EFFECTS OF *Cajanus cajan* AQUEOUS LEAF EXTRACT ON SERUM AMINO TRANSFERASE, ALKALINE PHOSPHATASE AND ELECTROLYTES CONCENTRATIONS OF NORMAL WISTAR RATS

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ABSTRACT

The present study was carried out to investigate the effect of oral administration of aqueous leaf extract of Cajanus cajan on the concentration of Na^+, K^+, Cl^- and HCO_3^- and the activities of amino transferases (ALT and AST) and alkaline phosphatase (ALP) in the serum of physiologically normal rats. Two different doses, 0.5 g/kg and 1.0g/kg body weight (BWT) were administered to rats for 14 days. The effect was assessed on the basis of comparative measures of the evaluated indices in non treated rats vis-à-vis those treated with the extract. With the exception of ALP, the extract at a dose of 0.5 g/kg produced no significant ($p < 0.05$) changes in ALT and AST activities and in the concentrations of the electrolytes in rats. Though the levels of serum electrolytes were not significantly raised, 1.0g/kg of the extract markedly ($p < 0.05$) increased the activities of serum enzymes. Overall, the data of the study suggests that aqueous leaf extract of Cajanus cajan at a dose of 0.5 g/kg did not cause obvious damage to the liver and kidneys of rats. However, 1.0g/kg bwt of the plant extract showed clear signs of hepatocellular derangement in rats. Hence, Cajanus cajan leaves may be hepatotoxic when consumed at concentrations about or above 0.5 g/kg of body weight.

Keywords: *Cajanus cajan*, Serum electrolytes, Enzymes, Hepatotoxicity, Hepatocellular derangement

INTRODUCTION

Plant materials are pivotal to ethnomedicine and have remained significant sources of new drugs. Orthodox drugs though, are generally preferred and acceptable, alternative medicine is still very much relied on all over the world (O'Brien, 2004; Leckridge, 2004), especially in the developing countries where the cost of western medicine is astronomical and unaffordable to a large size of the populace (Busia, 2005). According to the World Health Organization estimates, almost 80 % of the people in developing countries rely entirely on traditional medicine for their primary health care, and about 85 % of such traditional medicine involves the use of plant extracts (Farnsworth, 1988).

The toxicology of medicinal plants and their products is an important and integral part of the early and late phase of drug development (Gamaniel, 2000). Documented information on the toxicity and side effects associated with most plants used in ethnomedicine abound.

Cajanus cajan is popularly referred to as pigeon pea (English) and *Otili* (Yoruba, Nigeria). It belongs to the botanic family Fabaceae and grows in the forest and savannah regions of the world. The plant (different parts) is widely used either alone or in combination with other herbs to treat various kinds of diseases in many African countries. Some of the medicinal uses of *C. cajan* include treatment of cough, coronary heart diseases, menstrual disorder, cerebral infraction, jaundice and bronchitis (Morton,

1976; Duke, 1981). It is also used as antihelminthic, sedative and in child delivery. The aqueous leaf extract of *C. cajan* is consumed by pregnant women especially in southwest, Nigeria to aid easy delivery. *C. cajan* leaf extract has also been reported to induce uterine contraction in rats (Olatunji-Bello *et al.,* 2002). Its hypoglycaemic, antisickling and anti-plasmodial properties are well documented (Giri *et al*., 1987; Ogoda *et al.,* 2002; Duker-Eshun *et al.,* 2004). The furtherance of information on the biochemical and toxicological components of this important medicinal plant elucidated this study, on the effects of *C. cajan* aqueous leaf extract on serum amino transferase, alkaline phosphatase and electrolytes concentrations of rats.

MATERIALS AND METHODS

***Cajanus cajan* Extract:** Fresh *C. cajan* leaves were harvested as one batch in the month of June 2010 at Idishin, Ondo State, Nigeria. The botanical identification was confirmed at the Herbarium of the Department of Botany, University of Ibadan. The leaves were freed of extraneous materials, air-dried and ground into a uniform powdery form using a milling machine. The powdered leaf was extracted twice in distilled water and, on each occasion with 2.5L distilled water at room temperature for 48 hours, with occasional shaking, filtered using Whattman filter paper number 1. The obtained aquaeous extract filtrates were concentrated to dryness at 60 $\pm$ 1^{o}C in a rotary evaporator. Drying and solvent elimination finally gave a light brown extract. This crude aqueous extract was used, without further purification.

Animal Management: Ninety male albino rats (180g – 200g) of the Wistar strain were used for the study. They were purchased from the Institute for Advance Medical Research and Training (IMRAT), at the University College Hospital (UCH), Ibadan. The animals were handled humanely, kept in a plastic suspended cage placed in a well ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were provided rat pellets and served water *ad libitum* and subjected to natural photoperiod of 12 hours light and 12 hours dark cycle. The animals were randomly assigned into three (3) groups (n=10). Group I: Rats given 2ml of normal saline daily for 14 days (Control), Group II: Rats treated orally with 0.5g/kg body weight of *C. cajan* extract daily for 14 days and Group III: Rats treated orally with 1.0g/kg body weight of *C. cajan* extract daily for 14 days. All treatment groups were replicated thrice to minimize experimental error.

All animals were given access to normal laboratory chow and water *ad libitum* during the study. At the end of the administration, the animals (treated and non-treated) were fasted overnight and blood sample was collected separately from each rat via the retro orbital sinus of the eye by ocular puncture into non heparinized bottles for biochemical analysis.

Estimation of Enzyme Levels: The levels of alanine amino transferase (ALT: EC. 2.61.2.1) formerly known as glutamate pyruvate transaminase (SGPT) and asparte amino transferase (AST: EC. 2.6 1.1) formerly known as glutathione-oxaloacetate transaminase (SGOT) in blood samples were estimated by the use of end point colorimetric diagnostic kit (Randox Laboratories Limited, England) (Reitman and Frankel, 1957). Alkaline phosphatase (ALP) activity was determined by the use of sigma diagnostic kits (Sigma Diagnostic, USA) (Reitman and Frankel, 1957).

Serum Electrolytes Concentrations: Serum sodium and potassium concentrations were determined using flame emission photometry method (Magoshes and Vallee, 1956). Serum chloride concentration was determined using the titration method (Schales and Schales, 1941). The method is based on the precipitation of chloride ions in serum using mercuric nitrate. When chloride ion is titrated with standard solution of mercuric ion, undissociated but soluble mercuric chloride, $HgCl_2$, is formed. The excess mercuric nitrate reacts with diphenylcarbazone to produce a violet colour.

Table 1: Serum ALT, AST and ALP concentrations in *Cajanus cajan* aqueous leaf extract treated and non treated rats

Group/Extract dosage (g/Kg bwt)	Serum ALT activity (iu/l)	Serum AST activity (iu/l)	Serum ALP activity (iu/l)
Group I / 0.0	14.7 ± 5.0	18.6 ± 4.4	124.6 ± 5.9
Group II / 0.5	17.9 ± 4.9	21.0 ± 2.3	142.5 ± 8.1*
Group III / 1.0	41. ± 6.7*	36.1 ± 4.2*	224.0 ± 6.8*

*All values are presented as mean + standard deviation. (n = 30). Values with superscripts * means significant difference at P<0.05 when compared to non treated rats.*

Table 2: Serum potassium, sodium, bicarbonate and chloride ion concentrations in *Cajanus cajan* aqueous leaf extract treated and non treated rats

Group/Extract dosage (g/Kg bwt)	Serum K^+ concentration (mEq/L)	Serum Na^+ concentration (mEq/L)	Serum HCO_3^- concentration (mEq/L)	Serum Cl^- concentration (mEq/L)
Group I / 0.0	4.7 ± 2.0	138.2 ± 5.4	24.6 ± 0.8	99.0 ± 3.0
Group II / 0.5	4.6 ± 2.4	141.0 ± 2.3	24.2 ± 1.0	102.0 ± 0.1
Group III / 1.0	4.65 ± 1.7	142.1 ± 3.4	25.0 ± 0.8	100.8 ± 1.6

All values are presented as mean + standard deviation (n = 30)

Titration method of Van Slyke and Neil (1924) was employed in determining the concentration of bicarbonate ion in serum samples. The method is based on the release of carbon dioxide from bicarbonate ion in serum with dilute hydrochloric acid. The excess acid was then titrated with sodium hydroxide using phenol red as indicator.

Statistical Analysis: The data were compared by the use of one-way analysis of variance (ANOVA) followed by post hoc tests (Least Square Difference) and presented as mean ± S.D (standard deviation of the mean). The groups P value of less than 0.05 was declared as significant statistically.

RESULTS AND DISCUSSION

The aqueous leaf extract of *C. cajan* at all doses caused a significant increase in ALP concentrations from 4.7 ± 2.0 iu/l in the control group to 41. ± 6.7 iu/l in the group given 1.0 g / kg bwt of *C. cajan* leaf extract. Similar significant increases were noticed in the serum concentrations of AST from 18.6 ± 4.4 iu/l in the control group to 36.1 ± 4.2 iu/l in the group given 1.0 g / kg bwt of *C. cajan* leaf extract, and in the serum concentrations of ALP from 124.6 ± 5.9 iu/l in the control group to 224.0 ± 6.8 iu/l in the group given 1.0 g / kg bwt of *C. cajan* leaf extract. All treatments enzymes levels were significantly different (P < 0.05) (Table 1). The values of the enzymes (Table 1) of the control group were observed to remain within the normal physiological range: 44 – 147 iu/l for ALP, 8 – 37 iu/l for ALT and 10 – 34 IU/L for AST (Van Slyke and Neil, 1924; Schales and Schales, 1941; Magoshes and Vallee, 1956).

The mean concentrations of potassium, sodium, bicarbonate and chloride ions in non treated rats (control) were 4.7 mEq/L, 138.2 mEq/L, 24.6 mmol/L and 101.0 mmol/L respectively (Table 2). The values of the electrolytes (Table 2) were observed to remain within the normal physiological range: 3.7 – 5.2 mEq/L for K^+, 136 – 145 mEq/L for Na^+, 20 – 29 mmol/L for HCO_3^- and 101 – 111 mmol/L for Cl^- (Van Slyke and Neil, 1924; Schales and Schales, 1941; Magoshes and Vallee, 1956).

Significant increase in the concentration (P < 0.05) of alkaline phosphatase (ALP) following exposure of rats to $0.5gkg^{-1}$ of extract suggests that the consumption of *C. cajan* aqueous extract at a dose of 0.5g per kilogram of body weight may not be advisable and safe.

Administration of $1.0gkg^{-1}$ of body weight caused a significant increase in serum

enzyme activities (Table 1) but had no significant effect on the concentrations of the electrolytes (Table 2). The serum levels of all the selected electrolytes were minimally increased with the exception of potassium which was slightly decreased when compared to the control rats (Table 2).

Significant alteration in the concentration of the body electrolytes is indicative of poor renal functions or renal impairment. In the same light, a significant increase in concentrations of ALT, AST and ALP in the serum or plasma is an integral part of diagnosis for hepatocellular distortion. When the structural integrity of the liver is deranged or compromised, there is a leakage of these enzymes from the cytosol into the blood stream. This observation agreed with the report of Vermaulen *et al.* (1992) which stated that ALT, AST and ALP are normally located in the cytoplasm and released into circulation after cellular damage. The data of this study showed that the extract at the doses administered may not have interfered with kidney function but caused hepatocellular derangement in rats.

Conclusion: The present study showed that the aqueous leaf extract of *C. cajan* has the potential to be hepatotoxic when consumed at a daily dose of $0.5gkg^{-1}$ of body weight or more for 14 days. On this basis, we strongly recommend that lower dosage be considered in the local use of the plant leaf extract for treatment of ailment. Further investigation using lower dosages of aqueous leaf extract of *C. cajan* on its hepatotoxic and nephrotoxic effects is needful.

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