ejpmr, 2018,5(12), 388-393

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF METHANOLIC EXTRACT OF CAJANUS CAJAN ON BLOOD LIPIDS AND OXIDATIVE STRESS IN HFD AND FRUCTOSE INDUCED HYPERLIPIDEMIC RATS

Dr. M. Ganga Raju, Kumaraswamy K. Suvarchala Reddy NVL

Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana State.

***Corresponding Author: Dr. M. Ganga Raju**

Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana State.

Article Received on 15/10/2018 **Article Revised on 05/11/2018** **Article Accepted on 26/11/2018**

ABSTRACT

Objective: The present study was carried out to investigate antihyperlipidemic and antioxidant activity of methanolic root extract of *Cajanus cajan.* **Materials and Methods:** The antihyperlipidemic activity was evaluated by using high fat diet (HFD) and fructose induced hyperlipidemic rat models using simvasatin as standard. The extract was also screened for its antioxidant activity by reducing power assay and hydrogen peroxide assay using ascorbic acid as standard. **Results:** The methanolic root extract of *Cajanus cajan* (MECC) was evaluated at two dose levels namely 200 and 400 mg/kg bd.wt. MECC has shown significant ($p<0.05$) lipid lowering activity. The lipid lowering activity might be due to the presence of sterols, flavonoids, triterpenoids and saponins. The extract contains sterols like beta sitosterol and beta sigmasterol which reduces the absorption of cholesterol, increases the fecal excretion of steroids that results in reduction of body lipids. The extract also significantly ($p<0.05$) scavenged the free radicals. The phenolic constituents present in the extract might be responsible for its antioxidant activity as phenolics possess strong ability to inhibit oxidants and free radicals. **Conclusion:** From the above it is clear that MECC possess antihyperlipidemic and antioxidant activities.

KEYWORDS: *Cajanus cajan*, Antihyperlipidemic, Antioxidant activity.

INTRODUCTION

Hyperlipidaemia means abnormal increase in fat levels of blood. These fats include cholesterol and triglycerides. These are important for our body to function, but when their levels are high they can cause heart disorders. The lipids that are involved in hypercholesterolemia are cholesterol an essential component of cell membrane and a precursor of steroid hormone synthesis and triglycerides are important energy source, they are transported in blood as lipoprotein.[1,2]

The consequence of hyperlipidaemia is to cause atherosclerosis, thus the risk of coronary heart diseases and strokes. *C. cajan* being a forage crop has been utilized as an important remedy for various ailments. Chemical constituent investigations have indicated that *C. cajan* leaves are rich in flavonoids and stilbenes. They also contain saponins, conspicuous amount of tannins, and moderate quantities of reducing sugars, resins and terpenoids. Chemical studies reveal 2'-2' methyl cajanone, 2'-hydroxy genistein, isoflavones, cajanin cahanones etc., which impart antioxidant properties.[3] Roots are also found to possess genistein and genistin. It also contains hexadecanoic acid, α amyrin, β-sitosterol, pinostrobin, longistylin A and longistylin C which impart anticancer activity. Presence of cajanus lactone, a coumarin imparts antibacterial activity. Presence of cajaninstilbene acid, pinostrobin, vitexin and orientin is responsible for antiplasmodic activity.

Herbal formulations are preferred due to lesser side effects and their low cost. One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals. Thus, a drug having multi-fold properties such as antidiabetic, lipid lowering and antioxidant activities is in great demand.[4] Therefore, in the present study, an attempt has been made to explore hypolipidemic and antioxidant activities of *Cajanus cajan* root extract.

MATERIALS AND METHODS

Preparation of the extract

The root powder of *Cajanus cajan* was dried. Then the powder was extracted with methanol by simple soxhlet extraction technique.

Preliminary phytochemical screening

The extract was subjected to phytochemical investigation for plant secondary metabolites such as alkaloids, tannins, flavonoids, glycosides, saponins, carbohydrates, phenolic compounds, fixed oils, terpenoids, and steroids by utilizing standard methods.[5]

Experimental animals

Wistar rats (180-200 g) of either sex approximately the same age, procured from Albino labs, Hyderabad, India were used for the study. They were housed in polypropylene cages and fed with standard rodent pellet diet and water *ad libitum.* The animals were exposed to an alternate cycle of 12 h of darkness and 12 h of light. All the experimental works with the animals were carried out after obtaining approval from the Institutional Animal Ethics Committee (Reg. No. 1175/PO/Ere/S/08/CPCSEA).

Acute toxicity studies

An acute oral toxicity study was performed as per Organization for Economic Co-operation and Development 423 guidelines.

Experimental design

Antihyperlipidemic activity

a. High fat diet induced hyperlipidaemia

High Fat diet was prepared by mixing cholesterol 2%, sodium cholate 1%, and coconut oil 2%, with powdered standard animal food.[2,5] The diet which was prepared as pellets was placed in the cage carefully and was administered for 20 days and corresponding treatment for next 10 days.

Wistar rats (180-200 g) were selected and animals were divided into five groups with six animals per group.
Group 1: Normal control.
Group 2: HFD control (Vehicle 1 ml/100 g/day p.o).
Group 3: HFD with MECC (100 mg/kg, b.w./day p.o).
Group 4: HFD with MECC (200 mg/kg, b.w./day p.o).
Group 5: HFD with Simvastatin (10 mg/kg, b.w./day p.o).

On day 30th day, the blood was collected by retro orbital puncture and the serum was separated for estimation of total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and very low density lipoprotein (VLDL) levels. For estimation of triglycerides, cholesterol and HDL, kits (ARBA diagnostics kit, India) were used. All the estimations were carried out as per the instruction provided by the kit manufacturers. VLDL and LDL were calculated as per Friedewalds equation (mg/dl).[6]
VLDL = TG/5.0, LDL = TC - HDL – VLDL.

b. Fructose induced hyperlipidaemia

The animals of the all groups had free access to food pellet and water ad libitum. 10% fructose was used as inducing agent for hyperlipidaemia.[7]
Group I- Normal diet and distilled water.
Group II- Normal diet and water with 10% fructose
Group III- Normal diet and water with 10% Fructose + MECC (200 mg/kg, bd.wt *p.o*).
Group IV- Normal diet and water with 10% Fructose + MECC (400 mg/kg, bd.wt *p.o*).
Group V- Normal diet and water with 10% Fructose + Simvastatin (10 mg/kg, bd.wt *p.o*.).

All the animals were fasted for half an hour prior to drug administrations. On day 21st day, the blood was collected by retro orbital puncture and the serum was separated for estimation of total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and very low density lipoprotein (VLDL) levels.

***In vitro* Antioxidant Activity**

Determination of Reducing Power

An aliquot of samples (1 mL), with different concentrations, was mixed with 200 mM phosphate buffer (2.5 mL, pH 6.6) followed by of 1% potassium ferricyanide [K3Fe(CN)6, 2.5 mL]. The mixture was incubated for 20 min in a water bath at 50 °C. After incubation, 10% TCA (1 mL) was added, followed by centrifugation at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL). Then the Absorbance was measured at 700 nm against a blank.[8] EC50 value (µg extract/mL) is the effective concentration at which the Absorbance is 0.5 for reducing power and was obtained by interpolation from the linear regression analysis. Ascorbic acid were used as positive controls in these assays.

Hydroxyl radical scavenging activity

The scavenging activity of MECC at different concentrations (10, 20, 30, 40 and 50 µg/ml) on hydroxyl radical activity. The intensity of the colour formed was measured spectroscopically at 412 nm against reagent blank.[9] The Hydrogen peroxide radical scavenging activity of the sample extracts was evaluated as % of antioxidant activity.

Statistical analysis: All the values were expressed as mean ± standard error of mean. The data were statistically analysed by one-way analysis of variance followed by Dunnett's t-test, and values $p<0.05$ was considered to be significant.

RESULTS

Methanolic root extract of *Cajanus cajan* was explored for its antihyperlipidemic activity and its anti-oxidant activity using animal models. The preliminary phytochemical investigation of methanolic root extract of *Cajanus cajan* showed the presence of steroids, flavonoids, triterpenoids, tannins & phenolic compounds, saponins, glycosides, carbohydrates and proteins.

Acute Toxicity Studies

Methanolic root extract of *Cajanus cajan* was tested on female mice up to a dose of 2000 mg/kg bd. wt. *p.o.* All animals were safe even after 14 days of observation. Hence the extract is found to be safe even upto 2000 mg/kg bd. wt.

Table 1: Anti-hyperlipidemic activity for methanolic root extract *Cajanus cajan* on High fat diet induced hyperlipidemic rats.

Treatment	Lipid Profile (mg/dL)				
	Total Cholesterol	Triglyceride	HDL	LDL	VLDL
Normal control	113±2.26	72.5±0.1.33	65±2.08	31±0.52	14.5±0.26
Hyperlipidemic control	228±0.87^{b}	182±0.763^{b}	30.1±0.708^{b}	162±0.1.48^{b}	31±5.6^{a}
MECC (200 mg/kg)	125.5±0.763$^{a, *, B}$	83±0.577$^{a, *, B}$	47.83±0.830$^{b,**, A}$	61±1.38^{a,*,B}	16.6±0.11^{b,*,A}
MECC (400 mg/kg)	117±0.57$^{b, **, A}$	72±0.76$^{a, **, ns}$	52.5±0.76$^{a, *, B}$	50±0.2$^{b, **, A}$	14.4±0.15$^{a,*, B}$
Simvastatin (10 mg/kg)	105±1.36^{a,**}	75±0.76$^{a, **}$	55±0.57$^{b, **}$	34±1.36^{a,*}	15.06±0.15^{b,**}

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control group (a = p <0.01, b = p < 0.05), hyperlipidemic control (* = p<0.01, ** = p<0.05) and standard (A = p < 0.01, B = p < 0.05) and ns- non significant.

Table 2: Effect for methanolic root extract of *Cajanus cajan* on body weight by High fat diet induced hyperlipidemic rats.

Groups	Treatment	Initial Body weights of rats	Final Body weights of rats	Initial-Final Body weights of rats
I	Normal control	182±1.06	183±1.108	1g
II	Hyperlipidemic control	228±0.872	254±1.33	26g
III	MECC (200 mg/kg)	222.5±1.762	196±1.25	26.5g
IV	MECC (400 mg/kg)	220±0.88	195±1.20	25g
V	Simvastatin (10 mg/kg)	218±1.72	188.02±1.72	30g

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control group (a = p < 0.01, b = p < 0.05), hyperlipidemic control (* = p<0.01, ** = p<0.05) and standard (A = p < 0.01) and ns- non significant.

Table 3: Anti-Hyperlipidemic activity for methanolic root extract of *Cajanus cajan* on fructose induced Hyperlipidemic rats.

Treatment	Lipid Profile (mg/dL)				
	Total Cholesterol	Triglyceride	HDL	LDL	VLDL
Normal control	102.3±0.091	70.81±0.09	45.80±0.041	41.15±0.190	14.49±0.025
Hyperlipidemic control	179.60 ±1.809^{b}	140.4±0.023^{b}	27.60±0.501^{a}	110.350.270^{b}	28.80±0.170^{b}
MECC (200 mg/kg)	125.75 ±0.591$^{a, *, B}$	86.05±0.059$^{a, **, B}$	34.80±0.219$^{b, *, A}$	67.41±0.170$^{a, **, A}$	18.60±0.653$^{a, *, B}$
MECC (400 mg/kg)	115.6±0.062$^{b, *, A}$	77.76±0.012$^{a, *, ns}$	38.34±1.053$^{b, *, A}$	56.80±0.290$^{b, *, B}$	16.40±0.510$^{a,**, B}$
Simvastatin (10 mg/kg)	106.3±0.501$^{a, **}$	75.02±0.172^{b,*}	42.33±0.699^{a,*}	44.75±0.660^{a,**}	15.55±0.122$^{a, *}$

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control group (a = p < 0.01, b = p < 0.05), hyperlipidemic control (* = p<0.01, ** = p<0.05) and standard (A = p < 0.01, B = p < 0.05) and ns- non significant.

Table 4: Effect for methanolic root extract of *Cajanus cajan* on body weight by fructose induced Hyperlipidemic rats.

Groups	Treatment	Initial Body weights of rats	Final Body weights of rats	Initial –Final Body weights of rats
I	Normal control	184±1.02	185±1.108	1g
II	Hyperlipidemic control	225±1.7	235±0.9	10g
III	MECC (200 mg/kg)	213.5±1.9	197±1.45	16g
IV	MECC (400 mg/kg)	215±1.88	195±1.20	20g
V	Simvastatin (10 mg/kg)	210±1.9	187.02±1.2	23g

Values are expressed as Mean ± SEM, (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's test by comparing with control group (a = p < 0.01, b = p < 0.05) and ns = non significant and hyperlipidemic control (* = p<0.01, ** = p<0.05) and standard (A=p<0.01,B=p<0.05).

***In Vitro* Antioxidant Assays**

The methanolic root extract of *Cajanus cajan* was subjected to *in vitro* antioxidant activity. *In vitro* anti-oxidant activity was performed using reducing power assay & hydrogen peroxide scavenging assay.

a) Reducing power assay

Table 5: Anti-oxidant activity for methanolic root extract of *Cajanus cajan* by reducing power assay method.

S. No	Compounds	Concentration (µg/ mL)	% inhibition	IC_{50} value (µg/ mL)
1	MECC	10	26.49±0.16	35
		20	40.83±0.50	
		30	46.66±0.33	
		40	54.32±0.66	
		50	64.49±0.83	
II	Ascorbic acid	10	29.49±0.83	31
		20	45.49±0.50	
		30	49.66±0.67g	
		40	62.32±0.33	
		50	67.16±0.17	

Values are expressed as mean ± SEM.

From the above results it is clear that the MECC showed antioxidant activity. The reducing ability of a compound generally depends on the electron donating capacity and the reducing agent transfers electrons to another substance and thus itself oxidized forming Fe^{3+}-Fe^{2+} complex. The electron donating capacity of the methanolic extract might be responsible for its antioxidant potential. The reducing power activity of MECC might be due to presence of phenolics, triterpenoids and flavonoids with adequate number of hydroxyl groups.

b) Hydrogen peroxide Scavenging assay

Table 6: Anti-oxidant activity of methanolic extract of *Cajanus cajan* by H_2O_2 radical scavenging activity.

S. No.	Compounds	Concentration (µg/ mL)	% inhibition	IC_{50} value (µg/ mL)
I	MECC	10	28.99±1.0	40
		20	34.24±0.25	
		30	41.54±0.55	
		40	50.24±0.74	
		50	64.04±0.45	
II	Ascorbic acid	10	31.74±0.75	35
		20	35.99±0.50	
		30	44.04±0.95	
		40	56.99±1.5	
		50	68.99±1.0	

Values are expressed as mean ± SEM.

DISCUSSION

Phytochemical screening of methanolic extract of *Cajanus cajan* showed presence of different phytoconstituents like triterpenoids, phenolic compounds flavonoids, tannins, steroids, and saponins. Earlier studies also reported that phytoconstituents like triterpenoids, saponins, phenolics and flavonoids are known to possess antihyperlipidemic properties.

In the present study methanolic root extract of *Cajanus cajan* was evaluated for antihyperlipidemic activity by using high fat diet and fructose induced hyperlipidemic animal models. The various lipid profile parameters like total cholesterol, triglycerides, HDL, LDL, VLDL were evaluated. High-fat diet (HFD) is a most important risk factor for a plethora of severe ailments, including obesity, dyslipidaemia, cardiovascular diseases. HFD increases the amount of chylomicrons in the intestine. The later upon entry into the circulation leads to generate free fatty acid (FFA) that is taken up by the liver. These hepatic FFAs either enter mitochondria for beta oxidation or esterified to form triglycerides (TG). These TGs are either accumulated in the hepatocytes as tiny droplets or produces very low-density lipoprotein (VLDL) which in turn converts into LDL.[10]

There is considerable evidence supporting the ability of high fructose diets to upregulate the lipogenesis pathway, leading to increased TG production. Fructose induces hypertriglyceridemia, hyperinsulinemia and hypertension. After absorption in GIT, fructose is transported *via* portal circulation to the liver, where it enters hepatocytes via the glucose transporter GLU-T5 independently of insulin and is rapidly metabolized. Fructose is metabolized into "glycerol-3-phosphate" and "acetyl CoA". These two intermediate metabolites are then used as substrates for glycerides synthesis, contributing to VLDL-TG production in liver.[11] The exposure of liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and TG accumulation which in turn contributes to reduced

insulin sensitivity and hepatic insulin resistance/ glucose intolerance.[12]

The various phytochemicals identified in the methanolic root extract of *Cajanus cajan* are phenolics, polyphenolics, triterpenoids, flavonoids, tannins and saponins. Several studies suggested that the terpenoids could inhibit intestinal acyl-coenzyme A cholesterol acyl transferase activity and contribute to hypolipidemic activity. Also the plant extract contains sterols like stigma sterol and sitosterol which might also be responsible for lowering the lipids by reducing the absorption of cholesterol and thus increasing the fecal excretion of steroids. It has been reported that saponins have cholesterol-lowering activity either by inhibiting the absorption of cholesterol from the small intestine or by the reabsorption of bile acids.

During the study, the body weight of all the animals were elevated from the beginning to the end of the experiment in groups treated with HFD and fructose. But at the end of the study the body weight of the animals treated with extract and the standard drug were found to be decreased. Adipocytes hypertrophy due to increased triglyceride synthesis is responsible for the weight gain in animals fed with HFD and fructose. Upon treatment with the MECC and the standard drug the excess weight gain was decreased in the experimental animals. This study demonstrates that administration of MECC at a dose of 200 mg/kg & 400 mg/kg bd. wt significantly lowered the total cholesterol, triglycerides, LDL and VLDL levels in both high fat diet and fructose induced hyperlipidaemia.

Methanolic root extract of *Cajanus cajan* was explored for its antioxidant activity against reducing power assay and hydrogen peroxide scavenging assay. In reducing power assay, the methanolic root extract was tested at different concentrations. The reducing ability of a compound generally depends on the electron donating capacity and the reducing agent transfers electrons to another substance and thus itself oxidized forming Fe^{3+}-Fe^{2+} complex. The electron donating capacity of the methanolic extract might be responsible for its antioxidant potential. From the above results it is clear that the MECC showed antioxidant activity. The reducing power activity of MECC might be due to presence of various active constituents like phenolics, triterpenoids and flavonoids with adequate number of hydroxyl groups. Several studies reported the anti-oxidant activity of flavonoids which act by blocking the generation of free radicals in chain reaction.[9]

The methanolic root extract was tested for hydrogen peroxide scavenging assay at different concentrations and the results have shown that IC_{50} value for the methanolic root extract of *Cajanus cajan* was comparable with standard ascorbic acid. The MECC showed scavenging activity against hydrogen peroxide radicals. The scavenging capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, antioxidant activity has been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging. The preliminary phytochemical studies of the methanolic root extract of the *Cajanus cajan* showed the presence of phenolic compounds like simple phenolic acids, and polyphenolic compounds like flavonoids especially flavonol, flavones, isoflavones flavonones and isoflavanone. Scavenging of hydrogen peroxide by the extract may be attributed to their phenolic nature, which can donate electrons to H_2O_2, thus neutralizing it to water.[10]

MECC have shown dose dependent reducing power ability and inhibition of hydrogen peroxide which was comparable to standard ascorbic acid. Therefore it is suggested that further work could be done on the isolation and identification of antioxidative components in methanolic extract of *Cajanus cajan*.

From the above it is clear that the MECC possess significant antihyperlipidemic and antioxidant activity.

ACKNOWLEDGEMENTS

The authors are grateful to the Principal and the Management of the Gokaraju Rangaraju College of Pharmacy, for the constant support and encouragement during the course of the work.

CONFLICT OF INTEREST

The authors have no conflict of interest.

REFERENCES

1. Ankur R, Nidhi D, Seema R, Amarjeet D, Ashok K. Hyperlipidemia-a deadly pathological condition. International Journal of Current Pharmaceutical Research, 2012; 4: 15-8.
2. Brahma RD, Saileela CH. Anti-hyperlipidemic activity of methanolic extract of *Rhinacanthus nasutus*. International Journal of Research in Pharmacy and Chemistry, 2013; 3: 708-11.
3. Kong Y, Fu YJ, Zu YG, Chang FR, Chen YH, Liu XL. Cajanuslactone a new coumarin with Antibacterial activity from pigeon pea leaves. Food Chemistry, 2010; 121: 1150–5.
4. Dhaliya SA, Surya AS, Dawn VT, Betty C, Arun K, Dr. Sunil C. A review of hyperlipidemia and medicinal plants. International Journal of Applied pharmaceutical sciences and Biological Sciences, 2013; 2: 219-37.
5. Mohana T, Navin AV, Jayachandaran KS, Sivasitambaram ND. Protective effect of grape seed proanthocyanidins against cholesterol cholic acid diet-induced hypercholesterolemia in rats. Cardiovascular Pathology. 2011; 20: 361–8.
6. Friedewald WT, Lewy RI and Fredrickson DS. Estimation of low-density lipoprotein cholesterol in plasma, without use of the preparative centrifuge. Clinical Chemistry. 1972; 18: 499-504.

7. Ashok Kumar Gupta, Mansi Verma, Gajraj Singh Lodhi. Protective effect of Ficus infectoria plant extract against fructose induced hyperlipidemia and hyperglycemia in rats. The Journal of Phytopharmacology, 2014; 3(6): 431-435.
8. Allouche Y, Beltrán G, Gaforio JJ, Uceda M, Mesa MD. Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids. Food Chemistry and Toxicology, 2010; 48: 2885–2890.
9. Duh PD, Tu YY, Yen GC. Antioxidant activity of aqueous extract of harn jyur (*Chyrsanthemum morifolium* Ramat). Lebensmittel-Wissenschaft und Technologie, 1999; 32: 269–277.
10. Noorani AA, Dwivedi G, Kale MK. Antihyperlipidemic activity of Rimonabant on high cholesterol diet induced hyperlipidemia in rats. Pharmacology Online, 2011; 1: 1212–1220.
11. Rajasekar P, Kaviarasan S, Anuradha CV. L-carnitine administration prevents oxidative stress in high fructose-fed insulin resistant rats. Diabetologia Croatica, 2005; 34: 21-28.
12. Park OJ, Cesar D, Faix D, Ken WU, Shackleton CHL, and Hellerstein MK, Mechanisms of fructose-induced hypertriglyceridaemia in the rat. Biochemistry Journal, 1992; 282: 753-757.