

Effects of Fruits Extracts of Three Medicinal Plants on the Blood Glucose Level of Glucose-induced Hyperglycemic and Normal Rabbit Models

Ikram Ilahi (Corresponding Author)

Assistant Professor, Department of Zoology, University of Malakand

Chakdara, Dir (L), Khyber Pakhtunkhwa, Pakistan

E-mail: ikramilahi@yahoo.com

Asghar Khan

Student, Department of Biotechnology, University of Malakand

Chakdara, Dir (L), Khyber Pakhtunkhwa, Pakistan

E-mail: asghar444@yahoo.com

Ubaid Ullah

Student, Department of Biotechnology, University of Malakand

Chakdara, Dir (L), Khyber Pakhtunkhwa, Pakistan

E-mail: ubaid87_pk@yahoo.com

Imran Khan

Student, Department of Biotechnology, University of Malakand

Chakdara, Dir (L), Khyber Pakhtunkhwa, Pakistan

E-mail: 85@yahoo.com

Jawad Ali

Student, Department of Biotechnology, Faculty of Biological Sciences

Quaid-i-Azam University, Islamabad, Pakistan.

E-mail: ajwdli@gmail.com

Murad Khan

Lecture, Department of Biotechnology, University of Malakand

Chakdara, Dir (L), Khyber Pakhtunkhwa, Pakistan

E-mail: Pacifist88@yahoo.com

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Abstracts

The fruits of *Pistacia integerrima* (PI), *Withania coagulans* (WC) and *Withania somnifera* (WS) have been reported for their medicinal values. The aim of the present study was to compare the glucose lowering activities of the fruit extracts of these three medicinal plants in glucose induced non-diabetic hyperglycemic and normal rabbit models. During the first phase of experiments, the effects of oral administration of 70% methanol extract of each plant fruit (600 mg/kg body weight) and glibenclamide (5 mg/kg body weight) were studied on the glucose level of glucose induced hyperglycemic rabbits, while in the second phase of experiments, the effects of each fruit extract (600 mg/kg body weight) and glibenclamide (5 mg/kg body weight) were assessed on the glucose level of normal rabbits. Among the three fruit extracts, maximum reduction in the blood glucose level was caused by the PI extract ($P < 0.05$). The glucose lowering activity of the PI extract was greater than that of the standard, glibenclamide (5mg/kg), during the first phase of experiments. However during the second phase of experiments the hypoglycemic effect of the PI extract was comparable to that of the standard, glibenclamide (5mg/kg). In conclusion the present study reveals that the 70% methanol extract of *Pistacia integerrima* fruit can decrease glucose level significantly in glucose induced non-diabetic hyperglycemic and normal rabbit models.

Keywords: *Pistacia integerrima*, *Withania coagulans*, *Withania somnifera*, Glucose load, Glibenclamide

1. Introduction

Glucose is most important physiologically. Circulating glucose is derived from three sources: intestinal absorption during the fed state, glycogenolysis, and gluconeogenesis. Glycogenolysis and gluconeogenesis are partly under the control of glucagon, a hormone produced in the α -cells of the pancreas (Aronoff et al., 2004). The blood glucose level of humans varies between 80 and 100 mg/dl. Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted (Edem, 2009). In conditions of high blood glucose level, more glucose enters into the β -cells of islet of Langerhans. More glucose is metabolized and as a result more ATPs are produced (Matschinsky, 1995; Maechler, 2006;

Corkey, 2008). The increased concentration in intracellular ATP results in the closure of the ATP-sensitive K^+ channels (K_{ATP}) and decreased outward K^+ transport. The voltage-gated Ca^{2+} channels become opened which results in the influx of extracellular Ca^{2+} . The high concentration of intracellular Ca^{2+} , activates protein motors and kinases, which then mediate exocytosis of insulin-containing vesicles that leads to the increased insulin and decreased blood glucose levels (Fridlyand et al., 2003).

Diabetes mellitus (DM) should be suspected if venous plasma glucose level drawn irrespective of when food was last eaten (a “random” sample of blood glucose) is unequivocally elevated (i.e., ≥ 200 mg/dl), particularly in a patient who manifests the classic signs and symptoms of chronic hyperglycemia, polydipsia, polyuria, blurred vision, headaches, rapid weight loss, sometimes accompanied by nausea and vomiting (Marks et al., 1996).

Diabetes mellitus is characterized by high blood glucose level (Hyperglycemia). This is an important factor in the development and progression of diabetes and related complications (Luzi, 1998). More than 230 million people globally are affected with the disease (Arumugam *et al.*, 2008). Management of diabetes is a huge burden. While therapeutic insulin production is not adequate to meet demands, the recombinant DNA approach to diabetes management has faced several problems. It is hypothesized that the ultimate therapy for type I and type II diabetes lies in the herbal approach (Arumugam et al., 2008).

Herbal anti-diabetic medicines are gaining more acceptances amongst diabetic patients because it is generally believed that herbal medicines lack side effects on the patients’ health (Mahesh, 2010). More than 1000 plant species are being used for the treatment of diabetes (Marles & Farnsworth, 1995). The anti-diabetic property of medicinal plants is attributed to the presence of some phytochemical ingredients. The medicinal plants which are used against diabetes, are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents (Ji et al., 1998; Jung et al., 2006). Several species of anti-diabetic plants have been reported in the scientific and popular literature (Valianthan, 1998).

Pakistan is blessed with a variety of wild plants, which are being used for medicinal and aromatic purposes. Various preparations of medicinal plants are used for the treatments of diseases both at both community and user level. Many medicinal plants have still not been explored for their medicinal values (Ahmad & Hussain, 2008). The plants *Pistacia integerrima* (Stew), *Withania coagulans* (Dunal) and *Withania somnifera* (Dunal) are among the medicinal plants found in Pakistan. The plant *Pistacia integerrima* is a medicinal plant (tree) of family Anacardiaceae, found in Eastern Afghanistan and some parts of Pakistan. The plants *Withania coagulans* and *Withania somnifera* are shrubs, belong to the old genus *Withania* of the family Solanaceae and found in Iran, Afghanistan, Pakistan and India (Yasin & Nasir, 1985).

The fruit of *Pistacia integerrima* is known for its antimicrobial, antioxidant and hepatoprotective activities (Ahmad et al., 2008; Khan et al., 2008; Rahman et al., 2011;). Some plants of the family Anacardiaceae have been reported for their anti-hyperglycemic property. These include *Mangifera indica*, *Spondias mangifera* and *Anacardium occidentale*

(Aderibigbe et al., 2001; Tedong et al., 2006; Acharyya et al., 2010). The fruits of *Withania coagulans* and *Withania somnifera* have been reported for their antidiabetic activities (Jaiswal et al., 2009; Hoda, et al., 2010).

In the present study, 70% methanol extracts of the fruits of *Pistacia integerrima*, *Withania coagulans* and *Withania somnifera* were prepared and tested for their effects on the level of blood glucose in glucose induced non-diabetic hyperglycemic rabbits and normal rabbits.

Aims and objectives: The present study was aimed to compare the anti-hyperglycemic potentials of the fruits of *Pistacia integerrima*, *Withania coagulans* and *Withania somnifera* in non- diabetic glucose induced hyperglycemic and normal rabbit models.

2. Materials and Methods

2.1 Plant Material

The dry fruits of *Pistacia integerrima* (Stew), *Withania coagulans* (Dunal) and *Withania somnifera* (Dunal) were purchased from the local market of Mingora Swat, Khyber Pakhtunkhwa. The fruits were botanically authenticated by Mr. Mahboob-ur-Rahman, Associated Professor, Department of Botany, Government Post Graduate Jehanzeb College, Swat, Khyber Pakhtunkhwa, Pakistan.

2.2 Preparation of Crude Extract

The clean and dried fruits of *Pistacia integerrima*, *Withania coagulans* and *Withania somnifera* were coarsely ground separately. Then 333.3 gram of PI fruit powder was soaked in 1666.6 ml of 70% methanol, 182 gram of WC fruit powder in 600 ml of 70% methanol and 112 gram of WS fruit powder in 400ml of 70% methanol for 3 days with occasional shaking. The soaked material was filtered through a muslin cloth. The filtrate of each plant was then separately evaporated on a rotary evaporator (Heidolph Laborta 4000 efficient) under reduced pressure. The extracts in concentrated solution forms were then transferred to beakers and placed in a well-ventilated room for evaporating the remaining solvent and finally the extracts of three plant fruits were obtained in dry form. The amount and percentage yield (w/w) of dry extract obtained from *Pistacia integerrima* was 115 gram (34.5%), *Withania coagulans* 61.68 gram (33.51%) and *Withania somnifera* 32.64 gram (29.14%).

2.3 Preliminary Phytochemical Screening

A Preliminary phytochemical screening of 70% methanol extract of each plant extract was carried out by using standard procedures (Harborne, 1973).

2.4 Experimental Design

The experiments were performed in two phases. During the phase 1, the effects of plant extracts were studied on the glucose level of glucose induced hyperglycemic rabbits while in the second phase (Phase 2) of experiments the effects of plant extracts were assessed on the glucose level of normal rabbits.

Phase 1: Twenty five rabbits divided into five groups (5 rabbits each) were used in this experiment. The rabbits of each group were tagged with special color and figures on their ear pinnae. The detail is given below.

1. Control group, received glucose 500mg /Kg body weight, and treated with 3 ml of 2% dimethyl sulfoxide (DMSO) solution. This group was considered as hyperglycemic control group.
2. Standard group, received glucose 500 mg/Kg body weight and treated with glibenclamide 5mg/Kg body weight, dissolved in 3ml of 2% dimethyl sulfoxide (DMSO)
3. PI ext group, received glucose 500 mg/Kg body weight and treated with *Pistacia integerrima* extract 600 mg/Kg body weight, dissolved in 3ml of 2% DMSO
4. WC ext group, received glucose 500 mg/Kg body weight and treated with *Withania coagulans* extract 600 mg/Kg body weight, dissolved in 3ml of 2% DMSO
5. WS group, received glucose 500 mg/Kg body weight and treated with *Withania somnifera* extract 600 mg/Kg body weight, dissolved in 3ml of 2% DMSO

2.5 Procedure

Pre-treatment (0 hrs) blood glucose levels were measured after a 17 hours fast. Then the drug and plant extracts were administered orally by using oesophageal catheter. Then all the rabbits were orally administered with glucose at the dose of 2 g/kg body weight after 30 min of extract and drugs administration. Blood glucose levels were measured through an electronic glucometer (Accu check Advantage II, Roche Diagnostics, Germany) with a single drop of blood from marginal vein of ear rabbit after 1/2, 1, 2 and 3 hour of glucose load.

Phase 2: Twenty five rabbits divided into 5 groups (5 rabbits each) were used during this phase. This time the effects of plant extracts were studied on the glucose level of normal fasting rabbits, therefore the step of glucose load was skipped. The detail is given below.

1. Control group, treated with 3 ml of 2% DMSO solution. This group was considered as normal control group
2. Standard group, treated with glibenclamide 5mg/Kg body weight, dissolved in 3ml of 2% DMSO
3. PI group, treated with *Pistacia integerrima* extract 600 mg/Kg body weight, dissolved in 3ml of 2% DMSO
4. WC group, treated with *Withania coagulans* extract 600 mg/Kg body weight, dissolved in 3ml of 2% DMSO
5. WS group, treated with *Withania somnifera* extract 600 mg/Kg body weight, dissolved in 3ml of 2% DMSO

Pre-treatment (0 hours) blood glucose levels were measured after a 17 hours fast. Then the drug and plant extracts were administered orally by using oesophageal catheter. Blood glucose levels were measured through an electronic glucometer (Accu check Advantage II, Roche Diagnostics, Germany) with a single drop of blood from marginal vein of ear rabbit after 1/2, 1, 2 and 3 hour of drug and extract administration.

2.6 Statistical Analysis

Results were presented as mean + standard deviation. The means were compared by using Dunnett's Test of Post Hoc Multiple Comparisons in One Way Anova through SPSS 16 software for windows.

3. Results and Discussion

The preliminary phytochemical screening of each plant extract revealed the presence of tannins, saponins, glycosides, flavonoids and phenols. These ingredients have been reported for their anti-hyperglycemic activity (Sherman and Billing 1999; Sridhar et al., 2005; Anh and Kim, 2005; Zhou et al., 2009).

The effects of 70% methanol extracts of fruits of *Pistacia integerrima* (PI), *Withania coagulans* (WC) and *Withania somnifera* (WS) on the blood glucose level of glucose induced hyperglycemic rabbits studied during phase I are shown in table 1. The table shows that at zero hour there was no significant difference in glucose levels of all groups ($P>0.05$). After ½ hour of glucose load, an increase in the glucose levels of all groups was observed which later on gradually declined. High amount of glucose in the blood stimulate insulin secretion, which enhance peripheral glucose consumption and controls the production of glucose through different mechanisms. The stimulation of insulin secretion by glucose results primarily from depolarization of β cells plasma membrane (Meissner and Schmelz, 1974). When there occurs the depolarization of plasma membrane, the Ca^{+2} starts entry to beta cells through voltage sensitive Ca^{+2} channels (Satin & cook, 1985). The increase in intracellular Ca^{+2} activates protein motors and kinases, which then mediate exocytosis of insulin-containing vesicles which lead to increased insulin and decreased blood glucose level (Fridlyand et al., 2003).

The rabbit of drug and the fruit extracts treated groups showed non-significant reduction ($P>0.05$) in glucose level when estimated after ½ hour of glucose load. The Maximum decrease (10%) in glucose level was observed in PI extract treated group. After 1st hour of glucose load, the PI extract and the drug, glibenclamide caused 38% and 25.69% reduction in the blood glucose levels respectively. When the glucose levels of drug (119.70 ± 7.32) and the PI extract treated (99.00 ± 4.65) groups were compared to hyperglycemic control ($161.10 + 11.03$), the difference was significant ($P<0.05$). The mean glucose levels of WC extract (145.35 ± 8.10) and WS extract (129.6 ± 12.72) treated groups were also lower from the hyperglycemic control group, but the difference was non-significant ($P>0.05$). After 2nd hour of glucose load, a decrease in glucose levels of all groups was observed with time. A remarkable reduction in the blood glucose level was observed in the PI extract (37.66%) and glibenclamide treated group (28.74%). The difference in glucose levels from the control

group was significant ($P < 0.05$). After 3rd hour of glucose load, the glucose level of the drug, and each of the extract treated groups was significantly lower from hyperglycemic control group ($P < 0.05$). Maximum reduction in blood glucose level was caused by glibenclamide (33.65%) followed by PI extract (31.38%), WS (24.76%) and WC extract (23.08%). In the extracts and drug treated groups, the glucose levels have not exceeded more than the control group, giving an indication regarding the supportive action of extract and drug in glucose utilization. The effect of glibenclamide on glucose tolerance has been attributed to enhanced activity of β cells of pancreas resulting in secretion of large amount of insulin. The mechanism behind the anti-hyperglycemic activities of plant fruit extracts may involve insulin like effect by increasing peripheral glucose consumption or it may enhance the sensitivity of beta cells for glucose, resulting in an increased insulin release (Andrew, 2000).

The effects of 70% methanol extracts of fruits of *Pistacia integerrima* (PI), *Withania coagulans* (WC) and *Withania somnifera* (WS) on the blood glucose level of normal rabbits studied during phase II are shown in table 2. At zero hour there was no significant difference in glucose levels of all the groups ($P > 0.05$). After $\frac{1}{2}$ and 1st hour of drug and extract administration only glibenclamide caused significant reduction ($P < 0.05$) in the blood glucose level of normal rabbits. After 2nd and 3rd hours of drug and extract administration, the drug and the PI extract caused significant ($P < 0.05$) reduction in the blood glucose level. Glibenclamide caused 13.34% reduction after 2nd hour and 20.32% reduction after 3rd hour. The PI extract caused 10.37 % reduction after 2nd hour and 20.32% reduction after 3rd hour of drug and extract administration.

The WC and WS extracts also caused reduction in the blood glucose level of normal rabbits but the difference from the control group was non-significant ($P > 0.05$). During this study the drug glibenclamide and the PI extract resulted into a significant reduction of blood glucose level of normal rabbits. The hypoglycemic effects produced by the extract may be due to the increased insulin release resembling the mechanism of action of sulphonylurease (Miura et al., 2001; Okine et al., 2005).

The fruit of *Pistacia integerrima* is known for its antimicrobial, antioxidant and hepatoprotective activities (Ahmad et al., 2008; Khan et al., 2008; Rahman et al., 2011). Some plants of the family Anacardiaceae have been reported for their anti-hyperglycemic property. These include *Mangifera indica*, *Spondias mangifera* and *Anacardium occidentale* (Aderibigbe et al., 2001; Tedong et al., 2006; Acharyya et al., 2010). The fruits of *Withania coagulans* and *Withania somnifera* have been reported for their antidiabetic activities (Jaiswal et al., 2009; Hoda, et al., 2010).

In conclusion the present study reveals that the 70% methanol extract of *Pistacia integerrima* can decrease glucose level significantly in glucose induced non-diabetic hyperglycemic and normal rabbit models.

Table 1. The effects of 70% methanol extracts of fruits of three medicinal plants on the glucose level (mg/dl) of non-diabetic glucose induced hyperglycemic rabbits at a dose of 600 mg/kg body weight.

Group	0 hour	1/2 hour	1 hour	2 hours	3 hours
Control	87.75 ± 7.69	139.50 ± 8.28	161.10 ± 11.03	138.3 ± 9.9	118.70 ± 5.20
Standard	86.85 ± 6.47	132.30 ± 11.03 (-5.16%)	119.70 ± 7.32* (-25.69%)	98.55 ± 7.25* (-28.74%)	78.75 ± 8.2* (-33.65%)
PI ext	91.80 ± 8.18	125.55 ± 13.70 (-10%)	99.00 ± 4.65* (-38%)	86.25 ± 9.58* (-37.66%)	81.45 ± 7.72* (-31.38%)
WC ext	91.80 ± 8.18	140.4 ± 15.29 (+ 0.64%)	145.35 ± 8.10 (-9.7%)	125.8 ± 6.6 (-9.03%)	91.3 ± 6.1* (-23.08%)
WS ext	94.5 ± 9.32	138.6 ± 12.02 (-0.64%)	129.6 ± 12.72 (-19.55%)	114 ± 13.18 (-17.57%)	89.3 ± 7.14* (-24.76%)

Standard-glibenclamide, PI ext- *Pistacia integerrima* extract, WC ext- *Withania coagulans* extract, WS ext- *Withania somnifera* extract, *- Indicates significant difference in the blood glucose level when compared to the hyperglycemic control group ($P < 0.05$). Values in parentheses indicate percent increase (+) or decrease (-) in the blood glucose level of extract and drug treated animals from hyperglycemic control animals.

Table 2. Effects of 70% methanol extracts of fruits of three medicinal plants on the glucose level (mg/dl) of normal rabbits at a dose of 600mg/kg body weight. N= 5

Groups	0 hr	1/2 hr	1 hr	2 hr	3 hr
Control	102.7 ± 2.52	96.3 ± 5.23	92.5 ± 2.8	89.9 ± 5.34	84.5 ± 2.1
Glibenclamide	99.76 ± .74	88.45 ± 4.23*	81.25 ± 4.33* (-12.16%)	77.9 ± 2.86* (-13.348 %)	67.33 ± 4.13* (-20.32 0%)
PI	104.2 ± 3.3	90.02 ± 2.76 (- 6.521 %)	86.45 ± 5.27 (- 6.54 %)	80.57 ± 4.32* (- 10.37%)	67.33 ± 3.58* (-20.32 %)
WC	98.05 ± 4.3	93.12 ± 3.23 (-3.3%)	89.13 ± 3.24 (- 3.64 %)	86.3 ± 3.11 (- 4.04 %)	80.93 ± 6.22 (-4.22%)
WS	101.4 ± 3.40	94.3 ± 2.17 (- 2.07 %)	89.56 ± 2.45 (- 3.17 %)	87.23 ± 7.12 (- 2.97 %)	79.96 ± 3.114 (-5.37 %)

PI-*P. integerrima* extract, WC- *W.coagulans* extract, WS- *W.somnifera* extract, *- Indicates significant difference in blood glucose level from normal control group ($P < 0.05$). Values in parentheses indicate percent increase (+) or decrease (-) in blood glucose level of extract and drug treated animals from normal control animals after ½, 1, 2 and 3rd hours of glucose load.

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