Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(16):115-119
(http://www.derpharmachemica.com/archive.html)

Effect of Soy (Glycine max) Against Alcohol-Induced Biochemical Alteration in Liver of Male Albino Rat

Ikenna K Uchendu[1*], Chidozie E Agu[2], Oliver C Orji[1], Eluke B Chekwube[1], Ikechukwu J Chukwu[1], Nnedu E Bitrus[1], Tochi F Nwosu[3], Oluwanifemi P Apara[1]

[1]Department of Medical Laboratory Science, University of Nigeria, Enugu Campus, Enugu State, Nigeria
[2]Department of Medical Laboratory Science, University of Calabar, PMB 1115 Calabar, Cross River State, Nigeria
[3]Barnes Hospital and Cardiac Diagnostic Laboratories Ltd. 34b, Yesufu Abiodun Oniru Road, Dideolu Estates, Victoria Island Extension, Lagos

ABSTRACT

Alcoholic liver damage has become common among the populace. The economic situation and poverty level have made alcohol consumption high among the population; as people tend to resort to high alcohol consumption to give them succor. Soybean is known for its pharmacologic and nutritional properties. The aims of this study were to analyze the phytochemicals present in soybean and investigate the effect of soybean against alcohol-induced liver disease. In this study, twenty-four (24) male albino Wister rats weighing (200-220 gm) were divided into four (4) groups: group 1 served as normal control and received only distilled water, group 2 as positive control and was given ethanol plus vitamin C (200 mg/kg, oral),group 3, the test group was given ethanol plus soymilk (2000 mg/kg, oral), and group 4, the negative control was given ethanol alone (2000 mg/kg, oral) for twenty one (21) days. Albumin, total bilirubin, conjugated bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) levels were evaluated using Rx Monza analyzer and standard laboratory kits from Randox laboratories, UK. The results showed a significant decrease in levels of serum bilirubin, and a significant decrease ($P<0.05$) in levels of serum enzyme markers of liver damage from 101.00 ± 2.08, 100.00 ± 1.16, 576.00 ± 36.10 to 73.70 ± 6.98, 77.33 ± 1.76, 400.00 ± 20.80 for AST, ALT, and ALP respectively ($P<0.05$), when compared with the ethanol-only group (negative control group). Therefore, the results suggest that soymilk has the ability to significantly reduce/lower the effects of alcoholic damage to the liver.

Keywords: Liver damage, Alcohol, AST, ALT, ALP, Soybean, Albumin, Bilirubin, Albino rats

INTRODUCTION

The liver is the largest human organ after skin and it plays several vital functions which include metabolic, vascular, immunological, secretory, and excretory activities in the body [1]. Liver also provides a major function in the metabolism of fat, protein, and carbohydrate [1]. Numerous substances have been demonstrated to be toxic to the liver cells and alcohol was the most reported cause of liver disease worldwide [2].

The term alcoholic liver disease includes liver pathologies of various degrees due to direct and indirect effects of continuous alcohol ingestion [3]. Hepatic manifestations of alcoholism comprise, in increasing severity: alcoholic fatty liver, alcoholic steatohepatitis, as well as alcohol-induced hepatic fibrosis and cirrhosis, either with or without inflammation [4]. Furthermore, chronic alcohol consumption in patients with liver cirrhosis is a risk factor for the development of hepatocellular carcinoma [5]. Alcohol-induced liver damage is generally associated with the increasing level of free radicals which cause the development of liver cell peroxidation and would eventually lead to the oxidative stress on the liver cells [6,7]. One of the common histopathological observations for alcohol-induced liver damage is the development of steatosis whereby hepatocytes are occupied with lipid droplets [8].

Several modern medications and treatments are currently available for liver disease patients, but thus far none of them effectively/fully recovers the liver from its pathological condition [9]. Hence, the search for alternative medicines or supplements has gained more interest. Nutritional therapy is by far the most extensively investigated treatment and has proved effective with regard to important clinical endpoints including nutritional status, rate of infections, liver function and survival [3]. Some studies have shown that soybean (*Glycine max*) has potential bioactive substances that exhibit protective properties [9-11].

Yang et al., [12] have recently reported that soyasaponins-rich extract from soybean (*Glycine max*) may be able to improve on the acute alcoholic-induced hepatotoxicity in rats. A further research has to be done to evaluate the benefits of consuming soybean products against liver diseases so as to limit or minimize the financial burden on patients who suffer from liver disease. In this study, we hypothesize that soymilk has beneficial/curative effects against alcoholic liver disease and that it reduces liver damage due to alcohol intoxication. The aims of this research work were to identify the phytochemicals in soybean and to determine the effects of soy against alcohol-induced liver disease in male albino Wister rats.

MATERIALS AND METHODS

Soybeans

The soybeans *(Glycine max*) were obtained under special order from Bean warehouse of Ogbete main market Enugu, Nigeria.

Soymilk preparation

The soybeans (2 kg) were cooked to brownish coloration. The parboiled beans were mashed to remove the bean coat, weighed, and ground with grinder; tap water was added at a ratio of 4:1 with grinded bean and then filtered to separate soycake from soymilk. The soymilk was subsequently heated to 98°C. Afterwards the soymilk was cooled and preserved in a refrigerator at a temperature between 4-6°C until when needed.

Experimental animals and maintenance

Twenty-four (24) adult male albino wistar rats, with an average weight of (200-220 gm) were used in this study. They were obtained from the animal house of the College of Veterinary Medicine, University of Nigeria, Nsukka, Enugu state, Nigeria. The animals were housed in clean metallic cages in the animal house under ambient temperature (25 ± 3°C) and 12 h light and dark periodicity. They were adequately fed with commercial rat pellets (Neimeth Livestock Feeds Ltd., Ikeja) and water *ad libitum*. The animals were kept under observation for about 14 days before the onset of the experiment for acclimatization. All the animals used in this study were handled 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 [13]. 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.

Phytochemistry of soybean

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 were employed for the analyses [14].

Experimental design

The male albino Wister rats were divided into four (4) groups with six (6) rats each. Ethanol and soy treatment of rat was performed. To cause liver damage, the rats from all groups except those of the normal control group (group I) were treated with 50% (v/v) ethanol at a dose of 2000 mg/kg *via* gavage for one week. After that, rats in groups II and III were subjected to oral treatment once daily for 14 days *via* gavage. The groups are described as follows:

- Group I: normal control group received only rat feed and water.
- Group II: positive control group received 200 mg/kg b.wt. of vitamin C.
- Group III: treatment group received 2000 mg/kg b.wt. of soybean milk.
- Group IV: ethanol (negative) control group received 2000 mg/kg b.wt. ethanol alone.

Sacrificing of animal and sample collection

Blood samples were taken by cardiac puncture of the left ventricle of heart under chloroform anaesthesia. The blood sample collected in plain tube was centrifuged, and the supernatants (serum) were collected for the assay of biochemical parameters of liver function.

Biochemical analysis

Assessment of liver function
The 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 based on that described by Jendrassik and Grof [15].

Measurement of liver enzymes (Measurement of ALT and AST)
Determination of ALT and AST were by colorimetric method as described by Reitman & Frankel [16]

Measurement of ALP
Determination of ALP was by colorimetric method as described by Kind and King [17]

Measurement of albumin
Determination of aserum albumin was by Dye Binding method as described by Dumas et al. [18].

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

The result of the preliminary phytochemical analysis of soybean is represented in the Table 1 below.

Table 1: Preliminary phytochemical analysis

Constituent	Amount
Carbohydrate	++
Reducing Sugar	++
Alkaloids	+++
Glycosides	++
Saponins	+++
Tannins	++
Flavonoids	++
Resins	−
Proteins	+++
Oils	++
Acidic Compounds	++
Terpenoids	++
Steroids	+
Key: +++=More intensely present ++=Present +=Present (in trace amount) −=Absent	

Table 2 shows the results of liver biochemical parameters in four (4) groups of six (6) animals which received Vitamin C or soymilk for 2 weeks following 7 days of daily oral administration of ethanol (2000 mg/kg b.wt). From the results, soymilk showed similar strong hepatoprotection as vitamin C (a drug widely known for its anti-oxidant property). Soymilk significantly decreased the levels of AST, ALT and ALP (enzymes markers of hepatic injury) in the animals when compared with negative controls ($p<0.05$). Furthermore, it is worthy of note that soymilk also significantly decreased serum conjugated bilirubin levels in the animals when compared with negative control ($p<0.05$).

Table 2: Comparison of the liver biochemical parameters of treated groups with negative controls

Group	Albumin (g/dL)	Total bilirubin (mg/dL)	Conjugated bilirubin (mg/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal control	4.170 ± 0.088	1.130 ± 0.067	0.300 ± 0.058	75.700 ± 6.980	59.000 ± 7.000	302.000 ± 88.300
Vitamin C (200 mg/kg)	4.200 ± 0.066	1.230 ± 0.176	0.233 ± 0.033*	72.300 ± 5.040*	78.00 ± 2.080*	373.000 ± 38.400*
Soy milk (2000 mg/kg)	4.230 ± 0.086	1.130 ± 0.186	0.133 ± 0.033*	73.700 ± 6.980*	77.330 ± 1.764*	400.000 ± 20.800*
Alcohol (2000 mg/kg)	4.770 ± 0.219	1.730 ± 0.433	0.733 ± 0.145	101.000 ± 2.080	100.000 ± 1.155	576.000 ± 36.100
Values are given as Mean ± SEM *P<0.05 is significant when vitamin C (positive control) or soy is compared with negative control (alcohol alone).						

DISCUSSION

From our result, oral administration of rats with 2000 mg/kg body weight of 50% v/v ethanol for 7 days caused a significant increase in the levels of serum enzyme markers of liver damage (AST, ALT, and ALP). Increase in AST levels signified liver damage. This finding suggested that the mega doses of ethanol administration induced the production of free radicals, which caused damage to the hepatocytes of the rats. This correlates with the report by Sai et al. [19], that a significant increase in serum AST, ALT, and ALP levels suggests liver damage. Under physiological state, the excess metabolites of ethanol produced by the cytochrome P-450 system can be reduced by glutathione. However, the continuous administration of ethanol could cause a decrease in the hepatic content of reduced glutathione (GSH), which is an important biomolecule that affords protection, against chemically induced cytotoxicity [20]. The administration of ethanol increased alanine transaminase (ALT), aspartate transaminase (AST) levels and may have altered the reduced glutathione/oxidized glutathione ratio [21].

In other words, when ethanol is overdosed, the glutathione stores become depleted and the excessive metabolites will react with the liver macromolecules and cause hepatic cell death. The hepatic cellular enzyme ALP in serum will therefore increase [22]. In addition, the hepatic malondialdehyde level will increase invariably, hence resulting in the generation of free radicals in the body [23].

Liver damage was successfully induced in groups II, III and IV preceding ethanol withdrawal as indicated by increase levels of liver enzyme markers in rats fed only with alcohol (group IV). When the results of group II (positive control) were compared with that of

group IV (negative control), there was a significant reduction in the serum levels of the liver enzyme biomarkers indicating recovery from the alcohol toxicity on the liver. This is because of the antioxidant properties of Vitamin C as it has been reported to have antioxidant activities that prevent free radical production [24].

In a similar vein, the result from rats in the soymilk treatment group showed a significant reduction when compared to that of the negative control group (alcohol-only). Soymilk demonstrated similar antioxidant property as vitamin C. The antioxidant action of soymilk may be due to the polyphenol flavonoids contained in soy which have been reported to have antioxidant effects [23]. As documented by Messina [24], soybeans including the foods derived from the legume are considered as the richest dietary source of isoflavones. Isoflavones are a subclass of more ubiquitous flavonoids. Alia et al., [25], report that Flavonoids have antioxidant capacity that is much stronger than those of vitamins C and E, reportedly used to prevent free radical production. However, a work by Barnes, [26] shows that isoflavones are not strong antioxidants and may not be able to scavenge oxidants directly and they therefore are considered antioxidants because of their effects on gene expression of enzymes. But either way, isoflavones have been shown to possess antioxidant properties.

Similarly, saponins contained in soy (soyasaponins) might have contributed to the antioxidant and hepatoprotective effects of soy. Yoshiki and Okubo, [27] report that 1 mg of DDMP saponin per ml (2, 3-dihydro-2, 5-dihydroxy-6-methyl-4*H*-pyran-4-one moiety at C-22 position) scavenges superoxide at a degree equivalent to 17.1 units of superoxide dismutase per ml by the ESR spin trapping method. They documented that lack of this group didn't show the scavenging activity. This is in line with the study by Yakubu [22], that soybeans have strong antioxidant potential. Furthermore, a study by Ohominami et al., [28] showed that liver injury caused by peroxidized salad oil was inhibited by the addition of soyasaponin A_1 during peroxidation. Having similar properties as the soyasaponins, are the soyasapogenols. Soyasapogenols are aglycones of soyasaponins.

It seems reasonable to deduce that soy might have caused the significant reduction in the level of the liver enzyme markers (ALT, AST, ALP) when compared to the result of the negative control group. This could be attributed to high antioxidant potential of soybeans, which serve as an extracellular neutralizer of free radicals [29].

Increased levels of serum bilirubin in rats fed with reduced diet and ethanol could be viewed as a compensatory phenomenon in response to cellular peroxidative changes, which cause damage to the biliary tissue. This is because bilirubin functions *in vivo* as a powerful antioxidant, antimutagen, and an endogenous tissue protector [29]. Reduction of bilirubin and non-significant increase in albumin levels in rats treated with soymilk when compared to the normal control group were most effective, and stabilized the biliary cell function and endoplasmic reticulum leading to bile acid and protein synthesis [30]. This indicates hepatoprotection.

The oral administration of ethanol to the rats causing liver intoxication/injury stimulated an acute compensatory response by the liver leading to increase in the serum albumin levels. The non-significant increase in albumin levels in rats of groups II, III and IV might have resulted owing to the fact that the rats suffered from acute alcoholic liver damage. Stimulation has been advanced as a contributory hepatoprotective mechanism against hepatotoxin caused by alcohol intoxication.

CONCLUSION

The present study showed alcohol induced liver injury. However, the administration of oral soymilk reversed all the adverse effects. Thus our finding demonstrates that soy protein could be of health benefits to patients suffering alcoholic liver disease. We therefore recommend that further research on soy: (1) is needed to characterise the active antioxidant and anti-inflammatory principles and to elucidate its mechanism of action; (2) be carried out on its effect on haematological parameters such as haematocrit, haemoglobin level, red cell indices, and white blood cells on glycerol-induced acute renal failure.

ACKNOWLEDGEMENTS

The authors express deep sense of gratitude to Mr. Chris Ireoba, The head of department of the Laboratory Division at Eastern Nigeria Medical Centre, Enugu, and all the technical staff for their kind cooperation.

COMPETING INTERESTS

The authors declare no competing interests.

REFERENCES

[1] V. Mitra, J. Metcalf, *Anaes. Inten. Care. Med.*, **2009**, 10, 334-335.
[2] R. Saravanan, P. Viswanathan, K.V. Pugalendi, *Life. Sci.*, **2006**, 78, 713-718.
[3] F. Stickel, B. Hoehn, D. Schuppan, H.K. Seitz, *Nutr. Alcohol.*, **2003**, 18, 357-358.
[4] K.V. Menon, G.J. Gores, V.H. Shah, *Mayo. Clin. Proced.*, **2001**, 76, 1021-1029.
[5] F. Stickel, D. Schuppan, E.G. Hahn, *Gut.*, **2002**, 51, 132-139.
[6] A. Mishra, S. Paul, S. Swarnakar, *Biochimie.*, **2011**, 93, 854-866.
[7] P. Pramyothin, C. Ngamtin, S. Poungshompoo, C. Chaichantipyuth, *J. Ethnopharmacol.*, **2007**, 114, 169-173.
[8] J.K. Reddy, M.S. Rao, *Am. J. Physiol.*, **2006**, 290, G852-G858.
[9] A.R. Shivashankara, A. Azmidah, R. Haniadka, M.P. Rai, R. Arora, M.S. Baliga, *Food Funct.*, **2012**, 3, 2, 101-109.
[10] I.K. Uchendu, O.S. Onwukwe, C.E. Agu, O.C. Orji, B.E. Chekwube, T.F. Nwosu, *Int. J. Biomed. Res.*, **2016**, 7, (12), 822-828.
[11] I.K. Uchendu, O.S. Onwukwe, C.E. Agu, O.C. Orji, B.E. Chekwube, T.F. Nwosu, *J. Med. Allied. Sci.*, **2017**, 7(1), 34-40.
[12] X. Yang, C. Dong, G. Ren, *J. Agric. Food. Chem.*, **2011**, 59, (4), 1138-1144.
[13] American Physiological Society, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **2002**, 283, R281-R283.
[14] G.E. Trease, W.C. Evans, *Pharmacog*., 13th ed. Philadelphia: Bailliere Tindall, **1989**.
[15] H.T. Malloy, K.A. Evelyn, *J. Biol. Chem.*, **1937**, 119, 481-490.
[16] S. Reitman, S. Frankel, *Am. J. Clin.* Pathol., **1957**, 28, 56-58.

[17] P.R.H. Kind, E.J. King, *J. Clin. Path*., **1954**, 7, 322.
[18] B.T. Dumas, W.A. Watson, H.G. Biggs, *Clinica. Chimica. Acta*., **1971**, 258, 21-30.
[19] K. Sai, A. Takagi, T. Umemura, Y. Kurokawa, *J. Environ. Pathol.*, **1992**, 11, 139-143.
[20] S.K. Thakur, *Gastroenterol. Toda*., **2002**, 6, 78-82.
[21] N. Dixit, S. Baboota, K. Kohli, S. Ahmad, J. Ali, *Ind. J. pharmacol*., **2007**, 39, 172-179.
[22] N. Yakubu, G. Oboh, A.A. Olalekan, *Biotechnol. Res. Int.*, **2013**, 13, 2-6.
[23] E.E. Nwanna, G. Oboh, *Pakistan. J. Biol. Sci.*, **2007**, 10, 2682-2687.
[24] M. Messina, *J. Nutr.*, **2010**, 140: 1350S-4S.
[25] M. Alia, C. Horcajo, L. Bravo, L. Goya, *Nutri. Res.*, **2003**, 23, 1251-1267.
[26] S. Barnes, *Lymphatic. Res. Biol.*, **2010**, 8, 89–98.
[27] Y. Yoshiki, K. Okubo, *Biosci. Biotechnol. Biochem.*, **1995**, 59, 1556-1557
[28] H. Ohominami, H. Okuda, M. Yoshikawa, I. Kitagawa, *Wakanyaku Shinpojumu.*, **1981**, 14, 157-162.
[29] K. Pratibha, U. Anand, R. Agarwal, *Ind. J. Clin. Biochem.*, **2004**, 19, 128-131.
[30] S.V. Sureshkumar, S.H. Mishra, *Pharmacog. Magazine.*, **2007**, 3, 187-191.