

Full Length Research Paper

Evaluation of hypolipidemic and potential antioxidant effects of Pigeon pea (*Cajanus cajan* (L) mill sp.) leaves in alloxan-induced hyperglycemic rats.

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Antioxidant and hypolipidemic activity of the methanolic leaf extract from *Cajanus cajan* was carried out in alloxan induced hyperglycemic rats. A significant ($p < 0.05$) reduction in the levels of blood glucose, serum triglyceride, cholesterol, high density lipoprotein and low density lipoprotein cholesterol was observed in the hyperglycemic-extract treated rats. The administration of the extract (200 mg/kg) also caused a reduction in urea, creatinine and malondialdehyde levels in hyperglycemic rats. The extract was found to be able bring about 34.4 and 32.16% decrease in the activities of ALT and AST respectively in the hyperglycemic-treated rats compared to untreated group. Antioxidant activity of the extract was also confirmed through *in vitro* studies.

Key words: *Cajanus cajan*, hypolipidemic effect, antioxidant activity.

INTRODUCTION

Pigeon pea (*Cajanus cajan* (L) Mill sp) is a perennial plant belonging to the family Leguminosae. It is an important grain legume crop of rain-field agriculture in the tropic and subtropics. It is known to be useful in diverse ways (Chakraborty et al., 2007, Fu et al., 2006; Fu et al., 2007). And most commonly its leaves are used for the treatment of wound, bedsores, malaria as well as diet-induced hypercholesterolemia (Li et al., 2001; Luo et al., 2008). Protective effects of extracts from pigeon leaf against hypoxic-ischemic brain damage and alcohol-induced liver damage have also been reported (Huang et al., 2006; Kundu et al., 2008).

Hyperlipidemia is one of the common complications of diabetes mellitus with accompanied elevation in lipoprotein contents which ultimately accelerates atherogenesis in diabetes and contributes greatly to increase susceptibility to vascular complications. It has been established that free radicals and lipid peroxides are generated under various pathological conditions

including hyperglycemia. However, natural products like flavonoids and β -carotenoids have been reported to possess free radical scavenging action and antioxidant property by donating hydrogen or reacting with superoxide anions, thus eliciting free radical scavenging activity. *C. cajan* is known to contain flavonoidal glycoside (Nan, 2009). Thus, the present study was designed to determine the *in vitro* hypolipidemic and potential antioxidant property of the methanolic leaf extract containing flavone glycoside of *C. cajan* in experimental rats.

MATERIALS AND METHODS

Alloxan monohydrate, thiobarbituric acid, ascorbic acid and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma – Aldrich (Steinheim, Germany). All other chemicals and reagents used were of analytical grade with high purity.

Plant materials

The dried *C. cajan* leaves were collected from a local farm at Ife-Odan, Osun-State, Nigeria and authenticated by Dr Aworinde D.O

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(a plant taxonomist) in the Department of Biological Sciences (Botany unit), University of Agriculture, Abeokuta, Ogun-State, Nigeria. The leaves were pulverized to 40 mesh, then stored in a dry place at room temperature until used.

Preparation of the methanolic extract

The methanolic extract was prepared by extraction under reflux thus: 40 g of *C. cajan* leaves were added to 300 ml 80% methanol and extracted at 80°C for 1 h. The methanol was evaporated under vacuum to obtain the residue (yield 10.2% w/w). The residue was stored at 4°C in dark bottle until used.

Determination of antioxidant activity: DPPH radical scavenging assay

The hydrogen atom or electron donating ability of the methanolic extract and pure compounds were measured by bleaching of a purple colored ethanol solution of DPPH according to the method of Amarowicz et al. (2004) as described by Nan et al. (2009). This spectrophotometric assay uses the stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent. An aliquot of the sample (100 µl) was mixed with ethanol (1.4 ml) and then added to 0.004% DPPH in ethanol. The mixture was shaken vigorously and decrease in absorbance measured at 517 nm for 70 min using UV-vis spectrophotometer, (ascorbic acid, a stable antioxidant was used as a synthetic reference). The radical scavenging activities of the sample expressed as percentage inhibition of DPPH was calculated according to the expression given by Yen et al. (1994).

Animal materials

Hypolipidemic effects and some biochemical parameters were investigated using thirty (30) male albino rats (Wister), weighing about 180 to 200 g. These were purchased from the Department of Veterinary Medicine, University of Agriculture, Abeokuta, Nigeria. They were maintained under normal temperature (25°C) and relative humidity (50 to 60%). They were fed normal rat pelleted diet formulated by Ladokun and sons, Nig. Ltd, and allowed free access to water. After an acclimatization period of one week they were used for the study.

Experimental design

The rats were randomly divided into five groups of each containing six rats thus:

Group 1: Normal animal received only feed and water throughout the study

Group 2: Normal animal administered 200 mg/kg b.wt of extract.

Group 3: Hyperglycemic control animal.

Group 4: Extract (200 mg/kgbw⁻¹) treated hyperglycemic rats.

Group 5: Standard drug (glibenclamide, 0.5 mg/Kg) treated hyperglycemic rats.

The animals were made hyperglycemic by a single dose of alloxan monohydrate (150 mg/kg b. wt.) in physiological saline intraperitoneally. After 24 h of alloxan injection, the animals in Group 4 were treated with the daily dose of the extract (200 mg/kg b.wt) orally, while Group 5 rats received glibenclamide at a daily dose of 0.5 mg/kg b.wt. The treatment continued for a week. After the last treatment, the animals were made to fast overnight and then kept under mild diethylether anaesthetic in order to collect blood.

The blood samples were collected into two sets of tubes: for serum separation (without any anticoagulant) and another into tubes rinsed with heparin (anticoagulant); the whole blood samples collected with the anticoagulant rinsed tubes were precipitated with 10% trichloroacetic acid then centrifuged at 5000 rpm for 10 min, after which the supernatant was used for the estimation of glucose, urea and creatinine using Cypress Diagnostic Kits. The serum samples after separation were used for the estimation of total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol, activities of aspartate transaminase (AST) and alanine transaminase (ALT). However, the red blood cells obtained after centrifuging the whole blood were washed using normal saline and used for determination of malondialdehyde (MDA) concentration following the method of Draper and Hadley (1990).

Statistical analysis

Data were subjected to analysis of variance (ANOVA). The significance values were expressed as Mean ± SD at the p<0.05 level (Steel and Torrie, 1980). Differences were considered statistically significant at the p<0.05 level.

RESULTS

The results of the serum lipid-lowering effect of *C. cajan* leaves extract in Wister albino rats is presented in Table 1. There was a significant increase (p<0.05) in the levels of serum total cholesterol and triglyceride in the alloxan-injected hyperglycemic animals when compared to other groups. On treatment with the extract, the hyperglycemic animals (Group 4) had a significant (p<0.05) reduction in the serum cholesterol as well as significant alteration in the level of serum triglyceride when compared with the alloxan-injected hyperglycemic control animals (Group 3). A significant increase in HDL-cholesterol and LDL-cholesterol was observed in hyperglycemic (Group 3) rats. The mean ratio of HDL-cholesterol to total cholesterol (positive indicator) was found to be highest in Group 4 rats, followed by Group 2 rats and was least in Group 3. However, the negative indicator, that is, the ratio of LDL-cholesterol to HDL cholesterol was found to be highest in Group 3 when compared to other groups.

The results of blood glucose, urea, creatinine, bilirubin, MDA, ALT and AST activities are shown in Table 2. A significant increase (p<0.05) in blood glucose and MDA levels was observed in alloxan-injected hyperglycemic animal compared to other groups. However, administration of the extract to hyperglycemic animals was able to reduce these values close to that of the control. There was also an increase in the levels of urea and bilirubin concentration in Group 3 rats compared to other. However, there was no significant difference in these parameters in extract treated groups (Groups 2 and 4) compared to control and Group 5 (standard drug treated group)

On-set of hyperglycemia, as observed in Group 3 animals, significantly (p<0.05) increase the erythrocyte MDA levels compared to other groups.

Table 1. Lipid Profile and atherogenic risk predictor indices of different groups of albino rats treated with different concentration of *C. cajan* extract, alloxan and glibenclamide.

Group Parameter	Control	Group 2	Group 3	Group 4	Group 5
TC	101.51±3.21 ^a	105.62±3.10 ^a	214.3±2.76 ^b	116.75±3.17 ^a	110.17±2.40 ^a
TG	20.65±1.10 ^a	21.03±1.06 ^a	39.37±2.05 ^b	23.55±1.08 ^a	20.14±1.04 ^a
HDL-C	18.19±0.96 ^a	17.60±0.8 ^a	30.56±1.10 ^b	20.25±0.69 ^a	19.65±0.66 ^a
LDL-C	34.83±1.60 ^a	35.21±1.51 ^a	71.43±1.40 ^b	36.52±1.39 ^a	39.12±1.40 ^a
HDL-C/TC	0.18±0.02	0.17±0.01	0.14±0.01	0.19±0.02	0.18±0.02
LDL-C/HDL-C	1.91±0.03	2.00±0.03	1.90±0.03	1.80±0.03	1.99±0.04

Values are means of the six animals per group ± standard deviation. Values in the same row with different superscript are significantly different at $p < 0.05$. TC = total cholesterol (mg/dl), TG = triglyceride (mg/dl), HDL-C = high density lipoprotein-cholesterol (mg/dl), LDL-C = low density Lipoprotein (mg/dl).

Table 2. Effects of methanolic extract of *C. cajan* on some biochemical parameters in hyperglycemic rats.

Group Parameter	1	2	3	4	5
Blood glucose (mg/L)	91.32±3.1 ^b	90.51±2.45 ^b	140.0±3.60 ^a	101.22±3.96 ^b	92.66±3.2 ^b
Urea (mg/dl)	29.25±1.5 ^a	33.28±1.7 ^a	47.33±2.5 ^b	30.33±1.8 ^a	25.26±1.6 ^a
Creatinine (mg/dl)	0.93±0.03 ^g	1.01±0.05 ^g	2.3±0.07 ^f	0.97±0.05 ^g	0.79±0.03 ^g
Bilirubin (mg/dl)	0.69±0.03 ⁱ	0.73±0.03 ⁱ	1.09±0.02 ^h	0.72±0.03 ⁱ	0.65±0.32 ^j
MDA (nmol/mg)	0.80±0.02 ^r	0.83±0.03 ^r	2.75±0.05 ^s	1.12±0.10 ^r	0.90±0.11 ^r
ALT (U/L)	18.20±0.48 ^m	17.11±0.52 ^m	34.4±1.10 ^j	24.32±0.76 ^m	20.68±0.49 ^m
AST (U/L)	31.26±0.61 ^p	30.72±0.5 ^p	68.4±0.68 ^k	46.40±0.61 ^q	40.1±0.54 ^q

Values with different superscript along the same row are significantly ($p < 0.05$) different.

Administration of the extract at 200 mg/kg brought a decrease in the MDA values close to that of the control.

Moreover, the damage caused by the alloxan in the organs (liver and kidney) was clearly evidenced through the significant ($p < 0.05$) elevations in the levels of urea, creatinine and activities of ALT and AST in the alloxan injected hyperglycemic animals when compared with the control animals. Administration of the extract to these hyperglycemic animals showed a significant reduction in the levels of blood urea, creatinine, ALT and AST activities, when compared with the hyperglycemic control rats. In addition, these decreases were close to that of control and drug (glibenclamide) administered rats.

DISCUSSION

Several studies have been undertaken to determine the effective means of reducing hyperlipidemia and researchers have beamed their "search-light" mostly on natural products. It has also been proven that lowering the plasma lipids could minimize the complications associated with arteriosclerosis and cardiovascular events (Ghasi et al., 2000; Baradaran and Nasri, 2006). For instance, it has been reported that garlic and

preparations from it, is an effective hypoglycemic agent (Ziaei et al., 2001; Superko and Kraus, 2000). Sharma et al. (2007) also reported cholesterol and triacylglycerol lowering effects of the leaf extracts of *Aegle marmelos* while lipid lowering activity of *Globimetula braunii* was reported by Okpuzor et al. (2009).

The present study demonstrates that administration of *C. cajan* methanolic leaf extract to hyperglycemic rats could decrease the levels of blood glucose, cholesterol, triglyceride and creatinine concentrations. The increased urea and creatinine level in alloxan-induced diabetic rats is not surprising, in that, alloxan have been reported not only to be specific for pancreatic damage but also for causing damage to other organs like kidney and liver (Merina et al., 2010). The extract also reduced the MDA levels as well as decreased the activities of liver marker enzymes (namely AST and ALT) in experimentally induced hyperglycemic rats. This is in agreement with the reports of Asham et al. (2009) that protein fraction of *C. cajan* possess hepatoprotection by reducing the serum levels of ALT and AST compared to the CCl_4 -treated animals. Furthermore, similar to our results, natural products supplementations have been proven to be beneficial in decreasing hyperglycemia induced by alloxan or streptozocin in a variety of tissues in animals.

For instance, *Plumeria rubra* (L) flower extract was reported to possess both antioxidant and hypolipidemic effects in alloxan-induced hyperglycemic rats (Merina et al., 2010), while *Globimetula braunii* leaves exhibited lipid and blood pressure lowering activities in both *in-vivo* and *in-vitro* experimental studies (Okpuzor et al., 2000). Also, vasorelaxant properties of *Loranthus ferugineus* methanolic extract of some medicinal plants against CCl₄-induced hepatotoxicity in albino rats was reported by Asham et al. (2009), *Camellea sinensis* extract against tamoxifen-induced liver injury (El-Beshbishy, 2005).

The increase in the erythrocyte malonhydehyde levels in hyperglycemic conditions have been reported to be associated with oxidative stress which is usually manifested by increasing level of lipid peroxides with associated toxicological effects such as decreased membrane fluidity and functions, impaired mitochondria and golgi apparatus functions as well as inhibition of some enzyme activities. Therefore, it was assumed that the antioxidant effect of *C. cajan* extract could be related to its MDA concentration lowering effects by way of free-radical suppressing activity. This was further buttressed by the observed relatively high percentage inhibition value of (63%) obtained in the present study.

Possible potential lipid lowering effect of *C. cajan* leaves extract in hyperglycemic rat as measured by the atherogenic risk predictor indices (HDL-C/TC and LDL-C/HDL-C) in this study, revealed that extract treated hyperglycemic rats had the highest and least (though not statistically significant) values of HDL-C/TC and LDL-C/HDL-C respectively.

Conclusion

Although the mechanism for cholesterol and triglyceride lowering effect of *C. cajan* is not well spelt out in this study, we are able to establish that *C. cajan* leaf extract bring about a decrease in lipid levels and extent of peroxidation in hyperglycemic rats. Work is in progress to unravel the mechanistic mode of action of this plant extract using enzymatic inhibition studies on HMG-CoA reductase and fatty acid synthesis.

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