

DOSE DEPENDENT EFFECT ON HYPOGLYCEMIC AND ANTIHYPERGLYCEMIC ACTIVITIES OF CHLOROFORM EXTRACT OF *PHYSALIS MINIMA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

This study was undertaken to investigate the effect of chloroform extract of *Physalis minima* (CPM) whole plant on blood glucose levels and other biochemical parameters in streptozotocin (55 mg/kg. b. w., i. p) induced diabetic rats. The treatment was given at doses of (200, 300 and 400mg/kg. b. w., p. o), for 28 days. After the treatment with there is a significant reduction was observed in fasting blood glucose levels in treated diabetic rats. The CPM showed considerable lowering effect of total cholesterol, triglycerides, LDL & VLDL levels and considerably increase HDL cholesterol levels. There is a significant reduction of the SGOT, SGPT and ALP levels in diabetic rats treated with CPM, compared with disease control group. These results suggest that *Physalis minima* possesses antidiabetic activity in streptozotocin induced diabetic rats.

Keywords: Antidiabetic activity, *Physalis minima*, Oral glucose tolerance test, Streptozotocin, Triglycerides.

INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. Diabetes mellitus is a chronic disorder of metabolism caused by an absolute or relative lack of insulin. It is characterized by hyperglycemia in the postprandial and/or fasting state, and in its severe form is accompanied by ketosis and protein wasting (Bell, 1991). Besides drugs classically used for the treatment of diabetes (insulin, sulphonylureas, biguanides and thiazolidine-diones), several species of plants have been described in the scientific and popular literature as having a hypoglycaemic activity (Verspohl, 2002; De Sousa et al., 2004). Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown (Valiathan, 1998).

Physalis minima Linn., belongs to the family Solanaceae. It has been extensively used in Indian indigenous medicine. Four new steroidal lactones have been isolated from the leaves of *Physalis minima* (Solanaceae), along with the known physalin B, Withaphysalins A, B belong to the withanolide group showing most of the pharmacological responses (Mahendra S et al., 1984).

MATERIALS AND METHODS

Animals

The Wistar albino Rats (150–200g) were obtained from the Animal House (743/abc), PRIST UNIVERTIY, Tanjavur. Rats were maintained on standard pellet diet and tap water ad libitum. They were kept in clean cages under a 12 hour light/dark cycle and room temperature 22–24°C and were acclimatized to the environment for 2 weeks prior to experimental use. This study was conducted according to the guidelines approved by the Institutional Animal Ethics Committee. Diabetes was induced by injection of a single intra-peritoneal dose of Streptozotocin (freshly prepared in citrate buffer). Overnight fasted rats were injected with streptozotocin (55 mg/kg body wt., i. p) to induce diabetes. Diabetic was confirmed by glucose estimation. Animal with plasma glucose level > 250 mg / dl were selected for the study. Diabetic induced Animals were grouped for further study. After 3 days of streptozotocin induction, treatment was started.

Collection of plant material and treatment of animals

In the present study, the whole plant of *Physalis minima*. Linn., was collected from the out sides of road, Thanjavur, Tamil Nadu. The plant was authenticated (PARC/2009/469) by Dr P. JAYARAMAN,

National institute of herbal drugs, Chennai, Tamil Nadu. The dried whole plants were cut into equal pieces, and then ground plant. The pieces were extracted using soxhelt apparatus with chloroform 700 ml for 150 gms of coarse powder. The solvents were distilled condensed using rotary evaporator at ≤ 40 °C to get the brownish black semi solid mass (chloroform yield- 69%) and stored in desiccator. The semi solid mass of the extract was suspended in appropriate solvent system.

The rats were divided in to seven groups each group having six animals. Group I, normal rats; Group II, Streptozotocin (55mg/kg) treated rats; Group III, Standard drug as Glibenclamide (50mg/kg) treated rats; Group IV, CPM (200mg/kg); Group V, CPM (300mg/kg) and Group VI, CPM (200mg/kg) according to the body weight for the period of 28 days.

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Chemicals used

Streptozotocin was purchased from SIGMA Aldrich, St. LOUIS, MO, USA. All other chemicals used for this study were analytical grade.

Induction of Diabetes

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Acute toxicity studies

LD50 determination Acute oral toxicity (AOT) was determined using nulliparous, non-pregnant female mice. The animals were fasted for 3 hours prior to the experiment and were administered with single dose of CPM at dose of 2000 mg/kg and observed for mortality for up to 48 hours (short-term toxicity). No mortality was observed after 72 hrs. Acute toxicity was determined according to the method Litchfield and Wilcoxon (Litchfield and Wilcoxon, 1949).

Biochemical parameters

At the end of 28 days serum glucose levels were estimated by GOD/POD method. Oral glucose tolerance test (Bonner-Weir, 1988), Triglyceride, total cholesterol, HDL was measured by commercially available kits (Bucolo G *et al.*, 1973) (Nader R *et al.*, 2001). Determination of lipid profile (David Sohn *et al.*, 1974), SGPT, SGPT, ALP (Pari, L *et al.*, 2004), Bilirubin (Jendrassik, 1938), creatinine (Bowers L D, 1980), total protein (Tietz, N. W 1996), albumin (Doumas BT *et al.*, 1972). At the end of the study all the rats were dissected and pancreas was used for histopathological studies.

Statistical analysis were expressed as mean \pm SEM, n=6, p<0.001 compared with STZ induced group using Two way ANOVA followed by Bonferroni-post test.

Results

Serum Glucose levels

Table 1 shows the effects of three different doses of the CPM on serum glucose levels during the 28th days study period. The dose of 400 mg/kg reduced the hyperglycemia at 28th day of study (99.66 \pm 2.3) as compared to the diseased control group (316.83 \pm 6.1)

RESULTS

Table 1: Effect of CPM on Serum Glucose levels in streptozotocin induced diabetic rats

Treatment	0 DAY	7th DAY	14th DAY	21st DAY	28th DAY
Control	108.66 \pm 0.7	98.16 \pm 1.19	95.64 \pm 1.20	103.33 \pm 1.9	95.83 \pm 0.79
D. control	312.56 \pm 3.93	309.11 \pm 4.5	324.33 \pm 5.4	321.81 \pm 5.1	316.83 \pm 6.1
Standard	305.66 \pm 6.5	109.83 \pm 2.1	98.00 \pm 1.1	95.03 \pm 0.88	92.51 \pm 0.9
CPM-200mg/kg	311.88 \pm 169	115.83 \pm 1.2	104.16 \pm 0.7	100.33 \pm 0.4	95.66 \pm 0.6
CPM-300mg/kg	294.16 \pm 0.9	186.50 \pm 2.2	135.50 \pm 1.1	127.66 \pm 0.8	111.0 \pm 1.3
CPM-400mg/kg	304.16 \pm 1.6	134.83 \pm 1.3	123.16 \pm 0.6	119.66 \pm 0.7	99.66 \pm 2.3

Results are expressed as mean \pm SEM, n=6, p<0.001 compared with STZ induced group using Two way ANOVA followed by Bonferroni-post test.

Table 2: Effect of CPM on Oral glucose tolerance test in streptozotocin diabetic rats

Treatment	-30 (min)	0 (min)	30 (min)	60 (min)	90 (min)	120 (min)
Control	108.5 \pm 3.79	124.33 \pm 2.24	257.66 \pm 6.03	295.83 \pm 5.69	148.50 \pm 6.84	98.33 \pm 4.40
D. control	278.5 \pm 8.38	298.5 \pm 4.32	326.33 \pm 2.23	362.66 \pm 4.64	323.33 \pm 2.51	294.16 \pm 3.82
Standard	93.66 \pm 4.13	151.33 \pm 3.07	172.66 \pm 1.78	180.83 \pm 1.35	137.00 \pm 1.63	89.16 \pm 3.83
CPM-200(mg/kg)	90.83 \pm 0.87	84.66 \pm 1.021	162.33 \pm 1.47	195.33 \pm 1.62	141.23 \pm 1.12	104.33 \pm 1.05
CPM-300(mg/kg)	89.93 \pm 0.94	85.83 \pm 0.909	166.16 \pm 3.33	194.00 \pm 1.57	132.83 \pm 1.72	97.33 \pm 0.71
CPM-400(mg/kg)	89.58 \pm 1.38	84.55 \pm 1.14	161.64 \pm 2.34	187.16 \pm 1.81	135.60 \pm 1.42	91.66 \pm 1.14

Results are expressed as mean \pm SEM, n=6, p<0.001 compared with STZ induced group using Two way ANOVA followed by Bonferroni-post test

and near to the normal group (95.83 \pm 0.79). The CPM showed significant dose dependent (200, 300 and 400 mg/kg. b. w) decrease in hyperglycemia in STZ induced diabetic rats.

Oral glucose tolerance test

Glucose loading to normal rats increased serum glucose levels from 108.5 \pm 3.79 to 295.83 \pm 5.69 at 60 min and returned to normal at 120 min in normal rats. The CPM administration improved glucose tolerance significantly in a concentration-dependent manner with doses of 200, 300 and 400 mg/kg as 104.33 \pm 1.05, 97.33 \pm 0.71 and 91.66 \pm 1.14 respectively at 120 min. The CPM showed significant reduction in the blood glucose levels as compared to STZ treated diabetic group (294.16 \pm 3.82).

Serum lipid profile

Table 3 showed the effect of CPM on lipid profile. In STZ induced diabetic rats, CPM showed significant reduction in the total cholesterol and triglyceride levels at 28 day compared to control group. However, serum HDL levels had increased significantly (p < 0.001) in all the CPM treated groups. The CPM (200, 300 and 400mg/kg) increase in HDL levels at 28 day to 62.83 \pm 1.77, 59.5 \pm 0.703, 53.83 \pm 1.108 mg/dl respectively compared to diseased control group (34.2 \pm 0.54). In diabetic rats, LDL levels were decreased significantly to 127.66 \pm 1.86 and 125.54 \pm 0.55 to 119.5 \pm 1.47mg/dl in dose dependent manner compared to STZ induced diabetic rats 175.83 \pm 1.85.

Serum SGOT, SGPT and ALP levels

Table 4 shows the effect of CPM on liver enzymes. The three doses of the CPM decrease the elevated levels of enzymes by the inducing agent STZ. CPM showed dose dependent reduction when compared with STZ induced diseased group. There was a significant reduction in SGOT, SGPT and ALP levels were found to be 169.68 \pm 1.00, 76.08 \pm 0.26 and 149.54 \pm 0.60 189.18 \pm 0.28 respectively with CPM (400mg/kg) compared to the STZ induced diabetic rats. The effect of CPM was similar to that observed for glibenclamide.

Serum parameters

Table 5 shows the three different concentrations CPM in STZ inducing rats in a dose dependent manner. The CPM (400mg/kg) treated group showed decreasing the bilirubin levels 0.933 \pm 0.049 compared to STZ induced diabetic rats (1.80 \pm 0.044). The creatinine levels 0.768 \pm 0.013 for CPM (400mg/kg) compared to STZ induced rats (1.811 \pm 0.021). The albumin levels were found to be 4.016 \pm 0.065 (CPM 400mg/kg) and 7.466 \pm 0.08 (STZ rats). The effect of CPM was similar to that observed for glibenclamide.

Table 3: Effect of CPM on Lipid profile in streptozotocin induced diabetic rats:

Treatment	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	154.00±1.52	126.12±1.36	64.66±1.33	122.33±1.44	30.86±0.32
D. control	355.33±1.78	252.96±1.23	34.24±0.54	175.83±1.85	71.06±0.35
Standard	147.66±1.64	126.54±1.15	62.83±0.70	119.98±1.86	29.45±0.26
PM-200mg/kg	155.83±1.64	114.66±1.15	53.83±1.10	127.66±1.86	30.65±0.86
PM-300mg/kg	153.16±1.39	108.33±1.02	59.51±0.70	125.54±0.55	30.16±0.22
PM-400mg/kg	148.00±2.36	108.33±4.95	62.83±1.77	119.51±1.47	29.64±0.473

Results are expressed as mean±SEM, n=6, p<0.001 vs alloxan induced group using Two way ANOVA followed by Bonferroni-post test.

Table 4: Effect of CPM on SGOT, SGPT and ALP in STZ induced diabetic rats:

Treatment	SGOT(U/L)	SGPT(U/L)	ALP(U/L)
Control	178.50±0.24	77.86±0.22	122.75±0.23
D. control	189.18±0.28	89.58±0.18	176.55±0.50
Standard	170.13±0.14	77.60±0.29	153.40±0.18
CPM-200mg/kg	173.83±0.91	79.50±0.76	164.15±0.20
CPM-300mg/kg	172.60±1.55	78.33±0.44	154.13±0.21
CPM-400mg/kg	169.68±1.00	76.08±0.26	149.54±0.60

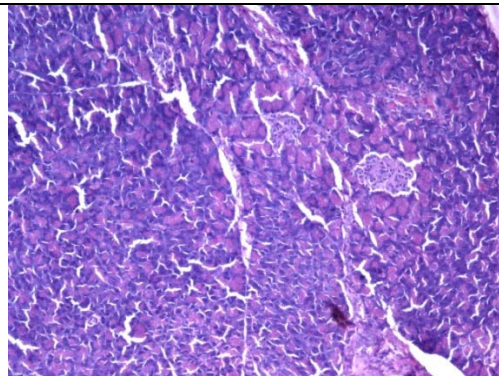
Results are expressed as mean±SEM, n=6, p<0.001 compared with STZ induced group using Two way ANOVA followed by Bonferroni-post test.

Table 5: Effect of CPM on Different kidney parameters in STZ induced diabetic rats

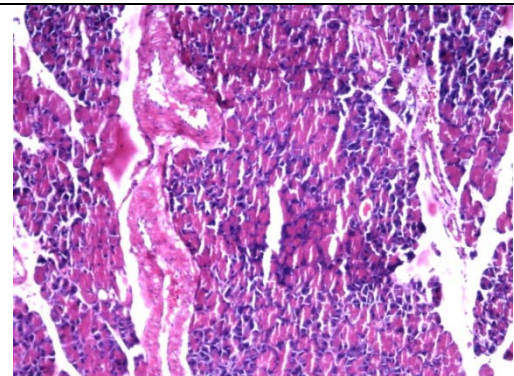
Treatment	Creatinine	Total bilirubin	Direct bilirubin	Total protein	Albumin
Control	0.68±0.017	0.98±0.06	0.75±0.06	7.5±0.096	4.05±0.67
D. control	1.81±0.021	1.80±0.044	1.21±0.074	9.75±0.042	7.46±0.08
Standard	0.72±0.004	0.95±0.036	0.71±0.047	6.56±0.076	4.11±0.13
CPM-200mg/kg	1.00±0.012	0.93±0.076	0.89±0.079	8.06±0.066	5.08±0.012
CPM-300mg/kg	0.86±0.004	0.96±0.061	0.81±0.070	7.25±0.099	4.68±0.03
CPM-400mg/kg	0.76±0.013	0.93±0.049	0.73±0.036	6.76±0.042	4.01±0.065

Results are expressed as mean±SEM, n=6, p<0.001 compared with STZ induced group using Two way ANOVA followed by Bonferroni-post test.

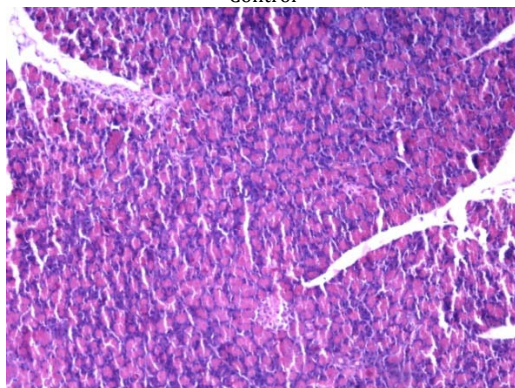
Effect of CPM and glibenclamide on histopathological studies of pancreas in STZ induced diabetic rats



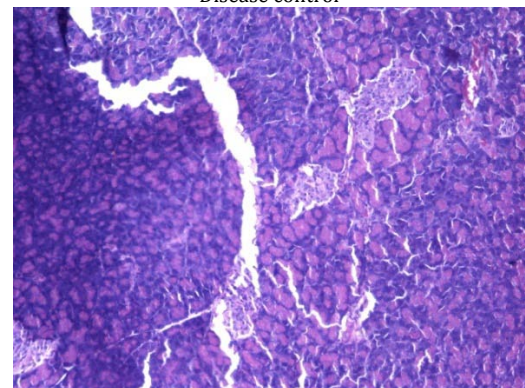
Control



Disease control



Standard



CPM (400mg/kg)

DISCUSSION

Diabetes mellitus is often referred to simply as diabetes (Ancient Greek: DIABETES "to pass through [urine]"), is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels (hyperglycemia). Diabetes develops due to a diminished production of insulin (in type I) and resistance to its effects (in type II and gestational) Both lead to hyperglycemia, which largely causes the acute signs of diabetes: excessive urine production, resulting compensatory thirst and increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism.

STZ Streptozotocin (STZ) administration generally causes the destruction of β -cells after three days and reaches its peak at three to four weeks in rats (Adeghate E., 2002). The β -cells are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes (Spinas GA., 1999).

Glucose estimations of serum were performed for the diagnosis and fall up of diabetes mellitus. In a normal healthy individual the fasting blood glucose level is between 70-100mg/dl. This level may increase up to 500mg/dl or more in diabetic person. This increase in glucose levels is referred as hyperglycemia. This occurs mainly due to deficiency of insulin. The continuous treatment for 28 days with the CPM showed significantly reduction in the blood glucose levels. This CPM was found to decrease the level of glucose significantly ($p < 0.05$) in STZ induced diabetic rats. The lower dose of CPM itself exhibits its activity and the effect was observed to be dose dependently.

Cholesterol is an essential structural element of the biological membranes. In addition, it is the precursor of many compounds such as the starting materials for the synthesis of bile acids, steroid hormones, and vitamin D among others. Despite this knowledge, high concentration of serum cholesterol increases the risk of developing CHD (Libby *et al.*, 2000). The present study demonstrated that rats treated with STZ showed a higher concentration of serum Triglycerides, total cholesterol, LDL and VLDL and Decreased levels of HDL compared to normal rats, while oral administration CPM (400mg/kg) was decreased the Lipid levels significantly and less with of CPM (300mg/kg and 200mg/kg). The observations of the present study indicate that the CPM has protected over the hypertriglyceridemia and hypercholesterolemia could be through its control of hyperglycemia. This is in agreement with the fact that the level of glycemic control is the major determinant of very low density lipoprotein and triglyceride concentrations (Markku, 1995, Shukla *et al.*, 2004). It is widely accepted that elevation of plasma LDL levels are major risk factors for CHD (Brliner *et al.*, 1997). Direct correlation between LDL and atherosclerosis and also the reversibility of the related pathological events by lowering the serum level of LDL have already been reported by many research groups (Ross, 1999). Our data indicated that, the high concentration of LDL in hypercholesterolaemic rats was significantly reduced by oral administration of CPM (400mg/kg) when compared to the other groups. Another risk factor for developing diabetes is reduced serum level of HDL. This effect of HDL is largely attributed to its central function in the reverse cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL particles for further delivery to the liver for metabolism (Martinez *et al.*, 2004). Therefore, it is logical that an increase in HDL level can contribute to lower risk of diabetes our results clearly showed that CPM is capable of increasing the serum level of good cholesterol (i. e HDL) in the CPM treated and glibenclamide treated rats compared to STZ induced diabetic rats.

The significant of SGPT, SGOT and ALT are the enzymes were found primarily in liver and heart is far greater enhanced and released into the blood stream is the result of liver abnormality. If therefore serve as a fairly specific indicator of liver status and it's elevated levels in serum indicates liver damage. The CPM (400 mg/kg) reduces the SGPT levels, when compared to the diseased group indicating it's protective effect on liver during diabetic conditions.

The raise in the levels of serum bilirubin is most sensitive and confirms the intensity of Jaundice (Sallie *et al.*, 1991). Rana *et al.* (1996) reported that the increase in plasma bilirubin (hyperbilirubinemia) may be resulted from the decrease of liver uptake, conjugation or increase bilirubin production from haemolysis and this finding coincided with the decrease in total erythrocyte counts. Also, the elevation in plasma bilirubin indicates liver damage as confirmed by the changes in the activities of liver (Table 4) enzymes. Furthermore, the improvement of the liver damage by oral administration of CPM (400mg/kg) could be confirmed through studying their effect on the level of plasma bilirubin. The results in (Table-4) showed that the experimentally induced diabetes increased ($p < 0.001$) the level of plasma bilirubin. Serum total protein and albumin levels are a selective marker of liver injury in rats (Mauer *et al.*, 1978). Treatment with CPM (400 mg/kg) prevented the rise in albumin levels in STZ induced diabetic rats. The effect was less with of CPM (300mg/kg), CPM (200mg/kg). The observations of the present study indicate that the CPM protect liver disorders effects of hyperlipidemia.

The light microscopic examination of pancreatic section of control group revealed that the normal structure of the exocrine and endocrine parts of the pancreas. Previous studies reported similar findings and added that the pancreas had a rich capillary network essential for the secretory process (Junqueira LC *et al.*, 2005). The light microscopic examination of endocrine part of pancreas of disease control group revealed the altered structure of both the exocrine and endocrine portions with significant decrease in the number of secretory cells. The treatment with the CPM extract and glibenclamide found to prevent the degenerative changes in STZ induced diabetic rats.

CONCLUSIONS

It is concluded that, The *Physalis minima* showed better hypoglycemic and antihyperglycemic activity against STZ induced diabetic rats. All the activities might be due to high levels of withanolide group present in chloroform extract of *Physalis minima*.

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