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Experimental Evaluation of Antidiabetic Effect of *Punica Granatum* Peel Extract against Streptozotocin Induced Diabetes in Albino Rats

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Abstract: Diabetes mellitus is a disease characterised by derangement in carbohydrate, protein and fat metabolism which is caused by the complete or relative insufficiency of insulin secretions. Plants and their bioactive constituents are used for the treatment of diabetes mellitus throughout the world; especially in countries where access to the conventional treatment of diabetes mellitus is inadequate. The present study was undertaken to investigate the anti-diabetic effect of *Punica granatum* ethanolic peel extract in STZ induced diabetic rat. STZ was used to induce diabetes in albino rats weighing 150-250 grams. The fasted diabetic rats were divided in to 6 groups of 6 animals each. Group I Normal control group received distilled water 5 ml/kg body weight orally daily, Group II were treated with STZ 50mg/kg body weight induced diabetic rats were served as diabetic control group and were treated with distilled water orally daily until diabetes occur, Group III i.e STZ induced diabetic rats were served as standard group and were treated with Glibenclamide at a dose of 0.5 mg/kg body weight orally daily. Group IV & Group V *Punica granatum* ethanolic peel extract was administered with 100mg/kg and 200mg/kg dose orally. This study was conducted over a period of 15 days with oral administration of drugs and the plant extract which was started on the 6th day of STZ treatment. The fasting blood glucose levels were determined on day 0, 5th, 10th, and 15th day by using glucometer. The maximum reduction in Blood Glucose level was observed at a dose of 200mg/kg body weight. Data were statistically analyzed by Student "t" Test. $P<0.001$ was considered as highly significant.

Keywords: Antidiabetic, Glibenclamide, Streptozotocin, Punica granatum

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1. INTRODUCTION

Diabetes mellitus is a spectrum of metabolic disorder, arising from myriad pathogenic mechanisms, all resulting in hyperglycemia[1,2]. Both genetic and environmental factors contribute to its pathogenesis, which involves insufficient insulin secretion, reduced responsiveness to endogenous or exogenous insulin, increased glucose production, or abnormalities in fat and protein metabolism. The resulting hyperglycemia may lead to both acute symptoms and metabolic abnormalities[3,4]. The worldwide prevalence of diabetes has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 451 million in 2017. Based on current trends, it is estimated that more than 693 million people will be suffering with the disease by year 2045[5,6]. The most dramatic increases have been recorded in developing countries such as India, and diabetes accounts for a major portion of healthcare expenditure, further emphasizing the need for newer and cheaper modalities of treatment. There is a need for more effective, durable and safer anti-diabetic agents. Statistical projection suggests that the number of diabetics will rise from 15 million in the year 1995 to 98 million in 2030 in India. This number is making India the country with the highest number of diabetics in the world[7]. There have been very strong traditional systems of medicine such as Chinese, Ayurvedic, and the Unani, born and practiced, more in the eastern continent over the last 2500 years. These traditions are still flourishing, since; almost 80% of the people in the developing countries depend on these systems of medicine for their primary health care needs[8]. The plants hold substances that can be used for therapeutic purposes, of which are precursors for the synthesis of drugs[9]. Over the past decade, pomegranate (*Punica granatum*) is titled as a wonder fruit because of its voluminous pharmacological properties. In 1830, *Punica granatum* fruit was first recognized in United States Pharmacopeia; the Philadelphia edition introduced the rind of the fruit. There are significant efforts and progress made in establishing the pharmacological mechanisms of peel (pericarp or rind) and the individual constituents responsible for them. However, the medicinal properties of Punica granatum as a fruit peel have very scanty studies. It contains many phytochemical components that contribute to antihyperglycemic[10], Nephroprotective[11], antihyperlipidemic [12] effect, and numerous other effects of wonderful, economic, and eco-friendly pomegranate peel extract[13] . The current study was focused on achieving antidiabetic property from a natural source with no or negligible side effects so that it can be used in patients with cardiac, hormonal or renal compromise with no fear of deleterious effects[14]. Hence the present study was planned. In the present study, ethanolic extract of dried fruit peel of *Punica granatum* was evaluated for antidiabetic activity in Streptozotocin induced diabetic albino rats.

2. MATERIALS AND METHODS

2.1 Animals

Adult Albino rats of either sex weighing between 150 to 250 gms were used in this study; laboratory bred albino rats were obtained from Sainath Agencies, Hyderabad, India and placed in individual cages in central animal house of department of Pharmacology, Chalmeda Anand Rao Institute of Medical sciences, Bommakal, Karimnagar, Telangana, India. The animals were stabilized for one week under standard conditions at temperature 25±1°C, 60±5% relative humidity and 12 hrs dark light cycles. They have been given free access to standard pellet diet and water ad libitum. The study was conducted according to the ethical norms approved by the IAEC, and was carried out in accordance with the recommendations of CPCSEA. Ref: **(**CAIMS/Academics/IAEC/PhD/1/2014/CPCSEA).

2.2 Extraction

Fresh Pomegranate ruits were collected from the local market of Karimnagar, Telangana, India. The authenticity of the *Punica granatum* was identified by the HOD, Department of Botany, S.R.R Degree and P.G. College of Sciences, Karimnagar, Telangana, India. Pomegranate peels were manually separated, shade-dried and grounded to coarse particles and extraction was carried out with Soxhlet apparatus using 50% ethanol. The extract was filtered to remove the peeled particles. The residue was re-extracted with the same solvent. The extracts were pooled and concentrated under vacuum at 45°C[15].

3. EXPERIMENTAL METHODS

Prior to the actual experiment, pilot studies were done to determine the:

Dose of Streptozotocin to be injected
Effective dose of *Punica granatum* peel extract.
Dose of Glibenclamide

3.1 Dose of streptozotocin [16]

To induce a stable hyperglycaemic state in rats, the dose of Streptozotocin suggested in literature is a single intraperitoneal injection at a dose of 50 and 60 mg/kg body weight. Hence, initially we administered 60 mg/kg intraperitoneal injection of streptozotocin to the animals and half of the animals died. Then, we tried 50 mg/kg injection and noted that all the animals developed diabetes with no mortality.

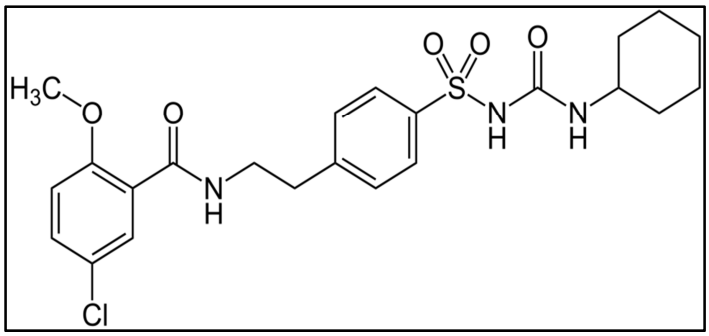
3.2 Effective dose of Punica granatum peel extract

As the dose of *Punica granatum* peel extract was not known, the effective dose in which it shows nephroprotective and antidiabetic activity at 50% of Gentamicin induced nephrotoxicity and STZ induced diabetic rats (ED50) was determined. The animals were administered orally with logarithmically graded dose increments of *Punica granatum* peel extract. The pairs of rats were given 10, 50, 100, 200, 400 & 500.

3.3 Dose of Glibenclamide [17]

Standard dose for glibenclamide is 0.5mg/kg. Glibenclamide (glyburide) belongs to sulfonylureas class of oral hypoglycaemics and is currently the most widely prescribed sulfonylurea[18].

3.4 Chemical Structure



5-chloro-N-(4-[N-(cyclohexylcarbamoyl)sulfamoyl]phenethyl)-2-methoxybenzamide

3.5 Mechanism of Action

Sulfonylureas stimulate insulin release by binding to a specific site on the cell K_{ATP} channel complex (the sulfonylurea receptor, SUR) and by inhibiting its activity. K_{ATP} channel inhibition causes cell membrane depolarization and the cascade of events leading to insulin secretion.

3.6 Acute Toxicity study

Seven groups of rats, two in each group were taken. Extract of *Punica granatum* peel was administered in doses of 10, 50, 100, 500, 1000, 5000 and 10000 mg/kg body weight orally and the animals were observed for toxic effects and mortality. No severe adverse effects or mortality was observed in any of the groups except diminished activity and the animals tolerated the drug well.

3.7 Induction of diabetes[19]

Streptozotocin was stored at 4 - 8° C. It was dissolved in sterile normal saline. It was always prepared freshly for immediate use. All rats were fasted overnight before diabetes was induced. STZ was given in the dose of 50 mg/kg body weight, single intraperitoneal injection. The animals were observed to be diabetic from the 3rd day onwards. The animals showing a blood glucose level of 200 mg/dl and above were considered diabetic and were included in the study.

3.8 Experimental design [20]

Animals were divided into 5 groups with 6 animals in each Group I (Control) received distilled water 5ml/kg body weight p.o, Group II STZ 50mg/kg body weight i.p induced diabetic rats were served as diabetic control group and were treated with distilled water orally daily until diabetes occur. Group III STZ induced diabetic rats were served as standard group and were treated with Glibenclamide at a dose of 0.5 mg/kg body weight p.o, daily. Group IV (test group-1) STZ induced diabetic rats were treated with *Punica granatum* extract at a dose of 100 mg/kg p.o, daily. Group V (test group-2) STZ induced diabetic rats were treated with *Punica granatum* extract at a dose of 200 mg/kg dose p.o, daily.

3.9 Collection of blood sample

Blood was drawn from the tail vein of the rat through a sterile tuberculin syringe. A very gentle aspiration was done, in order to avoid vein collapse.

3.10 Estimation of blood glucose

Blood glucose estimation was done by using One Touch - Horizon glucometer. The test strip was inserted into the glucometer and the sample was directly placed on the test strip. The result i.e., the blood glucose level will appear on the screen within five seconds in mg/dl.

3.11 Standard drug administration

Glibenclamide was administered through an oral feeding tube in diabetic rats for 15 consecutive days at a dose of 0.5 mg/kg body weight. The blood glucose concentrations were monitored on 0,5,10 and 15 days.

3.12 Test drug administration

The ethanolic extract of *Punica granatum* dried peel was administered orally at a dose of 100 mg/kg and 200 mg/kg body weight to groups IV & V respectively through an oral feeding tube for 15 consecutive days. The blood glucose concentrations were monitored on 0,5,10 and 15 days.

4. STATISTICAL ANALYSIS

All the values were expressed as Mean ± S.D., standard error of mean and percentage reduction were calculated. The test of significance was done by using student t-test.

5. RESULTS

The present study was carried out to evaluate the Antidiabetic effect of *Punica granatum* peel extract against Streptozotocin induced diabetes in albino Rats. They were divided into 5 groups with 6 animals in each. Blood glucose levels in each group of animals were monitored on 0, 5, 10 and 15th days, the results were shown in Table-I and the corresponding graphical representation was shown in Figure -I. The percentage reduction for various groups is as follows, Group III animals when compared with diabetic control groups were about 18.97% on the 0 day, 36.74% on the 5th day, 63.82% on the 10th day, and on day 15, it was about 73.14%. Whereas for Group IV animals the blood glucose levels were about 6.62% on the 0 day, 26.91% on 5th day, 45.49% on the 10th day and 69.14% on 15th day and the value recorded, And for Group V blood glucose levels were about 6.87% on the 0 day, 31.14% on 5th day, 52.88% on the 10th day and 71.67% on 15th day. The blood glucose levels of all groups with Mean±SD, standard error of Mean and

percentage reduction were compared together shown in Table-I and the differences were shown graphically in Graph-I. Simultaneously there was an increase in the body weight of rats when compared with the Diabetic group animal's i.e shown in Table-II and Graph-II. The results were recorded and presented in tabular forms as well as graphically. The outcome of the study was discussed and conclusions were drawn.

Table – I Mean blood glucose levels of all the groups

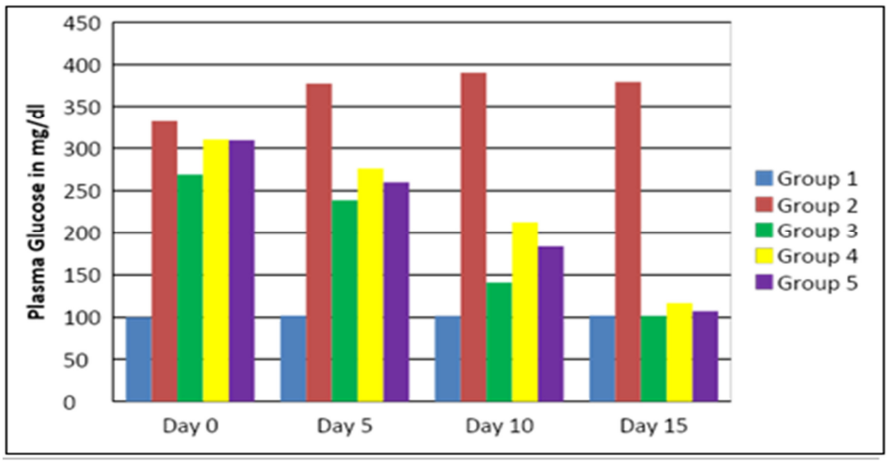
Group	Parameters	Day – 0	Day – 5	Day – 10	Day – 15
Normal Control	Mean ±SD	100.16±9.84	102.16±10.22	101.83±13.11	102.166±10.22
	SEM	4.020	4.174	5.35	4.174
Diabetic Control	Mean ± SD	332.16±41.82	377.50±45.36	390.166±60.04	379.166±33.43
	SEM	17.07	18.519	24.513	13.64
Diabetic Standard (Glibenclamide 0.5 mg/kg)	Mean ± SD	269.16±22.95	239.00±22.46	141.16±16.37	101.83±9.43
	SEM	9.37	9.172	6.68	3.850
	% reduction	18.97	36.74*	63.82**	73.14**
Diabetic Test-1 (*Punica granatum* 100mg/kg)	Mean ± SD	310.17±18.129	276.16±21.479	212.66±24.64	117.00±18.13
	SEM	7.40	8.76	10.062	7.40
	% reduction	6.62	26.91*	45.49**	69.14**
Diabetic Test-2 (*Punica granatum* 200mg/kg)	Mean ± SD	309.33±14.44	260.16±26.42	183.83±19.56	107.4±7.63
	SEM	5.897	10.787	7.989	3.117
	% reduction	6.87	31.14*	52.88**	71.67**

*P value<0.05, **P value<0.01. All the values are expressed as Mean ± SEM.

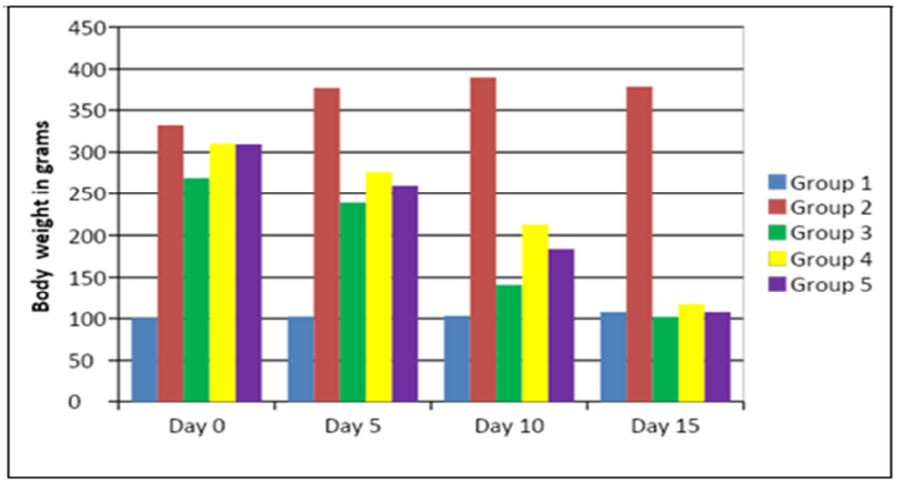
Table – II Mean body weight of all the groups

Group	Parameters	Day – 0	Day – 5	Day – 10	Day – 15
Normal Control	MEAN ± SD	211.67±20.14	213.33±18.36	211.33±20.35	214.33±17.41
	SEM	8.22	7.50	8.31	7.11
Diabetic Control	MEAN ± SD	190.83±6.11	173.0±6.164	160.83±6.33	141.83±3.48
	SEM	2.49	2.51	2.58	1.42
Diabetic Standard (Glibenclamide 0.5 mg/kg)	MEAN ± SD	188.16±5.87	178.83±4.53	164.16±3.06	151.83±4.44
	SEM	2.40	1.85	1.24	1.81
Diabetic Test-1 (*Punica granatum* 100mg/kg)	MEAN ± SD	191.5±5.04	179.33±4.08	160.33±3.26	149.16±2.85
	SEM	2.06	1.66	1.33	1.16
Diabetic Test-2 (*Punica granatum* 200mg/kg)	MEAN ± SD	189.16±5.11	178.66±5.11	171.0±4.979	153.16±4.02
	SEM	2.08	2.02	2.03	1.64

*P value<0.05, **P value<0.01. All the values are expressed as Mean ± SEM



Graph –I Mean blood glucose levels of all the groups



Graph –II Mean body Weight levels of all the groups

6. DISCUSSION

In the present study, ethanol extract of *Punica granatum* peel was given to different groups of animals at a dose of 100 and 200mg/kg body weight (groups IV and V) and the blood glucose lowering effect of these groups were compared with the diabetic control group. Statistical analysis of the test groups showed significant reduction in blood glucose levels, especially from the 5th day onwards. When compared to the diabetic standard group, the following observations were made.

- The diabetic test group I which were treated with 100 mg/kg extract showed anti-diabetic effect but it was not as significant as the diabetic standard group treated with glibenclamide.
- The diabetic test group II (200mg/kg) showed statistically comparable anti-diabetic effect as to the diabetic standard group and percentage reduction was also as effective as glibenclamide.
- There was a significant difference in glucose lowering effects of diabetic test group I (100 mg/kg) and diabetic test group II (200 mg/kg) which implies 200 mg/kg to be the ideal dose where the effect is significant and further increment in dose might increase its effect.

During this prolonged study, various physical parameters were also observed such as body weight[21], food intake, water intake, and weight of internal organs[22]. And also peel extract possess and stabilize free radicals[23] which reduce the stable radical DPPH to diphenylpicryl hydrazine[24]. Generally, body weights are reduced in diabetic animals, but in this study, the decrease in body weights was diminished by the extract treatment; thus this effect may be useful for the diabetic animals. The phytochemical study showed the presence of terpenoids such as ursolic acid and tannins[25], flavonoids[26], polyphenols, oleanolic acid compounds in the extracts, which might be a reason for the good activity of extract[27,28]. Sushil kumar middha et al[13] in his studies suggest that high content of phytochemical compound i.e phenolic active present in *Punica granatum* Aqueous peel has shown significant anti-diabetic activity and the mechanistic anti-diabetic activity of the extract is by stimulation, regeneration, and increased number of β-cells, by protecting pancreatic tissue and subsequent release of insulin[29]. One more study done by Das and Sarma also showed the anti-diabetic activity of fruit *PEPG* extract was given for 28 days significantly increased the body weight when compared with diabetic control in dose dependent manner[30,31]. *Punica granatum* can be considered as an important addition to the therapeutic armamentarium for the treatment of diabetes.

7. CONCLUSION

Diabetes mellitus is a globally prevalent chronic debilitating illness. The goals in management of diabetes are to alleviate various symptoms and signs, and to prevent or reduce the acute and chronic complications. There are a range of drug agents available for the treatment of diabetes but they are costly and have many adverse effects which warrant the continuous research for newer drugs. The result of the present study shows that *Punica granatum* peel extract brings back the blood glucose levels to normal in STZ – induced diabetic rats i.e. shows hypoglycemic activity and it has a greater antioxidant activity there by lowering the diabetic complications. Thus, instead of increasing the dose of the conventional oral hypoglycemic agent, which would result in more side effects, a combination of the low dose oral hypoglycemic agent with low dose or even higher dose of extract, which not only serves to control the blood glucose levels but also has many advantages as mentioned above, can be used in the treatment of diabetes mellitus.The hypoglycaemic activity of *Punica granatum* peel extract may be due to the insulin secretagogue effect of the active compound and also suggested that the effect may be due to the presence of terpenoids such as ursolic acid and oleanolic acid, which may help to scavenge the free radicals generated during diabetes, which are present in the extract and prolonged administration may stimulate the β cells of islets of langerhans to produce insulin The present study concluded that the *Punica granatum* peel extract has Anti-diabetic property and further studies are needed to evaluate its Anti-diabetic effect in different species and to find out the most probable mechanism of action. The reasons for the present study are

a) Only 1 or 2 studies are done exclusively over the antidiabetic property of *Punica granatum peel* extract.
b) Very easily available and economical.
c) Cost effective in treating multiple diseases (benefitting the poor)

Further studies can be undertaken at the cellular and molecular levels, which may further elucidate its mechanism in detail. The present investigation has also opened avenue for further research especially with reference to the development of potent formulation for diabetes mellitus from Punica granatum.

8. AUTHOR CONTRIBUTION STATEMENT

All authors discussed the results and commented the manuscript.

9. ACKNOWLEDGEMENT

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10. CONFLICT OF INTEREST

Conflict of interest declared none

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