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Anti-diabetic activity of Carica Papaya Linn in Alloxan-Induced diabetic rats.

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Abstract

Background: Type I Diabetes Mellitus (TIDM) can strike anyone at any age, however the majority of those affected are diagnosed in their mid-teenage years. Hyperglycemia is a symptom caused by the autoimmune death of insulin-producing pancreatic beta cells. Herbal diabetes treatment has become popular in recent decades because of its potential to improve diabetic complications such as nephrotic deterioration and oxidative stress. The goal of this study is to see if Carica Papaya leaf extract has anti-diabetic properties in Alloxan-induced diabetic rats.

Methodology: For this study, eighteen male Wistar rats between 150-200 g b.w were made into three groups (n=6), two of which (Group II and Group III) were induced DM through Alloxan, whereas control (Group I) remained untreated. Group III (diabetic) rats were given 250 mg/kg body weight of Carica papaya leaf extract (CPLE) after confirming hyperglycemia. On the 22ndday rats were sacrificed for biochemical analysis.

Results: The administration of papaya leaf extract resulted in a considerable drop in plasma glucose, AST, ALP, ALT, serum creatinine, blood urea nitrogen (BUN), and urea in the CPLE treated group as compared to the Alloxan-treated group. CPLE also increased plasma insulin levels and activity and concentration of important antioxidant enzymes such as CAT, SOD, and GSH significantly as compared with diabetic group.

Conclusion: According to the findings, the C. Papaya leaf extract has antidiabetic and antioxidative properties against alloxan-induced TIDM.

Keywords

Diabetes Mellitus, Alloxan, Carica Papaya Linn, Antioxidant.

Introduction

Diabetes Mellitus is a disorder that occurs when insulin's balance of glucose and lipid metabolism is disrupted. If the imbalanced homeostasis does not return to normal and persists for a longer time, it results in rise in blood glucose level, which in due path becomes a condition called diabetes mellitus[1]. The incidence of diabetes is increasing with time so much that it has emerged as a major socio-economic burden for developing countries[2]. Hyperglycemia caused by insufficient insulin production and/or receptor resistance to insulin is the major reason underlying diabetes[3]. The common forms of DM are Type I and Type II DM, the former is an autoimmune disorder, and the latter is linked with abnormal fat metabolism (obesity)[4]. Normally, blood glucose is lowered through glucose utilization by body cells, a function attributed to the hormone insulin[5]. Therefore, the insulin hormone can be considered a communicating bridge between the plasma and cellular glucose levels. Insufficient insulin production or improper response of cells to insulin can easily cause disturbance of glucose metabolism[6] which eventually leads to polyurea, polydipsia, polyphagia, and blurred vision[7]. Natural plant extracts have been shown to possess therapeutic benefits to the world of modern medicine.

Carica papaya (L), commonly referred to as Papaya, is an herbaceous plant belonging to a small family Caricaceae, typically grown in tropical or sub-tropical areas worldwide. Infusions usually are made from different parts of papaya, especially from leaves, for therapeutic uses due to its medicinal properties. Papaya leaf has many bioactive metabolites such as glutaminyl-cyclase, chitinase, and cysteine[8,9] and alkaloid compounds including Pseudo-carpaine, dehydro-carpaine-I and dehydro-carpaine-II and other important active compounds like flavonoids, papain, ascorbic-acid, chymo-papain, tocopherol, cystatin, cyanogenicglucoside or glucosinolate, Quercetin, and Kaempferol[10]. Papaya contains the enzyme Papain, a proteolytic enzyme that aids in protein digestion[11]. It also has been used to improve several symptoms of irritable bowel syndrome (e.g., constipation and bloating)[12]. Studies have shown several therapeutic uses of papaya leaf in treating conditions like Malaria and Dengue fever[13]. The anti-tumor, anti-microbial, and anti-inflammatory activities of papaya[14] are also reported in some studies. Some of the essential and useful compounds present in papaya help the body against oxidative stress as they can play an antioxidant role by scavenging the free radicals[15]. These antioxidants in papaya can prevent various degenerating conditions like leukemia, heart attacks, and premature aging.

The present study aimed to determine the anti-diabetic, hepatoprotective, and nephroprotective effects of Carica papaya leaf in Alloxan-induced diabetes.

Methodology

Animal-Selection and Acclimatization

Healthy male Albino Wistar rats weighing 150-200 g were acclimatised for two weeks. They were kept under the standard conditions (12 hrs light/dark and 22-24°C) and fed with the normal rat diet and water. The Institutional-Animal-Ethics-Committee has accepted the lab methodology used in this study.

Study Design

Animals were assigned into three groups (n=6). Group 1 served as untreated Control, Group 2 served as Diabetic (Alloxan-treated), and Group 3 served as CPLE treated group (Alloxan +CPLE), in which the rats, after being confirmed hyperglycemic, were treated with 250 mg/kg of CPLE for 21 days.

Extract-Preparation

The papaya leaves were cleaned, dried, and pounded into powder before being sieved then 10 g of it was cooked in 100 ml of water for 30 minutes before being filtered through cotton-gauze[16]. After that, the Carica papaya leaf extract (CPLE) was stored in airtight containers and kept at 4°C for five days. The extract was remade every other 5th day using the same method till the 21st day of the experiment.

Diabetes Induction

DM has been induced in Group II and III (after overnight fasting) by using a single injection of Alloxan (Intraperitoneally) (~120 mg/kg) dissolved freshly in the saline solution (0.9% NaCl). Hyperglycemia was then confirmed after three days of giving injection via estimation of fasting blood concentration. The rats with FBG levels above 250 mg/dL were chosen for the required objective. This process caused the condition of hyperglycemia in almost 75-80 percent of the animals after three days of giving injection[17].

Sample Collection

On the 22nd day, rats were decapitated, blood was drawn via heart puncture and collected in heparinized tubes. Plasma was procured after centrifugation, and the resultant specimen was stored at -70ºC. The organs (Pancreas, Liver, and Kidney) were collected in the saline-containing Petri-dishes and then were fixed in Formalin and placed in plastic bags to be stored at the same temperature.

Homogenate Preparation - Biochemical Assessment

The estimated parameters indicating the pancreatic functions are Glucose[18], Insulin[19]; liver functions: AST[20], ALT[21], ALP[22] and Kidney functions: Urea[23], Creatinine[24], BUN (Blood urea Nitrogen), and total protein[25] were analyzed. The Antioxidant status was also analyzed by estimating CAT[26], SOD[27], GSH[28] in tissues. Lipid peroxidation was assessed by estimating MDA[29] from the liver homogenate.

Statistical Analysis

Numerical values of results were presented as mean S.E.M., and the data were analyzed using SPSS version 16.0 and an independent T-test. The data was declared to be significant at p-value less than 0.05.

Results

The increase in bodyweight of the C. Papaya group were found as significant ($p<0.05$) in comparison to the Alloxan-treated group, as illustrated in (Table 1).

Table 1: Effect of Carica papaya leaf extract on body weights.

Groups	Day 0	Day 7	Day 14	Day 21
Group I	180.2±3.9	182.9±2.9	181.3±3.8	183.9±3.7
Group II	190.4±6.8	170.7±4.10	165.3±2.9*	151.6±1.8*
Group III	184.3±5.3	174±6.07	183.3±4.6**	188.3±4.4**

Data represented as Mean ± S.E.M.*: $p<0.05$

Group I (Control); Group II (Alloxan-Treated); Group III (Alloxan + C. Papaya- Treated)

*Group I compared with Group II; **Group II compared with Group III

A significant reduction of blood glucose levels in the C.papaya group has been observed ($p<0.05$) compared to the diabetes group. There is also a marked increase in the levels of plasma insulin ($p<0.05$) in the C. Papaya group when compared to the Alloxan (diabetic) group.

Table 2: Effects of Carica papaya leaf extract on pancreatic functions.

Groups	Glucose (mg/dl)	Insulin (IU/ml)
Group I	107.7±3.2	2.349±0.187
Group II	201.74±8.2*	0.23±0.0014*
Group III	116.93±3.79**	0.306±0.012**

Data is computed as Mean ± S.E.M. *: $p< 0.05$.

*Group I compared with Group II; **Group II compared with Group III

Administration of C. papaya leaf significantly reduced the activity of these serum biomarkers ($p<0.05$) with respect to the diabetic control (Alloxan-treated).

Table 3: Effects of Carica papaya leaf extract on liver functions.

Groups	AST(U/L)	ALT (U/L)	ALP (U/L)
Group I	36.4±0.64	20.77±2.5	243.6±9.13
Group II	79.7±4.2	35.17±1.59*	491.28±46.9*
Group III	67±7.3	21.99±3.6**	286.3±33.4**

Data is commuted as Mean ± S.E.M. *: p< 0.05

*Group I compared with Group II; **Group II compared with Group III

Papaya exhibited a nephroprotective effect that can cure renal dysfunction, significantly lower the serum Urea and BUN levels, and increase total protein in the C.papaya-treated group.

Table 4: Effects of Carica papaya leaf extract on kidney functions.

Groups	Urea (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)	Total Protein (g/dl)
Group I	4.85±1.62	0.169±0.025	2.26±0.75	7.20±0.23
Group II	23.95±3.69*	0.345±0.186	11.19 ±1.72*	2.032±0.915*
Group III	9.88±2.25**	0.118±0.023	4.61±1.05**	4.9±0.78**

Data is commuted as Mean ± S.E.M. *: p< 0.05

*Group I compared with Group II; **Group II compared with Group III

Administration of papaya leaf (p<0.05) significantly enhance the action of Catalase, GSH and SOD in the C. Papaya group. There is also a significant reduction in the levels of hepatic-MDA when compared to ALLOXAN (diabetic) group.

Table 5: Effects of Carica papaya leaf extract on antioxidant status.

Groups	Catalase (µmol/ gram tissue)	SOD (Unit/ gram tissue)	GSH (Unit/ gram tissue)	MDA(µmol/ gm tissue)
Group I	16.73±0.87	1.318±0.075	12.17±0.37	0.927 ± 0.022
Group II	6.08±2.45*	0.32 ±0.16*	5.72±5.41*	3.44 ± 0.41*
Group III	15.31±3.0**	0.919±0.081**	11.12±0.898**	1.1025 ± 0.23**

Data is commuted as Mean ± S.E.M. *: p< 0.05

*Group I compared with Group II; **Group II compared with Group III

Discussion

In the present study, Alloxan was used to induce DM in the experimental rats. Alloxan is a derivative of pyrimidine and a highly unstable compound with a structure like glucose. Alloxan and glucose are both hydrophilic, so they cannot penetrate the plasma membrane's lipid bilayer. Because of their structural similarity, Alloxan enters the cytosol by a Glucose-Transporter (GLUT2) in the plasma membrane of β-cells. It causes destruction via two mechanisms, accumulation in the beta cells by crossing through GLUT2 and production of ROS. The consequence of both mechanisms is β-cell necrosis and inhibition of the membrane protein, glucokinase (the glucose sensor of β-cells), which decreases glucose oxidation and ATP generation, further reducing insulin secretion[30].

When compared to the Alloxan-treated group, the C.Papaya-treated group had significantly lower blood glucose levels (p< 0.05). The level of plasma insulin in the C.Papaya group was similarly significantly higher (p< 0.05) than in the Alloxan-treated group. This action may be attributed to the extract's ability to decrease the rate of glucose absorption in the intestine or through increasing the consumption of glucose from the peripheral circulation. It may also activate remaining beta cells to release more insulin and regenerate them. The bioactive components such as alkaloids, saponin,

flavonoids, and tannins in the extract contribute to all the described mechanisms for the action of papaya-leaf extract[31-33].

Administration of Alloxan drug and the secondary effects of DM have produced significant adverse effects on liver functions, evidenced by increased plasma levels of liver enzymes ALT, AST, and ALP in the Alloxan-treated group compared to the control group and the Alloxan + C. Papaya-treated group. However, administration of C. papaya leaves extract significantly reduced the activity of these serum enzymes ($P<0.05$) compared to the Alloxan-treated group. These findings suggest that the extract contained some important bioactive compounds which curate hepatic parenchyma and regenerate liver cells (hepatocytes) via a hepatoprotective mechanism mediated by antioxidants[34].

Nephrotoxicity caused by Alloxan and secondary damage to the kidney due to DM lead to a marked increase in serum creatinine, urea, and BUN levels. Papaya leaves induced renoprotective effects by significantly lowering serum urea and BUN levels ($P<0.05$). Total Protein levels were also significantly enhanced in Alloxan+C. Papaya ($P<0.05$) group compared to the Alloxan-treated group.

The very first lines of defense toward oxidative injuries are Catalase, SOD, and GSH (Free-radical scavenging enzyme). Higher alloxan doses (120 mg/kg) cause liver toxicity and reduce antioxidant enzymes activity. Administration of papaya leaf ($p<0.05$) significantly enhances the action of Catalase, GSH, and SOD in the Alloxan+C. Papaya group compared to the Alloxan-treated group due to the bioactive components present in the leaves extract. A significant reduction was also seen in the levels of hepatic-MDA in the Alloxan+C Papaya-treated group compared to the Alloxan-treated group. Previous studies also found that papaya leaf compounds slow down the oxidation rate by direct splitting the peroxides into stable compounds which don't cause further oxidation or scavenge ROS. These activities are attributes of anti-oxidants present in extract or the ability to boost liver antioxidant-enzymes[34-36].

Conclusion

Findings from the present study indicate that Carica papaya leaf extract has significant anti-diabetic activity in the rat model. Therefore, C.papaya leaves could be a useful resource of pharmaceutical agents. However, further studies are required to better understand its possible therapeutic activities, the phytochemical components involved, and the exact mode of action.

Conflicts of Interest

The authors have declared that no competing interests exist.

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