ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

Antidiabetic Evaluation Of Isolated Compounds From Pomegranates (Punica Granatum) Peels In Alloxan-Induced Diabetic Rat Model

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ABSTRACT

Pomegranates fruits have innumerable health benefits and its implication in diseases cure have been widely recognized since ancient time. Moreover, pomegranate fruits, seeds and peels are intensively used in traditional medicine as a natural therapy. It contains numerous valuable ingredients such as flavonoids, ellagitannin, punicalagin, ellagic acid, vitamins and minerals. The principal constituents including punicalagins and ellagitannin are responsible for immeasurable health benefits due to its strong antioxidant activity. Additionally, constituents of pomegranate show health promoting effect through the modulation of physiological and biochemical pathways. Recent evidences suggested that pomegranates fruits, peels extract revealed the decrease in blood glucose level when compared with non-treated diabetic rodents. In this way, the current investigation work was affirmed that the extract has significant hypoglycaemic impact.

Keywords- Pomegranates, Punica granatum, Antidiabetic activity, Alloxan induced, hypoglycaemic activity.

Introduction

Punica granatum L (pomegranate) is a deciduous shrub, native to Iran. Pomegranate has extensively been used as a source of traditional medicine. Pomegranate fruit has medicinal properties such as anti-inflammatory and antibacterial activities. The pomegranate seed oil has inhibitory effect on skin and breast cancers. The pomegranate seed oil has phyto estrogenic compounds and the fruit is rich in phenolic compounds with strong antioxidant activity. The fruit and bark of pomegranate are used against intestinal parasites, dysentery, and diarrhoea. The juice and seeds are considered a tonic for throat and heart. It is used to stop nose and gum bleeds and treating haemorrhoids. Today, Punica granatum L. as a fruit

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not only attracts a lot of public interest but research is also focused on its medicinal properties and food industry. So a wide range of research studies have already been launched in this field. However, there are not enough studies performed on the medicinal properties of pomegranate. This review article presents the recently published findings on different aspects of this plant, with a focus on its medicinal properties

Material and Method

Fruits were procreated from market of Pune during the months of August and September and specimen deposited for taxonomic and ethno medicinal identification to Director, Botanical survey of India, Pune, Maharashtra. Fresh matured pomegranates (Punica granatum) peels was collected in bulk, initially rinsed thoroughly with distilled water, shade dried for 15 days. The shade dried materials were coarsely powder by a mechanical grinder and preserved in a nylon bag in a deep freezer, till further use.

Preparation of extract

The plant materials (1 kg) were initially defatted with petroleum ether and then extracted with alcohol using a Soxhlet apparatus. The yield of the plant extracts ethanol (95%) measured about 20 g after evaporating the solvent using water bath. The standard extracts obtained was then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening. Preparation of fractions of crude ethanolic extract then fractionated using Petroleum ether and Chloroform The ethanolic extract, chloroform fractions obtained from the plant was then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening. Petroleum ether fraction was not used in the study because of very less yield.

Investigational Model for Induction of Diabetes

Animals

Healthy adult Male albino Wistar rats, weighing 150–200 g was used for the Screening methods. Diabetes was induced by intra-peritoneal injection of Alloxan monohydrate (150 mg/kg b.w.) dissolved in the in normal saline. Blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia4 . The blood glucose level was checked before alloxanisation and after alloxanisation regularly in 24h intervals. Animals were considered diabetic when the blood glucose level was raised beyond 200 mg/100 ml of blood. This condition was observed at the end of 72 h after alloxanisation.

Preparation of Interventions

The measured quantity of extracts and fractions of Pomegranates and the standard drug glibenclamide (5 mg/kg) was suspended in 25% Tween-20 in distilled water. The solvent, test samples and standard drugs were administered by oral route based on dose and corresponding weight of the animals. For oral administration of test, standard as well as Solvent Feeding needle no 21 was used.

Maintenance of animals and Exposure Conditions

Earlier to the experiments, the selected animals were housed in acrylic cages in standard environmental conditions (temp: 20–25^0 C; relative humidity: 45-55 % under 12 hr light/dark cycle), feed with standard rat feed for 1 week in order to adapt to the laboratory conditions and water ad libitum. They were fasted overnight (12 hr.) before experiments, but

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were allowed free access to water. Six animals were used for each group of study. All the experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Animal Ethical Committee (IAEC/CPCSEA/INV/919/2021), Siddhant college of Pharmacy, Sudumbare, Pune.

Blood glucose level determination

Fasting blood glucose concentration was determined using a Glucometer (Optimum), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns.

Hypoglycaemic activity study of isolated compound on normoglycemic animals (Single dose treated)

The hypoglycaemic activity is important in the diagnosis of diabetes mellitus. It determines the ability of drug to decrease blood glucose level. This method permits for the effect of the drug to be tested in the animal with a whole pancreatic activity. The contrast may give some information regarding mechanism of action. The animals were fasted for 18 h, but were allowed free access to water before and throughout the duration of the experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. Plasma was separated following centrifugation the glucose was estimated by the GOD/POD method using a glucose estimation kit from M/s. Sigma Diagnostics (India) Pvt. Ltd., Baroda, India. The normal rats were then divided into six groups of six animals each. Group I served as solvent control and received only vehicle (2 ml/kg) through the oral route, Group II received glibenclamide (5 mg/kg) and served as reference control. Groups III to VI received the compound A and B of Pomegranates at a dose of 200 and 400 mg/kg, respectively, through the oral route. Blood glucose levels were examined after 1, 2, 4, 6, 8 and 10 h of administration of a single dose of the test and control samples.

Antihyperglycemic activity of isolated compound in glucose-loaded animals (oral glucose tolerance test)

The oral glucose tolerance test (OGTT) measures the body's ability to use main source of energy i.e. glucose. OGTT is to simplify and facilitate the diagnosis of diabetes this method is frequently referred to as physiological induction of diabetes mellitus because the blood glucose level of the animal is fleetingly increased with no damage to the pancreas. An oral glucose tolerance test (OGTT) was performed on diabetic rats by feeding glucose (5 g/kg) per os. Animals were deprived of food 18 h before and during the experiment, but were allowed free access to water. They were divided into 7 groups of 6 rats each. Group I served as normal control, Group II served as solvent control and received only vehicle (Tween + water - 2 ml/kg b.w.) through the oral route. Group III received glibenclamide (5 mg/kg b.w.). Groups IV to VII received the compound A and B of Pomegranates L at a dose of 200 and 400 mg/kg b.w., respectively, through oral route. The blood glucose level was determined before drug and glucose administration (1 and 0 h, respectively) and subsequently at 0.5, 1, 2 and 3h after.

Result and Discussion

Effect of Isolated compounds of Pomegranates on Blood Glucose Level of normoglycaemic rats (hypoglycemic activity)

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The effect of isolated compounds of Pomegranates on fasting blood glucose levels of normal rats are presented in table 1. The plant extracts at both the dose level of 250 and 500 mg/kg registered 77.42 to 85.32 mg/dl of fasting blood glucose level at the end of 10h of the study, while the standard drug, glibenclamide showed 71.63 mg/dl at the same time, with a low degree of significance while compared with solvent treated group. The percentage change of blood glucose of test extracts treated groups at the end of 10 h showed 4.27 to 15.10% fall when compared with initial BGL in a dose dependent manner. The potency order of the test extracts towards the falling of BGL is followed by ethanolic extract and chloroform fraction.

Effect of Isolated compounds of Pomegranates on BGL of glucose loaded hyperglycemic rats (oral glucose tolerance test, OGTT)

The blood glucose level (BGL) of isolated compounds of glibenclamide and vehicle treated albino rats after oral administration of glucose (5 g/kg) are summarized in table 2 The compound A and B at 250 mg/kg dose level registered 89.13, 92.50 mg/dl at the end of 3 h of the study, while it was 91.50, 94.51 mg/dl with dose level of 500 mg/kg. However, at the same time the standard drug glibenclamide at 5mg/kg showed 62.51 mg/dl of BGL. However, the calculated percentage fall of BGL demonstrated 9.28, 20.83 and 15.73, 25.89% with respect to 250 and 500 mg/kg dose levels when measured at the end of the 3 h of the study, while at the same time glibenclamide showed a 34.54% fall of BGL. The progressive fall of BGL of the test extracts, in the different test hour showed a statistically significant of $p< 0.05$ to $p< 0.01$, while analysed by using ANOVA followed by Dunnett's t-test. The aqueous extract possesses more BG lowering potency than that of the ethanol extract in a dose dependent manner. The test extracts at tested dose levels also showed a significant fall of BGL while compared with the solvent control group during the study period of 30, 60 and 120 min.

Table- 1: Effect of Isolated compounds of Pomegranates on Blood Glucose Level of normoglycaemic rats (hypoglycemic activity)

Group	**Treatment and Dose**	**Blood glucose level (mg/dl)**							
		0	1	2	4	6	8	10	**% decrease at 10th hr.**
I	control (tween+ water)	94.62 ± 1.1	87.22 ±4.62	91.42 ± 1.86	89.52 ±0.81	91.54 ± 2.23	89.65 ± 0.46	92.78 ± 3.22	--
II	Glibenclamide (5mg/kg)	91.44 ± 1.31	81.24 ±2.63	67.53 ±2.34*	58.14 ±2.61 **	54.74 ±2.44 **	73.86 ±1.42 **	71.68 ±2.81 **	21.66
III	Compound-4A (250mg/kg)	89.13 ± 1.2	87.6 ± 1.1	87.2 ± 2.65	86.73 ±1.46	86.33 ± 1.43	86.12 ± 0.89*	85.32 ± 1.51	4.27
IV	Compound-	88.4 ±	87.32	86.49	86.04	85.6 ±	84.4 ±	81.32 ±	7.94

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	4A (500mg/kg)	2.43	±2.16	± 1.87	±1.67	2.69	1.43**	2.49*	
V	Compound-4B (250mg/kg)	92.53 ± 1.27	91.46 ±1.68	89.94 ± 1.09	87.11 ±0.91	86.22 ± 2.13	84.11± 1.18**	82.11 ±1.89*	11.22
VI	Compound-4B (500 mg/kg)	91.18 ± 0.93	87.30 ±0.78	85.49 ± 2.61	85.21 ±1.37	84.3± 2.38*	82.6 ± 1.21**	77.42± 2.73**	15.10

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test (t-value denotes statistical significance at *p<0.05, **p<0.01 respectively, in comparison to group-I)

Table-2: Effect of Isolated compounds of Pomegranates on BGL of glucose loaded hyperglycemic rats (oral glucose tolerance test, OGTT)

Group	**Treatment and Dose**	**Blood glucose level (mg/dl)**					
		0 minutes	**30 minutes**	**60 minutes**	**120 minutes**	**180 minutes**	**% decrease at end of 3hr**
I	Normal control	83.74 ± 0.47	86.51 ± 0.98	88.50± 0.63	83.50 ± 0.63	86.50± 0.62	-
II	Solvent control (tween+ water)	90.50 ± 0.67	135.52 ± 0.64**	118.83 ± 0.85**	98.50 ± 0.63**	91.50 ± 0.44**	32.48
III	Glibenclamide (5mg/kg)	89.43 ± 0.40	95.50 ± 1.04**	81.53 ± 0.91**	72.50 ± 0.64**	62.51 ± 0.72**	34.54
IV	Compound-4A (250mg/kg)	83.62 ± 0.40	98.25 ± 0.85**	97.61 ± 0.91**	93.50 ± 0.64**	89.13 ± 0.34	9.28
V	Compound-4A (500mg/kg)	87.50 ± 0.64	107.31 ± 1.37**	102.32 ± 1.10**	94.50 ± 0.64*	91.50 ± 0.64	15.73
VI	Compound-4B(250mg/kg)	91.50 ± 0.64	116.84 ± 1.10**	109.83 ± 0.85**	102.65 ± 0.91*	92.50 ± 0.54**	20.83
VII	Compound-4B(500mg/kg)	82.97 ± 0.91	128.36 ± 0.85**	113.36 ± 1.10	101.51 ± 0.64**	94.51 ± 0.65**	25.89

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test (t-value denotes statistical significance at *p<0.05, **p<0.01 respectively, in comparison to diabetic control group).

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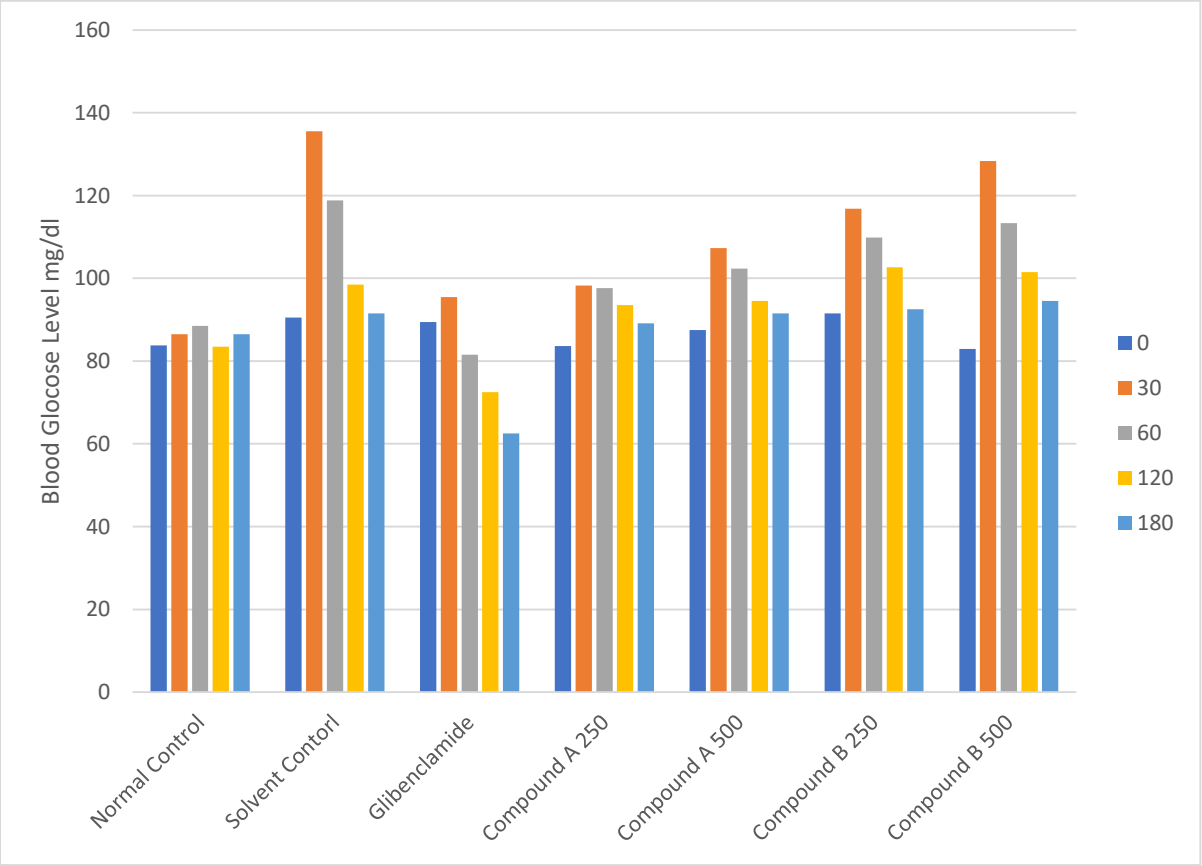

Conclusion

The research work focusses on anti-diabetic and hypoglycemic activity of Pomegranate for their possible to validate their folklore claim followed by chromatographic separation, isolation of presence of phytoconstituent named Compound A and B among the most potent fraction of plant. The dose levels of the isolated compounds were selected based on the results of the acute toxicity study and found as 250 & 500 mg/kg b. w. respectively. Since both Compound A and B showed good activity, hence the investigators think it may be more worth full in terms of its blood glucose lowering ability.

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