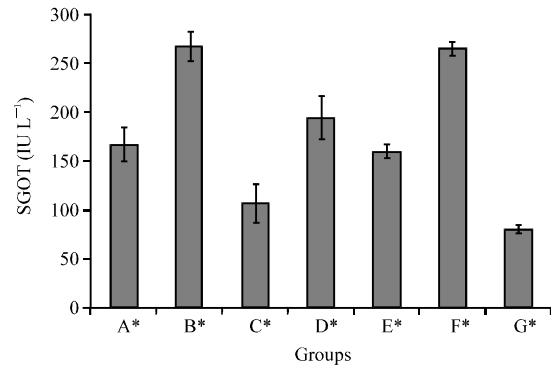


Fig. 2: Influence of ginger extract and its fraction D on serum SGOT level in CCl₄ induced hepatotoxicity

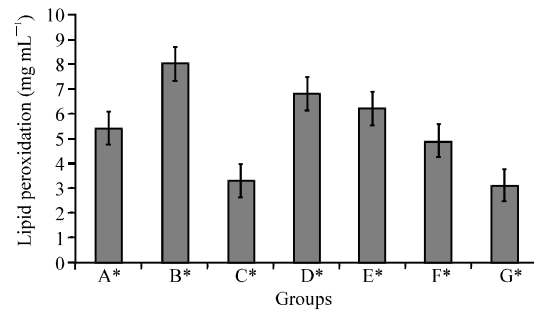


Fig. 3: Influence of ginger extract and its fraction D on liver homogenate lipid peroxidation level in CCl₄ hepatotoxicity (A-G represents the various group of rats and their CCl₄). Ethanolic extracts and Fraction D administrations

The administration of plant extract (1000 mg kg⁻¹ body weight intraperitoneally) before administration of CCl₄ 24 h later (Group E), gave the least observed elevation of the biochemical parameters tested viz: protein synthesis, inorganic phosphate, SGOT and SGPT. Group D, included rats injected with CCl₄ before administration of the extract 24 h later and there was more elevation in

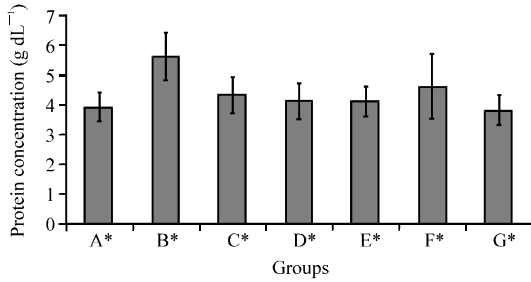


Fig. 4: Influence of ginger extract and its fraction D on serum protein synthesis level in CCl₄ induced hepatotoxicity

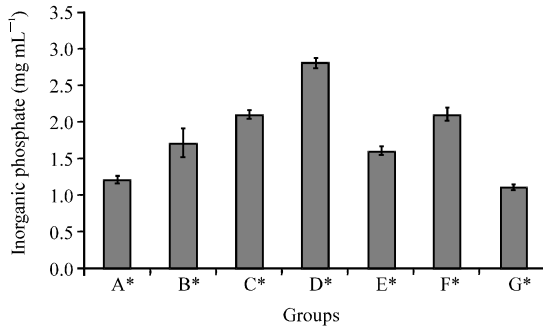
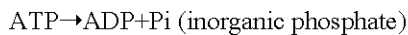


Fig. 5: Influence of ginger extract and its fraction D on serum inorganic phosphate level in CCl₄ hepatotoxicity

the serum enzymes (SGOT and SGPT, respectively) above those of group E. A significant ($p < 0.05$) increase in elevation of SGOT, SGPT and protein concentration as observed in the group F (administration of crude ginger ethanolic extract followed by CCl₄ immediately) (Fig. 4). Group F however had a slight reduction in the parameters mentioned in comparison to only group B (administration of CCl₄ only) which is not significantly ($p > 0.05$) different. Lipid peroxidation is highest in Group B (administration of CCl₄ only) the negative control followed by group D (CCl₄ administered followed by ginger ethanolic extract 24 h later).

Group A (administration of 10% tween 20 (10 mL kg⁻¹ body weight of rats) served as positive control and showed moderate level in all the biochemical parameters measured when compared to other groups especially the negative control (Group B). The production of inorganic phosphate in the liver is the index of the level of utilization of ATP (Lehninger *et al.*, 2000). Thus:



Therefore, groups D, F and C showed increased elevation of inorganic phosphate in an increasing order as shown in Fig. 5. This probably indicated the fact that high

level of CCl₄ and ginger (crude ethanolic extract) causes a proportional generation of energy for metabolic synthesis and subsequently production of inorganic phosphate. The observable protein synthesized in each group was within the normal range between 3.5-5 g dL⁻¹ except for group B (administration of CCl₄ only). The experimental results showed that increase in the dosage of CCl₄ as seen in group B and group F (ginger ethanolic extract administration followed by CCl₄ immediately) increases the synthesis of protein as seen in the concentration of protein serum. Administration of the fraction D alone showed significant ($p < 0.05$) reduction in all the biochemical parameters examined. Probably, fraction D of the ethanolic extract of *Zingiber officinale* Rosc contains the active agents (flavonoid) against hepatotoxicity. Flavonoid has hepatoprotective properties (Egbuna and Echetabu, 1996). This could be due to the fact that flavonoids have the ability to inhibit lipid peroxidation.

CONCLUSION

The conclusions derived from the experiment are that the fraction D of the ethanolic extract of ginger is highly hepatoprotective followed by the extract itself. This is seen in the administration of the ethanolic ginger extract followed by CCl₄ 24 h later (Group E) when compared to administration of CCl₄ followed by extract 24 h later as seen in group D. In other words, preventive use of ginger against toxic substances (CCl₄) is more effective than its treatment after hepatic damage had taken place due to exposure of the liver to such damages. Administration of ginger extract and CCl₄ simultaneously (Group F) had little or no significant effect in the antihepatotoxicity studies conducted.

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