Antidiabetic activity of aqueous and methanolic extract of *punica granatum* leaves in alloxan-induced swiss albino diabetic

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ABSTRACT

Diabetes mellitus is a most common endocrine disorder, affecting more than 300 million people worldwide. For these therapies developed along the principles of allopathic are often limited in efficacy, Carry the risk of adverse effects, and are often too costly, especially for the developing world. In order to identify complementary or alternative approaches to existing medications, we studied the anti-diabetic potential of leaves of Punica Granatum. The acute oral toxicity studies of the extracts revealed no toxic effects up to the levels of 2000mg/kg b.wt. The aqueous and alcoholic extracts of 20 and 30mg/kg body weight of Punica Granatum was screened for the presence of hypoglycemic and antidiabetic activity. In this study diabetes was induced by a single IP dose Alloxan monohydrate in 72hrs fasted rats. The FBGL was carried on 7^{th}, 14^{th} and 21^{st} day and OGTT was measured on 8^{th}, 15^{th} and 22^{nd} day. Glibenclamide was taken as the standard and the results are quite comparable with it. The studies were indicated that the leaves of Punica Granatum are effective in regeneration of insulin secreting β-cells and thus possess antidiabetic activity. The aqueous and alcoholic extracts showed significant effect in decreasing the Fasting blood Glucose level and oral glucose tolerance test of rats and it's also showed good hypoglycemic activity in normal glycemic rats. The preliminary phytochemical analysis of the extracts of Punica Granatum revealed the presence of Alkaloids, Tannins, Anthraquinones, Flavonoids, Saponins, Triterpenes, Sterols, Coumarin as the possible biologically active principles.

Keywords: Punica Granatum, Alloxan monohydrate, Glibenclamide, FBGL and OGTT.

I. INTRODUCTION

1.1-Diabetes Mellitus (DM):

Diabetes is one of the most common non-communicable diseases and a serious life-long condition appearing worldwide. The etiology of diabetes is a complex interaction of genetic and environmental factors. It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic beta cells and deficiency in insulin secretion or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other manifestable disorders. It is an endocrinological syndrome abnormally having high levels of sugar in the blood. This may be either due to insulin not being produced at all, is not made at sufficient levels, or is not as effective as it should be.

Diabetes is still a serious health problem all over the world since it is associated with increased morbidity and mortality rate. When compared with the general population, mortality and morbidity increase in diabetes is mainly due to the associated chronic complications both specific (microvascular) and nonspecific (macrovascular). Since the disease prevails in both genders and in all age groups, the general public has a concern about its control and treatment[1].

1.2-Classification of DM

Diabetes is classified by underlying cause. The most common forms of diabetes are categorized as

Type 1, or insulin-dependent diabetes mellitus (IDDM) - an autoimmune disease in which the body's own immune system attacks the pancreatic beta cells, rendering it unable to produce insulin and

Type 2, or non-insulin-dependent diabetes mellitus (NIDDM) - in which there is resistance to the effects of insulin or a defect in insulin secretion.

Type 2 diabetes commonly occurs in adults associated with obesity. There are many underlying factors that contribute to the high blood glucose levels in these individuals. An important factor is the resistance to insulin in the body essentially ignoring its insulin secretions. A second factor is the decreased production of insulin by the cells of the pancreas. Therefore, an individual with Type 2 diabetes may have a combination of deficient secretion and deficient action of insulin. In contrast to Type 2 diabetes, Type 1 diabetes most commonly occurs in children and is a result of the body's immune system attacking and destroying the beta cells. The trigger for this autoimmune attack is not clear, but the result is the end of insulin production[2].

Multiple risk factors for the development of Type 2 diabetes mellitus[3]:

- Family history (parents with diabetes).
- Obesity (i.e., ≥20% over ideal body weight or body mass index ≥25kg/m^2).
- Habitual physical inactivity.

- Impaired glucose tolerance.
- Hypertension (≥140/90mm Hg in adults).
- High density lipoprotein (HDL) cholesterol ≤ 35mg/dl and/or triglyceride level ≥ 250mg/dl.

1.3-History

The term "Diabetes" was first used around 250 B.C. It is a Greek word meaning "to syphon", reflecting how diabetes seemed to rapidly drain fluid from the affected individual. The Greek physician Aretaeus noted that affected individuals passed increasing amounts of urine as if there was "liquefaction of flesh and bones into urine". The complete term "diabetes mellitus" was coined in 1674 by Thomas Willis. Mellitus is Latin for honey, which is how Willis described the urine of diabetics[5].

Historical accounts reveal that as early as 700-200 BC, diabetes mellitus was a well recognized disease in India and was even distinguished as two types, a genetically based disorder and other one resulting from dietary indiscretion. Ancient Hindu writings document how black ants and flies were attracted to the urine of diabetics. The Indian physician Sushruta in 400 B.C. described the sweet taste of urine from affected individuals, and for many centuries to come, the sweet taste of urine was a key to the diagnosis.

Physicians have observed the effects of diabetes for thousands of years. One of the effects of diabetes is the presence of glucose in the urine (glucosuria). For much of the time, little was known about this fatal disease that caused weight loss of body, extreme thirst, and frequent urination. It was in 1922 that the first patient was successfully treated with insulin. Till the mid-1800s, the treatments offered for diabetes varied tremendously. A breakthrough in the puzzle of diabetes came in 1889. German physicians Joseph von Mering and Oskar Minkowski surgically removed the pancreas from dogs. The dogs immediately developed diabetes. Now that a link was established between the pancreas and diabetes, research focused on isolating the pancreatic extract that could treat diabetes. Dr. Frederick Banting succeeded in his experiments of isolating a pancreatic extract. The diabetic dog was kept alive for eight days by regular injections until supplies of the extract, at that time called "isletin", was exhausted. Experiments on dogs showed that extracts from the pancreas caused a drop in blood sugar, caused glucose in the urine to disappear, and produced a marked improvement in clinical condition.

A young boy, Leonard Thompson, was the first patient to receive insulin treatment in the year 1922 and lived for thirteen years. Over the next 70 years, insulin was further refined and purified. A revolution came with the production of recombinant human DNA insulin in 1978. Instead of collecting insulin from animals, new human insulin could be synthesized. In 1923, Banting and Macloed were awarded the Nobel Prize for the discovery of insulin. In his Nobel Lecture, Banting concluded the following about their discovery: "Insulin is not a cure for diabetes; it is a treatment."

1.4-Epidemiology[6]

Present status projects that incidence of diabetes is on rise. Present number of diabetics worldwide is 150 million and according to new estimates from researchers at the World Health Organization (WHO), there will be an increase of about 300 million or more by the year 2030 (Warner, 2004). Only in year 2001, about 441,004 deaths were registered and 49,855 of them provoked by diabetes, representing 11.2% of the total population. In United States, diabetes is the sixth leading cause of death. The prevalence of diabetes mellitus is rapidly increasing worldwide and India is estimated to have 31 million diabetics from the total population of the world. Diabetes is predicted to become one of the most common diseases in the world within a couple of decades, affecting at least half a billion people.

The driving force behind the high prevalence of diabetes is the rise of obesity, sedentary lifestyle, consumption of energy rich diet, etc. The diabetes epidemic is accelerating in the developing world, with an increasing proportion of affected people in younger age groups.

The prevalence of Type 2 diabetes is now at epidemic proportions. Type 2 diabetes has a significant impact on the health, quality of life, and life expectancy of patients, as well as on the health care system. Type 2 diabetes accounts for about 90-95 % of population while Type 1 diabetes accounts for about 5 -10% of the total population. In the past, Type 2 was rarely seen in the young, but recent reports describe Type 2 diabetes being diagnosed even in children and adolescent[7].

1.5-Sugar Regulation: Carbohydrate, protein and lipid metabolism[8]

Glucose is an essential fuel for the body and is the main source of energy for the tissue cell. The amount of glucose in the blood is controlled mainly by the hormones insulin and glucagon. The rise in blood glucose following a meal is detected by the pancreatic beta cells, which respond by releasing insulin. Glucose is transported into the beta cell by Type 2 glucose transporters (GLUT2). As glucose metabolism proceeds, ATP is produced which closes ATP-gated potassium channels in the beta cell membrane. Positively charged potassium ions (K^+) are now prevented from leaving the beta cell. The rise in positive charge inside the beta cell causes depolarization thereby opening the voltage-gated calcium channels and allowing calcium ions (Ca^{2+}) to flood into the cell. The increase in intracellular calcium concentration triggers the secretion of insulin via exocytosis.

Insulin increases the uptake and use of glucose by tissues such as skeletal muscle and fat cells. Once inside the cell, some of the glucose is used immediately via glycolysis. Any glucose that is not used immediately is taken up by the liver and muscle where it can be converted into glycogen (glycogenesis). When glycogen stores are fully replenished, excess glucose is converted into fat in a process called lipogenesis by increasing the number of glucose transporters (GLUT4) expressed on the surface of the fat cell, causing a rapid uptake of glucose. Glucose is converted into fatty acids that are stored as triglycerides for storage[9].

The rise in glucose also inhibits the release of glucagon, inhibiting the production of glucose from other sources, e.g., glycogen break down. Glucose may also indirectly contribute to protein synthesis by synthesis of amino acids. Glucagon is the main hormone opposing the action of insulin and is released when food is scarce. Glucagon also helps the body to switch to using resources other than glucose, such as fat and protein during starvation[10].

1.6-SIGNS AND SYMPTOMS[11]:

In both the types of diabetes, signs and symptoms are more likely to be similar as the blood sugar is high, either due to less or no production of insulin, or insulin resistance. In any case, if there is inadequate glucose in the cells, it is identifiable through certain signs and symptoms. These are quickly relieved once the diabetes is treated and also reduce the chances of developing serious health problems.

Type 1 Diabetes:

In type 1 the pancreas stops producing insulin due to autoimmune response or possibly viral attack on pancreas. In absence of insulin body cells don't get the required glucose for producing ATP (Adenosine Triphosphate) units which results into primary symptom in the form of nausea and vomiting. In later stage, which leads to ketoacidosis, the body starts breaking down the muscle tissue and fat for producing energy hence, causing fast weight loss. Dehydration is also usually observed due to electrolyte disturbance. In advance stages, coma and death is witnessed.

Type 2 Diabetes:

Increased fatigue: due to inefficiency of the cell to metabolize glucose, reserve fat of body is metabolized to gain energy. When fat is broken down in the body, it uses more energy as compared to glucose; hence body goes in negative calorie effect, which results in fatigue.

Polydypsia: As the concentration of glucose increases in the blood, brain receives signal for diluting it and, in its counteraction we feel thirsty.

Polyuria: Increase in urine production is due to excess glucose present in body. Body gets rid of the extra sugar in the blood by excreting it through urine. This leads to dehydration because along with the sugar, a large amount of water is excreted out of the body.

Polyphagia: The hormone insulin is also responsible for stimulating hunger. In order to cope up with high sugar levels in blood, body produces insulin which leads to increased hunger.

Weight fluctuation: Factors like loss of water (polyuria), glucosuria, metabolism of body fat and protein may lead to weight loss. Few cases may show weight gain due to increased appetite.

Blurry vision: Hyperosmolar, hyperglycaemia, nonketotic syndrome is the condition when body fluid is pulled out of tissues including lenses of the eye; this affects it's to focus, resulting blurry vision.

Irritability: It is a sign of high blood sugar of the inefficient glucose supply to the brain and other body organs, which make us, feel tired and uneasy.

Infections: The body gives few signals whenever there is fluctuation in blood sugar(due to suppression of immune system) by frequent skin infections like fungal or bacterial or UTI(urinary tract infection).

Poor wound healing: High blood sugar resists the flourishing of WBC, (white blood cell) which is responsible for body immune system. When these cells do not function accordingly, wound healing is not at good pace. Secondly, long standing diabetes leads to thickening of blood vessels which affect proper circulation blood in different body parts.

II. MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

6.1 Drugs and Chemicals

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Table No: 6.1 Drugs and Chemicals

S.No	*Materials*	*Company Name*
1.	Alloxan	Quali Kems Fine Chem Pvt, Ltd, Vadodara.
2.	Methanol	ChangshuYangyuan Chemicals, China.
3.	Alcohol	ChangshuYangyuan Chemicals, China.
4.	Glibenclamide	Orchid Pharma Ltd, Chennai.

6.3. Experimental animals

Healthy adult albino wistar rats weighing 200-250grams of either sex were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of experiment, after 72hours of fasting from the day of Alloxan introduction. Animals were housed within the departmental animal house and the room temperature was maintained at 27° C. Animal studies had approval of IAEC.

6.4. Plant Material Collection

The leaves of *Punica Granatum* were collected from the local market in Hyderabad in the month of January and was identified and authenticated from Department of Pharmacognosy. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

6.5. Preparation of plant extracts:

6.5.1 Preparation of Aqueous Extract:

Dried leaves of *Punica Granatum* were taken about 20gms into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled up to 80-90^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

6.5.2 Preparation of Alcoholic Extract:

Dried leaves of *Punica Granatum* were taken about 20gms into 250ml beaker containing 200ml of Alcohol. The contents were mixed well and then the mixture was boiled up to 50-60^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

6.6 Preliminary phytochemical analysis of the extracts

The extracts so obtained were subjected to preliminary phytochemical screening. Phytochemical studies were performed to identify the presence of various phytoconstituents as follows:

6.6.1. Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a. Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b. Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c. Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

6.6.2. Triterpenoids

a. Salkowski's Test: The extracts were treated with chloroform and filtered separately. The filtrate was treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, sterols are present. If the lower layer turns golden yellow triterpenes are present.

6.6.3. Saponins

a. Froth Test: The extractswere diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 mins. The formation of 1 cm layer of foam indicates the presence of saponins.

b. Liberman Burchard Test: The extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride boiled and cooled. Concentrated sulphuric acid was added through the sides of test tube. The formation of brown ring at the junction indicated the presence of steroidal saponins.

6.6.4. Flavonoids

a. Alkaline reagent Test: The extracts were treated with few drops of sodium hydroxide separately. Formation of intense yellow colour lesson addition of few drops of dilute acid indicates the presence of flavonoids.

b. Lead acetate Test: The extracts were treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

6.6.5. Phenolic and Tannins

a. Ferric chloride Test: The extract was treated with few drops of neutral ferric chloride solution. The formation of bluish black colour indicates the presence of phenolics nucleus.

b. Gelatin Test: To the extract, 1%gelatin solution containing sodium chloride was added. The formation of white precipitate indicates the presence of tannins.

c. Vanillin hydrochloride Test: the extracts were treated with few drops of vanillin hydrochloride reagent. The conformation of pinkish red colour indicates the presence of tannins.

6.7. Selection of dose for animal study

The dose considered for the experiment on rats was obtained from conversion of human dose of *Punica Granatum* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats (Ghosh 1984). Hence the calculated dose for the rats (considering human dose 0.3 and 0.5 g/kg) is 20 and 30 mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight.

6.8. Pharmacological evaluation

Preparation of extracts:

The aqueous and alcoholic extracts of *Punica Granatum* suspended in water in presence of 3%v/v Tween-80 solution.

All the drugs were administered orally for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

6.9. ACUTE ORAL TOXICITY:

The acute oral toxicity of aqueous and alcoholic extracts of *Punica Granatum* was determined by using Albino wistar rats (200-250g) which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed up to 7days for their mortality, behavioral and neurological profiles.

6.10. Assessment of Anti-diabetic Activity in Normal and Alloxan induced Rats

6. 10.1 Assessment of hypoglycemic activity on normal rats.

Table----.Group Classification:

Group	Treatment	Dose(mg/kg)
Group 1	Normal control received distilled water	10ml/kg

Group 2	Standard group received Glibenclamide	10ml/kg
Group 3	Aqueous extract of *Punica Granatum*	20 mg/kg
Group 4	Aqueous extract of *Punica Granatum*	30 mg/kg
Group 5	Alcoholic extract of *Punica Granatum*	20 mg/kg
Group 6	Alcoholic extract of *Punica Granatum*	30 mg/kg

Procedure:

Animals were divided randomly into six groups of four and each was fasted to overnight. The blood samples were withdrawn by tail vein at 0hour i.e. before I.P administration of extracts/standard/vehicle. Then blood was collected at an interval of 1, 2, 4, and 8 hour after the administration on 0^{th}, 7^{th}, 14^{th} and 21^{st} day respectively according to procedure blood glucose levels were measured by glucometer (ONE TOUCH glucometer).

- **Oral glucose tolerance test(OGTT) in normal rats:**

On the next day (1^{st}, 8^{th}, 15^{th} and 22^{nd} day) after the assessment of hypoglycemic activity OGTT was carried out in same normal animals.

Procedure:

All the animals in each group were administered 2g/kg of glucose one hour after extract/ glibenclamide/ vehicle administration. The blood samples were collected by tail vein at 0 hour, 0.5 hour, 1 hour, 1.5 hour and 2 hour after the administration of glucose load. Blood glucose levels were measured by glucometer on 1^{st}, 8^{th}, 15th and 22^{nd} day respectively.

6.10.2 Assessment of Anti-Diabetic Activity in Alloxan Induced Diabetic Rats:

Induction of Diabetes:

Albino wistar rats of either sex weighing 200-250 g were selected for the study. All the animals were allowed free access to water and pellet diet and maintained at room temperature in rat cages.

Alloxan was dissolved in normal saline immediately before use. Diabetes was induced in 16 hour fasted rats by single intraperitoneal injection of 120 mg/kg body weight of freshly prepared alloxan in normal saline.

The rats after alloxanization were given 5% w/v glucose solution in feeding bottles for next 24 hours in their cages to prevent hypoglycemia. After 72 hours rats with fasting blood glucose levels greater than 200 mg/dl were selected and used for further studies

All the animals were observed for seven days for consistent hyperglycemia (fasting blood glucose level greater than 200 mg/dl and lesser than 400 mg/dl) and such animals were selected and divided into six groups of four each and used for the study of the following experimental models.

Table-----.Group Classification:

Group	Treatment	Dose(mg/kg)
Group 1	Normal control received distilled water	10ml/kg
Group 2	Diabetic control received distilled water	10ml/kg
Group 3	Standard group received Glibenclamide	10ml/kg
Group 4	Aqueous extract of *Punica Granatum*	20mg/kg
Group 5	Aqueous extract of *Punica Granatum*	30mg/kg
Group 6	Alcoholic extract of *Punica Granatum*	20 mg/kg
Group 7	Alcoholic extract of *Punica Granatum*	30 mg/kg

Effect of Aqueous and Alcoholic extracts of *Punica Granatum* on blood glucose levels in alloxan induced diabetic rats:

All the animals of above groups were administered as per treatment protocol mentioned above. The blood samples were collected by retro orbital puncture at 0,1,2,4 and 8 hour after the administration. The treatment was continued for next 22 days. Again blood samples were also collected on 7^{th}, 14^{th} and 21^{st} day after 1 hour administration for sub acute study. Blood glucose level was measured by glucometer at various time intervals.

Oral glucose tolerance test (OGTT) in alloxan induced diabetic rats:

On the 8^{th}, 15^{th} and 22^{nd} day OGTT was carried out on the same alloxan induced diabetic animals used for assessment of anti-diabetic activity studies.

Procedure:

All the animals in each group were administered 2g/kg of glucose one hour after extract/ Glibenclamide/ vehicle administration. The blood samples were collected by retro orbital puncture at 0 hour, 0.5 hour, 1 hour, 1.5 hour and 2 hour after the administration of the glucose load. The Blood samples were collected by tail vein and its blood glucose levels were measured by using a glucometer apparatus.

Histopathological Examination

After collection of blood for hematology and serum for biochemistry, the animals were sacrificed for

histopathological examinations. Liver of each rat were examined grossly. Thereafter, liver tissues were removed for histological studies. The tissues were washed with normal saline and immersion fixed in 10% buffered formalin immediately upon removal. They were gradually dehydrated, embedded in paraffin, cut into 5 µm sections and stained with Hematoxylin and Eosin for histopathological examination

6.11. Statistical analysis

The values were expressed as mean ± SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparison had made. i.e.

1. Normal control Vs All treated groups.
2. Diabetic Control Vs All treated groups.

Differences between groups were considered significant at P<0.001 and *P* <0.05 levels.

III. RESULTS

7.1 Phytochemical screening of *Punica Granatum*.

The present investigation concluded that the isolated compounds from the plant *Punica Granatum* leaves shows the various Pharmacological effects was determined due to the presence of different phytochemical compounds. Further study is needed for the isolation of the constituents present in the plant and its individual pharmacological activity should need to consider and ultimately it should be implemented for the benefit to human beings.

Table 7.1. Phytochemical screening of *Punica Granatum*.

S.No.	Phytoconstituents	Aqueous	Alcoholic
1.	Alkaloids	+	-
2.	Tannins	-	+
3.	Anthraquinones	++	-
4.	Flavonoids	+	-
5.	Saponins	+	+
6.	Triterpenes	-	+
7.	Sterols	-	-
8	Coumarin	+	+

7.2 Acute toxicity testing

Acute toxicity studies revealed that the alcoholic extracts of*Punica Granatum were* safe up to 2000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any toxic reactions was observed up to the end of the study period.

7.3 HYPOGLYCEMIC ACTIVITY IN NORMAL RATS

Fasting Blood Glucose Levels (FBGL) were within the range of 90-105 mg/dl in all the groups at 0 day. Repeated treatment with the doses of aqueous and alcoholic extract (100 and 200 mg/kg) significantly decrease the blood glucose level on 7^{th}, 14^{th} and 21^{st} day, indicating that the extract produce significant hypoglycemic activity after repeated administration. Glibenclamide (10mg/kg) also significantly reduced Fasting Blood Glucose Level (FBGL) after repeated administration as compare to normal control group. Changes in FBGL in different groups after repeated dose administration are summarized in Table No 7.2.

Repeated administration of both aqueous and alcoholic extracts had significantly (p<0.005) reduced the FBGL on 7^{th}, 15^{th} and 21^{st} day, indicating these extracts can produce hypoglycemia on repeated administration. However hypoglycemic activity was more significant on 7^{th}, 14^{th} and 21^{st} day for Glibenclamide treated as compare with other groups. The results suggest that the both aqueous and alcoholic extracts possess significant hypoglycemic activity after repeated dose administration. The detailed results are summarized in Table No 7.2.

- **Effect of extracts of *Punica Granatum* on fasting blood glucose level (FBGL) in normal rats**

Table No 7.2: - Effect of extracts of *Punica Granatum on* fasting blood glucose level (FBGL) in normal rats.

Treatment	Dose (mg/kg)	Blood glucose level(mg/dl)		
		7^{th} day	14^{th} day	21^{st} day
Normal control	-	90.21±1.51	78.10±2.01	73.24±1.10
Glibenclamide	10	83.81±2.31	76.41±0.81	71.30±2.39
AQPG1	20	92.56±2.52	83.69±3.51	79.06±1.68
AQPG2	30	84.92±1.06	78.21±1.86	70.36±0.68
ALPG1	20	78.2±1.06	67.15±3.51	62.52±4.12

ALPG2	30	89.3±2.61	78.52±0.10	72.21±2.21

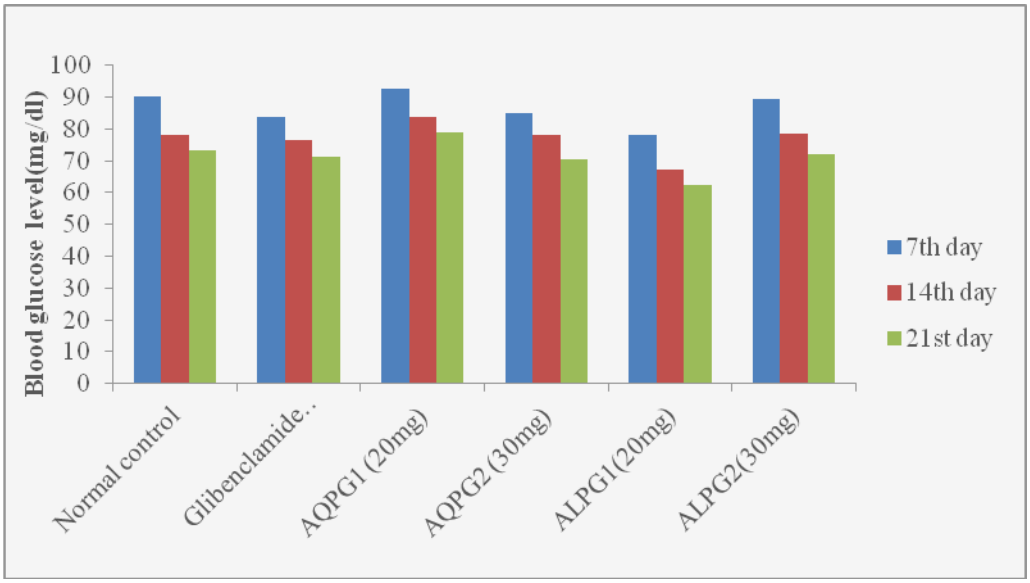

Fig 7.1: Effect of extracts of *Punica Granatum on* fasting blood glucose level (FBGL) in normal rats.

Values are expressed as mean± S.E.M. n=6. Significant values were compared with p<0.005, normal control Vs all groups. Parent thesis indicates % reduction in BGL.

➢ **Oral glucose tolerance test (OGTT) -**

Both the aqueous and alcoholic extracts of *Punica Granatum* significantly (P<0.005) suppress the rise in FBGL after glucose load (2g/kg) in rats, at first half-an-hour and up to 2hr time period as compare with other groups extract Glibenclamide on 8th, 15th and 22nd day. While aqueous and alcoholic extracts produced significant reduction in FBGL. Glibenclamide (10mg/kg) showed (P<0.005) significant suppression in FBGL rise at first half-an-hour, 1hr and normalized FBGL within 2hr. The detailed results are summarized in Table No: 7.3

Table No: 7.3- Effect of extracts of *Punica Granatum on* 8th, 15th and 22nd day in normal rats.

Treatment	**Dose (mg/kg)**	**Blood glucose level (mg/dl)**		
		8th day	**15th day**	**22st day**
Normal control	-	91.25±1.26	83.53±2.51	77.13±1.06
Glibenclamide	10	87.06±1.02	77.12±1.81	72.90±1.20
AQPG1	20	88.12±3.21	81.69±2.60	70.19±3.26
AQPG2	30	85.29±4.82	79.52±3.56	67.51±0.52
ALPG1	20	92.19±3.95	83.26±1.80	73.49±1.10
ALPG2	30	78.11±3.10	67.15±3.52	60.27±3.56

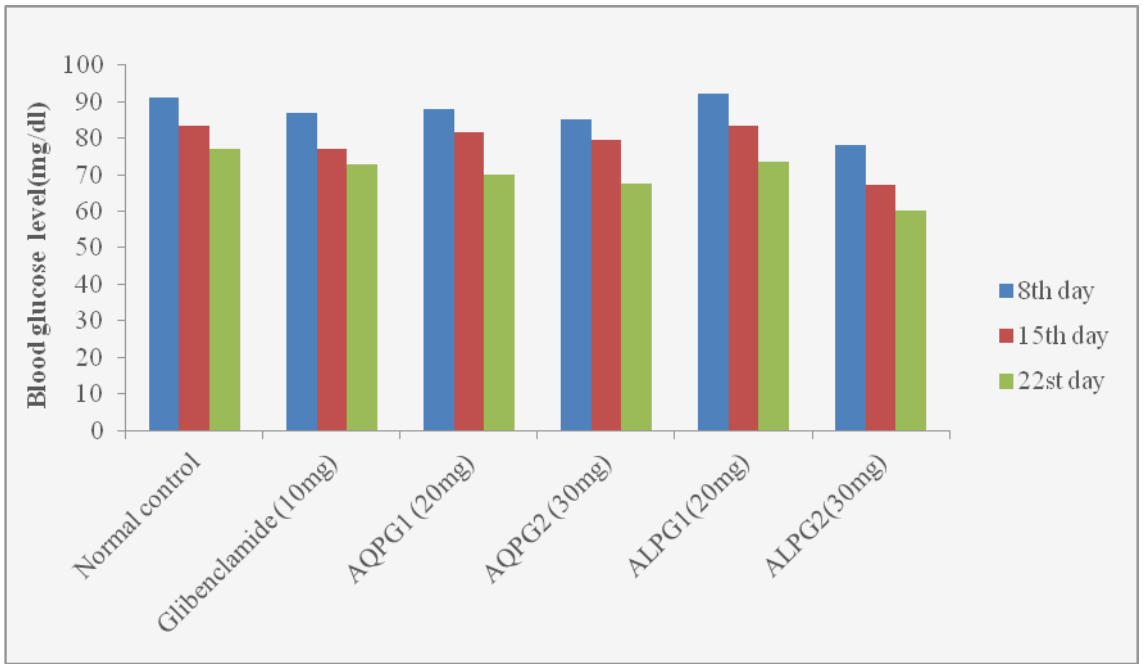

Fig 7.2: Effect of extracts of *Punica Granatum on* 8th, 15th and 22nd day in normal rats.

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.005. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

7.4 ANTI-DIABETIC ACTIVITY IN ALLOXAN INDUCED DIABETIC RATS

Changes in the fasting blood glucose levels in different groups are tabulated in Table No. This data shown that blood glucose level of normal control animals has maintained throughout the study period.

The diabetic control group has shown significant increase in fasting blood glucose levels during this 21st day study period. Glibenclamide (10mg/kg) treated group has shown (p<0.05) significant decrease in fasting blood glucose level during 7th, 14th and 21st day of study period.

- **Effect of *Punica Granatum* extractson antidiabetic activity in alloxan induced diabetic rats**

The animals treated with 100 and 200mg/kg of aqueous and alcoholic of different extracts shown significant decrease (P<0.05) in FBGL on 7th, 14th and 21st day of treatment when compare to other groups of animals. The aqueous extracts have reduced more (%) in FBGL when compared to alcoholic extracts except standard group. The detailed results are summarized in Table No: 7.4

Table No: 7.4- Effect of extracts of *Punica Granatum on* fasting blood glucose level (FBGL) in Alloxan induced diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level(mg/dl)		
		7th day	14th day	21st day
Normal control	**-**	88.12±4.01	77.12±1.92	69.34±1.62
Diabetic control	**10**	299.24±10.50	274.12±24.34	260.21±10.24
Glibenclamide	**10**	265.23±11.30	248.36±71.10	230.21±10.05
AQPG1	**20**	378.12±36.10	362.14±10.06	333.15±10.60
AQPG2	**30**	383.11±05.15	370.31±21.10	353.15±36.12
ALPG1	**20**	294.26±12.92	273.10±11.09	220.81±30.35
ALPG2	**30**	225.13±16.09	186.21±02.12	155.34±55.89

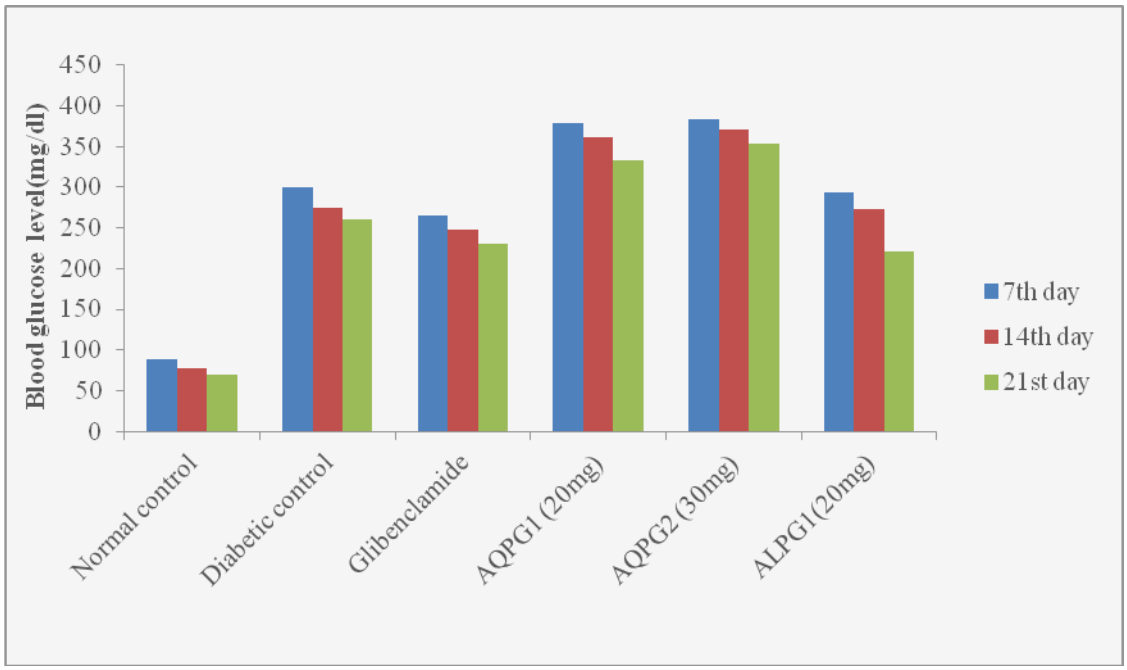

Fig 7.3: Effect of extracts of *Punica Granatum on* fasting blood glucose level (FBGL) in Alloxan induced diabetic rats

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

- **Oral glucose tolerance test (OGTT) on 8th, 15th and 22nd day-**

Both the aqueous and alcoholic extracts of *Punica Granatum* are significantly (P<0.05) suppress the rise in FBGL after glucose load (2g/kg) in rats, at first half-an-hour and up to 2hr time period as compare with other groups extract Glibenclamide on 8th,15th and 22nd day. While aqueous and alcoholic extracts produced significant reduction in FBGL. Glibenclamide (10mg/kg) showed (P<0.05) significant suppression in FBGL rise at first half-an-hour, 1hr and normalized FBGL within 2hr. The detailed results are summarized in Table No: 7.5.

Table No: 7.5- Effect of extracts of *Punica Granatum on* 8th, 15th and 22nd day in Diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level(mg/dl)		
		8th day	15th day	22st day
Normal control	**-**	85.12±2.85	75.28±1.91	63.14±2.89
Diabetic control	**10**	283.25±11.71	251.14±20.95	221.39±19.86
Glibenclamide	**10**	365.89±75.50	286.15±39.52	275.93±15.78
AQPG1	**20**	262.13±72.89	232.71±25.53	198.17±13.99
AQPG2	**30**	283.82±10.27	262.13±10.78	242.89±15.32
ALPG1	**20**	264.18±93.56	221.80±96.15	186.55±11.89
ALPG2	**30**	363.12±10.28	321.18±25.98	282.15±19.12

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

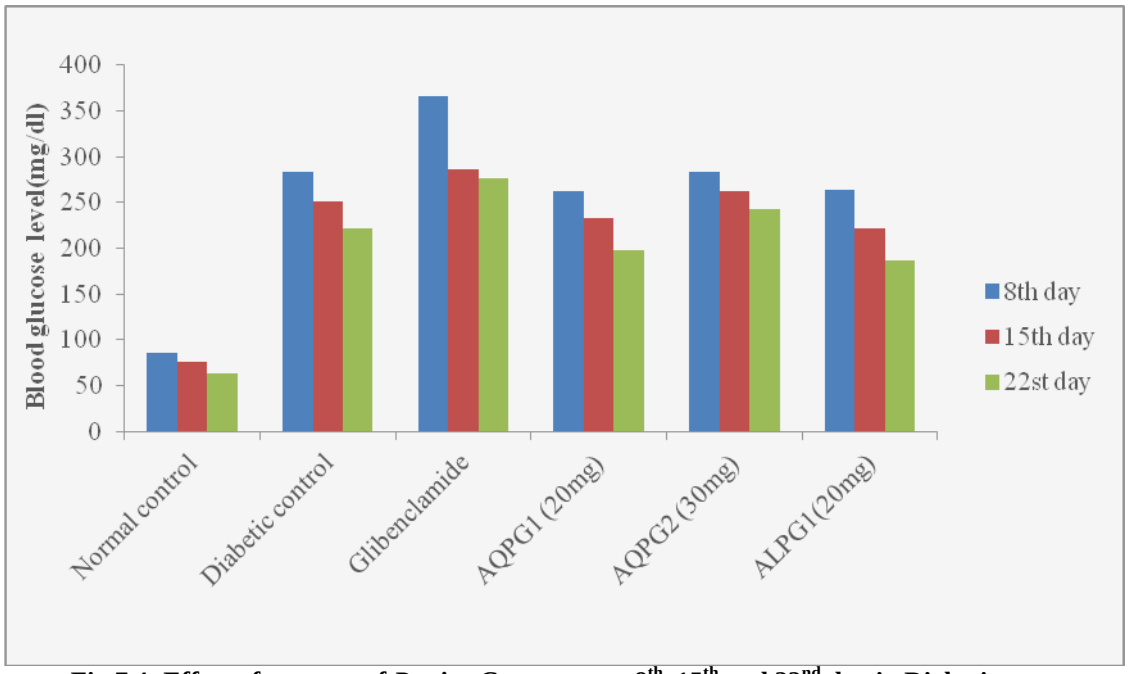

Fig 7.4: Effect of extracts of *Punica Granatum on* 8th, 15th and 22nd day in Diabetic rats.

DISCUSSION

Despite the fact that diabetes has high prevalence, morbidity and mortality globally, it is regarded as non curable but controllable disease. Different synthetic drugs, plant remedies and dietary modification play an effective role in the reduction of the suffering that it causes. The potential role of medicinal plants as antidiabetic agents has been reviewed by several authors. In order to identify the plants with antidiabetic properties various plants have been tested *in-vivo* using animal models, for example rats, against the complications caused by inducers of diabetes, and it has been established that many plants possesses the potential to lower the fasting blood glucose levels and besides help in improving other diabetic complications. The sustained reduction in hyperglycemia automatically decreases the risk of other major complications of diabetes. Effective glucose control is the key for preventing or reversing the diabetic complications and improving the quality of life of the diabetics.

Many natural active compounds have been isolated from plants of different species. These active principles are complex Alkaloids, Tannins, Anthraquinones, Flavonoids, Saponins,Triterpenes, Sterols, Coumarin and others. These compounds have been shown to produce potent hypoglycemic, anti-hyperglycemic and glucose suppressive activities. These effects might be achieved by facilitating insulin release from pancreatic ß-cells, inhibiting glucose absorption in gut, stimulating glycogenesis in liver and/ or increasing glucose utilization by the body.

Crude aqueous and alcoholic extracts of leaves of *Punica Granatum* at a dose of 20 and 30mg/kg showed significant effect on the glucose tolerance of rats and it also showed reduction in the fasting blood glucose levels of the normoglycaemic rats, thus revealing the hypoglycemic nature of the extracts. The effect was more pronounced for both extracts. These findings indicate that the extracts might be producing hypoglycaemic effect by a mechanism independent from the insulin secretion e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption.

Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus in animals. It induces diabetes by dose dependent destruction of β -cells of islets of langerhans. It is a generator of free radicals of oxygen which cause extensive DNA damage. It was observed that single intravenous dose of alloxan exhibited significant hyperglycemia. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues is the fundamental mechanism underlying hyperglycemia in the diabetic state. As the hyperglycemia induced by alloxan falls under category of mild diabetes and may reverse after a few weeks, the hypoglycemic effect of the plant in hyperglycemic rats was studied during 22 days treatment. The difference observed between the initial and final fasting serum glucose levels of extract treated hyperglycemic rat's revealed ant hyperglycemic effect of leaves of *Punica Granatum*throughout the period of study. The effect of the extracts was compared to that of reference standard, Glibenclamide and was found to be significant.

Phytochemical analysis of extracts of leaves of *Punica Granatum* revealed the presence of secondary metabolites that have been shown to possess antidiabetic effect in other plants. Flavonoids, alkaloids and Steroids which were responsible for the antidiabetic effect in other plants were also detected in the extracts of this plant. The presence of phenols in the plant could also be responsible for the antidiabetic effect have been shown to prevent the destruction of β-cells by inhibiting the peroxidation chain reaction and thus they may provide protection against the development of diabetes. Extracts of leaves of *Punica Granatum* appear to be attractive materials for further studies leading to possible drug development for diabetes.

Development of phytomedicine is relatively inexpensive and less time consuming; it is more suited to our economic conditions than allopathic drug development which is more expensive and spread over several years.

CONCLUSION

The study was performed to find out the beneficial effects of two different extracts of leaves of *Punica Granatum* in normoglycaemic rats and alloxan induced diabetic rats and the results reveal that the plant has beneficial effects on blood glucose levels.

In current scenario, herbs are the potent sources of medicines used in the treatment of various disease and disorders. Since, plants are used as medicine there is prompt need of evaluation of plant species, therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of leaves of *Punica Granatum*. Alkaloids,Tannins, Anthraquinones, Flavonoids, Saponins, Triterpenes, Sterols and Coumarin.

The aqueous and alcoholic extracts had hypoglycemic activity because the presence of flavonoids which are rich in treatment of hypoglycaemia with less side effects. Flavonoids might be producing hypoglycaemic effect by a mechanism independent from insulin secreation e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. The present study *Punica Granatum of* both aqueous and alcoholic extracts was showed significant effect on glucose tolerance and also showed reduction in fasting blood glucose levels in normal diabetic rats.

The data of the blood glucose level of rats treated with Alloxan (150mg/kg body weight) produced diabetes within 72 hours. After 72 hours of Alloxan administered the blood glucose levels of rats were observed. It was observed that significant lowering of sugar in aqueous and alcoholic extract. The administration of different extracts at a dose of 20 and 30 mg/kg showed significant anti-hyperglycaemic effect at 22^{nd} day which was evident from the 7^{th} day on wards as compared to standard. The aqueous and alcoholic extract of *Punica Granatum* has showed better anti-hyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats are shown in table. The decreasing blood glucose levels are comparable with that of 10 mg/kg of Glibenclamide. The Glibenclamide (10 mg/kg body weight) shows significant effect on compare to the initial and more significant effect on the 22^{nd} Day compare to the initial. The aqueous and alcoholic extracts of 20 and 30mg/kg body weight shows significant ($P^{*}<0.05$), effect.

Results of anti-diabetic activity in normal and alloxan induced rats the extracts established the scientific basis for the utility of these plants in the treatment of diabetes. The extracts have shown significant reduction in blood glucose levels in normal and alloxan induced diabetic rats and produced maximum anti-diabetic activity and are higher than the hypoglycaemic activity of Glibenclamide in the diabetic rats. In glucose loaded animals, the drug has reduced the blood glucose to the normal levels. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. In conclusion, these extract showed significant anti-diabetic effect in normal and diabetic rats after administration. Thus the claim made by the traditional Indian systems of medicine regarding the use of these plants in the treatment of diabetes stands confirms.

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