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Original Research Article

Anti-Hepatotoxic Effect of Soy (*Glycine Max*) Against Tetra Chloromethane (CCL_4)-Induced Liver Damage in Albino Rat

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ABSTRACT

Many liver injuries have been linked to drugs or alcohol. The hepatoprotective potential in Soy bean has remained understudied over the years. The aim of this study was to investigate the anti-hepatotoxic effect of *Glycine max* against tetra chloromethane (CCl_4)-induced liver damage in rats. Phytochemical tests were performed. A total of 24 male albino rats weighing(100±25g) were randomly divided into four groups (I-IV) with six rats per group. Group IV served as normal control and received no treatment. Group III received only CCl_4 (1ml/kg b. wt., 1:1v/v mixture of CCl_4 and liquid paraffin, i.p) and served as negative control. Group II received Vitamin C (200mg/kg b.wt, oral) and served as the positive control and Group I which served as test group received Soymilk (2000mg/kg, oral) in the presence of CCl_4challenge for 4 weeks.CCl_4 administration to the rats resulted in liver damage with AST, ALT and ALP levels: 162.00±2.000 IU/L; 93.330±2.404 IU/L and 508.333±30.046 IU/L respectively. The daily administration of Soymilk resulted in the mitigation of the CCl_4-induced liver damage with AST, ALT and ALP levels: 121.667±4.410 IU/L ($P<0.01$); 77.333±1.453 IU/L ($P<0.01$) and 456.333±22.048 IU/L ($P>0.05$) respectively. Histopathological result also revealed minor or no significant hepatocellular degeneration in soy group; hence hepatoprotection by *Glycine max*. *Glycine max* possesses liver protective properties against CCl_4-induced liver damage.

Keywords: ethnopharmacology, hepatoprotection, hepatotoxicity, liver, soybean, tetrachloromethane

INTRODUCTION

Soy consumption is increasing in Nigeria due to animal diseases such as mad cow diseases; global shortage of animal protein; strong demand for healthy cholesterol-free and low saturated fats foods; and economic reasons. [1] The natural antioxidants defence mechanisms can be insufficient and hence dietary intake of antioxidant components is important and well recommended. [2] Soy milk is rich in proteins; low in saturated fats, higher in polyunsaturated fatty acids; cholesterol free; and a good source of B-vitamin, minerals, isoflavones, and antioxidants such as carotenoids, vitamin C and E, phenolic and thiol(SH) compounds, and essential amino acids. [3] Some studies have shown that soy bean (*Glycine max*) has potential bioactive substances that exhibit protective properties. [4-13] It has been demonstrated that phenolic compounds are effective antioxidants, due to the formation of stable phenoxyl radical. [14, 15] Hence the strong indication that soy may help prevent or treat liver damage after one has been exposed to hepatotoxic drugs such as acetaminophen and CCl_4.

Tetrachloromethane (CCl_4) is well established as xenobiotics. Previous studies showed that both the liver and the kidneys are the target organs of CCl_4. Extensive evidence demonstrates that CCl_4 is

metabolized in the liver into a highly reactive trichloromethyl radical which initiate free radical mediated lipid peroxidation of the cytoplasmic membrane phospholipids. It also causes functional and morphological changes in the cell membrane leading to an accumulation of lipid derived oxidants causing liver injury. [16, 17] Interestingly, isoflavones and soy protein are the two major constituents responsible for the health benefits of soy food, and have most attracted the attention of researcher. [16,18] Other components in soy other than isoflavones; such as soy asaponins, phytic acid or plant sterols displays a wide range of bioactivities including hepatoprotective actions. [18, 19] Shivashankara *et al*. [4] report that soy bean (*Glycine max*) has potential bioactive substances that exhibit hepatoprotective properties.

Presently, only very few research works have been done to investigate the hepatoprotection of antioxidant-rich compounds against acute liver damage by tetra chloromethane, and also there is currently no literature on the hepatoprotective effect of Soy against CCl_4-induced hepatoxicity. The aims of this study were to evaluate the phytochemicals present in soybean and to investigate the anti-hepatotoxic effect of soy (*Glycine max*) against CCl_4-induced liver damage in rats. Hopefully, findings from this research maybe insightful in respect to alternative or safer ways to prevent and/or treat drug-induced liver damage.

MATERIALS AND METHODS

Experimental animals and management

A total of twenty-four (24) male albino rats weighing (100±25g) were obtained from the Animal House of the University of Nigeria Teaching Hospital (UNTH), Enugu State, Nigeria. The usage of animals was approved by the Institutional Animal Ethics Committee. The animals were housed in clean metallic cage at the animal house under ambient temperature (25±3° C) and 12-hour light/ dark periodicity. They were well fed with standard commercial rat pellets (Neimeth Livestock Feeds Ltd., Ikeja) and clean water *ad libitum* and allowed to acclimatize for 2 weeks. All the animals were handled in this study according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings. [20] In addition, proper care was taken as per the ethical rule and regulation of the concerned committee of the University of Nigeria, Nsukka, Enugu State, Nigeria.

Preparation of Soymilk

2kg of soybeans was purchased from Ogbete market, Enugu state, Nigeria. The soybeans were parboiled in boiling water for 30 minutes until the soybeans appeared brownish in colour. The soybeans were meshed and coat removed. The soybeans were then milled with a milling machine. Using a clean filter, the milled soybean was then sieved and the particles disposed. Tap water was added at a ratio of 4:1 with grinded beans and then filtered to separate soy cake from soymilk. The soymilk was subsequently heated to 98℃. afterwards; the soymilk was cooled and preserved in a refrigerator at a temperature between 4-6℃ until when needed.

Experimental Design

The 24 apparently healthy male albino rats were randomly placed into four (4) groups labelled I to IV according to their body weight. Each group comprised six (6) albino rats. Group I served as the test group. Group II and III were the Vitamin C (positive) and CCl_4 (negative) Controls respectively, while group IV served as normal control.

Group I received Soymilk (2000mg/kg, oral); Group II was given vitamin C (200mg/kg, oral) for 4 weeks. To induce hepatotoxicity, the animals in groups I to III were intraperitoneally injected with CCl_4 (1ml/kg, 1:1v/v mixture of CCl_4 and liquid paraffin) twice weekly during the same 4 weeks duration. Group IV was not given any form of treatment and it served as

normal control. After 4 weeks, all animals (group I-IV) were bled through the left ventricle of the heart under chloroform anaesthesiainto appropriately labelled plain bottles for biochemical analysis; and subsequently the liver tissues were excised for histopathological studies.

Phytochemical analysis

Preliminary phytochemical screening for the presence of glycosides, flavonoids, saponins, steroids, tannins, carbohydrates, proteins and terpenoids was carried out at Department of Pharmacognosy, Faculty of Pharmaceutical Science, University of Nigeria Nsukka. Procedures outlined by Trease and Evans [21] were employed for the analyses.

Biochemical analysis:

Assessment of Liver Function

Serum was used for the assay of liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP)], conjugated bilirubin and total bilirubin concentrations using Rx Monza Analyzer, and standard laboratory kits from Randox Laboratories Ltd. Albumin was also analyzed using standard Randox laboratory kits.

Measurement of Bilirubin (Total and Direct)

Colorimetric method as described by Malloy and Evelyn. [22]

Measurement of ALT and AST

Determination of ALT and AST were by colorimetric method as described by Reitman and Frankel. [23]

Measurement of ALP

Determination of ALP was by colorimetric method as described by Kind and King. [24]

Measurement of Albumin

Determination of serum albumin was by Dye Binding method as described by Dumas *et al*. [25]

Histopathological analysis

The excised liver tissues were fixed in 10% formal saline for 24 hr and further processed using the conventional paraffin wax embedding technique for light microscopic examination. The paraffin-embedded liver tissues were sectioned at 5 microns using the rotary microtome (Leitz 1520 Rotary Microtome, Leica Biosystems, Nussloch Germany). The tissue sections were stained using the haematoxylin and eosin technique as described by Baker and Silverton. [26] The histological sections were examined using an Olympus ™ light microscope.

Statistical analysis

Data was analyzed using SPSS software version 18. All data were expressed as mean ±SEM. Level Of Significance was determined by the student t-test or by the one way analysis of variance (ANOVA) followed by the Tukey's Post-HOC multiple comparison tests. $P<0.05$, $p<0.01$ or $P<0.001$ was considered significant.

RESULTS

Phytochemical results

The result of the preliminary phytochemical analysis of soy bean is represented in table 1.

Table 1:PRELIMINARY PHYTOCHEMICAL ANALYSIS

Constituents	Indication
Carbohydrate	++
Reducing Sugar	++
Alkaloids	+++
Glycosides	++
Saponins	+++
Tannins	++
Flavonoids	++
Resins	–
Proteins	+++
Oils	++
Acidic Compounds	++
Terpenoids	++

Steroids + Keys: +++ = More intensely present; ++ = Present + = Present (in trace amount); – = Absent

Biochemical results

Table 2 shows the results of liver biochemical parameters of four (4) groups of six (6) animals that received (Soy milk + CCl_4), (Vitamin C + CCl_4), CCl_4 alone or no treatment. From the results, vitamin C (a drug widely known for its high anti-oxidant property) showed better hepatoprotection than Soy milk; although Soy milk significantly decreased the elevated levels of AST and ALT (enzymes markers of hepatic injury) in the animals when compared with negative controls. Furthermore, it is worthy of note that soy milk non-significantly decreased serum

ALP levels in the animals when compared with negative control ($p>0.05$).

Table 2: STATISTICAL ANALYSIS OF LIVER BIOCHEMICAL CONCENTRATIONS IN DIFFERENT EXPERIMENTAL ANIMAL GROUPS.

Groups	Total Protein (g/dL)	Albumin (g/dL)	Total bilirubin (mg/dL)	Conjugated bilirubin (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Soymilk (2000mg/kg) (I)	7.033±0.203	3.700±0.116	0.900±0.058	0.400±0.058	121.667±4.410**	77.333±1.453**	456.333±22.048
Vitamin C (200mg/kg) (II)	6.770±0.376	3.530±0.176	1.100±0.100	0.533 ±0.067	110.000±2.887***	72.667±1.453**	395.000±24.667*
CCl_4 alone (1ml/kg) (III)	7.300±0.737	3.933±0.088	1.000±0.200	0.400 ±0.153	162.00±2.000	93.330±2.404	508.333±30.046
Normal control (IV)	6.800±0.200	3.800±0.058	0.900±0.208	0.333 ±0.088	78.333±1.667	47.000±0.577	235.667±7.513

Values given as Mean ± SEM. ***P< 0.001, **P<0.01 or *P<0.05 issignificant when vitamin C (positive control) or soymilk is compared with negative control (CCl_4 alone).

Histopathological result

Microscopical examination of the liver isolated from the rat at sacrifice revealed no histopathological alteration in group IV, control rats (Figure 1A). Presence of severe cell necrosis and severe hepatocellular parenchyma degeneration were observed in the liver of rats treated with intraperitoneal injection of CCl_4alone, group III (Figure 1B); however mild or no significant degenerations were observed in rats with co-administration of vitamin C, group II and soymilk (*Glycine max*), group I separately (Figure 1C and D, respectively). The livers of rats in group I and group II showed no significant histopathological alterations when compared with the normal control group.

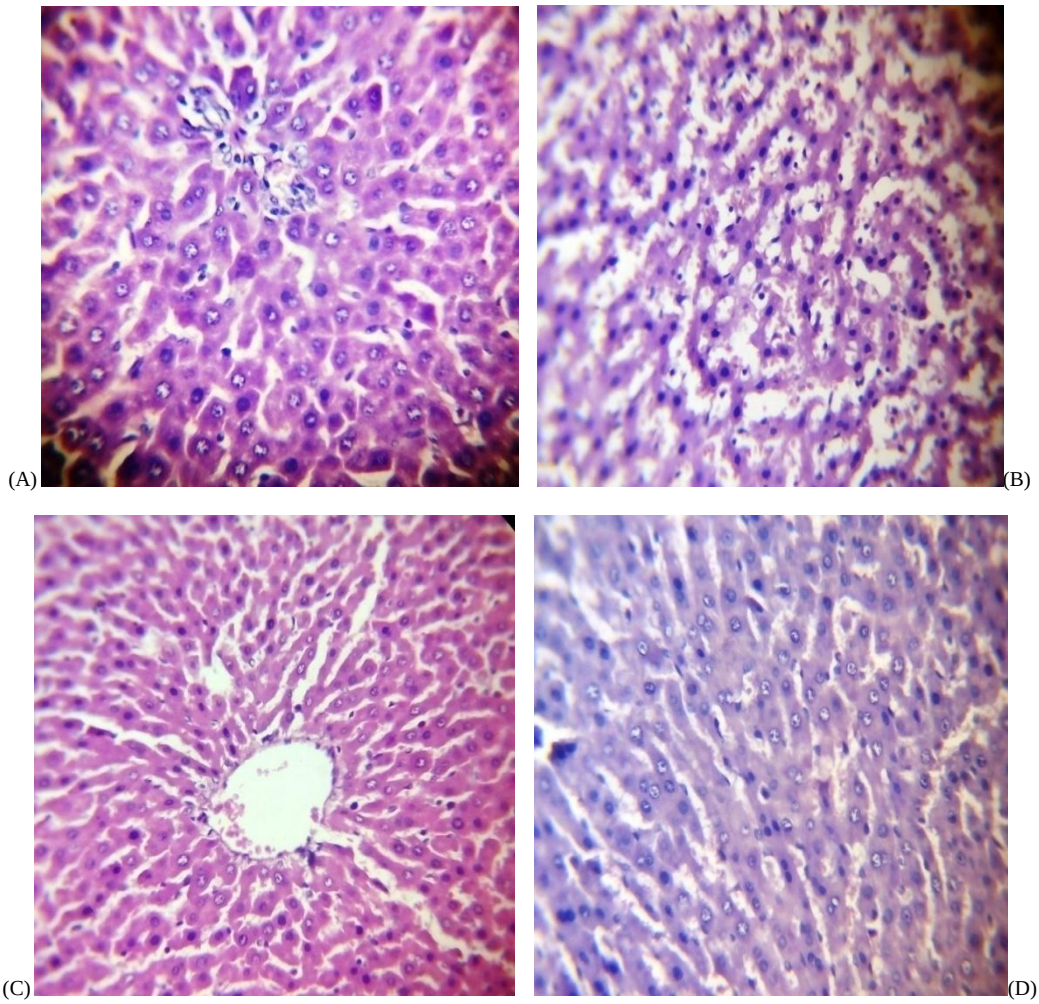

Figure 1(A-D): Histopathology and photomicrograph of liver from (A) normal control, (B) CCl_4alone-treated, (C)vitamin C-treated and (D) soymilk-treated rats [Stain: H and E; ×40]

DISCUSSION

Hepatotoxicity is a serious problem during drug development and for the use of many established drugs. For example, acetaminophen overdose is currently the most frequent cause of acute liver failure in the United States and the UK. Evaluation of the mechanisms of drug-induced liver injury indicates that mitochondria are critical targets for drug toxicity, either directly or indirectly through the formation of reactive metabolites. [27]

Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failure. More than 75% of cases of idiosyncratic drug reactions result in liver transplantation or death. [28] Compared to other health conditions, it is appalling how little attention is given to diseases of the liver, particularly considering the rising level of concerns in health and health-related environmental issues. Hepatoprotection (or protection of the liver) should be of high interest because the liver plays a critical role in all aspects of metabolism and overall health.

Due to the widely known adverse effect of drugs, scientists over years have researched on food with medicinal importance useful for treating various ailments. Few researches have been done on soy products; one of which is soymilk.Although several potential mechanisms were suggested to explain the therapeutic effects of antioxidants-rich plants or foods against hepatotoxicity by tetrachloromethane based on existing data and other recent studies, [15, 16, 29-31] the anti-hepatotoxicity by *Glycine max* has not been elucidated. The aim of this study was to investigate the hepatoprotective effect of Soy against tetrachloromethane (CCl_4)-induced biochemical and histological alterations in liver of albino rats.

Many people ingest various drugs without any precaution on whether the vital organs and glands are damaged or not. The chemicals, industrial solvents, or therapeutic drugs that can cause liver injury have been the subject of several reviews. Although the effects of the various hepatotoxicants in animals and man may differ quite markedly, similarity of actions do exist. Tetrachloromethane (CCl_4) which is a widely known hepatotoxicant was used in this study to induce acute liver damage and as experimental animal model of drug-induced liver injury.

The abnormal increase in liver enzymes level observed in this study is believed to be based on the mechanism of action of CCl_4. CCl_4 is well established as xenobiotics. Previous studies showed that both the liver and the kidneys are the target organs of CCl_4. Extensive evidence demonstrated that CCl_4 is metabolized in the liver into a highly reactive trichloromethyl radical which initiate free radical mediated lipid peroxidation of the cytoplasmic membrane phospholipids. It also causes functional and morphological changes in the cell membrane leading to an accumulation of lipid derived oxidants causing liver injury. [16,17]

The positive control drug, ascorbic acid (vitamin C) significantly reduced the elevated serum liver enzyme markers (AST, ALT and ALP). This was based on its mechanism of action as an antioxidant. Its primary role is to neutralize free radicals. Since ascorbic acid is water soluble, it can work both inside and outside the cells to combat free radical damages. Free radicals seek out an electron pair to regain their stability. Vitamin C is an excellent source of electrons; therefore it can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity. [29] Soymilk's mechanism of action has not been fully elucidated; however, the plausible explanationfor the protective action of soymilk could be attributed to the antioxidant properties of the phytochemical constituents of Soybeans similar to vitamin C.

The significant decrease in serum liver enzymes in the test group (soymilk

group) may be attributed to the singularor combined antioxidant or hepatoprotective action(s) of the bioactive phytochemicals present in soybean, possibly saponnins and flavonoids. Soyasaponins have been shown to possess hypocholesterolemic, anticarcinogenic, hepatoprotective properties and antioxidant activity.

Isoflavones, a putative health beneficial component in soy along with amino acid composition and fiber, may contribute directly to the antioxidant defense system in the body by scavenging Reactive Oxygen Species (ROS). It may also interact indirectly with other antioxidant defense system, such as enhancement of glutathione synthesis and sparing of vitamin C and E. [32-34] The ability of any potential hepatoprotective food constituents to inhibit the aromatase activity of cytochrome $P_{450,}$ favouring liver regeneration is an important factor. On that basis, it is suggested that isoflavones in soy could also be a factor contributing to its hepatoprotective ability through inhibition of cytochrome P_{450} aromatase and as well as membrane stability

CONCLUSION

Oral administration of soy under CCL_4 challenge significantly protected the liver of albino rats from severe hepatic damage. The attenuating or protective action of soy against hepatotoxicity by CCl_4 was evident by soybean's ability to prevent further biochemical changes which are indicators of severe hepatotoxicity. Soybean consumption may possibly be the safest treatment or management against any toxicity by drugs with similar mechanism of action as CCl_4.

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