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Protective Activity of Aqueous Pericarp Extract of *Punica granatum* Against Hyperglycaemia Induced by streptozotocin in Rats

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Diabetes mellitus is a chronic metabolic disorder characterized by raise in blood glucose levels known as hyperglycemia. In the present study, the aqueous pericarp extract of Punica granatum (*APPG*) was evaluated for hypoglycemia and its antihyperglycemic activity in streptozotocin-induced diabetic rats. The % inhibition of blood glucose levels in hypoglycemic activity of *APPG* 50 mg/kg and 100 mg/kg were found to be 26.91% (3hr) & 34.67% (6h) and 22.51% (3hr) & 25.24%(6 hr) hypoglycaemic normal rats. The antihyperglycemic activity of *APPG* with 150 mg/kg and 300 mg/kg in STZ induced diabetic rats were found to be 31.38% and 35.42% at 6hr respectively. The biphasic reduction was observed in both in normal and diabetic rats might be due to biphasic absorption or biliary secretion of the active principle present in *APPG*. At the end of 12 weeks study period the serum parameters were found to be increased in diabetic rats and treatment with *APPG* there is a significant reduction in all the parameters. In conclusion, the *APPG* found to have significant antihyperglycemic, antihyperlipedemia activity and significant protection against the damage to kidney. Which might be due to antioxidant potentials like flavonoids, polyphenols and tannins present in the *APPG*.

Key words: Diabetes mellitus, antihyperglycemic activity, Punica granatum

Diabetes mellitus the most common endocrine disorder of carbohydrate metabolism is affecting approximately 8.3% of the population worldwide (IDF Diabetes Atlas, 2013). In 1675 Thomas Willis added the word 'mellitus' to the disease , a word from Latin meaning 'Honey' a reference to the sweet taste of the urine (Chopra RN *et al*., 1956). Diabetes mellitus is not a single disease entity, but rather a group of metabolic disorders sharing a common underlying feature of hyperglycemia. Hyperglycemia in diabetes, results from defects in insulin secretion, insulin action or most commonly both (Robbins *et al*., 2004). Chronic elevation of blood glucose level leads to damage of blood vessels (angiopathy). The endothelial cells lining the blood vessels take in more glucose than normal, since they don't depend on insulin. They then form more surface glycoproteins than normal, and cause the basement membrane to grow thicker and weaker. In diabetes, the resulting problems are grouped under "microvascular disease" (due to damage to small blood vessels) like retinopathy, nephropathy & neuropathy and "macrovascular disease" (due to damage to the arteries) like cardiomyopathy.

Different groups of oral hypoglycemic agents are currently available with characteristic profiles of side effects (Prout 1974; Holman and Turner 1991; Williams and Pickup 1991; Kameswara, 1997). The search for antidiabetic

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agents with little or no side effects is continuous processes. The plant kingdom is a wide field to look for effective oral hypoglycemic agents. More than 300 species have been reported to display hypoglycemic activity (Rahman and Zaman, 1989) but only few of them have been investigated despite the World Health Organization (WHO) recommendation that traditional plant remedy for diabetics warrant further evaluation (WHO, 1980).

Effective control of blood glucose level is a key step in preventing or reverses diabetic complications and improving the quality of life in both type 1 and type II diabetic patients (Xie JT *et al*., 2005). In the history of Unani, Ayurveda, Siddha or Homeopathic has been well documented that illness can be managed purely by herbal preparations, thus the diabetic individual could lead a healthy life as non-diabetics. Experiments and clinical trials conducted worldwide have provided dependable evidences on the effects of various herbal formulations in the maintenance of normal blood sugar level. These invaluable findings are now conclusively processed in the backdrop of Ayurveda, Unani and Siddha system (Ameen Syed M. M *et al*., 2005).

Punica granatum L. (Punicaceae), commonly known as pomegranate. Pomagranate pericarp is a rich source of tannins, flavanoids, polyphenols and some anthocyanins. The potential therapeutic properties of Punica granatum are treatment and prevention for diabetes (Middha *et al*., 2012), cancer (Dikmen M *et al*., 2011; Hong M Y., 2008) , cardiovascular disease (Jurenka J *et al*., 2008), dental conditions (Viuda- Martos M *et al*., 2010), and erectile dysfunction (Kanatta S R *et al*., 2010), protection from ultraviolet (UV) radiation (Kanatta S R *et al*., 2010) and antimicrobial (Eswar Kumar *et al*., 2013).Other potential applications include infant brain ischemia, Alzheimer's disease (Middha *et al*., 2012), male infertility, arthritis (Kanatta S R *et al*., 2010), dermal wounds (Hayouni EA *et al*., 2011)and obesity (Kanatta S R *et al*., 2010).

MATERIALS AND METHODS

Chemicals

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

Plant Materials

The ripened pomagranate *(Punica granatum*) were obtained from local market. The pericarps were manually separated and shade dried. The pericarps were powdered in a grinder to get 40-mesh size powder. The moisture content of pericarp powder was found to be 13.5%. The powder was suspended in 2% gum acacia and used in the experimental studies.

Animals

Animals were obtained from the Tina laboratories, Hyderabad. Albino Wistar rats (180-200 g) of male were used in the present study. The animals were housed under standard environmental conditions (23±1ºC) with relative humidity of 50±10% and maintain 12:12 dark and light cycle, maintained with free access to water and *ad libitum* standard laboratory diet (70% carbohydrates, 25% proteins, 5% lipids (Hindustan liver Bangalore). After randomization before the experiment, the rats were acclimatized for a period of two weeks. The animal housing and handling were in accordance with CPSCEA guidelines. Our college was approved by CPCSEA for conducting animal experiments with the registration No. 516/01/A/CPCSEA. The prior permission for the study was obtained from our Institutional Animal Ethics Committee (IAEC).

Induction of Diabetes

The rats were fasted for 18 h prior to the experiment with water *ad libitum*. The rats were injected intraperitoneally with nicotinamide 100mg/kg. After 15 minutes streptpozotocin (STZ) were administered. STZ dissolved in citrate buffer at a dose of 55 mg/kg body weight. Animals were treated with 10% glucose to combat the early phase of hypoglycemia. Blood samples were collected after 72 hours of STZ treatment and the induction of diabetes mellitus was confirmed by estimation of fasting blood glucose levels (FBG). Only those rats with blood glucose levels e"250 mg/dl were included in the study (Day 0)

EXPERIMENTAL

Procedures

Control group was administered with distilled water, aqueous pericarp extract of *Punica granatum* (APPG) at 50mg/kg and 100 mg/kg of rat body weight to group-I, and group-II respectively.

Blood samples were withdrawn at 0, 1, 2, 3, 4, 6, 8, 10 & 12h intervals by retro-orbital puncture method and were analyzed for blood glucose by GOD/POD method using SCREEN MASTER 3000 autoanalyser.

After the induction of diabetes, the rats were grouped in to eleven different groups of each containing six animals. Group 1 contains control rats received distilled water and fed on normal diet, group 2 as diabetic control received vehicle only, group 3 contains diabetic rats treated with gliclazide at a dose of 1 mg/kg body weight, group 4 contains diabetic rats received *APPG* at a dose of 50 mg/kg body weight, group 5 contains diabetic rats received *APPG* at a dose of 100 mg/kg body weight for 12 weeks. Treatment with drugs was started after 72 hours of STZ treatment (i.e. Day 1) and was continued for 12 weeks. All drugs were given orally as a single oral dose. Blood glucose was measured before starting the treatment (day0) and 4 weekly thereafter up to the end of the treatment and estimated fasting blood glucose by glucose-oxidase-peroxidase (GOD-POD) method. All the treatment groups were compared with diabetic control group.

Biochemical assays

At the end of the 12 week study period, rats were fasted overnight and blood samples were withdrawn through the retroorbital plexus using glass capillary. Blood was allowed to clot and serum was separated by centrifugation at 4000rpm for 10 min. Serum glucose levels were estimated at 0, 1, 2, 3, 4, 6 and 8 hours intervals. Serum glycosylated haemoglobin, triglycerides, total cholesterol, HDL, LDL, VLDL, bilirubin, creatinine, albumin, total protein, urea, uric acid and BUN levels were estimated. Serum glucose levels were estimated by GOD/POD method. Triglyceride, total cholesterol, HDL was measured by commercially available kits (Bucolo G *et al*., 1973) (Nader R *et al*., 2001). Bilirubin ((Jendrassik, 1938), creatinine (Bowers L D, 1980), total protein (Tietz, N.W 1996), albumin (Doumas BT *et al*., 1972), uric acid (Tomas L *et al*., 1998)), urea (Fossati P *et al*., 1980). At the end of the study all the rats were dissected and pancreas was used for histopathological studies.

Statistical analysis

All the data were expressed as mean SEM. Statistical analysis was carried out using one way ANOVA followed by Dunnet's multiple comparison test.

RESULTS AND DISCUSSION

The WHO Expert Committee recommended the importance to investigate and explore hypoglycemic agents from plant origin because plants used in the traditional medicine have fewer side effects than synthetic drugs (Alarcon-Aguilara F J *et al*., 1998). So in the present study discussed about the hypoglycemic and antihyperglycemic effects of *APPG*. The doses of selected fruit pericarp extracts were fixed basing on their acute toxicity study in mice and preliminary hypoglycemic studies in Wistar albino rats. The doses that produce optimal and dose dependent reduction in blood glucose levels were selected for hypoglycemic and antihyperglycemic studies.

Some medicinal plants with hypoglycemic properties are known to increase circulating insulin level (pancreatic mechanism) in normoglycemic rats (Lamela *et al*., 1985). Another possible mechanism of action is that the extracts might stimulate residual pancreatic mechanism (extra pancreatic), probably increasing peripheral utilization of glucose as postulated by Erah *et al*., 1996. The *APPG* were shown significant hypoglycaemic activity with biphasic effect. The biphasic effect might be due to biphasic absorption or enterohepatic recirculation. We hypothesized that *APPG* could have a sulfonylurea-like mechanism since they significantly decreased the blood glucose levels in normoglycaemic rats. Sulfonylurea compounds lower blood glucose in normal and in diabetic animals by stimulating insulin release from pancreatic ^{2} cells and by peripheral utilization of glucose (Swathi p *et al*., 2014[a]). The *APPG* shown to have better hypoglycemic activity compared to standard Gliclazide in normal rats. Streptozotocin (STZ) is used to induce diabetes mellitus in albino Wistar rats, a poly ADP ribose inhibitor, nicotinamide was administered before 15 minutes of STZ administration to offer partial protection against the action of STZ in rats. So in the present study we used the Streptozotocin – nicotinamide model to prevent the excessive damage to the pancreas of diabetic rats. In this study, treatment with the *APPG* significantly reduced the elevated plasma glucose levels in STZ induced diabetic rats. The *APPG* shown delayed absorption in diabetic animals compared to normoglycemic animals, this might be due to delayed gastric absorption and

motility in diabetic condition as diabetes affecting the digestive processes, the motility and nervous control of the entire system of gastrointestinal tract. The effect of diabetes on digestive system can also cause malabsorption (Bener *et al.*, 2012; Bernstein, 2000). The *APPG* shown to have better antihyperglycemic activity compared to standard gliclazide in STZ induced diabetic rats.

Streptozotocin (STZ) induced diabetic rats enhanced the level of glycated hemoglobin (HbA1c) due to raise in levels of glucose in blood which further react with hemoglobin and produce the glycated hemoglobin formation (Pari L *et al.*, 2004). The *APPG* significantly lowered the blood glucose, which lead to the decrease in the levels of glycated hemoglobin.

The levels of serum lipids are usually elevated in diabetes mellitus (Pushparaj P *et al.*, 2000). This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots. It is reported that hypercholesterolemia (increased levels of total cholesterol) and hypertriglyceridemia (increased levels of triglycerides) occurs in STZ-induced diabetic rats (Pushparaj P *et al*., 2000; Pushparaj N P *et al*., 2001; Swathi P *et al*., 2014[b]). Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides (Taskinen M R 1987). However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. The *APPG* significantly reduced the levels of total cholesterol, triglycerides, LDL and VLDL and increased the levels of HDL. The *APPG* shown to have better antihyperlipidemic activity compared to standard gliclazide in STZ induced diabetic rats (table 3).

The serum bilirubin levels were found to be increased in STZ induced diabetic rats.Rana*et al.*, 1996reported that the increase in serum bilirubin (hyper-bilirubenimia) in STZ induced diabetic rats, may be resulted from the decrease of liver uptake, conjugation or increase total bilirubin, direct bilirubin production from hemolysis. The elevation in serum bilirubin indicates liver damage (Swathi P *et al*., 2012). The *APPG* shown to have better hepatoprotective activity than standard gliclazide. The estimation of total protein is useful for measuring gross changes in protein levels caused by various disease states. In diabetic conditions

Table 1. Effect of APPG on hypoglycemic activity in normal rats during 12 hours

	Time (hours)											
	0	1	2	3	4	6	8	10	12
Control	0.00±0.00	0.00±0.00	0.15±0.38	1.24±0.30	1.53±0.57	1.57±0.18	1.91±0.52	3.18±0.23	2.99±0.26
Standard	0.00±0.00	33.0±2.80	24.9±4.40	15.9±6.00	13.6±4.84	14.7±5.15	26.6±4.84	12.4±3.72	12.3±3.70
APPG (50mg/kg)	0.00±0.00	40.5±2.43	28.55±2.27	15.39±2.73	05.62±2.09	33.12±2.11	19.60±2.60	09.74±2.41	0.01±1.88
APPG(100mg/kg)	0.00±0.00	-0.54±2.02	44.58±1.30	30.02±0.58	12.98±1.20	35.78±0.61	22.70±1.00	10.82±1.26	01.04±1.53

Table 2. Effect of APPG on antihyperglycemic activity in STZ induced diabetic rats

	Time (hours)					
	0	1	2	4	6	8
Control	0.00±0.00 ^{ns}	0.96±0.35 ^{ns}	02.10±0.39 ^{ns}	02.95±0.28 ^{ns}	03.91±0.28 ^{ns}	05.20±0.30*
D.Control	0.00±0.00 ^{ns}	0.28±0.07	0.36±0.12	0.69±0.06	0.80±0.07	1.27±0.05
standard	0.00±0.00 ^{ns}	2.39 ±1.42 ^{ns}	13.22 ± 2.01$^{\$}$	32.00 ±1.03$^{\$}$	25.02 ± 0.7$^{\$}$	20.59 ± 0.94$^{\$}$
APPG (50mg/kg)	0.00±0.00 ^{ns}	0.04± 2.09 ^{ns}	9.9 ±1.4$^{\$}$	16.8± 1.7$^{\$}$	34.52± 1.08$^{\$}$	22.93± 1.3$^{\$}$
APPG(100mg/kg)	0.00±0.00 ^{ns}	07.15±1.69$^{\$}$	20.24±2.35$^{\$}$	41.58±1.20$^{\$}$	25.97±0.84$^{\$}$	12.58±1.58$^{\$}$

p>0.05^{ns}, p<0.05*, p<0.01$^{\#}$, p<0.001$^{\$}$ Significance followed by one way ANOVA followed by Dunnet's multiple comparison test when compared with disease control group.

Table 3. Effect of APPG on lipid profile of STZ induced diabetic rats

Groups	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
control	80.3 ±1.8*	127.8± 2.0 *	62.0±1.9 *	49.5±2.9 *	16.0± 0.3*
Diabetic Control	132.5± 3.0	241.8± 4.6	36.3 ±1.3	179.0±4.9	26.5± 0.6
Standard	82.5 ±2.6 *	130.6± 3.1 *	58.0 ±2.0 *	56.16±4.9 *	16.5± 0.5*
APPG(50mg/kg)	92.1± 2.3 *	135.8 ±1.9 *	60.5 ±2.1 *	56.9±2.3 *	18.4± 0.4*
APPG(100mg/kg)	83.8± 2.7 *	133.0±1.9 *	57.1 ±1.9 *	59.2±2.8 *	16.5± 0.5*

P>0.05^{ns}, P>0.001*, P>0.01$^{\#}$, P>0.05 $^{\$}$ two way ANNOVA followed by bonferronipost test when compared with toxicant group

the circulating protein binds with free reducing sugars leads to formation amadori products. The *APPG* able to increase the protein levels may be by breaking the link between the reducing sugars and amino acids of proteins. Urea is the major nitrogen containing metabolic product of protein metabolism, uric acid is the major product of purine nucleotides, adenosine and guanosine; creatinine is endogenously produced and released into body fluids and its clearance measured as an indicator of glomerular filtration rate (Burtis CA *et al*., 1996). The metabolism of protein is found to be increased in the diabetic rats as indicated by increase in the levels of serum urea, uric acid and decreased levels of proteins as explained above. The *APPG* shown to decrease the levels of urea and uric acid probably by decreasing the metabolism of proteins.

The treatment with the *APPG* found to be useful in reducing the damage caused due to hyperglycaemia induced by STZ. The *APPG* was shown to have better and comparable antihyperglycaemic activity with standard gliclazide. Serum creatinine and serum BUN levels measurement is taken as an index of altered GFR in diabetic nephropathy (Sugimoto H *et al*., 1999). Our results showed that the level of serum

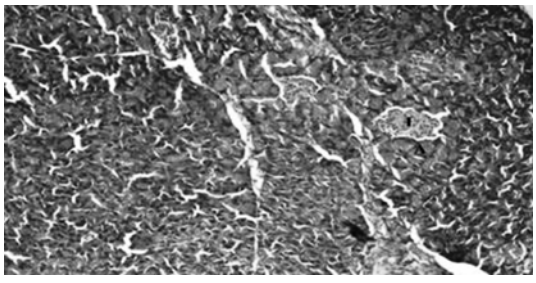

Fig. 1. Effect of control on histopathological studies on rat pancreas

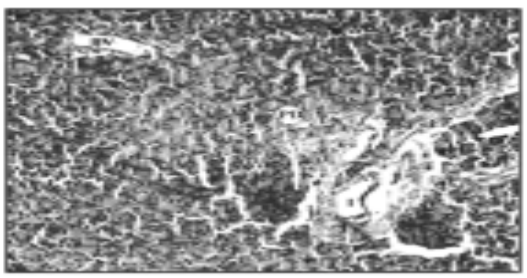

Fig. 2. Effect of disease control on histopathological studies on rat pancreas

Table 4. Effect of different concentrations of *APPG* on kidney parameters on normal and Streptozotocin induced diabetic and normal rats

Groups	HbA1c	T.bilirubin	D.bilirubin	Creatinine	Total protein	Albumin	Uric acid	BUN
Control	4.37±0.64*	0.75±0.018*	0.69±0.019$	0.69±0.02*	6.15±0.17*	3.72±0.10*	3.63±0.17*	21.95±0.45*
D.Control	11.68±0.41	1.75±0.055	1.22±0.013	1.90±0.03	3.95±0.23	7.20±0.10	8.14±0.21	36.20±0.20
Standard	03.88±0.34*	0.72±0.025*	0.69±0.021 $	0.78±0.02 *	5.87±0.67*	4.10±0.06 *	4.13±0.118	22.44±0.33*
APPG(50mg/kg)	4.92±0.29*	0.81±0.014 *	0.77±0.019 ns	0.91±0.04 *	6.90±0.24*	4.24±0.11*	4.98±0.10*	25.33±0.32*
APPG(100mg/kg)	3.58±0.30*	0.74±0.028 *	0.67±0.017 $	0.71±0.03	8.06±0.18*	3.94±0.12*	3.67±0.15*	23.26±0.38*

P>0.05^{ns}, P<0.001*, P<0.01#, P<0.05 $ two way ANNOVA followed by bonferroni post test when compared with toxicant group

creatinine and BUN levels was significantly elevated in diabetic animals. The treatment with *APPG* for 12 weeks shown significant reduction in the creatinine and BUN.

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 secretary 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

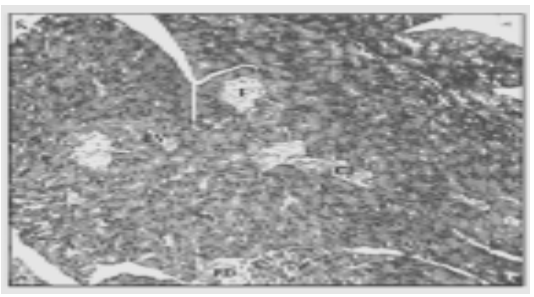

Fig. 3. Effect of standard on histopathological studies on rat pancreas

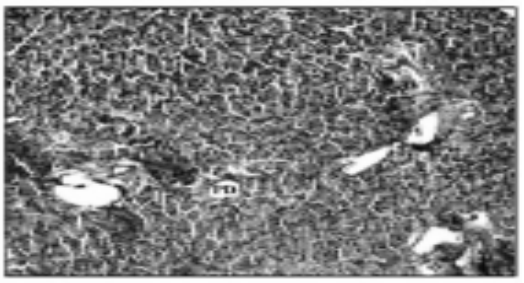

Fig. 4. Effect of APPG (50mg/kg) on histopathological studies on rat pancreas

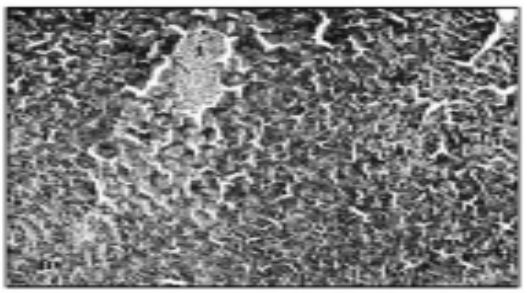

Fig. 5. Effect of APPG (100mg/kg) on histopathological studies on rat pancreas.

of secretary cells. The treatment with the *APPG* extract and Gliclazide for 12 weeks found to prevent the degenerative changes in STZ induced diabetic rats.

CONCLUSIONS

It is concluded that, The *Punica granatum* showed better hypoglycemic and antihyperglycemic activity against STZ induced diabetic rats. All the activities might be due to high levels of flavanoids, tannins and polyphenols present in aqueous pericarp extract of *Punica granatum.*

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REFERENCES:

1. Alarcon-Aguilara F J, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber C C, and Flores- Saenz J L. Study of the anti-hyperglycemic effect of plants used as antidiabetics. *Journal of Ethnopharmacology* 1998; **61**(2): 101–110
2. Ameen Syed M M. Traditional medicine was a valuable resource in the health care. *Niamath's Herbal Unani Med*, 2005; **6**(4): -5.
3. Bener A, Ghuloum S, Al-Hamaq A O, &Dafeeah E E. Association between psychological distress and gastrointestinal symptoms in diabetes mellitus. *World Journal of Diabetes* 2012; **3**(6): 123–129.
4. Bower L D & Wong E T. Kinetic serum creatinine assays. A critical evaluation and review, *Clin.Chem* 1980; **26**: 555-561.
5. Bucolo G, David M. Quantitative determination of serum triglycerides by the use of enzymes. *Clin .Chem* 1973; **19**: 476-482.
6. Burtis CA, Ashwood ER. Teitz. Fundamentals of clinical chemistry. *Enzymes* 1996; **4**: 312–35.
7. Chopra RN, Nagar SL, Chopra IC. Glossary of indian medicinal plants. CSIR, Newdelhi, 1956.
8. Dikmen M, Ozturk N, and zturk Y O. The antioxidant potency of *Punica granatum*L. fruit peel reduces cell proliferation and induces apoptosis on breast cancer," *Journal of Medicinal Food* 2011; **14**(12): 1638–1646.
9. Doumas BT, Arends RI. Pinto PC in standard *methods of Clinical Chemistry*. 1972; **7**: 175-189.
10. Erah P.O, Osuide G.E, Omogbai E.K.I. Hypoglycemic effect of the extract of Solenostemon monostachysleaves. *Journal of West Africa Pharmaceutics* 1996; **10**: 21-27.
11. Fossati P, Prgncipe L, Berti G. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin Chem* 1980; **26**: 227–31.
12. Fossati, Prencipe L, Berti G. Enzymic Creatinine Assay: A New Colorimetric Method Based on Hydrogen Peroxide Measurement. *Clin. Chem* 1980; **26**: 227-231.
13. Holman RR, Turner RC., Oral agents and insulin in the treatment of NIDDM. In: Pickup JC, Williams G (eds) Text book of Diabetes. Blackwell, Oxford. 1991; 467-469.
14. Hong MY, Seeram NP, and Heber D. Pomegranate polyphenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells overexpressing the androgen receptor, *Journal of Nutritional Biochemistry* 2008; **19**(12): 848–855.
15. IDF Diabetes Atlas, International diabetes federation. 6th Edition, 2013.
16. Jendrassik L & Grof P. Colorimetric Method of Determination of bilirubin. *Biochem.*Z 1938; **297:** 81-82.
17. Junqueira LC and Carneiro J., Basic histology: Text and atlas. 11thed. McGraw-Hill, 2005.
18. Jurenka J. Therapeutic applications of pomegranate (*Punica granatum*L.): a review, *AlternativeMedicine Review* 2008; **13**(2): 128-144.
19. Kameswara RB, Giri R, Kesavulu MM, Apparao Ch. Herbal medicines in the treatment of diabetes mellitus. *Manphar Vaidya Patrika* 1997; **1**(45): 33–35.
20. Eswar Kumar K, Swathi Putta, Nagendra sastry. Y. *In-vitro* antimicrobial and antioxidant activities of aqueous pericarp extract of *Punica Granatum. Journal of Applied Pharmaceutical Science*. 2013; **3**(08): 107-112.
21. Lamela M, Cadavid I, Gato A, Calleja JM. Effect of Lythrumsaricariain normoglycemic rats. *Journal of Ethnopharmacology* 1985; **41**: 83-91.
22. Middha S K, Usha T, and RaviKiran T. Influence of *Punica granatum* L. on region specific responses in rat brain during alloxan-induced diabetes, Asian Pacific *Journal of Tropical Biomedicine* 2012; **2**(2): 905–909.
23. Nader R, Bachorik P S, Albers J J., Lipids,

Lipoproteins, and Apolipoproteins. Tietz Fundamentals of Clinical Chemistry, 5th Ed W.B. Saunders eds, Philadelphia USA 2001; 463-469

24. Pari L, Saravanan R. Antidiabetic effect of diasulin, an herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats. *Diabetes Obes Metab* 2004; **6**:286–292.
25. Prout TE, Malaisse WJ, Pirart J., Procedings (VIII) of Congress of International Diabetes Federation. *Excerpta medica* 1974; 162.
26. Pushparaj NP, Tan HKB, Tan HC. The mechanism of hypoglycemic action of the semi-purified fractions of Averrhoabilimbiin streptozotocin diabetic rats. *Life Sci* 2001; **70**: 535-47.
27. Pushparaj P, Tan CH, Tan BKH. Effects of Averrhoabilimbileaf extract on blood glucose and lipids in streptozotocin-diabetic rats. *J Ethnopharmacol* 2000; **72**: 69-76.
28. Rahman AU, Zaman K. Medicinal plants with hypoglycemic activity. *J ethnopharmacol* 1989; **26**: 1-33.
29. Robbins and Cotran. The endocrine pancreas. Pathologic basis of disease. *Elsevier* 2004; 1189-1207.
30. Sugimoto K, Sakamoto K, Fujimura A. Decrease in oral bioavailability of cyclosporin A by coadministration of probucol in rats. *Life Sci* 1997; **60**: 173–179.
31. Swathi P, Eswar Kumar K, Jagadeesh Kumar T, vijay CH. Evaluation of anti hyperglycemic and anti hyperlipidemic activity of ethonolic extract of Tylophora indica in alloxan induced diabetic rats. *Int j curr pharm res*. 2012; **4**(1): 25-31.
32. Swathi P[a], Eswar Kumar K. Effect of hydroalcholic pericarp extract of Momordica chochinchinenesis on hypoglycemic and antihyperglycemic activities in normal and streptozotocin induced diabetic rats. *World Journal of Pharmaceutical Research*. 2014; **3**(8): 1-13.
33. Swathi P[b], Eswar Kumar K. Effect of methonolic pericarp extract of Feronia limonia on hypoglycemic and antihyperglycemic activities in normal and streptozotocin induced diabetic rats. *J. Phamac. Toxicol*. 2014; 1-19.
34. Taskinen MR. Lipoprotein lipase in diabetes. *DiabMetab Rev* 1987; **3**: 551–70.
35. Tietz N W. Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders Company 1996; 240.
36. Tomas L. Clinical laboratory diagnostics, 1st ed., Frankfurt: TH-Books Verlagsge sells chaft 1998; 208–14.
37. Viuda-Martos M, Fernandez-Lopez J, and Perez J A. "Pomegranate and itsmany functional components as related to human health: a review," *Comprehensive Reviews in Food Science and Food Safety*. 2010; **9**(2): 635–654.
38. Williams G, Pickup JC. New drugs in the management of diabetes mellitus. Text book of Diabetes. Blackwell, Oxford 1991; 977–993.
39. Xie JT, Mehendale SR, Li X, Quigg R, Wang X, Wang CZ, Wu JA, Aung HH, Rue PA., Anti-diabetic effect of ginsenoside Re in ob/ob mice , *Biochimica et Biophysica Acta*. 2005; 319-325 (1740).