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**Blood pressure lowering effect of  
*Pennisetum glaucum* in rats**

## Blood pressure lowering effect of *Pennisetum glaucum* in rats

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### Abstract

The present study was carried out to evaluate the seeds of *Pennisetum glaucum* for its blood pressure lowering effect in rats. Aqueous-methanolic extract of *P. glaucum* seeds in 250, 500 and 1000 mg/kg doses was studied in normotensive, egg-feed diet and glucose-induced hypertensive rats using non-invasive technique. The extract significantly ( $p < 0.5$  -  $p < 0.001$ ) decreased blood pressure and heart rate with maximum effect at 1,000 mg/kg dose. The extract was found to prevent rise in blood pressure of egg and glucose fed rats as compared to control group in 21 days study. The extract was safe in mice up to dose of 4 g/kg and sub-chronic toxicity study showed that there was no significant alterations in blood chemistry of extract treated rats. It is conceivable, therefore, that aqueous-methanolic extract of *P. glaucum* seeds has exerted considerable antihypertensive activity which may be due to the presence of phytochemical constituents.

### Introduction

*Pennisetum glaucum* (L.) R. Br. (pearl millet) belongs to family Poaceae and is abundantly grown in tropical semi-arid regions of Africa and Asia. It serves as a major staple food for many populations around the globe. It is locally known as Bajra in Province Punjab of Pakistan. It is recommended for several therapeutic purposes in folklore. It has been reported to reduce the cholesterol and blood glucose level (Nani et al., 2011). It is also used for the treatment of severe constipation, stomach ulcers, and weight loss because of high fiber content. It is considered a repository of dietary antioxidants, especially flavonoids and phenolic acids. It has got the anti-ageing property and is useful in neurodegenerative diseases (Dicko et al., 2002). The presence of naturally occurring antioxidants has been thought inversely related to mortality due to coronary heart disease. The incidence of heart attacks is low in the people who consume it as their food (Nambiar et al., 2010). It is effective in the cardiovascular diseases as it controls the blood pressure and plasma low density lipo-protein cholesterol levels (Nils-Georg, 1996). In the present study *P. glaucum* has been evaluated for its

blood pressure lowering effect in normal, egg feed diet and glucose treated rats to provide scientific basis for its use in hypertension.

### Materials and Methods

**Drugs and chemicals:** All chemicals used were of analytical grade. Methanol and glucose were purchased from Sigma Chemical Co.

**Animals:** Sprague-Dawley rats (200-300 g) and albino mice (23-40 g) of either sex were used. Animals were housed in controlled environment (23-25°C) at animal house of Faculty of Pharmacy, University of Sargodha, Sargodha.

**Plant material and extraction:** The seeds of *P. glaucum* were used in the study. Aqueous methanolic extract of *P. glaucum* was prepared. The seed powder of the plant was soaked in mixture of distilled water (30%) and methanol (70%) for 72 hours at room temperature. It was filtered through muslin cloth and then through filter paper. The solvent was evaporated under reduced pressure using a rotary evaporator. The extract was



placed in petri dishes for further drying and then it was lyophilized. The percentage yield of the dried extract was 15%. The extract was stored in well closed cellophane bags at 4°C in refrigerator.

*Hypotensive activity in normotensive rats:* Normotensive rats of either sex were randomly assigned into three groups (n = 6). Aqueous-methanolic extract of *P. glaucum* seeds was administered at doses 250, 500 and 1000 mg/kg b.w to group 1, 2 and 3 respectively. The blood pressure and heart rate of animals were measured at 0, 2, 4, 6 and 8 hours after administration of extract doses using non-invasive technique. For estimation of blood pressure, rats were placed in the NIBP restrainers and appropriate cuff with sensor was then mounted on its tail and warmed to about 33–35°C. The tail cuff was inflated to a pressure well above the expected systolic blood pressure i.e. 250 mmHg and slowly released during which the pulses were recorded by using Power Lab data acquisition system and Lab chart 5.0 software. Systolic blood pressure (SBP), mean blood pressure (MBP) and heart rate (HR) were measured directly using pulse tracing while DBP was calculated from SBP and MBP using formula:  $DBP = (3MBP - SBP) / 2$  (Alamgeer et al., 2013).

*Determination of antihypertensive activity in glucose-induced hypertensive rats:* Sprague Dawley rats of either sex were randomly assigned into three groups (n = 6) and were provided 10% glucose solution instead of tap water for 21 consecutive days. At 22<sup>nd</sup> day, Animals in group 1, 2 and 3 were treated with 250, 500 and 1000 mg/kg b.w of the extract. The blood pressure and heart rate of animals were measured at 0, 2, 4, and 8 hours after drug treatment.

*Egg feed-induced hypertensive rats:* Rats of either sex were divided into two groups (n = 6). Group 1 was given a specially prepared egg feed diet for 21 consecutive days in order to produce cholesterol-induced hypertension. The diet was prepared by the addition of yolk of 12 eggs to 500 g standard rat diet. Animals in group 2 were given egg feed diet and crude extract of *P. glaucum* seeds in 1,000 mg/kg dose for the same time period. Animals in all the groups were given normal saline instead of tap water. Blood pressure and heart rate of each of these groups were measured at week 0, week 1, week 2, and week 3 (Saleem et al., 2005).

*Glucose fed hypertensive rats:* Rats were randomly assigned into two groups (n = 6). Group 1 received 10% glucose solution instead of tap water for 21 consecutive days while Group 2 were given 10% glucose solution and crude extract of *P. glaucum* for 21 days. Animals were fed on standard diet. Blood pressure and heart rate of these groups were measured at week 0, week 1, week 2, and week 3 using NIBP measuring apparatus (Alamgeer et al., 2013; Saleem et al., 2005; Reaven and Ho, 1991).

*Acute toxicity test:* Acute toxicity was performed as previously described with some modifications (Iqbal et al., 2014). Mice of either sex were randomly divided into five groups of 2 animals each. Group 1 served as control and received normal saline (10 mL/kg) while other groups (Group 2, Group 3, Group 4 and Group 5) were given different doses of aqueous methanolic extract of *P. glaucum* seeds in an ascending order i.e., 500, 1000, 2000 and 4000 mg/kg orally. The mortality rate and signs of toxicity were observed for 24 hours in mice allowed them free access to feed and water.

*Sub-chronic toxicity study:* Rats of either sex were randomly divided into two groups (n= 6). The first group was the control and received normal saline (5 mL/kg body weight p.o.) and group 2 was given 1,000 mg/kg of aqueous methanolic extract of *P. glaucum* seeds for 29 days. Food and water intake of animals were observed during this period. At 30<sup>th</sup> day, blood was collected from overnight fasted rats of each group by cardiac puncture for the determination of serum biochemical parameters. For the determination of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total cholesterol, triglycerides, low-density lipoprotein (LDL) and high density lipoprotein (HDL) levels, blood samples were collected in clot activator gel tubes. The serum was separated by centrifuging the blood samples at 2,000 rev/min for 10 min. Serum biochemical parameters were then measured by using commercially available reagent kits (Biswas et al., 2010).

*Statistical analysis:* The results were expressed as means  $\pm$  standard error of mean (S.E.M) and statistical analysis was carried out by student's t-test.  $P < 0.05$  was considered statistically significant.

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## Results

*Effect of crude extract of P. glaucum seeds on blood pressure and heart rate of normotensive rats:* The crude extract of *P. glaucum* seeds showed a significant decrease in the systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP) and heart rate (HR) of normotensive rats at 500 and 1,000 mg/kg doses. The dose 250 mg/kg showed non-significant results in normal rats. The maximum decrease in all these parameters was observed at 1,000 mg/kg dose (Table I).

*Effect of crude extract on blood pressure and heart rate of glucose-induced hypertensive rats:* The extract produced significant decrease in SBP, DBP, MBP and heart rates of glucose induced hypertensive rats in dose dependent fashion as shown in Table II. The dose 1,000 mg/kg exhibited maximum effect by decreasing the blood pressure and heart rate persistently for 8 hours both in normotensive and glucose induced hypertensive rats

Table I

Effects of aqueous-methanolic extract of *Pennisetum glaucum* seeds on blood pressure and heart rates in normotensive rats

Time (hour)	Treatment with extract of <i>Pennisetum glaucum</i> seeds											
	250 mg/kg				500 mg/kg				1,000 mg/kg			
	SBP	MBP	DBP	HR	SBP	MBP	DBP	HR	SBP	MBP	DBP	HR
0	127.1 (0.6)	102.9 (0.3)	90.7 (0.6)	384 (2.0)	124.8 (0.5)	106.8 (0.8)	97.7 (1.3)	378 (2.1)	127.1 (0.7)	105.6 (0.8)	94.7 (1.1)	387 (2.1)
2	125.1 (0.5)	101 (0.4)	88.9 (0.8)	383 (1.0)	120.4 (2.1)	100.4 (0.4)	90.4 (0.7)	373 (1.5) <sup>a</sup>	121.4 (2.1) <sup>a</sup>	99.0 (1.7)	87.7 (3.1)	373 (2.4) <sup>a</sup>
4	124.5 (0.9)	99.6 (0.7)	87.2 (1.0)	382 (1.3)	118 (0.4)	94.3 (1.0) <sup>a</sup>	82.0 (1.7) <sup>a</sup>	366 (2.2) <sup>b</sup>	112.7 (2.1) <sup>b</sup>	91.5 (1.5) <sup>a</sup>	80.1 (2.7) <sup>a</sup>	357 (4.4) <sup>b</sup>
6	123.2 (1.2)	98.5 (1.7)	86.1 (2.8)	381 (1.8)	116.7 (1.7) <sup>a</sup>	85.0 (1.8) <sup>b</sup>	69.0 (1.0) <sup>c</sup>	354 (2.6) <sup>b</sup>	104.2 (2.4) <sup>c</sup>	86.6 (1.4) <sup>b</sup>	77.9 (2.5) <sup>b</sup>	341 (3.1) <sup>c</sup>
8	125.6 (0.6)	101 (0.8)	88.8 (1.1)	383 (0.9)	121.4 (0.7) <sup>a</sup>	91.0 (2.1) <sup>a</sup>	75.0 (3.0) <sup>b</sup>	359 (2.3) <sup>b</sup>	101.5 (2.0) <sup>c</sup>	83.6 (1.5) <sup>c</sup>	74.6 (2.7) <sup>c</sup>	332 (2.7) <sup>c</sup>

Results are expressed as means (S.E.M); n = 6; SBP means systolic blood pressure; MBP means mean blood pressure; DBP means diastolic blood pressure; HR means heart rate; <sup>a</sup> = (p<0.05), <sup>b</sup> = (p<0.01), <sup>c</sup> = (p<0.001) vs. control (0 hour)

Table II

Effects of aqueous-methanolic extract of *Pennisetum glaucum* seeds on blood pressure and heart rates in glucose-induced hypertensive rats

Time (hour)	Treatment with extract of <i>Pennisetum glaucum</i> seeds											
	250 mg/kg				500 mg/kg				1,000 mg/kg			
	SBP	MBP	DBP	HR	SBP	MBP	DBP	HR	SBP	MBP	DBP	HR
0	160.0 (2.2)	141.0 (0.7)	133.0 (0.9)	396 (4.0)	166 (0.8)	155.0 (0.7)	150.0 (0.8)	398 (3.8)	165.4 (2.0)	144.0 (0.6)	133.0 (0.8)	400 (2.3)
2	154.3 (1.0)	140.9 (0.6)	131.0 (0.8)	383 (2.6) <sup>a</sup>	150.5 (1.2)	143.0 (0.6)	139.0 (0.7)	386 (4.1) <sup>a</sup>	145.0 (3.2) <sup>a</sup>	129.0 (2.4) <sup>b</sup>	121 (2.5) <sup>a</sup>	371 (3.7) <sup>b</sup>
4	151.1 (1.6)	130.0 (1.5) <sup>a</sup>	120.0 (1.8)	376 (3.7) <sup>b</sup>	143.0 (1.8) <sup>a</sup>	137.3 (1.2) <sup>a</sup>	134.0 (1.1)	360 (2.7) <sup>b</sup>	132.0 (2.6) <sup>b</sup>	109.4 (1.8) <sup>c</sup>	98.0 (1.1) <sup>b</sup>	363 (3.1) <sup>c</sup>
6	141.0 (1.8) <sup>b</sup>	124.0 (3.5) <sup>b</sup>	116.0 (0.7)	363 (2.1) <sup>b</sup>	139.0 (2.8) <sup>a</sup>	130.0 (2.5) <sup>b</sup>	125.0 (0.7) <sup>a</sup>	350 (4.2) <sup>b</sup>	123.4 (1.3) <sup>c</sup>	97.0 (1.2) <sup>c</sup>	84.0 (2.5) <sup>b</sup>	340 (4.2) <sup>c</sup>
8	138.0 (1.0) <sup>b</sup>	126.0 (2.6) <sup>b</sup>	120.0 (1.9)	367 (4.0) <sup>b</sup>	130.0 (2.3) <sup>b</sup>	122.0 (2.2) <sup>c</sup>	118.0 (1.1) <sup>b</sup>	348 (3.2) <sup>b</sup>	119.4 (1.2) <sup>c</sup>	89.4 (1.0) <sup>c</sup>	74.4 (3.1) <sup>c</sup>	327 (3.2) <sup>c</sup>

Results are expressed as means (S.E.M); n = 6; SBP means systolic blood pressure; MBP means mean blood pressure; DBP means diastolic blood pressure; HR means heart rate; <sup>a</sup> = (p<0.05), <sup>b</sup> = (p<0.01), <sup>c</sup> = (p<0.001) vs. control (0 hour)

so, it was selected for further experiments.

**Effect of crude extract on blood pressure and heart rate of egg feed diet fed rats:** The Figure 1 and 3 shows the effect of aqueous-methanolic extract on blood pressure and heart rate of rats receiving the egg feed diet for 21 days. It has been clearly depicted that the treatment with extract of *P. glaucum* seeds significantly prevented SBP, DBP, MBP and heart rates to increase in egg feed treated rats as compared to control.

**Effect of crude extract on blood pressure and heart rate of glucose induced hypertensive rats:** The effect of aqueous-methanolic extract on blood pressure and heart rate has been shown in Figure 2 and 3. The extract exhibited the significant antihypertensive effect by preventing the SBP, DBP, MBP and heart rate to increase in the rats that were administered 1,000 mg/kg dose of extract at daily basis for 21 days.

**Acute and sub-chronic toxicity:** The crude extract of was

found safe in mice up to the dose of 4 g/kg. After 24 hours no mortality or any other apparent behavioral abnormalities were observed. In sub-chronic toxicity studies, the extract did not cause significant alterations in serum ALT, AST and ALP levels. Total cholesterol, triglycerides, LDL levels and HDL levels were also not changed in the extract treated rats as compared to control group.

## Discussion

The last few decades have witnessed over the growing interest of researchers towards medicinal plants to explore their pharmacological uses in the treatment of various diseases. This is due to the extraction and development of several successful drugs and chemotherapeutic agents from plants. Herbal drugs are widely used today due to their minimal adverse effects and low costs (Khanet al., 2014). *P. glaucum* seeds have

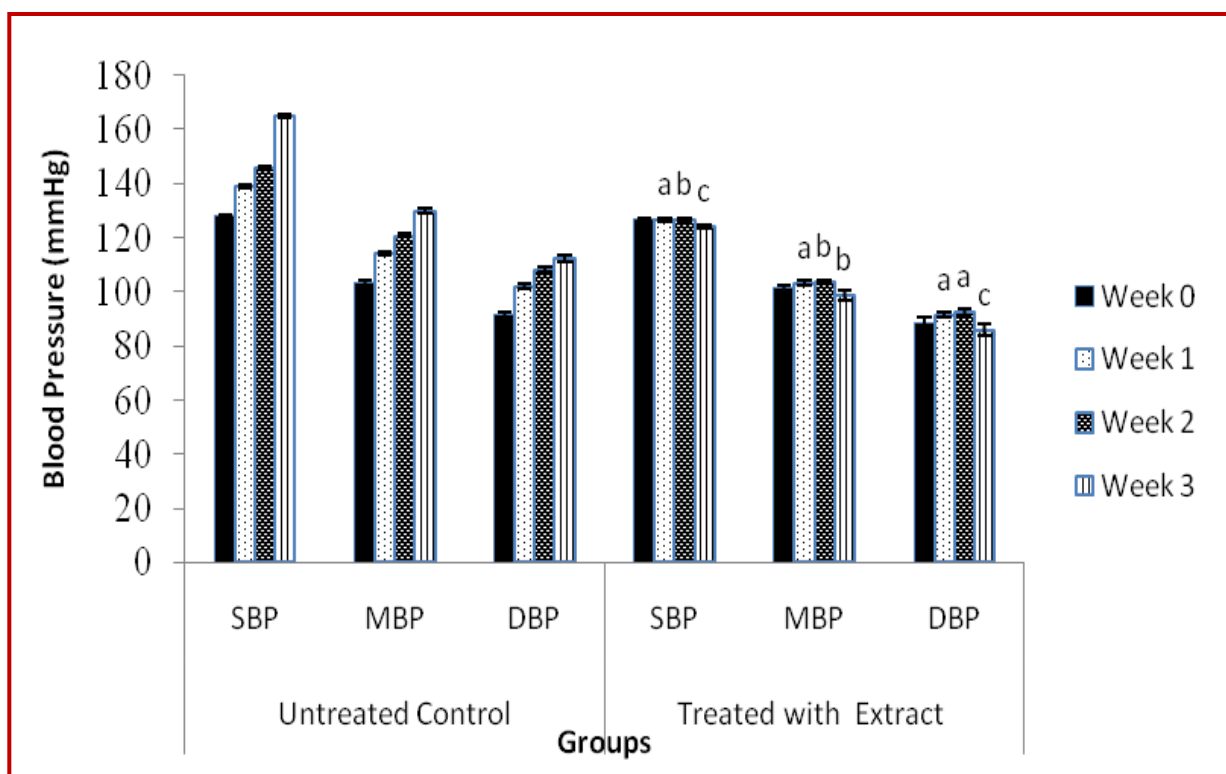


Figure 1: Antihypertensive activity of aqueous-methanol extract of *Pennisetum glaucum* seeds in egg feed treated rats. a = ( $p < 0.05$ ), b = ( $p < 0.01$ ) and c = ( $p < 0.001$ ), compared to control

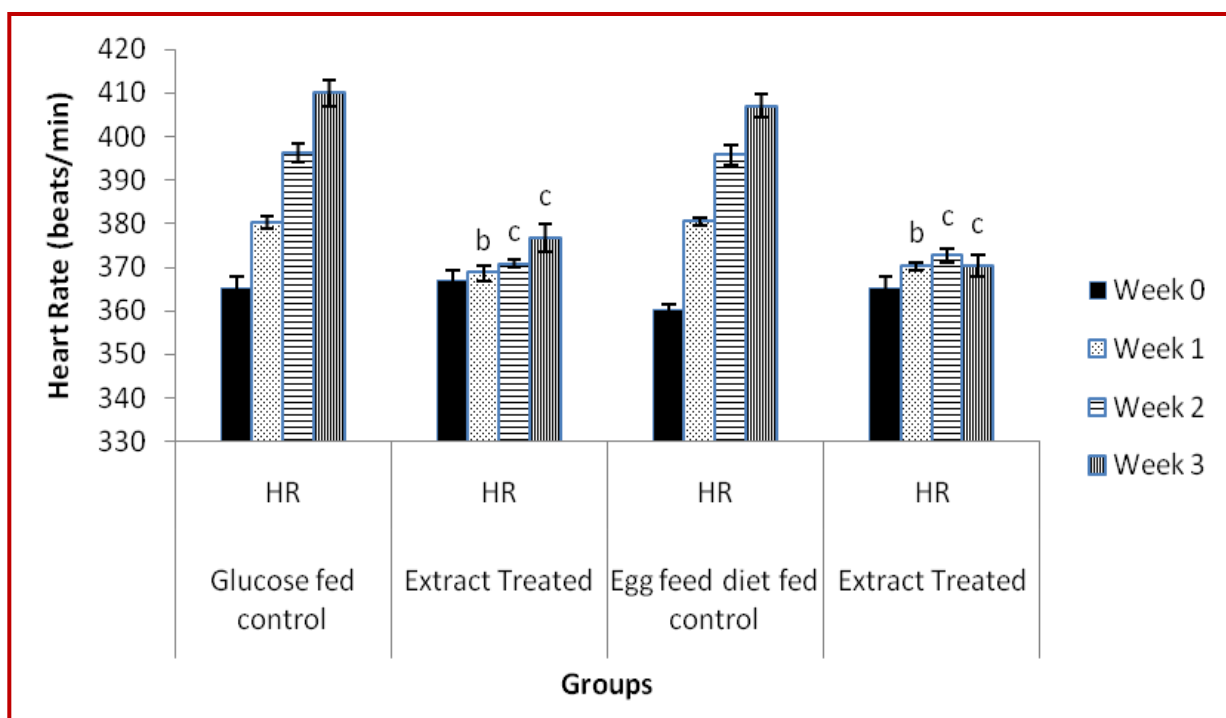


Figure 2: Effect of aqueous-methanol extract of *Pennisetum glaucum* seeds on heart rate of egg feed and glucose fed rats. b = ( $p < 0.01$ ) and c = ( $p < 0.001$ ), compared to control

been used for the treatment of various diseases in folklore. In the current study, the seeds of this plant were investigated to rationalize its use in cardiovascu-

lar disorders. The crude extract of *P. glaucum* seeds produced the decrease in blood pressure and heart rate in concentration dependent fashion in normotensive

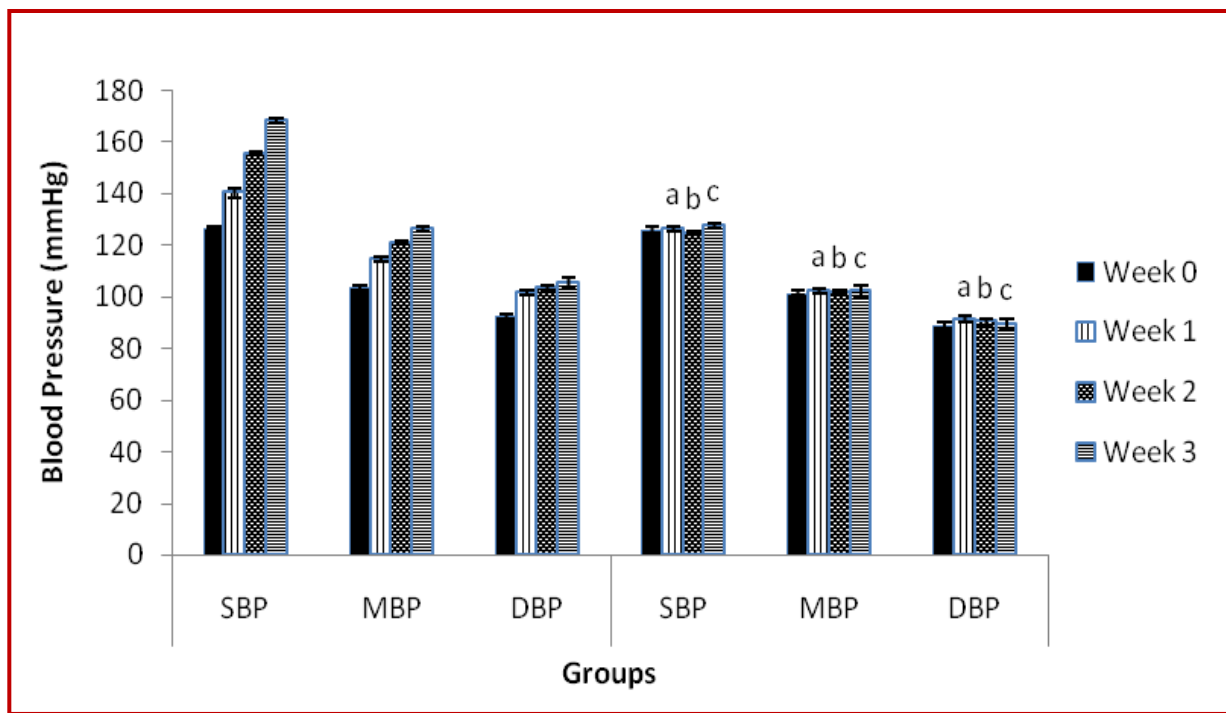


Figure 3: Antihypertensive activity of aqueous-methanol extract of *Pennisetum glaucum* seeds in glucose fed rats. a = ( $p < 0.05$ ), b = ( $p < 0.01$ ) and c = ( $p < 0.001$ ), compared to control

and glucose treated rats. The dose 1,000 mg/kg showed the more pronounced effect and further experiments were carried out at this dose. The results have shown that the extract reduced the blood pressure more in hypertensive rats as compared to normotensive rats. The results are in line with our previous study (Alamgeer et al., 2013).

The extract also prevented the increase in blood pressure in glucose and egg feed diet treated rats in 21 days study. The use of glucose for an extended time period has been reported to produce free radicals. The reactive oxygen species (ROS) are prime responsible for increasing blood pressure (Midaoui and Champlain, 2002; Reaven and Ho, 1991). The seeds of *P. glaucum* have been reported to contain significant amount of antioxidant phytochemical constituents including flavonoids, flavanol, various phenolic acids (Nambiar et al., 2012). It is suggested that the lowering blood pressure effect of the plant might be due to the presence of these phytochemical constituent which counter act the reactive oxygen species. This is in consistent with the previous studies (Alamgeer et al., 2015). Egg feed induced hypertensive model in rats has also been used to study the antihypertensive effect of the plants. The increased blood pressure in egg feed diet fed rats is due to high cholesterol level and dyslipidemia which lead to hypertension (Ayele et al., 2010). The administration of the crude extract in high cholesterol diet treated rats prevented the rise in blood pressure as compared to untreated control. The previous studies have shown that secondary metabolite attenuate the endothelial

dysfunction by decreasing the level of bad cholesterol (LDLs) and production of nitric oxide (Wing-Hung et al., 2000). Flavonoids have been shown to modify eicosanoid biosynthesis and protect low density lipoprotein (LDL) from oxidation which promotes relaxation of cardiovascular smooth muscle leading to its antihypertensive effect.

The acute toxicity study shows that the extract is safe for use as no sign of toxicity were observed up to 4 g/kg b.w dose in mice. The extract was also studied by administering it for one month for its possible adverse effects in liver enzymes and lipid profile of rats. There was no significant change observed in liver enzyme levels and lipid profile of the extract treated rats. However, a decreasing trend in LDL-cholesterol level was observed which shows the possible beneficial effect of the extract in lowering the bad cholesterol. These effects of the extract may be attributed due to the presence of phytochemical compounds. However, further studies are required for the isolation of active constituent (s) and elucidation of exact mechanism of action.

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### Conflict of Interest

Animals were fed standard diet and tap water and were treated according to the standard procedures guided by

National Research Council.

## Conflict of Interest

Authors declare no conflict of interest

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