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Effect of soy milk on alcohol induced liver disease in male *albino Wistar* rats

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Abstract

The study was carried out to determine the effect of soymilk (soy extract) on alcoholic liver of male albino rats. Fifty four Male albino rats divided into six groups of 9 rats each were orally administered 20% alcohol, 20% alcohol and 25 mg/kg silymarine, 20% alcohol and 100 mg/kg of soymilk extract, 20% alcohol and 200mg/kg of soymilk extract and 20% alcohol and 400mg/kg of soymilk extract while the last group was fed water and rat diet *ad libitum* to serve as control for 28 days. The Aspartate aminotransaminase (AST), Alanine aminotransaminase (ALT), Alkaline Phosphatase (A LP), Total bilirubin, Direct bilirubin, Total Protein and albumin were determined using Colorimetric, Phenolphthalein Monophosphate by Jendrasik and Grof, Biuret and Bromocresol by Green methods respectively. The result showed there was significant difference ($P<0.05$) in activities of AST (U/L) of 42.7±1.60, 84.0 ±3.86, 45.2±3.94 and 34.9±1.22, ALT (U/L) of 30.6±2.59, 105.6±6.36, 39.4±3.1 and 31.5 ±1.23 and ALP (U/L) of 55.5±3.20, 110.7±5.18, 78.9±6.16 and 65.6±2.33 in Control, Alcohol, Silymarine drug and Soy Extract respectively. There was significant difference ($P<0.05$) in Total Bilirubin (umol/l) of 2.4±0.05, 3.0±0. 17, 2.6±0.15 and 2.41±0.05 while there was no difference ($P>0.05$) in Direct bilirubin (umol/l) of 1.4±0.75, 1.4±0.10, 1.3±0.08 and 1.25±0.03 in Control, Alcohol, Silymarine drug and Soy Extract respectively. There was no significant difference ($P>0.05$) in total protein (g/l) was 66.6±1.18, 64.7±2.55, 60.4±7.37 and 66.8±1.21 and albumin (g/l) of 33.7±1.35, 32.9±1.08, 34.1±0.92 and 35.7±0.54 in Control, Alcohol, Silymarine drug and Soy Extract respectively. The result obtained in the study suggested that Soymilk extract caused reversal to liver dysfunction caused by alcohol.

Keywords: Soymilk, Alcohol, Liver.

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1. Introduction

Soy milk is a plant based drink produced by soaking dried soybeans and grinding them in water. It is a staple of East Asian cuisine. Soymilk is a stable emulsion of oil, water and protein. It can be produced at home using a soymilk machine and can be used as substitute for dairy milk. Soybean contains estrogenic isoflavones and other substances such as coumestans and lignans. Soybeans are thought to be beneficial in preventing osteoporosis because they contain estrogenic isoflavonones such as genistein, daidzein and their glycans.

Alcohol liver disease (ALD) is a result of long term alcohol consumption and involves fatty liver, steatosis, fibrosis and cirrhosis. Alcohol induces hepatic fat accumulation, tissue damage and liver dysfunction. The liver is primarily susceptible to alcohol related injury because it is the primary site of alcohol metabolism. Epidemiological studies revealed that a threshold dose of alcohol must be consumed for serious injury to become apparent.[1] The Cytosolic alcohol dehydrogenase (ADH) pathway catalyses reversible oxidation of ethanol to acetaldehyde which is about 80%-90% ethanol in normal

condition while the pathway through cytochrome P_{450} 2E1(CYP2E1) is of minor quantitative importance but is induced by chronic alcohol consumption. The role of Peroxisomal ethanol metabolism mediated by catalase is less important under physiologic conditions in human except in the fasted state [2]. Chronic alcohol consumption by rats has been shown to increase H_2O_2 production in per central regions of the liver and increased catalase activity [3]. Ethanol oxidation may produce pathogenic effect as a result of altered redox state, toxic products made by the induced CYP2E1 and the direct cellular toxicity of acetaldehyde.

The aim of this study is to evaluate the effect of soymilk on alcohol induced liver disease of albino rats using aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKPHOS), Total Protein (Tprot), Albumin(Alb), Total Bilirubin (Tbil) and Direct Bilirubin (Dbil) as indicator.

2. Materials and Methods

2.1 Materials

2.1.1 Soy Milk: Soymilk was purchased from the Eke Ukwu Owerri Market Owerri, Imo state.

2.1.2 Alcohol: Absolute alcohol used in this experiment was manufactured by Guanddong Guanghua Sci-Tech Co. Ltd, Shantou, China was purchased in Port Harcourt.

2.1.3 Silymarine: The silymarine used in this study was manufactured by Alexander Co Pharmaceuticals, Alexandria, Egypt was purchased from Madonna University Teaching Hospital (MUTH), Elele, Rivers State.

2.1.4 Test Animals

Fifty four Wistar albino rats of 0.195kg average body weight on normal rat diet were obtained from the animal house of the department of Pharmacology and Toxicology, University of Port Harcourt. These rats were fed *ad libitum* with normal rat pellet and water and acclimatized to laboratory conditions for a period of 14days prior to commencement of study.

2.1.5 Reagents

Commercially prepared aspartate aminotransferase, alanine aminotransferase, Total Protein, Albumin and Bilirubin reagents were obtained from Randox Diagnostics, London while alkaline phosphatase reagents was obtained from Quimica Clinica Applicada Spain.

2.1.6 Preparation of Soymilk

Glycine max (Soy Milk) was dried at room temperature on a laboratory bench and pulverized into coarse powder. The powder soy material was extracted using cold maceration method in 80% methanol for 48hours with intermittent shaking at 3hour interval. The extract was filtered by using Whatman No. 1 filter paper. The filtrate was concentrate d in a hot air oven at 40^{O}C and the hydromethanoic glycine max extract was stored in a refrigerator at 4^{O}C until required for the experiment.

2.2 Experimental design

Fifty four Male albino rats were divided into six groups of 9 rats each. The rats were administered 20% alcohol, 20% alcohol and 25mg/kg silymarine, 20% alcohol and 100mg/kg of soymilk extract, 20% alcohol and 200mg/kg of soymilk extract and 20% alcohol and 400mg/kg of soymilk extract respectively through the oral route while the last group was fed water and rat diet *ad libitum* to serve as control for 28days. The albino rats were sacrificed and their blood samples collected.

2.3 Biochemical Studies

2.3.1 Determination of ALT and AST:

Determination of ALT and AST was done by monitoring the concentrations of pyruvate hydrazone formed with 2, 4 dinitrophenyl hydrazine. Five hundred microlitre (0.5ml) of buffer solution was dispensed into test tubes labeled blank, sample, control blank and control respectively for AST and ALT respectively. One hundred microlitre (0.1ml) of sample and control was dispensed into their respective test tubes. All the tubes were incubated at 37ºC for 30minutes. Five hundred microlitre (0.5ml) of 2, 4 dinitrophenyl hydrazine was dispensed into all test tubes. One hundred microlitre (0.1ml) of sample and control was dispensed into their respective blank test tube. The contents of each test tube was mixed and allowed to stand for 20minutes at 25ºC. 5ml of 0.4N sodium hydroxide was added to each tube, mixed and read at 550nm against the respective blank prepared. The activity of the unknown was extrapolated from the calibration curve already prepared[4].

2.3.2 Determination of Alkaline Phosphatase:

Alkaline Phosphatase activity was determined by Phenolphthalein Monophosphate method. The test tubes were respectively labeled sample, standard and control. One millilitre (1.0ml) of distilled water was pipette into each tube followed by a drop of the substrate into each test tube. All the test tubes were incubated at 37ºC for 5minutes. Ten microlitre (0.01ml) of sample, standard and control were dispensed into their respective test tubes. The test tubes were incubated at 37ºC for 20minutes. Five milliliter (5ml) of colour developer was added to each test tube, mixed, and read at 550nm using water as blank. The activity of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard [5].

2.3.3 Determination of Total Protein concentration:

Total Protein concentration was determined using Biuret method as described by Canon *et al*.[6]. 5ml of Biuret reagent was pipetted into tubes labeled blank, standard, test, and control respectively. 10ul of distilled

water, standard, sample and control were pipetted and dispensed into the respectively labeled test tubes. All the tubes were mixed and incubated at 25^{O}C for 30minutes. The absorbance of each tube was measured at wavelength of 546nm against the reagent blank. The concentration of total protein was calculated by dividing the absorbance of sample against absorbance of standard multiplied by concentration of standard as shown below.

Total Protein (g/l) =

$$\frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

2.3.4 Determination of Albumin estimation:

Albumin estimation was done by Bromocresol green (BCG) method described by Doumas *et al*.[7]. 3ml of Bromocresol green reagent was pipette and dispensed into test tubes labeled blank, standard, sample and control respectively. 10ul of distilled water was dispensed into the tube labeled blank while 10ul of standard, sample and control was pipette into their respectively labelled tubes. The tubes were mixed and incubated at 25 ºC for 5minutes. The absorbance of each tube contents were measured at wavelength of 578nm against the reagent blank. The concentration of Albumin was determined by dividing the absorbance of sample against absorbance of standard multiplied by concentration of standard as shown below.

Albumin (g/l) =

$$\frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

2.3.5 Determination of Total bilirubin concentration:

Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction which diazotized sulphanillic acid. 200ul of sulphanillic acid was dispensed each into two different test tubes labeled sample blank and sample followed by the addition of 1 drop (50µl) of nitrite reagent and 1000 µl of caffeine reagent . 200 µl of the test serum was dispensed into each of the test tubes and the mixtures incubated for 10 minutes at 25^{0}C. This was followed by the addition of 1000 µl of tartarate reagent and the mixture incubated again at 25^{0}C for 10 minutes. The absorbance of the sample (ATB) was then read against the sample blank at 578nm wavelength. The total bilirubin concentration (mg/dl) was then calculated by multiplying the absorbance of total bilirubin (578nm) with a constant (10.8) as shown below [8]:

Total Bilirubin (mg/dl)= Absorbance (578nm) X 10.8

2.3.6 Determination of serum Direct Bilirubin concentration:

Serum Direct bilirubin concentration was determined using the Randox Kit (Randox laboratories limited UK) based on the method described by Jendrassik and Grof [8]. Direct bilirubin reacts with diazotized sulphanillic acid in alkaline medium to form a blue coloured complex. 200ml of sulphanillic acid was dispensed each into two different test tubes labeled sample blank and sample followed by the addition of 1 drop (50µl) of nitrite reagent and 2000 µl of 0.9 % NaCl. 200 µl of the test serum was the dispensed into each of the test tubes and the mixtures incubated for 10 minutes at 25^{0}C. The absorbance of the sample was then read against the sample blank at 546 nm wavelength. The direct bilirubin concentration was then calculated by multiplying absorbance of Direct bilirubin (mg/dl) with a constant (14.4) as shown below;

Direct Bilirubin (mg/dl) = Absorbance (546nm) X 14.4

2.2.6 Statistical Analysis

Biochemical data values were reported as Mean± Standard error of Mean (SEM). Analysis of Variance (ANOVA) was done on the data obtained using Statistical Package for Social Sciences (SPSS) version 21.

3. Results

The AST (U/L) was 42.7±1.60, 84.0 ±3.86, 45.2±3.94, 40.9±1.87, 34.0±1.22 and 30.0±1.42 in Control, Alcohol, Silymarine drug, 100mg/kg of soy extract, 200mg/kg of soy extract and 400mg/kg of soy extract respectively. The ALT (U/L) was 30.6±2.59, 105.6±6.36, 39.4±3.1, 36.3±2.44, 30.9±1.16 and 27.2±1.52 in Control, Alcohol, Silymarine drug 100mg/kg of soy extract, 200mg/kg of soy extract and 400mg/kg of soy extract respectively. The ALP (U/L) was 55.5±3.20, 110.7±5.18, 78.9±6.16, 73.4±4.80, 67.59±2.50 and 56.0±2.05 in Control, Alcohol, Silymarine drug 100mg/kg of soy extract, 200mg/kg of soy extract and 400mg/kg of soy extract respectively. Total Bilirubin (umol/l) was 2.4±0.05, 3.0±0. 17, 2.6±0.15, 2.5±0.10, 2.5±0.09 and 2.3±0.06 in Control, Alcohol, Silymarine drug 100mg/kg of soy extract, 200mg/kg of soy extract and 400mg/kg of soy extract respectively while the Direct bilirubin (umol/l) was 1.4±0.75, 1.4±0.10, 1.3±0.08, 1.2±0.06, 1.3±0.05 and 1.3±0.05 in Control, Alcohol, Silymarine drug 100mg/kg of soy extract, 200mg/kg of soy extract and 400mg/kg of soy extract respectively. The total protein (g/l) was 66.6±1.18, 64.7±2.55, 60.4±7.37, 64.6±1.86, 69.2±1.86 and 66.7±2.46 in Control, Alcohol, Silymarine drug 100mg/kg of soy extract, 200mg/kg of soy extract and 400mg/kg of soy extract respectively while the albumin (g/l) was 33.7±1.35, 32.9±1.08, 34.1±0.92, 35.2±1.46, 36.9±0.67 and 35.01±0.16 in Control, Alcohol, Silymarine drug 100mg/kg of soy extract, 200mg/kg of soy extract and 400mg/kg of soy extract respectively as shown in table 1.

Table 1: Liver Function in alcohol induced rats treated with various concentrations of Soymilk

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	TBIL (Umol/L)	DBIL (Umol/L)	T Prot (g/L)	Alb (g/L)
Control	42.7±1.60	30.6±2.59	55.5±3.20	2.4±0.05	1.4±0.75	66.6±1.18	33.7±1.35
20% Alcohol	84.0 ±3.86	105.6±6.36	110.7±5.18	3.0±0. 17	1.4±0.10	64.7±2.55	32.9±1.08
Silymarine drug	45.2±3.94	39.4±3.1	78.9±6.16	2.6±0.15	1.3±0.08	60.4±7.37	34.1±0.92
100mg/kg of soyextract	40.9±1.87	36.3±2.44	73.4±4.80	2.5±0.10	1.2±0.06	64.6±1.86	35.2±1.46
200mg/kg of soyextract	34.0±1.22	30.9±1.16	67.59±2.50	2.5±0.09	1.3±0.05	69.2±1.86	36.9±0.67
400mg/kg of soyextract	30.0±1.42	27.2±1.52	56.0±2.05	2.3±0.06	1.3±0.05	66.7±2.46	35.01±0.16
f	56.404	81.296	22.988	5.253	0.882	0.701	1.813
p	0.000	0.000	0.000	0.001	0.500	0.626	0.128

The AST (U/L) was 42.7±1.60, 84.0 ±3.86, 45.2±3.94 and 34.9±1.22 in Control, Alcohol, Silymarine drug and Soy Extract respectively. The ALT (U/L) was 30.6±2.59, 105.6±6.36, 39.4±3.1 and 31.5 ±1.23 in Control, Alcohol, Silymarine drug and Soy Extract respectively. The ALP (U/L) was55.5±3.20, 110.7±5.18, 78.9±6.16 and 65.6±2.33 in Control, Alcohol, Silymarine drug and Soy Extract respectively. Total Bilirubin (umol/l) was 2.4±0.05, 3.0±0. 17, 2.6±0.15 and 2.41±0.05 in Control, Alcohol, Silymarine drug and Soy Extract respectively while the Direct bilirubin (umol/l) was 1.4±0.75, 1.4±0.10, 1.3±0.08 and 1.25±0.03 in Control, Alcohol, Silymarine drug and Soy Extract respectively . The total protein (g/l) was 66.6±1.18, 64.7±2.55, 60.4±7.37 and 66.8±1.21 in Control, Alcohol, Silymarine drug and Soy Extract respectively while the albumin (g/l) was 33.7±1.35, 32.9±1.08, 34.1±0.92 and 35.7±0.54 in Control, Alcohol, Silymarine drug and Soy Extract respectively as shown in table 2.

Table 2: Liver Function in alcohol induced rats treated with Soy milk

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	TBIL (Umol/L)	DBIL (Umol/L)	T Prot (g/L)	Alb (g/L)
Control	42.7±1.60	30.6±2.59	55.5±3.20	2.4±0.05	1.4±0.75	66.6±1.18	33.7±1.35
Alcohol only	84.0 ±3.86	105.6±6.36	110.7±5.18	3.0±0. 17	1.4±0.10	64.7±2.55	32.9±1.08
Silymarine drug	45.2±3.94	39.4±3.1	78.9±6.16	2.6±0.15	1.3±0.08	60.4±7.37	34.1±0.92
Soy Extract	34.9±1.22	31.5 ±1.23	65.6±2.33	2.41±0.05	1.25±0.03	66.8±1.21	35.7±0.54
f	79.673	129.563	31.259	8.049	1.256	0.903	2.375
p	0.000	0.000	0.000	0.000	0.299	0.446	0.081

4. Discussion

The result showed that 20% alcohol caused microsoma induction of AST, ALT, and ALP.The most prominent result of liver damage is the released of the intracellular enzymes AST, ALP and ALT from the liver into the blood. The serum concentrations of AST, ALP and ALT can serve as indicators of the state of the liver. Higher levels of AST, ALP and ALT are indicators of liver damage [9]. Therefore, the increased in the serum concentration of AST, ALP, ALT and Total bilirubin after ethanol administration suggestive of liver damage caused by ethanol in Wistar rats in this study.

The study shows that administration of ethanol causes increase in the serum concentrations of AST, ALP and ALT which are biomarkers for liver damage and it also shows increase in serum concentration of total bilirubin. Chronic alcohol consumption is an established risk factor for the development of hepatocellular carcinoma in patients with liver cirrhosis [10].

The result of the study further showed that treatment with various doses of soymilk caused reduction in inducible liver ezymes caused by 20% alcohol. The decreased in the serum concentration of ALP, AST and ALT in groups treated with soy milk extract shows the antioxidant potentials of soy milk.

The results obtained above revealed that soy milk have a strong antioxidant potential in ameliorating the oxidative damage caused by ethanol on the liver. Soya bean decrease the risk of various diseases and pathological conditions, including various types of cancers, osteoporosis, menopause symptoms and coronary heart diseases. Flavonoids which are one of the components of soya bean have gained importance as scavengers of free radicals and a potent inhibitor of lipid peroxidation. It has been reported that population having high intake of isoflavones show lower incidence of cardiovascular diseases, osteoporosis, kidney disease and cancer risk [11].

Administration of soy milk ameliorated alcoholic induced liver injury in rats, as evidenced by biochemical findings.

Similar protective effects were also observed in rats receiving silymarine, which was used as a positive control, although the mechanism of action for these effects may not be the same. Silymarine is a polyphenolic flavonoid isolated from the fruit and seeds of the milk thistle (*Silybum marianum*). Various studies indicate that

silymarine exhibits strong antioxidant activity [12] and shows protective effects against hepatic toxicity induced by a wide variety of agents by inhibiting lipid peroxidation [13,14]. Serum ALP and total bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis, in the presence of increasing biliary pressure [15].

In the study, the extract has been found to reduce serum bilirubin in the soymilk treated groups compared with alcohol treated groups. The results also showed that rats exposed to ethanol significantly decreased serum total protein levels. Hence the decline in total protein content can be deemed useful index of the severity of cellular dysfunction in chronic liver diseases and may be associated with the decrease in the number of hepatocytes, which in turn may result in the decreased hepatic capacity to synthesize protein and consequently decrease liver weight. Stabilization of serum protein levels in the pre-treatment groups administered with soy milk extract is further a clear indication of the improvement of the functional status of liver cells. The site specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis [16].

5. Conclusion

The study above showed that Soy milk had potent hepatoprotective effects against ethanol-induced hepatotoxicity in rats. Soy milk inhibited the hepatic damage accompanied by decreased activity of serum liver enzymes. Treatment with soymilk resulted in restoration of the antioxidant defence system, which was impaired by ethanol exposure. Therefore, soymilk is a potential candidate for the prevention of ethanol-induced liver damage in rats.

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