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RESEARCH ARTICLE

Anti-diabetic and Hypolipidemic Effect of Aqueous and Methanolic Root Extracts of *Physalis angulata* in Streptozotocin (STZ) Induced Diabetic Rats

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ABSTRACT

The present study was carried out to evaluate the anti-diabetic and hypolipidemic effect of aqueous and methanolic root extracts of *Physalis angulata* in Streptozotocin (STZ) induced diabetic rats. Aqueous and methanolic root extracts of *Physalis angulata* were administered orally for 14 days at the dose level of 200 mg/kg and 400 mg/kg to the diabetic rats. The lipid metabolic profiles, like Triglycerides (TG), Total Cholesterol (TC), HDL Cholesterol, VLDL and LDL Cholesterol and serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and oxidative markers like malondialdehyde (MDA), reduced glutathione (GSH), and levels were evaluated in serum. The elevated serum levels of glucose, cholesterol and MDA were normalized in *Physalis* extracts treated rats, while the HDL cholesterol and GSH levels were increased. These findings reveals that the anti-diabetic and hypolipidemic potential of *Physalis angulata* root extracts.

KEYWORDS

Physalis Angulata, Diabetes, Lipid profiles, Rats

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia (high blood sugar), due to defects in insulin secretion, insulin action, or both, associated with the development of long-term vascular and neuropathic complications.[1] This blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia. Diabetes is the most common metabolic disorder in our Indian community.

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There is a need to search for new drugs with minimum side effects and good therapeutic activity.[2]

Medicinal plant sector has traditionally occupied an important position in the socio cultural and medicinal arena of rural and tribal lives of India. Survey of literature and interactions with village healers revealed that, the *Physalis angulata* plant accredited with medicinal properties and contain different classes of secondary metabolites of pharmacological importance. *Physalis angulata* commonly called in Andhra Pradesh (India) as budda kodisha, belongs to the family Solanaceae.[3]

In India the plant is used as antiasthama in the areas of Madhya Pradesh, Uttar Pradesh and Utharkand.[4] It also acts as antibacterial[5], antiviral[6], immunomodulatory[7], anti-inflammatory activity[8]. It is also considered as antipyretic, antinociceptive, anti-diuretic, and anti-inflammatory drug for hepatitis and cervicitis.[7,9,10,11]

Phytochemical studies on *Physalis angulata* reveal that it contains many types of biologically active, naturally occurring chemicals including flavonoids, alkaloids and steroids known as physalins, B, D, F and G, withanolides and secosteroids many of which have never been seen in science before.[12]

The aim of the present study was to investigate the anti-diabetic potential and hypolipidemic properties of *Physalis angulata* in diabetic rats. Hence, the present study was conducted to know the impact of *Physalis angulata* on lipid metabolic profiles, SGOT, SGPT levels and oxidative markers in diabetic rats.

MATERIALS AND METHOD

Animals

Wistar strain albino rats weighing 180 to 220 grams were purchased from Suresh agencies, Hyderabad, India and used for study. The protocol was approved by Institutional Animal Ethical Committee (IAEC/03/UCPSc/KU/10). The animals were housed in standard polypropylene cages and maintained under standard laboratory conditions (12:12 hour light and dark cycle; at an ambient temperature of 25 ± 5^{O}C, 35-60% of relative humidity). They were fed with standard rat pellet diet and water *ad libitum.*

Chemicals

Streptozotocin was purchased from Sigma-Aldrich Company, USA. While, assay kits (GOD-POD), Triglyceride kit, Total cholesterol kit, HDL kit, Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT) were purchased from Kamineni Life Sciences, Hyderabad, India. Pioglitazone was purchased from Dr. Reddy's foundation, Hyderabad, India. All other chemicals and solvents used were of analytical grade.

Preparation of *Physalis Angulata* Extracts

The plant *Physalis angulata* (with roots) were collected from the rural areas of Warangal district (Andhra Pradesh, India), during September-October 2009. The plant was identified and authenticated by comparing with the voucher specimen by Prof. V. S. Raju, Department of Botany, Kakatiya University, Warangal. The collected plant material (roots) was dried in shade for 15 days and the dried roots were broken into small pieces with an axe, and powdered in electrical grinder into coarse powder. The powder obtained were passed through sieve plate No.10 mesh and then used for extraction. The root extracts were prepared with solvents methanol and water by maceration technique.

Induction of Diabetes

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ) (60 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5). The animals were allowed to drink 15% glucose solution overnight to overcome the drug- induced hypoglycemia. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day after STZ injection, the animals were acclimatized one week in diabetic condition. After one week the extracts of *Physalis* were given to the diabetic rats for 14 days.

Experimental Design

Rats were fasted over night before experiment and divided into 7 groups of six rats each.

Group I: Normal control group rats received 0.1% Na carboxy methyl cellulose (**NC**).

Group II: Diabetic control rats received 0.1% Na CMC (**DC**)

Group III: Diabetic rats treated orally with *Physalis angulata* root aqueous extract 200 mg/kg (**D+PAA1**).

Group IV: Diabetic rats treated with *Physalis angulata* root aqueous extract 400 mg/kg (**D+PAA 2**).

Group V: Diabetic rats treated with *Physalis angulata* root methanol extract 200 mg/kg (**D+PAM 1**).

Group VI: Diabetic rats treated with *Physalis angulata* root methanol extract (PAME) 400 mg/kg (**D+PAM 2**).

Group VII: Diabetic rats treated with Pioglitazone 15 mg/kg (Standard group) (**D+Pt**).

Blood samples were withdrawn from the retro-orbital plexus of the rats on 14th day after the treatment. The samples were analyzed on spectrophotometer for serum glucose content using glucose oxidase-peroxidase method.[13]

TG, TC, HDL, VLDL, LDL, SGOT and SGPT were estimated by the standard kits. Whereas GSH levels are measured by spectrophotometrically by Ellman GL, 1959, Beutler et al., 1963. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid-reactive product Malondialdehyde (MDA), using the method of Ohkawa et al (1979).

Statistical Analysis

All the experimental values were expressed as mean ± SD and were compared with positive control value in each group. One-way analysis of variance (ANOVA) followed by Dunnet test to compare means from the control groups and each of the group treated with extracts and the statistical significance was judged at the 0.05 probability level.

RESULTS AND DISCUSSION

A significant increase in serum glucose levels, Triglycerides (TG), Total Cholesterol (TC), VLDL, LDL Cholesterol, SGOT, SGPT and MDA in the diabetic control rats, when compared to the normal control rats. Whereas HDL Cholesterol and reduced glutathione (GSH) levels are decreased in diabetic rats. Oral administration of *Physalis angulata* for 14 days period exhibited down regulation of serum glucose levels, Triglycerides (TG), Total Cholesterol (TC), VLDL, LDL Cholesterol, SGOT, SGPT and MDA, except HDL Cholesterol and GSH. This reflects restoration of the levels of lipid metabolic profiles and oxidative stress markers to the near-normal values. (Table 1, 2, 3, 4).

Table 1: Effect of aqueous and methanolic root extracts of *Physalis angulata* on Body weight changes (grms/kg) and Serum glucose (mg/dl) levels in Streptozotocin induced diabetic rats.

Groups	Body weight		Serum glucose	
	0 Day	14th Day	0 Day	14th Day
Group I	195±3.162	235±8.50	81±1.414	88±4.42
Group II	200±4.242	214.7±6.2*	253±2.60^{V}	253±9.26*
Group III	206±13.03	236.3±11.93**	81±1.414^{b}	165.7±11.7**
Group IV	198±3.162	248±9.62**	82±1.42^{b}	136.4±7.89**
Group V	210±2.48	234.4±6.5*	81±2.36^{b}	115.8±8.3**
Group VI	208±13.03	238.5±6.8**	84±2.38^{b}	106.5±6.8**
Group VII	189±3.71	248.8±13.77**	258±4.75^{V}	120.3±3.64**

Table 2: Effect of aqueous and methanolic root extracts of *Physalis angulata* on Total Cholesterol (mg/dl), Triglycerides (mg/dl), LDL Cholesterol (mg/dl) in Streptozotocin induced diabetic rats.

Groups	Total Cholesterol(mg/dl)	Triglycerides (mg/dl)	LDL Cholesterol (mg/dl)
Group I	121.2±5.8	75±5.2	63.18±5.40
Group II	189.2±3.5*	149.4±4.3*	160.8±18.2 V
Group III	149.4±3.99**	108.9±6.04*	115.9±5.73 V
Group IV	130.2±10.31**	89.34±4.59**	83.74±9.86 V
Group V	139.40±3.6*	98.6±5.8**	95.80±5.3 V
Group VI	122.50±5.1**	85.4±4.3**	73.24±9.2 b
Group VII	125.90±4.9**	86.07±1.3**	65.65±51 b

Table 3: Effect of aqueous and methanolic root extracts of *Physalis angulata* on VLDL Cholesterol (mg/dl), HDL Cholesterol (mg/dl), in Streptozotocin induced diabetic rats.

Groups	VLDL Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)
Group I	14.98±1.19	43.24±0.2
Group II	29.89±0.95*	27.27±1.9*
Group III	21.8±1.24**	36.05±1.1**
Group IV	17.89±0.88**	39.3±1.42**
Group V	18.5±1.2**	39.4±1.2**
Group VI	13.6±0.7**	42.5±1.5**
Group VII	17.21±0.26**	40.59±0.7**

Table 4: Effect of aqueous and methanolic root extracts of *Physalis angulata* on SGOT (IU/L) SGPT (IU/L), GSH (nmol/ml) and MDA (nmol/ml) in Streptozotocin induced diabetic rats

Groups	SGOT (IU/L)	SGPT (IU/L)	GSH (nmol/ml)	MDA (nmol/ml)
Group I	27.94±7.9	36.45±4.1	39.33±1.25	7.2±1.20
Group II	119.6±7.8*	143.5±6.3*	21.83±0.53 V	14.05±0.50 V
Group III	79.01±5.01*	85.99±8.12**	28.53±0.43*	12.05±0.33 V
Group IV	54.18±4.58**	58.05±6.27**	30.28±0.31**	10.99±0.89*
Group V	63.4±4.1**	65.84±6.3**	31.58±0.36**	10.50±0.30*
Group VI	44.18±3.5**	48.12±5.2**	35.62±0.25**	8.70±0.60**
Group VII	40.16±5.1**	46.09±7.4**	35.33±0.52**	8.50±0.80**

All the values are expressed in mean ± SD of six individual observations.
*p<0.001 compare to normal control (Group-I),
**p<0.01 compare to diabetic control (Group-II),
v=p<0.01 compare to normal control (Group-I),
b= not significant compare to control (Group-I)

DISCUSSION

This study was undertaken to evaluate the anti-diabetic activity, hypolipidemic (Triglycerides, total cholesterol, HDL cholesterol, and VLDL & LDL cholesterol) effect of *Physalis angulata* root extracts in streptozotocin (STZ) induced diabetic rats.

In Streptozotocin-induced diabetic rats, serum glucose levels were increased. Serum glucose levels of untreated diabetic rats were significantly higher than those in normal rats. This may be due to over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis.[14] Whereas the *Physalis angulata* treated diabetic rats showed lower levels of serum glucose. This may be due to the free radical scavenging activity of *Physalis angulata*. Which inhibits lipid peroxidation, prevents streptozotocin-induced oxidative stress and protects β-cells resulting in decreased serum glucose levels (Table 1).

In the current study, the decrease in body weight of diabetic rats was observed. The characteristic loss of body weight associated with diabetes is due to increased muscle wasting in diabetes.[15] This indicates polyphagic condition and loss of weight due to excessive break-down of tissue proteins.[16,17] Hakim et.al. have stated that decreased body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins. Increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight gain by diabetic rats.[18] When *Physalis* was administered to diabetic rats, the weights seemed to be increased, as was the ability to reduce hyperglycaemia. However, it could not normalize the body weight completely. The increase in body weight of the treated animals support the antidiabetogenic effect of *Physalis angulata* root extracts as diabetic condition is associated with fluctuation of body weight. An increase in the body weight of diabetic animals, probably due to improvement in insulin secretion and glycemic control[19] (Table 1). Similar kind of effect i.e., body weight gain was previously reported with other plants, such as, *Ficus* bengalensis[20] and *Trigonella foenum-graecum*[21] and ginger[22] well known for their antidiabetic activity. (Table 1).

Therefore, the plant extracts may have insulin like effect on several tissues as in the case of oral hypoglycemic agents used. The present study suggest that, the antihyperglycemic activity of *Physalis angulata* under study could also be due to insulinogenic activity by stimulating insulin secretion from the remanant or/and regenerated beta cells. Similar effects were also observed in insulinogenic activity with the treatment with leaves of *Gymnema sylvestrae*[23], roots of *Clausana anisata*, fruits of *Momordica charantia* and *Momordica cymbalaria*[24].

Hyperlipidemia is a recognized consequence of diabetes demonstrated by the elevated levels of tissue cholesterol, phospholipids and free fatty acids.[25] In the present study revealed an increase in total cholesterol, triglyceride, LDL, VLDL cholesterol with decrease in HDL cholesterol[26] in Diabetic rats. Lipids play an important role in the pathogenesis of diabetes mellitus. Hyperlipidemia is a recognized consequence of diabetes mellitus demonstrated by the elevated levels of TG, TC, LDL, VLDL, and depleted levels of HDL.[27] The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagon, catecholamine, and other hormones enhance lipolysis. The level of serum lipids is usually raised in diabetes.

The levels of total serum cholesterol, triglycerides, LDL, VLDL were lowered and HDL levels are increased after the treatment with *Physalis* root extracts and reference drug. It indicates that the *Physalis* is more useful in the treatment of diabetes as it has hypolipidemic effect. Moreover, its hypolipidemic effect could represent a protective mechanism against the development of diabetic complications. (Table 2, 3, 4).

In another study oral administration of *Pterocarpus marsupium* heartwood resulted in a significant reduction of serum lipid levels in rats

with hyperlipidemia viz. serum triglycerides and total cholesterol, LDL and VLDL cholesterol levels without any effect on HDL cholesterol and confirmed its hypocholesterolemic and hypolipidemic effects. Flavonoids are known for their diverse biological activities including hypolipidemic activity. *Physalis,* thus possess both hypoglycemic and hypolipidemic activity.

Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. SGOT and SGPT are reliable markers of liver function. Liver was necrotized in STZ- induced diabetic rats.[28] Therefore an increase in the activities of SGOT and SGPT in serum might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream[29], which gives an indication of the hepatotoxic effect of STZ. Increased gluconeogenesis and ketogenesis are observed in diabetes which may be due to high level in the activities of these liver markers.[30] Similarly, many scientists have reported that STZ increased the activities of SGOT, SGPT in both liver and serum of diabetics. This increased level of SGOT, SGPT, which is active in the absence of insulin because of the availability of amino acid in the blood of diabetics is responsible for the increased gluconeogenisis and ketogenisis metabolism in diabetics.[31] Supplementation of *Physalis* showed a significant decrease in enzyme level. However, after treatment with *Physalis* the level of these enzymes were reduced, when compared with diabetic rats. Hence, the decrease of SGOT and SGPT level after supplementation of *Physalis* further strengthen the antidiabetogenic and hepatoprotective effect of the extracts.

Glutathione (GSH) serves as a sensitive marker of oxidative stress and it plays an important role in maintaining the integrity of the cell system. GSH is involved in several reactions in the body and is one of the most prominent non-enzymatic antioxidants.[32] In the current investigation, GSH level was decreased in serum of diabetic rats. Depletion of serum GSH levels enhances cellular damage caused by oxidative stress. Significant depletion of GSH (*$p<0.001$) in diabetic rats suggests its increased utilisation against reactive oxygen species. The marked depletion of GSH observed in the tissue of diabetic condition, may be due to the utilization of this compound by two antioxidant enzymes, GPx and GST as their substrate.[33] However, *Physalis* treatment in diabetic rats, reversed the GSH to normal levels, this shows that *Physalis* has an antioxidant property. (Table 4)

In the present study, MDA levels were increased in diabetic rats. In general, increase in MDA in the diabetic situation suggests that hyperglycaemia induces the peroxidative reactions in lipids. Increased lipid peroxidation under diabetic conditions can be due to increased oxidative stress in the cells as a result of depletion of antioxidant enzymes. This study shows that MDA, a lipid peroxidation product and a marker of oxidative stress was significantly lower in the diabetic group treated with *Physalis*. Similar result was also found in diabetic patients by Mahboob *et al.*[34] Treatment with *Physalis* brought back lipid peroxidation markers to near normal levels, which could be as a result of improved glycemic control and antioxidants status. This normalization of MDA may be accomplished by the antioxidant and free radical quenching nature of *Physalis.* (Table 4)

CONCLUSION

Administration of *Physalis angulata* root extracts produced a significant reduction in serum glucose, total cholesterol, triglyceride, LDL, VLDL, SGOT, SGPT, MDA and increased HDL and GSH in diabetic rats. This study shows the antidiabetic, hypolipedemic and antioxidant potential of *Physalis* root extracts. However, comprehensive chemical and pharmacological research work is required to find out the exact mechanism of this root extracts for its antidiabetogenic and hypolipedemic effect and to identify the active constituents responsible for this effect.

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