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Antihyperglycemic and hypolipidemic activities of aqueous extract of *Carica papaya* Linn. leaves in alloxan-induced diabetic rats

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Abstract

Background:

India is considered as the diabetic capital of the world. The study of plants having antihyperglycemic and hypolipidemic activities may give a new approach in the treatment of diabetes mellitus.

Objective:

The study was intended to evaluate the antihyperglycemic and hypolipidemic activity of aqueous extract of leaves of *Carica papaya* Linn. (AECPL) in alloxan-induced diabetic albino rats.

Materials and Methods:

Diabetes was induced in albino rats by administration of alloxan monohydrate (120 mg/kg, i.p.). Rats were divided into 6 groups of 6 animals each. First group served as non-diabetic control, second group as diabetic control, third group as standard and was treated with 0.1 mg/kg/day of glibenclamide. Group 4, 5, and 6 received 100, 200, and 400 mg/kg body weight of AECPL. Blood samples were analyzed for blood glucose on day 0, 1, 7, 14, 21 and lipid profile on day 21.

Results:

The AECPL showed significant reduction ($P<0.01$) in blood glucose level and serum lipid profile levels with 400 mg/kg body weight in alloxan-induced diabetic rats as compared with the control.

Conclusion:

It is concluded that AECPL is effective in controlling blood glucose levels and in improving lipid profile in diabetic rats.

Keywords: Aqueous leaf extract, hypoglycemia, hypolipidemia, *Carica papaya* Linn

INTRODUCTION

Diabetes is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbance of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action or both.[1] India leads the world with largest number of diabetic patients and is termed as the diabetic capital of the world. According to International Diabetes Federation the number of people with diabetes in India is currently around 40.9 millions and is expected to rise to 69.9 million by 2025 unless

urgent preventive steps are taken.[2] Diabetes mellitus has been identified by the Indian Council of Medical Research as one of the refractory diseases for which satisfactory treatment is not available and suitable herbal preparation has to be investigated. In India, there is documentation of about 150 plants in various families with antihyperglycemic activity.[3] More than 1200 plant species are used worldwide in diabetes phytotherapy, and experimental studies support the antihyperglycemic activity of large number of these plants.[4] In addition to correction of blood glucose levels, several plants have the potential to ameliorate lipid metabolism abnormalities of diabetes mellitus.[5] In order to reduce the number of diabetes complications and to postpone their development, Savickiene[6,7] recommended the use of biologically active components and plants. Thus the study of such plants having antihyperglycemic and hypolipidemic activities may give a new approach in the treatment of diabetes mellitus.

Carica papaya (CP) Linn. (family: Caricaceae) is a tropical tree, which is native to South America but now widely cultivated in other tropical regions of the world. It is a small unbranched tree, single stem growing up to 5–10 m tall. The leaves are large, 50–70 cm in diameter, deeply palmate lobed with 7 lobes. The fruits are rich in vitamin A and vitamin C. The fruit, leaf, latex of CP are used for the treatment of typhoid fever, wound infection, asthma, fever, diarrhea, boils, hypertension, and so on.[8,9] Recently, antifertility[10] anthelmintic,[11] and anti-inflammatory activity[12] have been reported. CP seeds possess moisture, proteins, fatty acids, and phospholipids, such as phosphatidylcholine and cardiolipin. Other compounds present in seeds are carpaine, benzyl isothiocyanate, benzyl glucosinolate, beta-sitosterol, caricin, enzyme myrosin. The most well-studied proteinases from papaya are papain, chymopapain, caricain, and glycyl endopeptidase. Papain occurs in all parts of the tree except the root.[13] Fruit and seed extracts have antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. [14,15] The juice is used for curing warts, cancer, and tumors. Leaves have been poulticed into nervous pains, elephantoid growths.[16] The antihyperglycemic effect of unripe mature fruits and seeds of CP have also been reported.[17,18] There is dearth of reports on the antihyperglycemic and hypolipidemic effects of the leaves of this plant.

In view of this, the current study was designed to evaluate the antihyperglycemic and hypolipidemic activity of the aqueous extract of leaves of *Carica papaya* Linn. (AECPL) in diabetic rats.

MATERIALS AND METHODS

Plant material

The leaves of CP were collected from the garden of S. Nijalingappa Medical College, Bagalkot, Karnataka, India, from the period May to June 2009. Identity of the plant was authenticated by botanist Prof. Jadimath and voucher specimen was deposited in the Herbarium of Department of Pharmacology of S. Nijalingappa Medical College, Bagalkot, Karnataka, India.

Preparation of the extract

The leaves of the plant were subjected to surface sterilization using 30% alcohol, and then dried in shade. The dried leaves were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. The powdered sample (50 g) was boiled in hot water for 30 min after which it was filtered using a piece of white cotton gauze. The filtrate was evaporated to dry at 40°C producing brown color solid residue (yield: 35% w/w). The residue was weighed and stored in air and water proof containers, kept in refrigerator at 4°C. From this stock, fresh preparation was made whenever required.

Animals

Healthy Wistar albino rats of either sex weighing about 150–200 g were used. The animals were housed in poly propylene cages, maintained under standard conditions (12:12 h light:dark cycle; 25 ± 2°C, 35%–60% humidity). They were fed with standard rat pellet diet (Hindustan Lever Ltd. Mumbai, India) and water ad libitum. The Institutional Animal Ethical Committee of S. Nijalingappa Medical College (IAEC-reg No: 627/02/a CPCSEA), Bagalkot, Karnataka, India, approved the study protocol.

Phytochemical screening

The extract obtained was subjected to various qualitative tests for identification of the constituents present, by using simple and standard qualitative methods described by Trease and Evans.[19]

Sample collection

Blood samples were collected by the retro orbital plexus puncture method from overnight fasted rats under light ether anesthesia and blood glucose levels were estimated using Accu-Check Active[®] glucose strips and test meter device (Accu-Chek Extra Care, Roche Diagnostics India Pvt. Ltd), which measures the blood glucose level by GOD–POD method (Glucose oxidase-peroxidase method).

Determination of LD₅₀ of the extract

For acute oral toxicity study and LD₅₀ determination, the Organization for Economic Co-operation and Development (OECD) guideline 425[20] was followed.

Induction of diabetes

A single dose (120 mg/kg, b.w., i.p.) of alloxan monohydrate (Sigma Ltd., USA) dissolved in normal saline was used for induction of type 2 diabetes in rats after overnight fasting. After 1 h of alloxan administration, the animals were fed standard pellets and water ad libitum. The animals were stabilized for a week and animals showing blood glucose level more than 250 mg/dL were selected for the study.

Experimental design

Rats fasted overnight for 12h were randomly (simple random sampling technique) divided into 6 groups of 6 rats per group. Group 1 served as normal control or nondiabetic group was treated with 10 mL/kg/day of distilled water orally. Group 2 served as untreated diabetic control received 0.5 mg/100 g of vehicle (2% gum acacia). Group 3 served as standard group and was treated with 0.1 mg/kg/day of glibenclamide. Groups 4, 5, and 6 were treated orally with 100, 200, and 400 mg/kg/day of AECPL, respectively, based on their acute oral toxicity study. Fasting blood glucose estimation was done at 0, 2, 4, and 6 h after treatment. Treatment was continued for 21 consecutive days. Fasting blood glucose levels were estimated at 0, 1, 7, 14, and 21 days.

Estimation of biochemical parameters

On day 21, blood was collected from overnight fasted rats under ether anesthesia by retro orbital plexus puncture method and was kept aside for 30 min for clotting. By centrifuging the same sample at 6000 rpm for 20 min, the serum was separated and was analyzed for total proteins (Biuret method),[21] cholesterol by CHOD–PAP method (Cholesteroloxidase-Phenol+amino phenazone),[22] and triglycerides by GPO method (Glycerol-3-phosphate oxidase).[23]

Statistical analysis

All the values were expressed as mean±SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. $P < 0.05$ was considered significant.

RESULTS

Phytochemical analysis

Phytochemical analysis of the extract showed the presence of tannins, alkaloids, flavonoids, saponins, anthraquinones, anthocyanosides, and reducing sugars.

Determination of LD₅₀ of AECPL

Administrations of single dose of extract (500 mg/kg, b.w., and p.o.) did not produce any mortality. All 5 animals were alive, healthy, and active during the observation period of 14 days. AOT 425 software was used to obtain higher doses for LD₅₀ determinations as per the OECD guidelines. In case of AECPL, the computer program suggested doses 550, 1750, and 2000 mg/kg. Results indicate that the dose up to 2000

mg/kg were nonlethal. All the animals were found to be alive, healthy, and active during the observation period of 14 day postadministration of highest dose. The computer program showed $LD_{50} > 2000$ mg/kg.

Effect of AECPL on diabetic rats

The antihyperglycemic activity of AECPL on the fasting blood sugar level of diabetic rats is shown in [Table 1](#). Acute and chronic treatment in the dose of 400 mg/kg b.w., in alloxan-induced diabetic rats showed a significant ($P < 0.01$) decrease in the elevated blood glucose level as compared with the control. The doses 100 and 200 mg/kg b.w. of the extract did not reduce the sugar levels to being statistically significant in acute treatment. But the extract in the dose of 100 mg/kg b.w. showed significant ($P < 0.01$) antihyperglycemic activity on day 14 and the dose 200 mg/kg b.w., showed significant ($P < 0.01$) result on day 7.

Other biochemical parameters

Since reduction in elevated blood glucose was seen more significantly with the extract dose 400 mg/kg b.w. and the same dose of AECPL when given in diabetic rats has shown a decrease in serum lipid profile and serum protein ($P < 0.01$) when compared with diabetic control and normal rats as shown in [Table 2](#).

DISCUSSION

The present study showed the antihyperglycemic and hypolipidemic effect of AECPL in alloxan-induced diabetic rats. The diabetic rats when treated with the CP extract in the dose 400 mg/kg b.w., showed 4.15%, 6.52%, and 8.56% decline in the blood glucose level in the initial 2, 4, and 6 h, respectively. Then they showed 12.63%, 22.63%, 30.14%, and 38.19% decline in the blood glucose level on day 1, 7, 14, and 21, respectively. There were statistically significant differences in the serum cholesterol, triglycerides, and total protein levels when diabetic rats received the CP extract at a dose of 400 mg/kg b.w. During this study because lower doses, such as 100 and 200 mg did not show significant effects over lipid profile and protein parameter, the results of those have not been tabulated.

Alloxan induces diabetes by destroying the insulin-producing beta cells of the pancreas.[24,25] *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to induction of cell necrosis.[26] This action is mediated by reactive oxygen species with a simultaneous massive increase in calcium concentration leading to a rapid destruction of beta cells.[27] The use of lower dose alloxan (120 mg/kg b.w.) produced partial destruction of pancreatic beta cells even though the animals became permanently diabetic.[28] Thus these animals have surviving beta cells and regeneration is possible.[29]

Glibenclamide, the second generation sulfonylurea is known to mediate the antihyperglycemic effect by stimulating insulin release from pancreatic beta cells, reducing the hepatic clearance and suppressing the secretion of glucagon.[30] Sulfonylurea have been shown to suppress gluconeogenesis.

The antihyperglycemic effect of the aqueous extract may be due to the enhanced secretion of insulin from the beta cells of pancreas or may be due to increased tissue uptake of glucose by enhancement of insulin sensitivity.

Elevated plasma cholesterol and triglyceride levels are major risk factors of cardiovascular disease. The existing antihyperglycemic agents allow a sharp control of blood glucose levels but insufficient correction of lipid abnormality, especially in hypertriglyceridemia.[31] Diabetic rats showed elevated plasma cholesterol and triglyceride levels due to hyperglycemia and insulin resistance.[32] Aqueous extract in the dose of 400 mg/kg b.w., reduced the triglyceride and cholesterol levels along with reduction in the blood glucose levels. Some studies have reported similar hypolipidemic activity in experimentally induced diabetic rats.[33,34]

The active constituents responsible for antihyperglycemic and hypolipidemic activities are not known. Phytochemical analysis showed the presence of alkaloids, tannins, saponins, flavonoids, anthraquinones, anthocyanosides, and reducing sugars. The presence of any of these phytochemicals might be responsible for the antihyperglycemic and hypolipidemic activity in diabetic rats. Some studies have also reported that these biological activities might be because of the presence of flavonoids, alkaloids, and tannins in CP

extract.[35] Again these studies would require experimental validation.

Since many antidiabetic drugs do not correct dyslipidemia, the observed hypolipidemic effects of this plant extract in diabetic rat makes CP quite important in the management of diabetes. Further investigations are needed to elucidate the mechanism of action, particularly the bioactivity-guided fractionation, isolation identification, and enzymatic study of constituents of the plant extract responsible for the observed pharmacologic activities. Since there is a strong, well-established link between diabetes mellitus, dyslipidemia, obesity, hypertension, and ischemic heart disease, effect of this plant extract on weight loss/gain and organ histopathologic studies need to be explored on scientific base.

In conclusion, this preliminary study has been able to demonstrate the antihyperglycemic, hypolipidemic potentials of AECPL in diabetic rats. And further scientific evaluation is needed to derive its molecular level mode of action.

Footnotes

Source of Support: Nil

Conflict of Interest: None declared.

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Figures and Tables

Table 1

Group	Treatment	Blood glucose conc. (mg/dL)							
		0 h	2 h	4 h	6 h	Day 1	Day 7	Day 14	Day 21
1	Normal control (Vehicle 2% gum acacia)	87.5 ± 1.6	87.6 ± 1.3	87.9 ± 1.9	88.2 ± 1.5	88.3 ± 1.5	88.8 ± 1.3	89.7 ± 1.9	90.4 ± 1.3
2	Diabetic control (vehicle 2% gum acacia)	262.1 ± 5.3	266 ± 5.1**	269.5 ± 3.7**	270.6 ± 3.1**	272.6 ± 2.7**	280 ± 1.2**	293 ± 2.2**	313.2 ± 3.6**
3	Alloxan (120 mg/kg i.p.) +0.1 mg/kg Glibenclamide	272.2 ± 6.4	239.6 ± 1.9*	228.4 ± 2.3**	221.3 ± 1.5**	208.1 ± 3.6**	165.3 ± 4.2**	107.7 ± 3.2**	95.5 ± 1.4**
4	Alloxan (120 mg/kg i.p.) +AECPL 100 mg/kg.	272.3 ± 5.8	271.7 ± 5.5	270.7 ± 5.4	270.3 ± 5.8	269.7 ± 5.5	270.1 ± 6.1	270 ± 5.2*	269.3 ± 4.9**
5	Alloxan (120 mg/kg i.p.) +AECPL 200 mg/kg	278.7 ± 7.9	273 ± 7.0	269.4 ± 7.3	266.2 ± 7.6	256.8 ± 6.4	242.4 ± 4.8**	230.7 ± 5.2**	221.4 ± 4.3**
6	Alloxan (120 mg/kg i.p.) +AECPL 400 mg/kg.	245.3 ± 11.8	235.1 ± 10.4**	229.3 ± 10.1**	224.3 ± 9.5**	214.3 ± 11.2**	190.4 ± 9.7**	170.7 ± 5.8**	151.6 ± 2.6**

All values are expressed as mean ± SEM (n=6), Group 2 was compared with group 1, Groups 3–6 were compared with group 2; *P<0.05, **P<0.01, AECPL: Aqueous extract of leaves of *Carica papaya* Linn.

Effect of AECPL on blood glucose level in alloxan (120 mg/kg. i.p.) induced diabetes rats

Table 2

Group	Treatment	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Total protein (g/dL)
1	Normal control (vehicle)	86.65 ± 1.83	85.62 ± 3.22	7.20 ± 0.23
2	Diabetic control (vehicle)	153.88 ± 4.19**	189.86 ± 1.74**	4.73 ± 0.53**
3	Alloxan (120 mg/kg i.p.) +Glibenclamide (0.1 mg/kg)	92.03 ± 1.09**	94.13 ± 1.20**	6.95 ± 0.07**
4	Alloxan (120 mg/kg i.p.) +AECPL (400 mg/kg)	135.32 ± 4.04**	145.18 ± 3.67**	6.05 ± 0.8**

All values are expressed as mean±SEM (n=6), Group 2 is compared with Group 1. Groups 3 and 4 are compared with Group 2. **P<0.01, AECPL: Aqueous extract of leaves of *Carica papaya* Linn.

Effect AECPL on biochemical parameters in alloxan-induced diabetic rats

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