

Protective Effects of Ethanol Leaf-Extract of *Cajanus Cajan* (Linn) in Alcohol Induced Nephrotoxicity in Albino Rats

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Abstract

The protective effects of ethanol leaf-extract of *Cajanus cajan* in alcohol induced nephrotoxicity in albino rats were investigated. Thirty six albino rats were assigned into six (6) experimental groups (A,B,C,D,E,F) with six rats in each group. Group A (Normal control) was administered normal saline only. Group B (standard control) was administered 5mg/kg body weight of standard drug (Silymarin); group C (positive control) was administered 3.7g/kg body weight of 99.7% ethanol while rats in groups D,E and F (test groups) were administered graded doses of 200mg/kg, 400mg/kg and 600mg/kg body weights of ethanol leaf-extract of *Cajanus cajan* respectively. Rats in standard control and test groups were administered 3.7g/kg body weight of 99.7% ethanol, once a day, 3 hours after administration of standard drug and ethanol leaf-extract of *Cajanus cajan* respectively. The administration lasted for 14 days through oral intubation. The result of our investigation showed that creatinine, uric acid and urea were significantly ($P<0.05$) increased in positive control group compared to the levels observed in the normal and standard control groups. However, groups administered ethanol leaf-extract of *Cajanus cajan* at the doses of 200mg/kg, 400mg/kg and 600mg/kg body weight all showed a significant ($P<0.05$) reversal in the trends of these parameters to concentrations comparable to those observed in the positive and normal control groups. The findings of this study suggest that ethanol leaf-extract of *Cajanus cajan* may have potentials that could be exploited for development of chemotherapies against renal toxicity.

Keywords: *Cajanus Cajan*; Alcohol; Nephrotoxicity; Kidneys; Albino rats; Creatinine; Uric acid; Urea

Introduction

Cajanus cajan is a tropical woody herb with yellow flowers and is a perennial plant belonging to the family Leguminosae. It is commonly known as Pigeon pea in English, “Fiofio in Igbo”, “Waken turawa” in (Hausa) and “Otili” in Yoruba Nigeria. It has a highly nutritious seed of the tropical Pigeon pea plant Luo et al. [1]. It has been cultivated in ancient Egypt, Africa and Asia. This plant was introduced in America and in other several tropical countries and it is mainly produced in India Mc Cuskey [2]. It is a nutritious non-toxic edible tropical legume with several desirable characteristics, widely used in Indian folk medicine for prevention of various liver disorders Luo et al. [1]. The pigeon pea leaf extracts may be valuable natural antioxidant sources and are potentially applicable in both medicine and healthy food industry Wu et al. [3].

Alcoholism is the most serious addictive disease prevalent in our society and accepted to be a worldwide drinking related health problem. Despite the obvious consequences of acute and chronic alcohol toxicity, the natural quest for alcohol consumption has made it “a free for all drink” Nwodo [4] and Kundua et al. [5]. The morbidity and mortality of diseases associated with alcohol intake is both a racial, social and health problem and the complication of kidney failure may be a double tragedy for alcoholics. Fatty liver, cirrhosis, kidney failure and hepatitis have been associated with high intake of alcohol Ewa & Arthur [6] and Nwodo [4]. Alcohol-induced organ pathogenesis is due to the accumulation of toxic substances generated during alcohol metabolism, which in turn generates Reactive Oxygen Species (ROS) and other free radicals Moncada et al. [7] and Albano et al. [8]. This results to covalent modification of cellular macromolecules, morphological changes leading to tissue damage and kidney dysfunction Mc Cuskey [2] and Kundua et al. [5].

Residual products (urea, creatinine, uric acid, etc.) formed as a result of metabolic reaction of substances incoming into the body are removed from the blood by kidneys which are natural treatment system of the body Brenner & Floyd [9]. In addition to this, kidneys can perform many vital functions such as regulation of water balance in the body and protection of body liquids (acidity/alkalinity). Kidneys can filtrate about 200 liters of blood per day and remove toxic materials and excess liquid via 1.5 liters urine. Within this period, any imbalance occurred might cause disorder of kidney functions rapidly Agbafor et al. [10]. Kidney function disorder is resulted as accumulation of toxic substances in the blood that should be removed from the body Henry [11]. In practice, blood and urine tests are carried out for the assessment of kidney functions and determination of degree and level of function disorders. Such tests indicate to what extent kidneys are successful to filtrate these residual products and excess liquid in the body Shivaraj et al. [12]. Creatinine and Blood Urine Nitrogen (BUN) can be searched in a blood sample. As filtration process of kidneys decreases, the levels of these residual products increase in the blood. Abnormal results obtained are generally first indicators of kidney diseases Mehta & Chertow [13].

Also, Creatinine is a breakdown product of creatine phosphate in muscle and is usually produced at a fairly constant rate by the body (depending on muscle mass). Serum creatinine (a blood measurement) is an important indicator of renal health because it is easily measured as a by-product of muscle metabolism that is excreted unchanged by the kidneys Lujambio et al. [14]. The typical human reference ranges for serum creatinine are 0.5-1.2mg/dl (about 45-90 μ mol/l) for adults male, 0.4-1.1mg/dl (about 60-110 μ mol/l) and 0.0-0.7mg/dl for children up to 12 years of age. Urine creatinine ranges for adult male is 20-25mg/kg/day and for adult female 15-20mg/kg/day Hille [15]. Increased creatinine levels are seen in impaired renal function, chronic nephritis, shocks and muscle atrophy can result in decreased creatinine level. Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen and hydrogen with the formula $C_5H_4N_4O_3$. It forms ions and salts known as urates and acid urates such as ammonium acid urate Heining & Johnson [16]. In human blood plasma, the reference range of uric acid is typically 3.4-7.2mg/dl (200-430 μ mol/l) for an adult male, 2.4-6.1mg/dl for an adult female (140 - 360 μ mol/l) and children range from 3-4mg/dl. Drugs such as Thiazide diuretic can increase uric acid level by interfering with renal clearance Slims [17]. Urea (Carbamide) is an organic compound with the chemical formula $CO(NH_2)_2$. The molecule has two- NH_2 groups joined by a carbonyl (C=O) functional group. The liver forms urea by combing two ammonia molecules (NH_3) with a carbon dioxide (CO_2) molecule in the urea cycle. 7 to 20mg/dl (2.5 to 7.1mmol/l) is considered normal range. For adult male is 8 to 20mg/dl, adult female 6 to 20mg/dl and children 5 to 18mg/dl.

Extract from *Cajanus cajan* leaves have been reported to have antimicrobial, antibacterial, antidiabetic, anthelmintic, anticancer, antimalarial and neuroactive activities Wu et al. [3]. Sharafati & Sherafati [18] also reported the antimicrobial activities of *Cajanus cajan* leaf extract against eight microbial strains (*Staphylococcus*

epidermidis, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*; and *fungi*, *Aspergillus niger* and *Candida albicans*). Despite the reports above, there are still very limited publications on the nephroprotective effects of *Cajanus cajan* leaf extracts. The data generated from this present study would go a long way to increasing the volume of information about the potential uses of this plant, especially as it relates to its protection against renal toxicity.

Cajanus cajan preparations are often recommended for the treatment of several organ disorders by most herbalists in Nigerian traditional societies and quite often claimed to have offered significant relief to patients. In attempt to get more scientific evidence for this traditionally reported herbal drug, our attentions were aroused to carry out this study to investigate the protective effects of ethanol leaf-extract of *Cajanus cajan* in alcohol induced nephrotoxicity in albino rats.

Aim/Objectives

The aim of this study was to investigate the protective effects of ethanol leaf-extract of *Cajanus cajan* (Linn) in alcohol induced nephrotoxicity in male albino rats.

Material and Method

Material

Fresh leaves of *Cajanus cajan* were collected from Ndegu-Obu village in Ameffia Ngbo in Ohaukwu Local Government Area of Ebonyi State, Nigeria in the month of July. The experimental animals used in this study were albino rats purchased from the animal unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu state, Nigeria. All chemicals and reagents used were of analytical standard.

Method

Urea was measured according to the method described by Allian et al. [19]. Creatinine was measured according to the method of Burtis [20]. Uric acid was measured according to the method of Barham [21].

Experimental Design

A total of thirty-six (36) albino rats were used in this study. The rats were randomly assigned into six (6) experimental groups of A,B,C,D,E and F with six (6) rats in each group after acclimatization for 7 days: Group A (normal control) was given normal saline only. Group B (standard control) was administered standard drug (silymarin) at the dose of 5mg/kg body weight and co-administered absolute ethanol (3.7g/kg body weight) 3 hours later. Group C (positive control) was administered absolute ethanol of 3.7g/kg body weight of the rats only. Groups D,E and F (Test groups) were administered graded doses of 200,400 and 600mg/kg body weight of ethanol leaf extract of *C. cajan* respectively and absolute ethanol of 3.7g/kg body weight 3 hours later. This administration lasted for 14 days through oral intubation. They were all fed with grower's marsh and water ad libitum.

Collection of Blood Sample

After 14 days of administration, the rats were mildly anesthetized using chloroform, blood sample were collected from the albino rat by femoral vein puncture method.

Discussion and Conclusion

Result

Data are shown as Mean \pm Standard Deviation (n=6). Mean values with different alphabet showed significant difference at $p < 0.05$ in the figures.

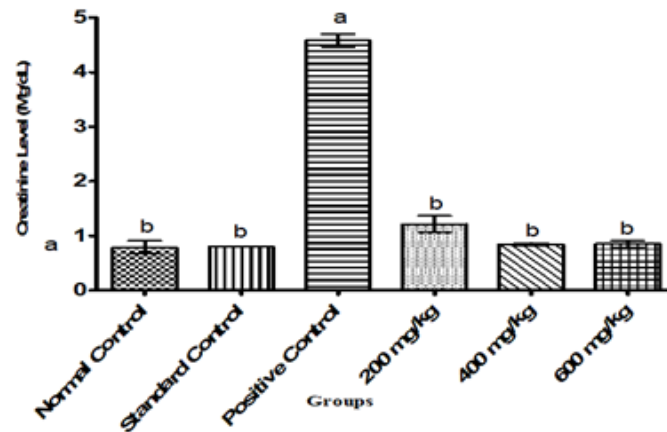


Figure 1: Creatinine level in Albino Rats Administered Ethanol-Leaf Extract of *Cajanus cajan*.

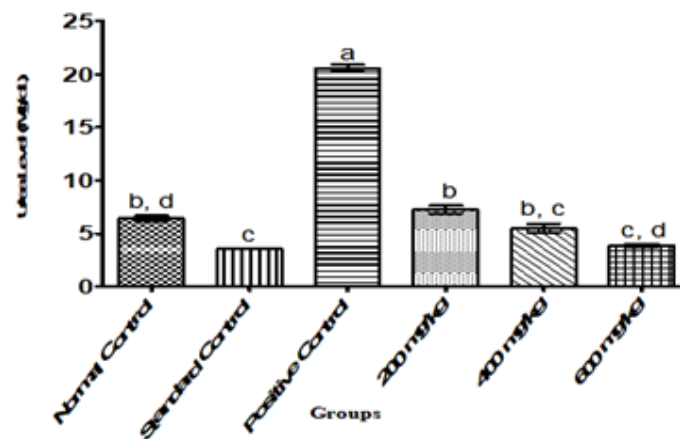


Figure 2: Urea level in Albino Rats Administered Ethanol-Leaf Extract of *Cajanus cajan*.

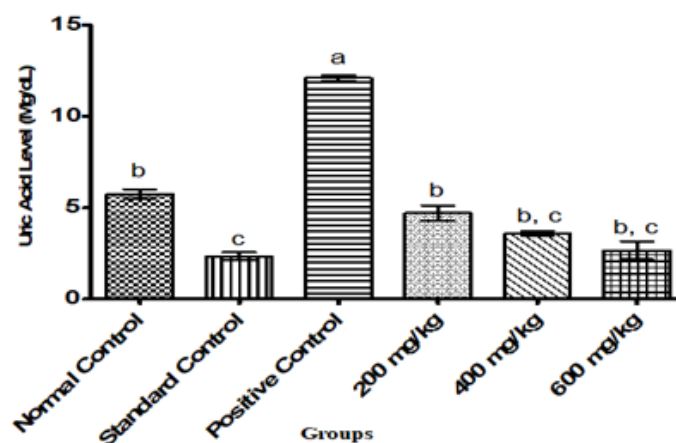


Figure 3: Uric acid level in Albino Rats Administered Ethanol-Leaf Extract of *Cajanus cajan*.

In general, parameters of renal function and fluid homeostasis investigated in this study gave some significant results. The positive control showed a significant ($P < 0.05$) increase in the levels of creatinine, urea and uric acid relative to normal and standard control groups. However, administration of ethanol leaf extract of *Cajanus cajan* at the graded doses of 200, 400 and 600 mg/kg body weight of the rats showed a significant ($P < 0.05$) reversal in the trend of these kidney biomarkers to levels comparable to those observed in normal and positive control groups as shown in Figure 1-3 respectively.

The positive control showed a significant ($P < 0.05$) increase in the levels of creatinine relative to levels seen in normal and standard control groups. However, administration of ethanol leaf extract of *Cajanus cajan* at the graded doses of 200, 400 and 600 mg/kg body weight of the rats showed a significant ($P < 0.05$) reversal in the trend of creatinine to levels comparable to those observed in normal and positive control groups as shown in Figure 1. Agbafor et al. [22] showed an insignificant decrease ($P > 0.05$) in concentrations of creatinine in the animals given the leaf-extract when compared to the control group. These effects were found to be in dose dependent manner. Ahamefule et al. [23] reported a study on rabbits fed with raw pigeon pea seed based diets in a completely randomized design to ascertain remarkable changes in haematological and biochemical profile for weaner rabbits. The result of their study showed that among the parameters not affected ($P > 0.05$) by diets were blood urea, creatinine, bilirubin, total protein, SGOT and SGPT concentrations. The variations in the results of Ahamefule et al. [23] with that obtained in the present study may be attributed to the difference in methodology, experimental animal model, doses of the extract or different species of the plant used. Serum creatinine is an indicator of renal health because it is an easily measured by product of muscle metabolism that is excreted unchanged by the kidney. If the kidney is deficient in its filtration ability, creatinine blood level rises Ahamefule et al. [23]. Since the result of this research showed a decrease in creatinine level due to administration of *Cajanus cajan* leaf-extract, the extract could be assumed to be nephroprotective.

Madinah et al. [24] used *Carica papaya* to investigate the protective effect of aqueous extract of *Carica papaya* seeds in paracetamol induced nephrotoxicity in male Wistar rats which showed a decrease in concentration of uric acid, creatinine and urea. From this present study, the result obtained showed a significant ($P < 0.05$) decrease in urea concentrations of uric acid, creatinine and urea in the test groups when compared to the positive control as shown in Figure 2. The result of this study therefore suggests that *Cajanus cajan* leaf-extract has more potential to decrease urea level than *Carica papaya* as reported by Madinah et al. [24]. Urea plays an important role in the metabolism of nitrogen-containing compounds by animal and is the main nitrogen-containing substance in the urine of mammals, when urea level is high in the blood tissue breakdown tissue can result e.g. hemorrhage Murray et al. [25]. Urea arises principally as a by-product of amino acid deamination and is the main route of nitrogen excretion Agbafor et al. [22].

The biochemical explanation for the significant decrease ($P < 0.05$) in the level of uric acid in test groups when compared to positive control (Figure 3) is that the decrease may be as a result of the presence of some compounds such as flavonoids and anthocyanins, a chemical constituent present in the leaf extract of *Cajanus cajan* reported by Agbafor et al. [22].

In conclusion, the present study showed that the ethanol-leaf extract of *Cajanus cajan* is capable of lowering kidney function parameters such as creatinine, urea and uric acids [26]. The result of this present investigation therefore suggests that ethanol leaf-extract of *Cajanus cajan* may have potentials that could be exploited for development of chemotherapies to manage and/or treat renal toxicity.

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