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Comparative evaluation of aqueous leaf extract and dried latex powder of Carica papaya Linn. for their antidiabetic and antihyperlipidemic activities in Wistar rats

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Abstract

The present study was undertaken to evaluate and compare the effect of dried latex powder (DLPCP) and aqueous leaf extract (ALECP) of Carica papaya Linn. in streptozotocin induced diabetic rat model for a period of 21 days for their antidiabetic and antihyperlipidemic properties. A total of 32 male Wistar rats, divided into four groups with eight animals in each group. The groups include normal control (Group-I), diabetic control (Group-II), diabetic rats treated with DLPCP @ 400mg/Kg b.wt (Group-III) and diabetic rats treated with ALECP @ 400 mg/Kg b.wt (Group-IV). Serum was collected on 0th , 4th, 14th and 21st days from all the experimental rats for serum biochemical analysis. On 21st day, the animals were sacrificed for histopathological examination of pancreas and liver. ALECP treatment group has shown antihyperglycemic activity as early as the 4th day (327.7 + 18.65) and hepatoprotective activity on the 4th day (252.9 + 4.6, 155.3 + 4.8), whereas, DLPCP treatment group has shown the similar affect on 21st day (311.7 + 28.83) and on 14th day (228.8 + 10.6, 179.8 + 0.9) of experiment respectively. Antihyperlipidemic affect was exhibited by both the treatment groups with reduced triglycerides, total cholesterol, LDL, VLDL and increased HDL levels, when compared to diabetic control groups. Histopathological examination has endorsed the biochemical findings. ALECP treatment when compared to DLPCP treatment has shown a better antidiabetic activity on the early days of experiment by ameliorating its associated affects. The present study indicate that ALECP acts as an alternative remedy for diabetes milletus.

Keywords: Carica papaya- Antdiabetic property- Antihyperlipidemic effect- Aqueous leaf extract- Dried latex powder- Histopathlogy.

Introduction

Diabetes mellitus is a multifaceted metabolic disorder involving chronic hyperglycemia with associated disturbances in carbohydrate, protein and lipid metabolism due to inadequate insulin secretion or poor sensitivity of target tissues to the metabolic effect of insulin (Guyton and Hall 2006). The incidence of diabetes mellitus (DM) in animals particularly in non-ruminants is on par with humans. In particular, female dogs and male cats are among the most affected with less observed in bovines, equines, pigs, sheep and birds species (Kaoud 2017). Genetics, age, obesity and environmental factors influence the incidence of DM in feline and canine species.

DM is broadly categorised as two types, type I diabetes is also called as insulin dependent diabetes mellitus (IDDM) is caused by insufficiency of insulin secretion. Dogs, commonly get affected with type I diabetes, breeds like Samoyed, Australian terrier, Keeshond, and Cairn terriers are genetically predisposed (Nelson and Reusch 2014). In developing countries like India, rearing dogs as companion animals is rising gradually, accompanying rapid increase in dogs succumbing to type I diabetes, which makes type I DM a major concern to animal health practice. Type II diabetes is also called as non-insulin dependent diabetes mellitus (NIDDM), which is characterized by the presence of insulin resistance with an inadequate compensatory increase in insulin secretion. High carbohydrate diets in cats will increase the blood glucose and insulin levels, which may predispose them to obesity associated type II diabetes, the commonly observed type in cats (Rand et al. 2004). The risk for development of diabetes increases about 2-fold in overweight cats and about

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4-fold in obese cats (Laflamme 2012). The therapeutic management of DM with minimal side effects either in humans or in animals remains a clinical challenge.. There is an obligatory need in finding alternative drugs of plant origin for DM as they are cheaper, least toxic and with fewer side effects (Nissen and Wolski 2007). A herbal remedy which is having antidiabetic and antihyperlipidemic properties will address the this disease comprehensively.

Carica papaya Linn, commonly known as ‘papaya plant’, the products of this palnt are commonly available and vastly consumed across the country. Evaluating and establishing the antidiabetic properties of this plant shall make it an alternate choice for existing synthetic drugs. In this perspective, numerous scientific investigations have been conducted to evaluate the antidiabetic activities of various parts of *C. papaya*. *Carica papaya* leaves (Rojop et al. 2012; Airaodion et al. 2019), seeds (Venkateshwarlu et al. 2013; Ebenezer et al. 2019), the unripe pulp of fruit (Ezekwe et al. 2014) and root extract (Nimenibo-Uadia and Nwachukwu., 2020) has been reported to be effective in ameliorating the damage caused by diabetes in experimental rats. These studies has revealed that different parts and extracts of *Carica papaya* is having moderate to good antidiabetic and antihyperlipidemic activity, of which aqueous leaf extract is one of the best to have such activity (Maniyar and Bhixavatimath 2011). However, certain components of the plant like latex derived from unripe mature fruit of *Carica papaya*, which is hypothesised to exhibit antidiabetic properties (Ajani and Ogunbiyi 2015) has not been tested comprehensively. With this background the present study was taken up to study and compare the antidiabetic and antihyperlipidemic effects of dried latex powder of *Carica papaya* Linn (DLPCP) and aqueous leaf extract of *Carica papaya* Linn (ALECP).

Materials and methods

The leaves of *C. papaya* plant were collected from the local premises (Tirupati). 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 (400 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: 15% w/w). The residue was weighed and stored in an air and water proof containers at 4°C. From this stock, fresh preparation was made whenever required for the experimental study.

Papaya plant contains specialised cells (laticifers) dispersed throughout the plant tissues that secrete latex. Latex is a thixotropic fluid, with a milky appearance that contains about 85% water. Latex was collected in the early hours of a day as the flow of latex is low as day progresses. Collection was done by making 1-2 mm deep incisions on the skin of unripe mature fruit. The collected latex is passed through 50 mesh sieve to remove dirt. The latex was then dried at room temperature till it became crumbly and non-sticky. The dried latex was triturated using a mortar and pestle, and the same is stored in air tight containers at 4ºC.

A pilot study using dried latex of three doses *viz* 100 mg/Kg b.wt, 200 mg/Kg b.wt and 400mg/Kg b.wt was conducted intially on diabetic rats for antidiabetic dose selection. Nine streptozotocin induced diabetic rats were randomly divided into 3 groups. DLPCP was administered to group D1, D2 and D3, daily for 7 days @ 100mg/Kg b.wt., 200mg/Kg b.wt and 400mg/Kg b.wt. respectively. The selected three doses are lesser than or equal to 1/10th value of the proposed LD50 (4000mg/Kg) of dried latex of *Carica papaya* (Kumar et al. 2018). From the results obtained, the proven best dose was selected for the present study.

The present study was carried out in the Department of Veterinary Biochemistry,College of Veterinary Science, Sri Venkateswara Veterinary University (SVVU), Tirupati, Andhra Pradesh. The experimental procedures were approved by the Institutional Animal Ethics Committee (281/go/ReBi/S/2000/CPCSEA/CVSc/TPTY/011) of College of Veterinary Science, SVVU, Tirupati.

The experimental rats selected for induction of diabetes were fasted overnight before the administration of streptozotocin (STZ). The experimental induction of diabetes in rats was carried out by a single intraperitoneal injection of STZ at the rate of 50mg/Kg b.wt. The induction of diabetes was done for the animals in all the experimental groups except for the animals in normal group to which normal saline was injected..

From the total 32 male Wistar rats weighing 150-200 g, 24 rats were injected with STZ (50 mg/Kg b.wt) through intraperitoneal route, while 8 rats were injected with normal saline. The group of animals which received normal saline served as group I (Normal Control). After 72 hours of STZ administartion, the blood glucose levels of the rats were estimated and the rats with blood glucose levels >200 mg/dl were treated as diabetic animals. Those animals with diabetes (blood glucose levels >200 mg/dl) were distributed into three groups Group II, Group III (DLPCP treatment) and Group IV (ALECP treatment). All the groups were fed on basal diet *ad libitum*.

Blood samples were collected from all the experimental rats on 0th day, 4th day, 14th day and 21st day of the experiment. The collected serum was stored at -20° C for further analysis. Serum biochemical parameters such as, glucose, SGOT , SGPT, triglycerides, total cholesterol and HDL were estimated in automatic analyser (BioSystems A15 analyser), VLDL and LDL levels are calculated based on levels of the triglycerides, total cholesterol and HDL cholesterol.

The rats were sacrificed by cervical dislocation after the routine blood collection on the 21st day of the experiment under deep anaesthesia. The tissues liver and pancreas were collected and preserved till further processing. The liver and pancreas tissues collected were processed and stained with Haematoxylin and Eosin (H & E) stains, slides were observed under light microscope

The group wise and day wise comparison of the biochemical parameters was done statistically by analysing the data with two-way-analysis of variance (ANOVA) followed by bonferroni post-hoc test in graph pad prism version 5.0.

Results and Discussion

The mean blood glucose values in Group II (DC) were consistently increased from 0th day to 21st day with a high of 380.3 ± 23.05 mg/dL on day 21 (Table I). Significant increase in blood glucose values in diabetic control group compared to normal control group was in accordance with previous findings using streptozotocin to induce diabetes mellitus (Deepak *et al.*2020 and Azad and Sulaiman., 2020). Among the groups, ALECP treated group (group IV) has shown decreased serum glucose levels from the 4th day itself but the decrease is more significant on 14th and 21st day. Similar results were reported by Maniyar and Bhixavatimath (2011), with significant decrease in serum glucose levels by ALECP treatment as early as day 7 of the experiment. Whereas, DLPCP treated group (group III) has not shown effective reduction of glucose levels in the earlier days of the experiment, but moderate reduction was observed on 21st day. In a similar pattern, the day wise comparison within the group has indicated the superiority of the ALECP which has shown significant decrease of serum glucose levels from 0th day to 21st t day of the experiment, which is not observed with DLPCP in any of the days of the experiment. Although, DLPCP has not shown significant decrease in the serum glucose levels from 0th day (360.0 + 54.63) to 21st day (311.7 + 28.83), it has been found to be effective in ameliorating the increasing trend of serum glucose observed in diabetic group from 0th day (356.1 + 30.82) to 21st day (380.3+ 23.05). The results with DLPCP were similar with findings of antidiabetic effect showed by ethyl acetate extract of *Atylosia albicans* of 100mg/Kg b.wt in streptozotocin induced diabetic rats (Bhava *et al.*2020). This antihyperglycemic effect of ALECP & DLPCP may be due to the presence of flavonoids, alkaloids and tanins as reported earlier (Buch *et al.*2000 and Maniyar and Bhixavatimath., 2011).

Table I: Serum glucose levels in different experimental groups of rats

GLUCOSE (mg/dL)	Day of treatment			
Groups	**0**	**4**	**14**	**21**
Group-I Normal Control (NC)	91.0 ± 6.24^{a,p}	92.6 ± 6.41^{a,p}	93.6 ± 1.47^{a,p}	92.3 ± 4.56^{a,p}
Group-II Diabetic Control (DC)	356.1 ± 30.82^{b,p}	360.7 ± 16.86^{b,p}	374.3 ± 22.07^{c,p}	380.3 ± 23.05^{c,p}
Group-III (DM+DLPCP @ 400mg/Kg b.wt)	360.0 ± 54.63^{b,p}	351.0 ± 32.11^{b,p}	320 ± 30.32^{bc,p}	311.7 ± 28.83^{b,p}
Group-IV (DM+ALECP @ 400mg/Kg b. wt)	363.2 ± 30.20^{b,q}	327.7±18.65^{b,pq}	293.7 ± 13.01^{b,p}	267.0 ± 5.62^{b,p}

Values are mean ± SE (n=8); Two way ANOVA (Graph pad prism, version 5.0)

a, b, c Means sharing different superscripts in a column differ significantly (P ≤ 0.05)

p, q Means sharing different superscripts in a row differ significantly (P ≤ 0.05).

The serum SGOT and SGPT activities in the group III (DM+DLPCP) and group IV (DM+ALPCP) has decreased significantly when compared to diabetic control group from 14^{th} day and 4^{th} day respectively (Table II). Further, day wise comparison within the group has showed that DLPCP shown significant decrease of SGOT and SGPT activities from the 14^{th} day whereas, ALECP shown this effect from 4^{th} day itself, which indicates superiority of ALECP than DLPCP in terms of hepatoprotective action. Hepatoprotective affect shown by treatment groups may be the manifestation of their hypoglycemic activity, which would have effectively reduced the fatty acid infiltration into the liver and thereby protecting from hepatocyte damage (Juurinen *et al.*2007). Our results with ALECP were similar with findings of hepatoprotective effect showed by aqueous leaf extract of *Carica papaya* of 1.5 g/100ml in streptozotocin induced diabetic rats (Rojop *et al.*2012) and results with DLPCP were similar with findings of hepatoprotective effect showed by hydroethanolic extract of *Artemisia amygdalina* of 250 mg/Kg b.wt in streptozotocin induced diabetic rats (Ghazanfar *et al.*2014).

Table II: SGOT and SGPT (IU/L) activity in different experimental groups of rats

SGOT(IU/L) Day of treatment					SGPT (IU/L) Day of treatment			
Groups	**0**	**4**	**14**	**21**	**0**	**4**	**14**	**21**
Group-I Normal Control (NC)	151.5 ± 5.3^{a,p}	152.3 ± 1.5^{a,p}	155.4 ± 3.6^{a,p}	162.0 ± 2.0^{a,p}	54.3 ± 5.9^{a,p}	57.2 ± 6.6^{a,p}	55.5 ± 5.4^{a,p}	56.6 ± 3.9^{a,p}
Group-II Diabetic Control (DC)	274.7 ± 6.8^{b,p}	297.7 ± 9.1^{c,p}	336.3± 2.1^{c,q}	356.0 ± 8.2^{d,r}	213.9 ± 3.6^{b,p}	240.1 ± 3.2^{d,q}	264.3 ± 8.9^{d,r}	293.0 ± 8.9^{c,s}
Group-III (DM+DLPCP @ 400mg/Kg b. wt)	292.8 ± 4.4^{b,q}	279.9 ± 10.6^{c,q}	228.8± 8.5^{b,p}	213.0 ± 3.6^{c,p}	223.6 ± 6.9^{b,r}	200.0 ± 5.2^{c,r}	179.8 ± 0.9^{c,q}	170.9 ± 2.9^{b,p}
Group-IV (DM+ALECP @ 400mg/Kg b. wt)	282.7 ± 8.9^{b,s}	252.9 ± 4.6^{b,r}	212.8 ± 13.3^{b,q}	190.3 ± 7.1^{b,p}	224.9 ± 10.5^{b,s}	155.3 ± 4.8^{b,r}	103.9 ± 4.0^{b,q}	63.9 ± 6.9^{a,p}

Values are mean ± SE (n=8); Two way ANOVA (Graph pad prism, version 5.0) a,b,c,d Means sharing different superscripts in a column differ significantly ($P \leq 0.05$) p,q,r,s Means sharing different superscripts in a row differ significantly ($P \leq 0.05$)

Among the groups, both ALECP and DLPCP treated groups has shown the significant decreased levels of serum triglycerides, total cholesterol, LDL, VLDL and increased HDL levels (Table III & Table IV) from the 4^{th} day of the experiment, but the ALECP is more effective compared to DLPCP. Within the group comparison, group IV (ALECP + DM) has shown significant decrease in serum levels of triglycerides, total cholesterol, LDL, VLDL and significant increase in HDL levels from 0^{th} day to 21^{st} day of the experiment, which is not observed in the DLPCP treated groups in any of the days of the experiment. Although, DLPCP has not shown significant decrease in the serum levels of serum triglycerides , total cholesterol, LDL, VLDL and significantly increased HDL levels from 0^{th} day to 21^{st} day, it has been effective in ameliorating the increasing trend of serum triglycerides , total cholesterol, LDL, VLDL and decreasing HDL levels observed in diabetic group from 0^{th} day. Our results with DLPCP and ALECP were similar with findings of antihyperlipidemic effect shown by hydroethanolic extract of *Kaemferia galanga* rhizome of 250 mg/Kg.b.wt and 500 mg/Kg.b.wt respectively in streptozotocin induced diabetic rats (Subbaian and Ragavan., 2020). Antihyperlipidemic effects of treatment groups may be due to increased insulin secretion, which could inhibit lipolysis, thereby decrease the levels of lipids in the serum (Jothivel *et al.*2007). These antihyperlipidemic effects could be attributed to saponins present in DLPCP and ALECP, which inhibits the absorption of dietary lipids from the intestine (Marrelli *et al.*2016).

The above biochemical finding are also endorsed by histopathological studes, where in the section of pancreas from Group III (DM+DLPCP) showed mild regeneration β cells, slight reconstructive appearance of ILH and exocrine acini (Fig 1). The section of liver of Group III (DM+DLPCP) has shown mild reconstructive appearance of hepatocytes along with diminution in inflammatory changes (Fig 2). The histopathological studies with ALECP supplemented Group IV were suggestive of marked regeneration of β cells, moderate reconstructive appearence of ILH and almost normal appearance of acini of pancreas (Fig 1). The sections from liver of Group IV (DM+ALECP) showed normal number of hepatocytes and minute inflammatory changes resembling almost normal liver (Fig 2). The above results showed that the ALECP has been superior in terms of antidiabetic activity, hepatoprotective activity and antihyperlipidemic activity, when compared to DLPCP. Hence, the aqueous leaf extract of *Carica papaya* may be considered as better alternative remedy than dried latex of *Carica papaya* in regulating diabetis and its complications. However, further research is needed to gain a better understanding of their potential therapeutic action, the implicated phytochemical constituents and the exact mechanism of action.

Table II: SGOT and SGPT (IU/L) activity in different experimental groups of rats

SGOT(IU/L) Day of treatment					SGPT (IU/L) Day of treatment			
Groups	**0**	**4**	**14**	**21**	**0**	**4**	**14**	**21**
Group-I Normal Control (NC)	151.5 ± 5.3[a,p]	152.3 ± 1.5[a,p]	155.4 ± 3.6[a,p]	162.0 ± 2.0[a,p]	54.3 ± 5.9[a,p]	57.2 ± 6.6[a,p]	55.5 ± 5.4[a,p]	56.6 ± 3.9[a,p]
Group-II Diabetic Control (DC)	274.7 ± 6.8[b,p]	297.7 ± 9.1[c,p]	336.3± 2.1[c,q]	356.0 ± 8.2[d,r]	213.9 ± 3.6[b,p]	240.1 ± 3.2[d,q]	264.3 ± 8.9[d,r]	293.0 ± 8.9[c,s]
Group-III (DM+DLPCP @ 400mg/Kg b. wt)	292.8 ± 4.4[b,q]	279.9 ± 10.6[c,q]	228.8± 8.5[b,p]	213.0 ± 3.6[c,p]	223.6 ± 6.9[b,r]	200.0 ± 5.2[c,r]	179.8 ± 0.9[c,q]	170.9 ± 2.9[b,p]
Group-IV (DM+ALECP @ 400mg/Kg b. wt)	282.7 ± 8.9[b,s]	252.9 ± 4.6[b,r]	212.8 ± 13.3[b,q]	190.3 ± 7.1[b,p]	224.9 ± 10.5[b,s]	155.3 ± 4.8[b,r]	103.9 ± 4.0[b,q]	63.9 ± 6.9[a,p]

Values are mean ± SE (n=8); Two way ANOVA (Graph pad prism, version 5.0) a,b,c,d Means sharing different superscripts in a column differ significantly ($P \leq 0.05$) p,q,r,s Means sharing different superscripts in a row differ significantly ($P \leq 0.05$)

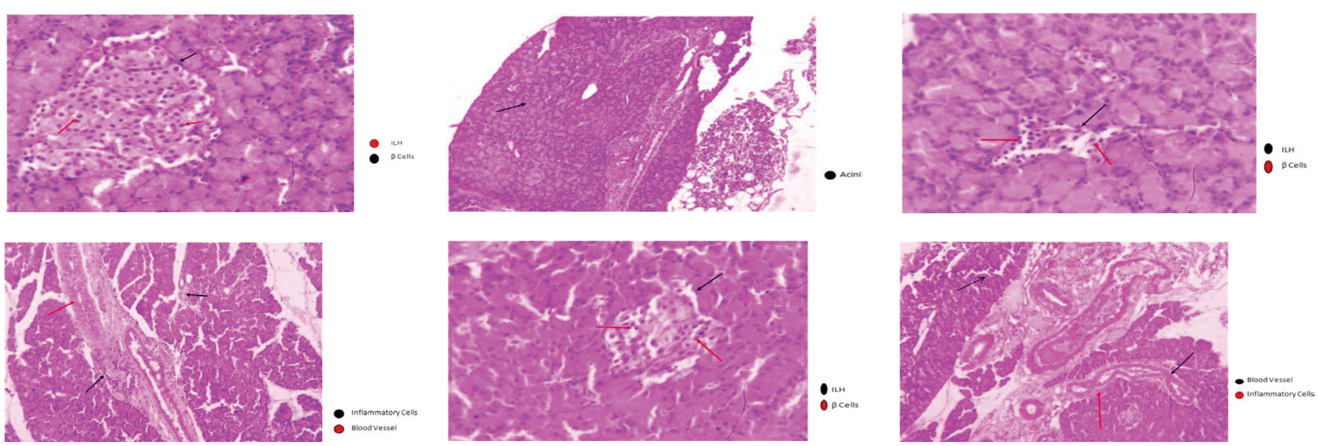

Fig. 1a) Histopathological section of pancreas of group I (NC) rats exhibiting normal sized ILH and normal number of β cells. H&EX400. b) Histopathological section of pancreas of group I (NC) rats showing normal appearance of acinar cells. H&EX100. c) Histopathological section of pancreas of group II (DC) rats showing shrunken ILH with less number of β cells. H&EX400. d) Histopathological section of pancreas of group II (DC) rats showing thickened & dilated blood vessels with severe infiltration of inflammatory cells in acini and around blood vessels. H&EX100. E) Histopathological section of pancreas of group III (DM+DLPCP) rats showing reconstructive appearance of ILH with mild regeneration of β cells. H&EX400. F) Histopathological section of pancreas of group IV (DM+ALECP) rats showing prominent ILH with marked regeneration of β cells. H&EX400.