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Attenuation of Glycerol-Induced Acute Renal Failure in Albino Rats by Soy Beans (*Glycine max*)

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Abstract : The health benefit of soybean consumption has currently gained the attention of researchers. The aim of this study was to investigate the effect of soymilk onrenal biochemical parameters and histopathology of glycerol-induced acute renal injury in rats. In this study, twenty-four (24) albino wistar rats weighing (200-250g) were divided into four (4) groups with six (6) animals per cage: group A served as normal control group and received only distilled water, group Bas positive control and was given glycerol plus vitamin C (200mg/kg, oral), group C, the negative control was given 50% glycerol alone (10ml/kg, i.m.), and group D, the test group was given glycerol plus soymilk (2000mg/kg, oral). Renal injury was assessed by analysing renal histopathology and biochemical parameters such as: Na^+, K^+, Cl^-, HCO_3^-, creatinine and blood urea nitrogen (BUN) levels. The Glycerol treatment resulted in marked elevation of:K^+ (8.66±0.64mmol/l); creatinine (1.47±0.23 mg/dl);and BUN (44.39±5.78 mg/dl) and caused deranged renal functions which were significantly attenuated after soymilk treatment: K^+ (6.51±0.33 mmol/l); creatinine (0.88±0.005 mg/dl); and BUN (19.67±1.45 mg/dl) with[$P < 0.05$].Based on these results, this study demonstrates the potential beneficial effects of soy bean against glycerol-induced acute renal failure in rats.
Key words : Renal injury, myoglobinuria, glycerol, soy bean, soymilk, attenuation.

Introduction

Acute kidney injury remains associated with high morbidity and mortality, despite progress in medical care [1]. It is an abrupt reduction in kidney function based on an elevation in serum creatinine level, a reduction in urine output, the need for renal replacement therapy (dialysis), or a combination of these factors [2]. A diagnosis of acute kidney injury is currently made on the basis of the presence of increased serum creatinine and/or blood urea nitrogen levels and/or a decreased urine output, despite their well-known limitations [3]. The pathogenesis of acute renal failure especially those of glycerol-induced myoglobinuric acute renal failure involves, among other causes, ischemia, vascular congestion, and reactive oxygen metabolites [4]. Intake of leguminous foods such as soy bean or its constituent slows or prevents development of kidney disease in several different animal models [5].

Soybeans are leguminous plants that bear their fruit in pods, which are castings with two halves, or hinges [6]. Legumes are a very healthy food because it is low in fat and high in protein. Legumes are also very high in fibre and other nutrients [6, 7].Few studies have shown that renal function can be affected by consuming legumes, particularly soybean protein and fibre, and also other bean fibre [5]. Soybean seeds *Glycine max*

*(L)Merr*is a member of family Fabaceae [8]. Soybean is known as the "Golden bean" of the twentieth century, it contains very small amounts of saturated fatty acids but do not contain any trans fatty acids [8]. Omega-6 and omega-3 fatty acids are present in soybean, which are also rich in iron, phosphorus, magnesium, vitamin B12 and folate [8].It is one of the best vegetarian sources of total proteins containing all essential amino acids required in the human dietand also has many beneficial effects on the body tissues [5, 8].

The effect of soy bean or its constituents on renal function parameters is not adequately investigated. Only few studies have shown that renal function could be affected by consumption of soy, and most of the works were mostly in Asia. There is also increase in the incidence of acute renal failure due to poor feeding habits of individuals and in addition are the side effects of the treatment drug. Therefore, we hypothesize that soy or its constituents could improve cases of acute renal disease. The aims of this study were to analyze the phytochemistry of soy bean, evaluate the effect of soy on renal biochemical and histopathological alterations on glycerol-induced acute renal injury in albino rats.

Materials and Method

Seed collection

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

Soy milk preparation

This was prepared as described by Kingsley *et*al[9]. Briefly, the soy beans (2kg) were boiled till a brownish coloration was observed.The parboiled beans were drained, weighed, and ground with a grinder; tap water was added at a ratio of 4:1 with grinded bean 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.

Preparation of reagents

The Vitamin C was purchased from EMZOR pharmaceutical, Nigeria and a stock of 100mg per 100ml was prepared. All other reagents used were of analytical grade.

Experimental animals

Twenty-four (24) adult male albino wistar rats, with an average weight of (200-250g)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[10].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.

Experimental design and conduct

Animals were divided into 4 groups;

Group A (normal control group): no treatment was given.

Group B (positive control group): Glycerol (50%, 10ml/kg) was administered to rats intramuscularly distributed equally in both hind limbs in a single dose to induce acute renal failure, then each animal was given oral daily dose of vitamin C (200mg/kg) for 21 days.

Group C (negative control group): Glycerol (50%, 10ml/kg) was administered to rats intramuscularly distributed equally in both hind limbs in one dose.

Group D (test group) Glycerol (50%, 10ml/kg) was administered to rats intramuscularly distributed equally in both hind limbs in one dose, then each animal was given oral daily dose of soy milk (2000mg/kg) for 21 days

Sacrificing of animal and sample collection:

Blood samples for biochemical analysis were taken by cardiac puncture of the left ventricle of heart under chloroform anaesthesia and subsequently kidney was excised. For histopathological studies, the right kidney was isolated immediately after sacrificing the animal and washed with saline and then processed.

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 were employed for the analyses [11].

Biochemical analysis

The levels of serum electrolytes, blood urea nitrogen (BUN) and creatinine were estimated.

Determination of serum electrolytes:

Serum electrolytes were determined usingPerlong Medical PL1000A Electrolyte Analyser. The electrolyte analyser applies the principle of advanced ion-selective electrode, which gives the instrument a stable and reliable measurement. It measures the ion concentrations of K^+, Na^+, Cl^-, Ca^{++}, HCO_3, and pH values in the whole blood, serum and urine sample.

Determination of Blood Urea Nitrogen:

Serum urea concentration was determined using the diacetylmonoxime method with protein precipitation according to Natelson*et al*[12].

Calculation:

Calculation of Blood Urea Nitrogen

BUN = Urea / 2.14

Determination of serum creatinine concentration

Serum creatinine concentration was determined using the Jaffe Reaction according toFabing and Ertingshausen [13].

Histopathological analysis

The excised kidneys 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 Kidney tissues were sectioned at 5 microns and stained using the Haematoxylin and Eosin [H and E] Staining procedure [14]. 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

Serum Na, K, creatinine and BUN levels in all groups are shown in table 2. The levels of K and BUN were highly elevated significantly in the affected group (glycerol alone). Administration of Soymilk (2000mg/kg) and vitamin C (200mg/kg) separately after glycerol challenge significantly lowered the elevated levels of K ($p<0.05$) and BUN ($p<0.01$) when compared to the affected group. Furthermore and note-worthy, the levels of creatinine was non-significantly elevated in the affected group (glycerol alone); however the administration of soymilk (2000mg/kg) after glycerol challenge significantly lowered the elevated levels of creatinine ($p<0.05$) when compared to the affected group.

Serum Ca, HCO_3^- and Cl levels; and blood pH in all groups are shown in table 3. The level of Cl was elevated non-significantly in the affected group (glycerol alone). Administration of Soymilk (2000mg/kg) and vitamin C (200mg/kg) separately after glycerol challenge non-significantly lowered the elevated levels of Cl ($p>0.05$), when compared to the affected group. Furthermore and note-worthy, there were no significant differences or changes in HCO_3^- level and blood pH (renal/acid-base parameters) among the groups ($p>0.05$). However, the level of Ca was elevated significantly in the affected group; administration of soymilk and vitamin C separately after glycerol challenge non-significantly lowered the elevated level of Ca ($p>0.05$).

Table 2: Statistical Analysis of Renal Biochemical Concentrations in Different Experimental Animal Groups.

Group	Na (mmol/L)	K (mmol/L)	Creatinine (mg/dL)	BUN (mg/dL)
Normal control	144.90±1.19	6.15±0.35*	0.97±0.09	17.63±1.33**
Vitamin C (200mg/kg)	144.17±2.48	6.51±0.33*	0.88±0.05	19.67±1.45**
Glycerol alone (10ml/kg)	137.73±1.42	8.66±0.64	1.47±0.23	44.39±5.78
Soy milk (2000mg/kg)	143.33±0.75	6.51±0.35*	0.80±0.06*	16.67±1.45**

Values are given as Mean ± SEM. **P<0.01 or *P<0.05 is significantly different when normal control, vitamin C (positive control) or soymilk is compared with negative control (glycerol alone).

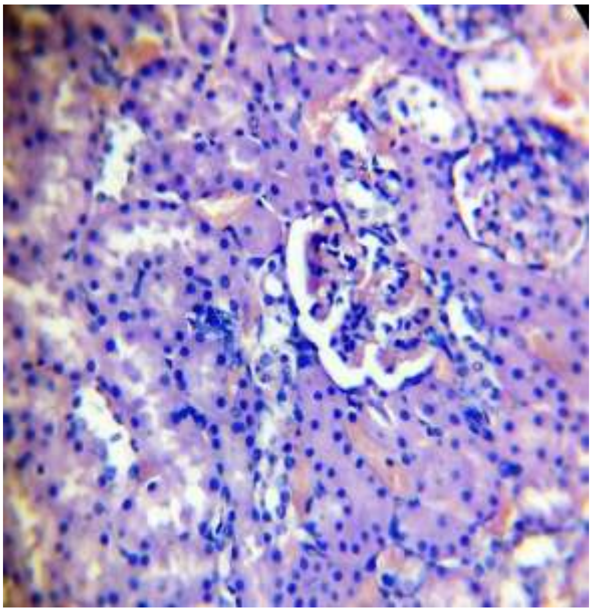
Table 3: Statistical Analysis of Renal Biochemical Concentrations in Different Experimental Animal Groups.

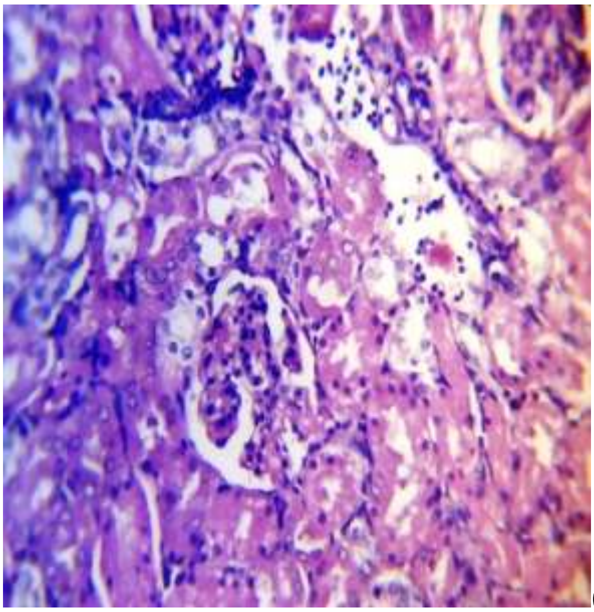
Group	Ca (mmol/L)	HCO_3^- (mmol/L)	Cl (mmol/L)	Blood Ph
Normal control	1.09±0.01*	21.33±1.33	103.4±2.75	7.27±0.02
Vitamin C (200mg/kg)	1.13±0.04	22.00±1.16	102.40±3.80	7.39±0.03
Glycerol alone (10ml/kg)	1.21±0.01	19.33±1.76	106.20±2.88	7.30±0.01
Soy milk (2000mg/kg)	1.15±0.03	20.33±0.88	100.53±2.40	7.39±0.05

Values are given as Mean ± SEM. *P<0.01 is significantly different when normal control, vitamin C (positive control) or soymilk is compared with negative control (glycerol alone).

Histopathological result

Microscopical examination of the kidney isolated from the rat at sacrifice revealed no histopathological alteration in the control rats. Presence of severe interstitial degeneration and tubular necrosiswere observed in the kidney of rats treated with intramuscular injection of glycerol(Figure 1C); howeverno significant degenerations were observed in rats treated with vitamin C (positive control) and soy milk separately (Figure 1B and D, respectively). The kidneys of rats in group B and D showed apparently normal histological features when compared with the control group.

(A) 

 (B)

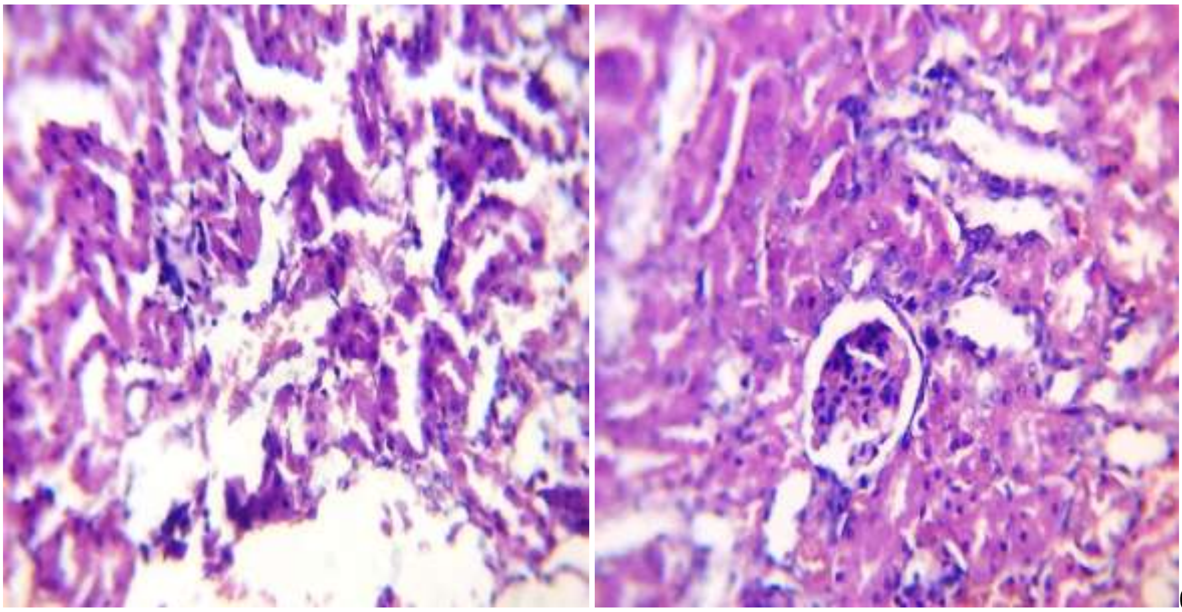
(C)  (D)

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

Discussion

Acute renal failure has remained associated with a high mortality and morbidity in societies, considering major advances made in the field of medicine and medical care as stated by Vanmassenhove *et al.* [1]. There are other sources of medicine that are found in the under-developed and developed countries alike. The sources and composition of these medicines are mainly herbs and other botanicals as described by Mohamed *et al*.[7], soy bean being inclusive. The aims of this study were to analyze the phytochemistry of soy bean, evaluate the effect of soy on renal biochemical and histopathological alterations on glycerol-induced acute renal injury in albino rats.

A model for studying acute renal failure is obtained by a single intramuscular injection of glycerol evenly distributed in both limbs of the rat [15]. This method of inducing renal failure resembles that of rhabdomyolysis and ischaemia reperfusion-induced acute renal failure [16]. Myoglobinuric acute renal failure has three pathogenic mechanisms which include tubular obstruction, renal vasoconstriction and oxidative stress [17]. Mohamed *et al*. [7] describe the mechanism of generation of oxidative stress as through the release of iron from heme group of the myoglobin. Iron induces the generation of superoxides and high activity oxides such as hydrogen peroxide and oxygen molecules that increase oxidative stress and provoke lipid peroxidation and cellular death.

The renal biochemical parameters, which include serum creatinine, blood urea nitrogen (BUN), were elevated in the group administered with glycerol alone (affected group) 24 hrs after glycerol administration, in this study. From the result, it was observed that BUN and serum creatinine levels were elevated when compared to normal group. Serum creatinine is influenced by gender, age and the amount of muscle mass[18]. These factors were kept constant, as the rats were of the same sex; same weight range (200-250g) and all had restricted movement. Therefore the observed increases in serum creatinine and BUN were due to renal injury by glycerol. The possible damage to proximal tubular cells mighthave led to the obtained data.

The BUN and serum creatinine levels were seen to be significantly reduced in the group that was administered with glycerol and vitamin C (positive control group), when compared with that of the affected group (glycerol alone). This was due to the antioxidant and anti-inflammatory properties of the drug as was documented by Kumar [19]. Vitamin C also absorbs iron by its ability to reduce ferric iron to the ferrous form as described by Iqbal*et al*. [20].

The mechanism of action of the antioxidant and anti-inflammatory effect of soy is not elucidated in this study; however, activities comparable to the antioxidant and anti-inflammatory actions of vitamin C were observed. It was also observed that the group that received glycerol and subsequently oral administration of soy milk had a significant reduction in the BUN and serum creatinine levels. This result shows a protective or an ameliorating effect by soy milk, which correlates with the statement by Anderson [5] who states that soy, has protective effects in animal models of kidney disease. This renal-protection, could be as a result of reported properties of the phytochemicals in soy, such as; the effect on gene expression of enzymes that enhance antioxidant defenses by isoflavones; scavenging of hydroxyl and free oxygen radicals by soyasaponins, sapogenols, phenols and lignins; and the anti-inflammatory activity of the triterpenes and phytosterols [21, 22, 23].

The level of K^+ was also shown to be elevated significantly, in the affected group (glycerol alone), this was as a result of the damage to the proximal convulated tubule [24]. This agrees with the work of Leuhardt*et al.* [25], who reported that acute renal insufficiency leads to increased serum K^+, however, these levels were shown to be decreased significantly, in the positive control group as a result of the antioxidant and anti-inflammatory property of vitamin C to ameliorate the damage to the tubules. These levels were also observed to be decreased significantly, in the group that was given an oral administration of soy milk after glycerol injection, which might be as a result of the antioxidant and anti-inflammatory properties of the phytochemical constituents of soy as stated earlier. The HCO_3^- levels were not significantly altered in any of the groups, but showed slight elevation in the affected group but not in the vitamin C or the soy milk administered groups, as observed. Therefore, these results are in agreement with Anderson *et al*[5], who report that using soy protein reduces development of kidney diseases in animal.

Conclusion

The present study demonstrates thatglycerol induces acute renal failure and that soymilk reversed all the adverse effects. Thus our finding demonstrates that soy protein could be of health benefits to patients suffering rhabdomyolysis or ischaemia reperfusion-induced acute renal failure.

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