

Antidiabetic and Hypolipidemic Activity of *Punica granatum* Linn on Alloxan Induced Rats

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Abstract: *Diabetes mellitus* (DM) is a metabolic disorder in which the carbohydrate and lipid metabolism is improperly regulated by insulin. Many indigenous Indian medicinal plants have been found to be successfully used to cure diabetes. Herbal drugs are considered to be less toxic and free from side effects than synthetic ones. Keeping this view, the study aimed to evaluate the antidiabetic and hypoglycemic activity of *Punica granatum*. Diabetes & hyperlipidemia was induced by the intra peritoneal injection of alloxan mono hydrate (120mg/kg) for 2 consecutive days. Diabetes was confirmed 2days after the last alloxan dose administration by determining the blood glucose concentration. Treatments were started after confirmation of diabetes in rats. During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. So the total hemoglobin level is lowered in alloxan induced diabetic rats. Alloxan induced diabetes has been observed to cause a massive reduction of the beta cells of the islets of pancreas leading to hyperglycemia. Rats treated with alloxan (120mg/kg), for 2 consecutive days, showed an increase in the concentration of glucose, triglycerides, cholesterol, LDL cholesterol, VLDL cholesterol and a decrease in the level of HDL cholesterol and hemoglobin content. Administration of crude powder of *Punica granatum* husk reduced the concentration of glucose, triglycerides, cholesterol, LDL cholesterol, VLDL cholesterol and raised the level of HDL cholesterol and hemoglobin content in the blood of both group-I normal and group III alloxan diabetic rats treated.

Key words:

INTRODUCTION

Diabetes is a disease in which the body either fails to generate insulin (type 1, also called insulin-dependent or juvenile-onset), or the insulin that formed is unable to convert the food into energy (type 2, also called non-insulin-dependent or adult-onset). The inefficiency of insulin production enhances the level of glucose in the blood [1]. Diabetes without proper treatments can cause many short time complications include hypoglycemia, hyperglycemia, ketoacidosis and hyper-osmolar syndrome. Long term complications such as cardiovascular disease, kidney disease, neuropathy, diseases of the eyes, peripheral vascular disease and more can seriously compromise the diabetic patient. Certain syndromes (for example, Prader-Willi, Down's, Progeria

and Turner's) may result in a hyperglycemic state. If this state is prolonged, the result can be permanent diabetes [2].

Diabetes can be treated by antioxidants due to their various therapeutic benefits. They include phytochemicals, vitamins and other nutrients that protect our cells from damage caused by free radicals. Antioxidants can fit in two broad categories - enzymatic and non-enzymatic. They provide the necessary defense against the OS generated by ROS. It has an array of non-enzymatic antioxidants-ascorbate, urate [3], vitamin - E [4], pyruvate [5], glutathione [6], albumin, vitamin-A, ubiquinol [7], taurine and hypotaurine [8]. Most of the fruits, vegetables, culinary herbs and medicinal herbs contain high levels of antioxidants. The antioxidant level of herbs can be as high as 465mmol per 100g [9].

A botanical diversity of fruits and vegetables plays a role in the biological effect of antioxidant phytochemicals and foods that contain antioxidants in our daily diet.

Punica granatum L. (Family-Punicaceae), commonly known in India as 'Anar' or 'Dalim', is a highly ornamental large deciduous shrub or small tree widely distributed and cultivated in many parts of India [10]. Pomegranates have a high level of antioxidants. Studies have shown that pomegranate contains more antioxidants than green tea, cranberries and even red wine. Pomegranate in our daily diet can improve blood flow, help to prevent heart diseases such as heart attacks, stroke or clogged arteries and it also promotes healthy blood pressure levels and low cholesterol. Pomegranate juice can be helpful for men as well as studies have concluded that it may help prevent prostate cancer and growth.

Antibacterial effect of *Punica granatum* leaves [11], hypoglycemic action of flowers [12] seeds [13] and rind [14] have been reported. Literature reviews indicated that no studies showing the antidiabetic and hypolipidemic effect of the fruit husk of this plant on alloxan-induced diabetes have so far been undertaken. Considering the drawback, the study was aimed to evaluate the antidiabetic and hypolipidemic activity of the crude powder of *Punica granatum* husk.

MATERIALS AND METHODS

Male albino rats weighing about 120-180g were used in the study. The animals were housed in polypropylene cages and maintained in controlled temperature with 12hrs period of light and dark and fed with standard rat feed and water. The animals were grouped as group-I (normal) rats, group-II alloxan induced diabetic rats without treatment (Control) and group-III alloxan induced diabetic rats (treated) with husk extract of *Punica granatum* 1g/kg body weight per day for 10days. Diabetes & hyperlipidemia were induced by an intra peritoneal injection of alloxan mono hydrate (120mg/kg) dissolved in distilled water for 2 consecutive days. Diabetes was confirmed 2days after the last alloxan dose administration by determining the blood glucose concentration. Treatments were started after confirmation of diabetes in rats [15]. Husk of *Punica granatum* was collected, shade dried and powdered and was used as drug in the crude form. After the completion of experimental regimen, the rats were fasted over night and blood samples were

collected by puncturing the retro orbital plexus under mild ether anesthesia. The collected samples (Serum & blood) were used for analysis of different biochemical parameters. Six parameters were analyzed and the methods used for analysis were serum glucose [16], serum total cholesterol [17], serum triglyceride [18], serum HDL Cholesterol [17], the blood hemoglobin (cyanmethemoglobin method; [19], LDL and VLDL Cholesterol in serum [20]. The results were presented as mean \pm standard deviation (SD). Student's 't' was used to analyze statistical significance.

RESULT AND DISCUSSION

Diabetes is said to be one of the most important and critical health crisis in the future. Adequate treatment of diabetes is thus important. Medicinal plants play an essential role in the management of diabetes mellitus. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities. In this aspect, the study was carried out to evaluate the antidiabetic and hypolipidemic activity of husk of *Punica granatum*. Several plant species have been described as hypoglycaemic. These include *Opuntia streptacantha* Lem, *Trigonella foenum graecum* L, *Momordica charantia* L, *Ficus bengalensis* L, *Polygala senega* L, *Gymnema sylvestre* R, *Allium sativum*, *Citrullus colocynthis*, myrrh, black seeds, helteet, fenugreek, aloe and *Artemisia*. Other species are less well known [21-24]. The insulin-like and insulin releasing action of ingredients of many herbal plants have previously been reported. [25, 26]. The fruit of *Citrullus colocynthis* is traditionally used as anti-diabetic and its aqueous extract showed dose-dependent increase in insulin release from isolated islets [27]. Drugs such as steroids, Dilantin and others may elevate the blood sugar through a variety of mechanisms. Certain other drugs, such as alloxan, streptozocin and thiazide diuretics, are toxic to the beta cells of the pancreas and can cause diabetes. In our study, alloxan monohydrate used to induce diabetic and hyperlipidemic state. Alloxan, a beta cytotoxic agent, rapidly and selectively accumulates in pancreatic beta cells [28] and causes beta cell death and apoptosis by generation of reactive oxygen species (ROS), super oxide radicals and hydrogen peroxide [29]. Beta cell death causes hyperglycemia due to insulin deficiency which further aggravates the oxidative stress induced by alloxan [30].

Punica granatum includes innumerable health benefits. It consists of antioxidant, antidiabetic, antiviral and anti-tumour properties. It also include cure of stomach disorders, cancer, dental care, osteoarthritis and anaemia According to a research study stated in American Journal of Clinical Nutrition, *Punica granatum* being rich in antioxidants can prevent the oxidization of LDL 'bad' cholesterol. Antioxidants are phytochemicals, vitamins and other nutrients that protect our cells from damage caused by free radicals. Antioxidants provide numerous health benefits. Samples were collected and six different parameters were determined from the collected samples (serum & blood). The observations made on different groups of normal, experimental and treated animals were discussed.

Diabetes mellitus is characterized by constant high levels of blood glucose (sugar). Human body has to maintain the blood glucose level at a very narrow range, which is done with insulin and glucagon. The function of glucagon is causing the liver to release glucose from its cells into the blood, for the production of energy. In our study we have observed, a decrease in blood glucose level in both normal and alloxan diabetic rats treated with crude powder of *Punica granatum* husk. The possible mechanism by which *Punica granatum* brings about its hypoglycemic action may be stimulating the insulin effect of serum by increasing either the pancreatic secretion of insulin from the beta - cells of islets of langerhans or its release from bound insulin. In this context several other plants have also been observed to have hypoglycemic effects [31]. *Artemisia pollens* is a shrub used in folk medicine as a treatment for diabetes mellitus in parts of south India [32]. Oral administration of methanol extract of aerial parts of *Artemisia pollens* showed a dose dependent (100- 500 and 1000mg/kg) anti hyperglycemic effect in glucose fed hyperglycemic and alloxanized rats (60mg/ kg intravenous). Subcutaneous administration of alkaloid fraction of *Areca catechu* (0.05 - 0.5mg/kg) in alloxanized rabbits (140mg/kg) showed significant hypoglycemic effect lasting for 4-6hrs [33]. Oral administration of *Capparis deciduas* fruit powder for 3weeks to alloxanized (80mg/kg intraperitoneal) diabetic rats (blood glucose, 450mg %) also showed significant hypoglycemia (blood glucose, 120-130mg %)[34].

In our study, we have also noted a reduce in level of total hemoglobin and HDL cholesterol of alloxan diabetic rats. During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated

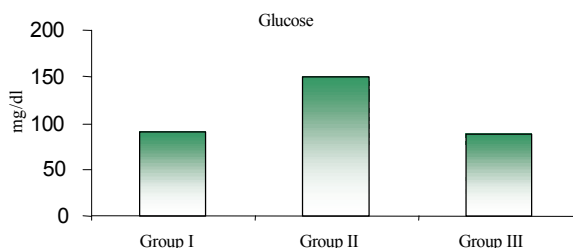


Fig. 1: Effect of crude powder of *Punica granatum* husk on serum level of glucose in normal and experimental rats

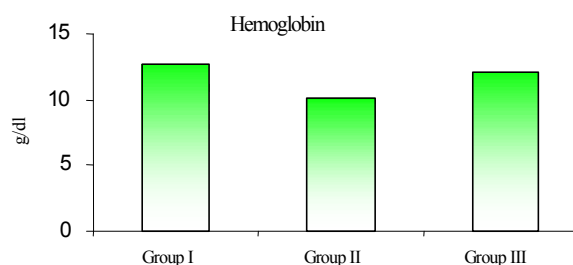


Fig. 2: Effect of crude powder of *Punica granatum* husk on hemoglobin content in normal and experimental rats

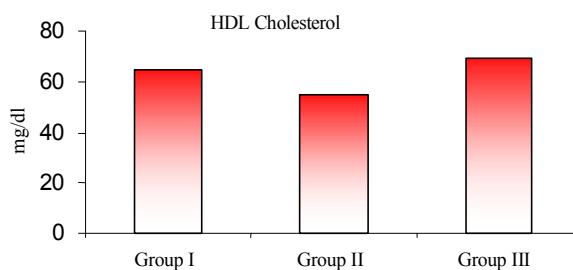


Fig. 3: Effect of crude powder of *Punica granatum* husk on HDL Cholesterol content in normal and experimental rats

hemoglobin. So the total hemoglobin level is lowered in alloxan diabetes rats [35] whereas the administration of husk extract of *Punica granatum* shows a normal level in group I. The administration of the extract of *Capparis deciduas* also produced a significant increase in the level of High-density lipoprotein-cholesterol (HDL-C) [36].

Lipids play an important role in the pathogenesis of Diabetes mellitus. The level of serum lipids are usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease [37]. Hyperlipidemia is a recognized consequence of Diabetes mellitus [38]. The abnormal high concentration of serum lipids are mainly due to increase in the mobilization of free fatty acids from the peripheral depots. Since insulin inhibits the hormone sensitive lipase; on the other hand, glucagons,

catecholamine and other hormones enhance lipolysis. The marked hyperlipidaemia that characterizes the diabetic state is the consequence of the uninhibited actions of lipolytic hormones on the fat depots [37].

We have noted a significant increase in total cholesterol, LDL, VLDL level in both normal and alloxan diabetic rats treated with crude powder of the husk of *Punica granatum*. The fruit of *Momordica cymbalaria* (250mg/kg for 15 days) caused significant reduction in fasting blood glucose levels in alloxanized rats (150mg/kg intraperitoneal) and its hypolipidemic activity has been described in the literature[39].oral administrations of the aqueous extract of *Capparis spinosa* L. (CS) at a dose of 20 mg/kg caused a significant decrease in plasma cholesterol levels 4 days ($p < 0.05$) and 1 week ($p < 0.05$) and plasma triglycerides levels 1 week ($p < 0.05$) and 2 weeks ($p < 0.01$) were observed in normal and streptozotocin-induced diabetic rats[40].The administration of the extract of *Capparis deciduas* produced a significant ($p < 0.05$) dose-dependent decrease in the levels of total cholesterol (TC), Triacylglycerol (TG), low-density lipoprotein-cholesterol (LDL cholesterol), with a significant increase in the level of High-density lipoprotein-cholesterol (HDL-C). The extracts of *C. deciduas* prove to have a hypolipidemic potential [36].

The results for total cholesterol, triglycerides, LDL and VLDL were summarized in a table

Parameters (mg/dl)	Group-I	Group-II	Group-III
Total Cholesterol	152 ± 6.531	192 ± 6.531*	112 ± 6.531#
Triglycerides	120.75 ± 0.9574	261.25 ± 1.5*	138.25 ± 1.5#
LDL	26.085 ± 1.259	43.905 ± 1.260*	25.319 ± 1.49#
VLDL	24.15 ± 0.1914	52.25 ± 0.229*	27.65 ± 0.3#

*P < 0.001 significantly different from group - I rats.

#P < 0.001 significantly different from group - II rats.

It had been concluded that in our study, decrease in the concentration of glucose, triglyceride, cholesterol, LDL cholesterol, VLDL cholesterol and increase in HDL cholesterol and hemoglobin content were observed in both normal and alloxan diabetic rats treated with crude powder of the husk of *Punica granatum*. The antidiabetic and hypolipidemic activity of the plant source is due to the phyto chemical constituents present in the husk of *Punica granatum*.

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