



Original Research Article

Evaluation of Antihyperglycemic activity of aqueous extract of *Carica papaya* linn. leaves in Alloxan induced diabetic Albino rats

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Abstract

Background: *Diabetes is everywhere a most common growing health concern in the world and is now emerging as an epidemic world over. Currently, more attention is being paid to the natural products study as potential antidiabetics. The study of plants that possess Antidiabetic activity may give a new idea in the treatment approaches of diabetes mellitus.*

Objective: *The present study was aimed to evaluate the antihyperglycemic effect of Aqueous extract of *Carica papaya* linn. Leaves (AECPL) in alloxan - induced diabetic albino rats.*

Methods: *Albino rats weighing 150- 250 grams were grouped into 6 equal groups taking 6 rats in each. Group A served as control(normal), Group B as diabetic control, Group C, D & E was received alloxan+ AECPL suspension at a doses of 100, 200 & 400 mg/kg orally respectively, Group F was given alloxan + standard drug Glibenclamide (0.1mg/kg b.w.) suspension for 21 days & the AECPL effect on blood sugar was measured at regular intervals. At the end of this research study blood samples were collected from all rats for biochemical estimation.*

Results: *The present study revealed that the AECPL has statistically significant ($P < 0.05$) and sustained Antihyperglycemic effect which is comparable with the hypoglycemic effect of standard drug Glibenclamide.*

Conclusion: *It is concluded that the AECPL has shown significant antihyperglycemic effect at a doses of 100, 200 & 400 mg/kg. b.w. in alloxan induced diabetic albino rats.*

Keywords: *AECPL, Glibenclamide, Antidiabetic, Aqueous extract, albino rats, blood sugar, glucometer, herbal remedy.*

Introduction

In many countries Medicinal plants are used to treat and control diabetes mellitus. The hypoglycemic action of these medicinal plants is still being studied.^[1] Diabetes is a group of metabolic diseases which is characterized by hyperglycemia resulting from defective insulin action or insulin secretion or both. Broad research work on diabetes resulted to synthesis of a number of synthetic oral hypoglycemic agents like thiazolidinedione's, biguanides, & sulphonylureas being used to treat diabetes. But all of these synthetic oral hypoglycemic drugs have their side effects which are associated with their uses. On the other hand, traditional medicinal plants along with their various biological constituents have been used effectively by the different communities since long time to treat diabetes. Several natural products such as polysaccharides, glycosides, alkaloids, saponins, terpenoids, flavonoids are isolated from these medicinal plants & are being reported to have or possess anti-diabetic active-ties. In addition, herbal drugs are extensively used for the treatment of various diseases due to their effectiveness, minimal side effects and their relative low cost. Therefore, it is very much important to isolate the bioactive molecules from traditional anti-diabetic plants.

Regarding the management of this disease concerned which may include lifestyle modifications, exercise, diet, long – term use of insulin therapy or oral hypoglycaemic agents. The search for medicinal plants with the hypoglycaemic property is an area that draws attention of research workers globally reviewed 45 medicinal plants and their products that have been used in the Indian traditional system of medicine. Diabetes management without any one of side effect is yet a big challenge to the medical community whoever on oral hypoglycemic agents like thiazolidinedione's, biguanides, & sulphonylureas. There is continuous search for alternative drugs for the treatment of diabetes mellitus. Even though

herbal medicines have been effectively used from a long time in treatment of diseases in Asian communities and throughout the world, it is prudent to look for more herbal medicines for diabetes. From ancient times, some of these herbal medicinal plant preparations have been used in the diabetes treatment. So, for the diabetes treatment so many traditional plants were used. The active compounds of these medicinal plants play an important role in the diabetes mellitus management especially in developing countries. Even though, during the past few years some of the new bioactive drugs & bioactive molecules as biological active medicines isolated from the medicinal plants showed antidiabetic activity with more efficacy than the oral hypoglycemic agents used in clinical therapy.

Carica papaya (CP) Linn. (family: Caricaceae) is a tropical tree, which is native plant of South America but now widely cultivated in other tropical regions of the world. It is a small unbranched tree, single stem which is growing almost up to 5–10 m tall. The leaves of *Carica papaya* are large, 50–70 cm in diameter, deeply palmate lobed with 7 lobes. The fruits are rich in vitamin A & C. The fruit, leaf, latex of CP are used for typhoid fever treatment, wound infection, asthma, fever, boils, diarrhea, hypertension, and so on.^[2,3] Recently, antifertility^[4] anthelmintic,^[5] & anti-inflammatory activity^[6] have been reported. CP seeds possess moisture, proteins, fatty acids, & phospholipids, such as phosphatidylcholine and cardiolipin. Other compounds which are present in seeds are carpain, benzyl isothiocyanate, benzyl glucosinolate, beta-sitosterol, caricin, enzyme myrosin. The most well-studied proteinases from papaya are papain, chymopapain, caricain & glycyldipeptidase. Papain occurs in all parts of the tree except the root.^[7] Fruit and seed extracts have antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* (E.coli) & *Pseudomonas aeruginosa*.^[8,9] The juice is used for curing warts, cancer & tumors. Leaves have been poulticed into nervous pains, elephantoid

growths.^[10] Dried papaya seeds actually look quite similar to peppercorns and can be used in just the same way. Grinding a couple over a meal, especially protein rich meals is a simple way to add extra enzymes to your diet & improve your digestive health^[11]. Papaya markedly increases iron (Fe) absorption from rice meal, which was measured in parous Indian women, using the erythrocyte utilization of radioactive Fe method. The black seeds edible and have a sharp, spicy taste. They are sometimes ground up and used as a substitute for black pepper. In some parts of Asia the young leaves of papaya are steamed and eaten like spinach^[12] The fermented papaya fruit is a promising nutraceutical as an antioxidant. It improves the antioxidant defence in elderly patients even without any overt antioxidant deficiency state at the dose of 9 g/day orally. The papaya lipase, a hydrolase enzyme tightly bonded to the water insoluble fraction of crude papain, is considered as a “naturally immobilized” biocatalyst^[13].

Materials and Methods

Chemicals

Alloxan monohydrate (sigma Aldrich., Mumbai), Tab Glibenclamide 5mg, glucometer.

Animals

Both sexes of the animals (Albino rats) with a weight between 150gm – 250 gm were used in this experiment. Albino rats were housed in a group of 6 in polypropylene cages at a controlled room temperature of $25 \pm 2^\circ\text{C}$, and a relative humidity of 55% & 12 hrs. light: dark cycle. Albino rats were fed with the supplied standard food pellet diet & water ad libitum during this experiment. Prior to this experiment the wistar rats were fasted for a exact time period of 12 hrs with water ad libitum given & weighed. All the study protocols of this research study were cleared and approved by CPCSEA (Committee for the Purpose of Control & Supervision of Experiments on Animals) & were cleared by Institutional Animal Ethics

Committee (IAEC) clearance at Mamata Medical College, Khammam, Telangana state.

Plant Material and Preparation of Test Extract

The *Carica papaya* (CP) leaves was collected from local regions near Khammam city at rotary nagar in the month of October – November and authenticated by Assistant Professor and Head, Department of Botany, Govt. SRBJNR PG College, Khammam. The collected plant leaves were thoroughly washed with distilled water & shade dried, powdered and stored in air tight containers. 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.

Experimental Induction of Diabetes

Rats were fasted overnight and blood is withdrawn from tail vein of the rats of each group before the treatment i.e (initial) day (0 day) and on 7th, 14th, 21st day of the given test drug AECPL from rats of each group using glucometer and BS levels were analyzed and were observed. Serum of collected blood was used for estimation of biochemical parameter like blood sugar. Animals were fasted upto 18 hrs. before Diabetes induction. A single dose (120 mg/kg, b.w., i.p.) of alloxan monohydrate was dissolved in normal saline was used for induction of type -2 diabetes in rats after overnight fasting After 72 hrs of alloxan administration, the animals were fed standard pellets and water ad libitum. The animals were stabilized for a week and animals showing blood sugar level >250 mg/dL were selected for the study. Fasting blood sugar [FBS] level was monitored in blood samples with a glucometer before administration of the drugs.

Experimental study Design

36 albino rats were divided into 6 groups (A, B, C, D, E) of 6 animals (n=6) each. Group A served as normal control or nondiabetic received only 10 mL/kg/day of distilled water orally for 21 days on routine diet. Group B was served as diabetic control received single dose of alloxan monohydrate (120 mg/kg, b.w., i.p.) dissolved in 0.5 mg/100 g of vehicle (2% gum acacia) was used for type 2 diabetes induction in rats after overnight fasting. Whereas the Group C, D & E (Experimental Groups) were treated as Diabetic and received alloxan (120 mg/kg, b.w., i.p.) With AECPL suspension at a doses of (100, 200 & 400 mg/kg b.w. /day respectively) for 21 consecutive days. Group F was received alloxan (120 mg/kg, b.w., i.p.) plus suspension of Glibenclamide (0.1mg/kg b.w./day orally for 21 consecutive days. After 30 minutes of treatment, rats of each group were given glucose (5gm/kg) in distilled water orally. Blood samples were collected on day before the experiment i.e. (0 day (initial day) of the experiment, and experimental days like on day 0 (initial day), 7th, 14th, 21st days of the given test drug AECPL from rats of each group with a glucometer and blood sugar levels were analyzed and blood sugar (FBS) values were observed.

Statistical Analysis

Results of biochemical estimation like blood sugar are reported as mean \pm SD of six animals in each group (n=6). The data were subjected to one-way analysis of variance (ANOVA) for multiple comparisons followed by Dunnett's test was applied for determining statistical significance of difference in blood serum glucose. P value of less than 0.05 (p<0.05) were considered statistical significant.

Results

The present investigation revealed that the AECPL has antidiabetic activity against alloxan induced diabetic albino rats on intra peritoneal injection of alloxan at a dose of 120mg/kg body weight. This study also confirms that on intra peritoneal injection of alloxan at a 120mg/kg b.w. dose causes significant raise in blood sugar level in untreated albino rats (diabetic control) groups when compared to control group was shown in (Table 2). Treatment of diabetic rats with AECPL for 21 days caused dose dependent fall in blood sugar levels (FBS) in diabetic albino rats. Glibenclamide treated diabetic rats also showed significant (P < 0.00) fall in blood sugar levels (FBS) after 21 days of treatment as shown in (Table 2).

Table 1: Blood glucose levels of albino rats of normal control group

S.NO.	Animal (albino rats) weight (in grams)	Normal control group albino rats blood glucose levels (mg/dl)			
		0 th day	7 th day	14 th day (final)	28 th day (final)
1.	172	82	85	81	85
2.	160	83	87	82	80
3.	168	80	82	86	84
4.	180	80	85	86	82
5.	170	80	83	82	85
6.	165	80	86	86	81

Figure 1 Carica papayalinn. leaves



Figure 2: Effect of Carica papaya linn. leaves Aqueous extract (AECPL) on fasting blood sugar levels in alloxan induced diabetic albino rats.

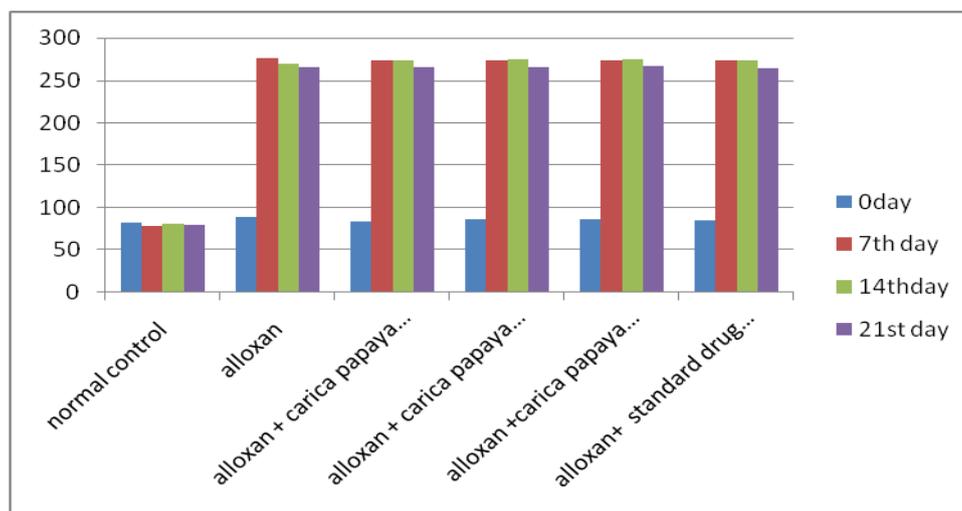


Table1: Effect of Carica papaya linn. leaves Aqueous extract (AECPL)on fasting blood sugar levels in alloxan induced diabetic albino rats.

Treated groups Fasting blood serum glucose (mg/dl)

	0 th day	7 st day	14 th day	21 st day
Normal control group given only 10 mL/kg/day of distilled water orally	82.6±1.8	78.8±1.3	81.8±2.4	80.8±2.1
Diabetic control (given 0.5 mg/100 g of vehicle (2% gum acacia). + Alloxan (120 mg/kg) in a single i.p. dose	89.3±7.4	276.8±5.1	269.3±4.3	265±3.9
Diabetic+Carica papaya linn. leaves Aqueous extract (AECPL) (100mg/kg/ b.w. /day) orally	83..5±5.6	273±6.5*	271±6.4**	263±2.6**
Diabetic+Carica papaya linn.leaves Aqueous extract (AECPL) (200mg/kg/ b.w. /day) orally	83.5±5.6	273±5.3*	274±5.6**	265±3.2**
Diabetic+Carica papaya linn. leaves Aqueous extract (AECPL) (400mg/kg/ b.w. /day) orally	86.5±5.4	274±5.3*	275±5.6**	267±3.2**
Diabetic+standard drug glimiperide(0.1mg/kg b.w.day)orally	85±6.1	273±6.3*	273±7.0**	264.5±2.6**

All values are expressed in Mean±SD. Analyzed by one way ANOVA followed by Dunnet’s test for multiple comparison tests. * = p<0.05 when compared to normal control group (significant p value). ** = p<0.00 when compared to diabetic control group (Highly significant p value).

Discussion

Alloxan-induced diabetes is a most commonly employed experimental model to produce permanent diabetes in experimental animals such as rabbits, rats. Alloxan (2,4,5,6 tetraoxypyrimidine) is actually an oxygenated pyrimidine derivative which is usually present as alloxan hydrate in aqueous solution. Alloxan is the diabetogenic agent which is very well known diabetic inducer all over the world and is used as a diabetogenic agent to induce type-2 diabetes in experimental animals. Alloxan-induced diabetes is the one of the most popular and most potent method of experimental diabetes (chemically induced experimental diabetes).^{[14],[15],[16]} In our present research study, alloxan caused a marked increase in fasting serum glucose levels in diabetic rats. As it is a highly reactive molecule which is readily reduced to diuleric acid, rapidly which is then gets auto-oxidized back to alloxan which ultimately results in the free radicals production which actually damaged the DNA of β – cells of the pancreatic islets & cause cell death.^[17]

In addition, alloxan has also been most widely used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used for experimental diabetes induction.^{[18],[19]}

As the alloxan has been widely accepted diabetic inducer & a diabetogenic agent, it actually destroys the insulin-producing β – cells selectively which are usually found in the pancreas, hence it is used to induce diabetes in the laboratory animals. The toxic action of this alloxan on pancreatic β – cells which usually involve the oxidation of essential sulphhydryl (-SH groups), inhibition of glucokinase enzyme and generation of free radicals & disturbances in intracellular calcium homeostasis.^{[20],[21],[22]} This plant leaves are used in the present research study to check the effectiveness of the drug in the treatment of Alloxan induced diabetes, on blood sugar levels in experimental albino rat model.

Treatment of diabetic rats with Carica papaya leaves Aqueous extract (AECPL) at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg for 21 days caused a significant reduction in fasting blood sugar level in a dose dependent manner when compared to diabetic control group. Most of the studies of Carica papaya on diabetes revealed that there is remarkable improvement in the condition of damaged β -cells in histological study of the pancreas in Carica papaya linn. leaves Aqueous extract (AECPL) treated diabetic albino rats. However, the Carica papaya linn. leaves Aqueous extract (AECPL) did not restore the disturbed biochemical parameter like blood glucose levels to normal value in diabetic rats. Hence, the extract can be used in combination with other established anti-diabetic drugs or herbal formulations for more effective outcomes.

Conclusion

In the present research study, our experimental data finding clearly confirmed and also revealed that the test extract of our study, i.e. Carica papaya linn. leaves Aqueous extract (AECPL) at a dose of 100, 200 and 400 mg/kg b.w. possess potent antihyperglycemic effect. Thus, Carica papaya leaves can be used as potential antidiabetic drug. It could be a novel antidiabetic agent and also a dietary adjunct for the management of type 2 diabetes and its complications. However, the exact mechanism of the antidiabetic property of Carica papaya linn. leaves Aqueous extract is unknown. However, further studies are required to identify the probable mechanism of action to establish its antidiabetic activity. Further human studies are necessary and found the active component of Carica papaya and role of these herbal drugs in controlling diabetes and its complications.

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Declarations

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee.

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