Protective Effect of *Cajanus cajan* in Hepatotoxic Rats

Evbakhavbokun, Winifred O. and Iweala E.J. Emeka

Department of Biochemistry, Covenant University, Ota. Nigeria.

Winifred.evbakhavbokun@stu.edu.ng

Abstract. Hepatotoxicity results from overload of chemicals and drugs including N-Nitrosodiethylamine (NDEA), a nitrosamine found in smoke, meat and food products. This study examined the hepatoprotective potential of *Cajanus cajan* in male Wistar rats. Hepatotoxicity was induced by administration of NDEA at 200mg/kg while *Cajanus cajan* was administered at 200mg/kg, 400mg/kg and 800mg/kg for 28 days. Body weight, liver weight and markers of hepatotoxicity including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Albumin (ALB) were evaluated. NDEA treated group showed a marginal increase in body weight and a significant (p < 0.05) increase in liver weight. The *Cajanus cajan* treated groups showed a significant (p < 0.05) increase and decrease respectively in body and liver weights. NDEA treated group significantly (P<0.05) increased ALT and AST and significantly reduced ALB. *Cajanus cajan* significantly (P<0.05) decreased ALT and AST and significantly (P<0.05) elevated ALB. The results indicate that *Cajanus cajan* ameliorates NDEA-induced hepatotoxicity.

1. Introduction

N-Nitrosodiethylamine (NDEA), a nitrosamine compound is a popular hepatic carcinogen causing liver damage [1]. They can be seen in industrial processes, food stuffs e.g. meat and milk, pharmaceuticals and tobacco smoke [2, 3]. NDEA leads to cellular injury and oxidative stress due to ROS [4]. Liver being the major site for metabolic biotransformation of NDEA, generation of ROS can lead to oxidative stress causing damage to the liver [5]. These ethyl radicals and other reactive oxygen species interact with DNA which leads to mutations, increase in blood markers such as ALT and AST, and also lead to neoplastic transformation in tissues of the liver [6, 7]. Various animal studies proved that increased intake of plant products are linked to decrease in the development of liver diseases and different types of cancers [8].

*Cajanus cajan* is commonly known as Pigeon pea. It’s a therapeutic plant used for treating wounds, bedsore and malaria [9]. Chemical constituents’ and antioxidant activities have shown *Cajanus cajan* leaves to be high in flavonoids, stilbenes, saponins and alkaloids [10]. Four essential compounds which include pinostrobin, vitexin, cajaninstilbene acid and orientin gotten from the leaves contain antioxidant properties [11]. It also contains various medicinal properties such as antioxidants [11], anthelmintic [7], protection against alcohol induced liver damage [12] and so on. Studies have shown the protective potential of *C. cajan* against CCl4 hepatotoxicity in rats [13]. Also, anticancer activity of *C. cajan* was examined towards MCF-7 breast cancer and it showed that the plant inhibited MCF-7 cell growth [13]. The aim of this present work was to study the hepatoprotective effect of ethanol extract of *C. cajan* against NDEA hepatotoxicity so in future an efficient formulation could be produced or developed which will be specific for imparting hepatoprotection.

2. Materials and methods

1. Chemicals
N-Nitrosodiethylamine which was of analytical grade was obtained from Sigma chemical company, USA. Silymarin was purchased from Micro Labs and other chemicals/reagents were of analytical grade, supplied by Merck (India).

2. **Animals**

Male albino rats (n=35) which weighs 150-200g body weight were bought from the University College Hospital, Oyo State, Nigeria and then housed in cages and fed *ad libitum* with rat chows and distilled water under 12-hours light/dark cycle at room temperature. All experimental procedures carried out were approved by Covenant University Health Research Ethics Committee (CHREC/015/2019).

3. **Preparation of plant extract**

*Cajanus cajan* was obtained in June, 2018 from a market in Ogun State, Nigeria and properly identified and authenticated by a qualified taxonomist. It was plucked from the branches, washed and air dried at room temperature (26°C) for four weeks. The leaves (349.1g) were then pulverized using electric blender and soaked in 80% ethanol (600g) as solvent for 72 hours using two successive extractions. It was then sieved out using muslin cloth and cotton wool. Rotary evaporator was used at 50°C and water bath at 40°C to obtain a yield of 32g semi-solid crude substance.

4. **Research design**

Male albino Wistar rats (thirty five) were separated into seven groups consisting having five animals each in different cages. Rats were acclimatized for two weeks. Group A served as the control and received no treatment. Group B was the negative control and was given intraperitoneal injection (i.p.) of only NDEA mixed in saline at 200mg/kg, Group C, D and E received i.p. of NDEA 200mg/kg followed by oral intubation of *Cajanus cajan* at doses of 200, 400 and 800 mg/kg respectively, Group F was the positive control and was given i.p. of NDEA followed with a standard drug Silymarin at 50 mg/kg, Group G was given by oral intubation of *Cajanus cajan* at 800mg/kg only. The experiment lasted for 28 days. The intraperitoneal injection of N-Nitrosodiethylamine was done following the procedure described by Mathur *et al.* [14].

5. **Measurement of body weight**

The initial body weights of the rats were weighed on the first day and the final body weight measured on the last day of the experiment using a weighing balance and the unit expressed in grams (g).

6. **Collection of Blood**

Blood obtained from the animals through cardiac puncture was put into heparinized tubes and spun for 15 minutes at 3500 rpm to get the plasma used for biochemical analyses [15].

7. **Measurement of liver weight**

After sacrificing the rats, their livers were excised and weighed using a weighing balance and units expressed in grams (g).

8. **Measurement of ALT and AST activity**

AST and ALT which are vital markers of liver function were measured using commercial Randox diagnostic kits according to the instructions of the manufacturer. A Spectrophotometer was used for measuring the absorbance at 340nm and unit expressed in U/L.

9. **Measurement of Albumin**

Albumin was measured using commercial Randox diagnostic kits according to the instructions of the manufacturer. A Spectrophotometer was used for measuring the absorbance at 578 nm and unit expressed in g/dl.
3. Results
The NDEA treated group showed a marginal increase in final body weight and a significant (p < 0.05) increase in liver weight in comparison to the control (Table 1). In the *Cajanus cajan* treated and Silymarin groups, there were significant (p < 0.05) increases in the body weights and significant (p< 0.05) decreases in the liver weights compared to the NDEA treated group.

Table 1. Effect of *Cajanus cajan* on body and liver weights

| Groups | Treatment         | Initial body weight (g) | Final body weight (g) | Liver weight (g) | Relative Liver weight (liver/100g b.w) |
|--------|-------------------|-------------------------|-----------------------|------------------|---------------------------------------|
| A      | Control           | 168 ± 12.90             | 198 ± 10.72           | 7.08 ± 0.24      | 3.58 ± 0.11                           |
| B      | NDEA              | 160 ± 14.0              | 170 ± 11.60           | 9.93 ± 0.44      | 5.98 ± 0.36                           |
| C      | NDEA + 200mg/kg   | 166 ± 11.49             | 182 ± 13.24           | 8.16 ± 0.35      | 4.48 ± 0.25                           |
| D      | NDEA + 400mg/kg   | 164 ± 12.46             | 185 ± 13.26           | 7.55 ± 0.26      | 4.19 ± 0.16                           |
| E      | NDEA + 800mg/kg   | 163 ± 11.47             | 189 ± 13.29           | 7.04 ± 0.22      | 4.01 ± 0.13                           |
| F      | NDEA + Silymarin  | 161 ± 11.41             | 186 ± 15.08           | 7.58 ± 0.11      | 3.91 ± 0.25                           |
| G      | 800mg/kg Only     | 167 ± 11.06             | 195 ± 11.38           | 6.97 ± 0.35      | 3.57 ± 0.13                           |

Values are expressed as mean ± SEM.

*Values significantly differ from Control group.*

NDEA induction significantly (p < 0.05) increased AST and ALT activity when compared with the control (Table 2). Treatment with *C. cajan* significantly (p < 0.05) reduced ALT and AST when compared with NDEA treated group. This reduction was comparable to Silymarin.

Table 2. Effect of *C. cajan* on AST and ALT activity

| Groups | Treatment         | ALT (U/L)      | AST (U/L)     |
|--------|-------------------|----------------|---------------|
| A      | Control           | 69.73 ± 5.19   | 106.67 ± 5.47 |
| B      | NDEA              | 124.73 ± 22.06 | 227.15 ± 25.5 |
| C      | NDEA + 200mg/kg   | 109.23 ± 8.39  | 180.34 ± 8.25 |
| D      | NDEA + 400mg/kg   | 98.34 ± 6.81   | 145.05 ± 6.75 |
| E      | NDEA + 800mg/kg   | 74.86 ± 5.63   | 112.87 ± 5.80 |
| F      | NDEA + Silymarin  | 90.59 ± 6.77   | 132.12 ± 5.96 |
| G      | 800mg/kg Only     | 70.33 ± 5.22   | 109 ± 5.76    |

Values are expressed as mean ± SEM.

*Values significantly differ from Control group.*

The NDEA treated group had a significantly (p < 0.05) reduced albumin when compared with control (Figure1). Significantly (p < 0.05) increased albumin in the *Cajanus cajan* treated and Silymarin groups with 800mg/kg being the highest when compared with the NDEA treated groups was seen.
Discussion

Hepatotoxicity means dysfunction of the liver due to overload of chemicals and drugs that are toxic to the body. NDEA is known to produce ROS that results to liver damage. It is known that DNA, lipids and proteins are the main targets of oxidative injury. Liver serves to filter out toxic substances from the bloodstream. When there are excessive chemicals filtering through the liver, it becomes overloaded and can lead to hepatotoxicity. The activities of particular enzymes are quickly changed in any sort of liver damage and transaminases are known to be vital markers of liver function. They sensitively reflect the status of liver damage [16]. This study showed that increase in marker enzymes were signs of cellular damage due to administration of NDEA. Treatment with ethanol extract of Cajanus cajan brought these enzymes back to near normal by protecting the functional integrity of the hepatocytes, showing its hepatoprotective property against NDEA induced liver damage. Also, Silymarin which is now widely used to treat hepatic damage also helped in protecting the liver.

This study showed that for AST, NDEA induction increased plasma AST activity compared to the control. This finding is similar to a previous study [17, 18]. Treatment with Cajanus cajan significantly (p < 0.05) reduced the activity to near normal levels compared with NDEA treated group. This reduction was comparable with Silymarin which confirms earlier observations [19, 20, 21].

This study showed that NDEA induction significantly increased plasma ALT activity in comparison with the control. There was a reduction in the Cajanus cajan treated and silymarin group when compared with NDEA treated group [17, 18]. This indicates protective activity of the Cajanus cajan extract revealing that the plant has the ability of protecting NDEA-damaged cells.

NDEA induction marginally increased the final body weight and elevated liver weight when compared to the control. This marginal increase in body weight in NDEA treated animals could predominantly be due to reduction of skeletal tissue following exposure to the chemical. This result is in line with a previous study which reported body weight loss after administration of N-nitrosodiethylamine [7, 22].

Albumins (ALB) are globular proteins made by the liver. From this study, the NDEA treated group recorded a significantly (p < 0.05) reduced albumin which may be associated with reduction of hepatocytes [23]. This observation is in agreement with a study which indicated that NDEA induced hepatic damage in experimental animals and reduced plasma albumin level [24, 25]. There was increased ALB in the Cajanus cajan and Silymarin treated groups indicating their protective role against liver cell damage.

Conclusion

The antioxidant and free radical scavenging properties of C. cajan on NDEA induced liver toxicity in rats was seen. Various reports have shown that ‘alkaloids, flavonoids, steroids and also triterpenoids’ have protective effects on the plasma and also liver as a result of their antioxidant properties. These phytochemicals are present in Cajanus cajan and may be responsible for the protective effect in NDEA induced hepatotoxicity.
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