

# Antidiabetic activity of extracts of *Pistachia khinjuk* on alloxan monohydrate induced diabetic mice

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**Abstract.** Diabetes is spreading all over the world day by day. There are many ways to treat diabetes mellitus which mainly includes synthetic drugs, homeopathic medicine or Unani medicine etc. These Medicines have many side effects. Natural products; which include herbs, shrubs and large plants, cure diabetes mellitus and prove excellent hypoglycemic activity. These herbal medicines have no reported side effects. Many species of family Anacardiaceae show hypoglycemic activity and used from centuries to cure diabetes. Various pistachio species show hypoglycemic activity and have a very long history of herbal remedies. The present study evaluates the hypoglycemic effect of methanolic extract of *Pistachia khinjuk*. Six groups of Swiss albino mice were made for extract (80:20 Methanol: water) of *Pistachia khinjuk* and each group contains six albino mice. All the mice were injected alloxan monohydrate except normal group of wax and extract. Group 1 was treated as normal group and receives no treatment, group 2 receive 5mg/kg of glibenclamide after alloxan monohydrate induction, group 3 receive no treatment after alloxan monohydrate induction, group 4 and 5 receive 500 and 250mg/kg of *Pistachia khinjuk* extract, while group 6 receives 500mg/kg *Pistachia khinjuk* wax after alloxan monohydrate treatment. All the mice for extract (*Pistachia khinjuk*) of group 4, 5 and 6 show hypoglycemic activity and decreases blood glucose level. There may be many factors behind this activity which needs more research on it by isolating and analyzing specific secondary metabolites which causes this effect. The methanolic extract due to phenolic constituents proves to be excellent antidiabetic medicine.

## 1 Introduction

Natural products are derived from living organisms, including plants, animals, insects and microbes to get the compounds, while natural product studies include structural investigation, composition, their use and purpose of the organism (Farrell 1998). Humans use plants to treat a variety of diseases from thousands of years. According to the World Health Organization, most of the population still relies on traditional medicines for mental and physical requirements because they cannot afford the cost of allopathic medicines with

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their side effects and lack of health care facilities. In rural areas where many developing countries rely on traditional medicine to meet the needs of primary health care, they still find a place in their daily lives. These drugs are safer and cheaper than synthetic drugs or modern medicine. (Fatima et al., 2012). Anacardiaceae family includes more than 700 species of 82 belongings in the first tropical, some species, however, extend to the temperate zone. Family members of Anacardiaceae growing around the world for their results and good seed to eat medicinal cares, precious timber, and natural attraction. Some Anacardiaceae products include mango and pistachio, cashew nuts, pink pepper and enjoy the rest of the world. It mainly includes Anacardiaceae trees and shrubs, and genus with a clear milky white resin milk channel. Leaves are estipulate usually choosen, but may be simple or complex. In general, the flower is not very high, horizontally or essentially connected. Fruit varieties are very high in shape with family found thousands of species (Pell 2004)

Pistachio is usually found in the Mediterranean region of Pakistan. The subspecies from Anacardiaceae's family known as terebinthaceae is also Pistaciaceae. (Abdelkrim et al., 2015) Gender pistachio (Anacardiaceae) is widely distributed in the Mediterranean region. It can grow up to 25 meters in *khinjuk* and thrive in arid and semi-arid areas. All species are Dioius, but *Pistachia khinjuk* are monosios. *Pistachia khinjuk* promotes gingival secretions, breathe the offspring of the air, treats hackers, colds and stomach diseases (Barrero et al., 2005). Different parts of pistachia species have been used for a variety of uses, such as supplements, disinfectants, antihypertensive, dental and digestive system management, liver and urethral and respiratory problems, as part of traditional medicine. Logical findings also show pharmacological exercises and extensive parts of these species, such as enhanced cells, antimicrobial, antiviral, dilute, anti-coke and anti-tumor. *Pistachia khinjuk* have beneficial effects for Digestive system (Bozorgi et al., 2013). *P. lentiscus* in the treatment of peptic ulcer and is also used in cosmetics, perfumes and flavor foods are useful. This factor is traditionally used for the treatment of peptic ulcer and mouth infection (Delazar, Reid, and Sarker 2004). They play an important role in folk medicine and are used in throat infections, kidney stones, asthma, stomach pain, anti-inflammatory, chest, treatment of asthma, antipyretic, antibacterial, antiviral, and stimulants (Tsokou et al., 2007). The plant can be used as the root of pistachio. Used in furniture and wood processing industry (Ahmed et al., 2017). The plant of Oleoresin is also used in the treatment of peptic ulcer and mouth infection. This traditional use of the plant extract is wrapped in the Iran (Haghdooost et al., 2013). *Pistachia khinjuk* oil is also well known in the cosmetics industry, and its benefits are moisturizing the skin. With a variety of essential oils it makes both the ideal treatment for both skin and hair (Ahmed et al., 2017). Cases of diabetes mellitus increases day by day because of gradual increase in population, increasing urban areas, obesity, lake of physical activities, increase in production of food additives, and hereditary causes are also included (King and Rewers 1993). The effects of diabetes mellitus are blindness, dehydration, increase hunger, excessive weight loss or gain, and increase or decrease blood sugar levels (Ramachandran et al., 1999). Aim of this study was the collection of plant extracts (*Pistachia khinjuk*) and carry out extract of *Pistachia khinjuk* from fruit to evaluate the hyperglycemic activity of extract in animals.

## 2 Materials and Methods

All the reagents such as Alloxan monohydrate and standard drug (glibenclamide) were purchased from Atlas Chemicals Co. (United Kingdom). While the other reagents like normal saline, olive oil, formaline, and alcohol were purchased from Sigma brands of Pakistan.

## 2.1 Collection of plant material

Plant fruit of *Pistachia khinjuk* were collected from the local area of Sibi; located in *Baluchistan*, province of Pakistan. The local name of *Pistachia khinjuk* is Shiney. The plant was collected and identify by the botanist of university of Balochistan, Quetta. The identification number was NCBI 434236.

## 2.2 Animals

All the animals are Swiss Albino mice and their weight ranges are from 23-25g are collected from the animal house of Government college university, Lahore Pakistan and are physically fit for the treatment. All albino mice are divided into 6 groups according to the treatment procedure.

## 2.3 Acute toxicity treatment

Toxicity indicates the property of a substance or a drug that how it is poisonous or harmful. According to pharmacological review, a drug or substance is toxic or poisonous when someone take too much of dose and its amount increase in its blood stream that leads to the dangerous level in the body. To prepare dose, 0.9% saline solution were prepared. After saline solution preparation, 10, 100, 500, 1000, 3000 and 6000 mg/kg of crude extract of plant (*Pistachia khinjuk*) according to body weight of mice were mixed in it. Each concentration was making 3ml of saline solution for each mice. After dose administration, the mice were closely observed for 48h with time interval of 3, 7, 18 and 24h, in that time of duration the mice were observed by checking their behavior ( behavior includes shivering , aggression, depression etc.) and mortality rate.

## 2.4 Alloxan monohydrate induced diabetes

All the mice were preparing for inducing alloxan monohydrate into them. First of all 150 mg/kg of alloxan monohydrate were dissolved in saline solution to make 3ml of total solution. Like this 3ml of alloxan monohydrate solution were prepared for each mice of every group except group 1 because group 1 were served as normal group and receive no diabetic content. After preparation of alloxan monohydrate solution in saline, dose were injected into the abdomen of mice with help of plastic syringes of 1cc. The abdomen place where plastic syringe were injected, cleaned with ethyl alcohol with the help of cotton to avoid any kind of infection, All the mice were fasted for 18h before injecting alloxan monohydrate to make them diabetic. After one hour of injecting alloxan monohydrate, the mice were served with standard diet pellets and fresh water. Blood glucose level of all the mice were checked for 72h after 24h of interval and their data is given in the table 1.

## 2.5 Grouping of animals

After 72h of injecting of alloxan monohydrate to every mice, their blood glucose level was checked by tail tipping method. The mice were holding in steel net holder carefully. The tail was puncture with the help of glucometer puncture and drop of blood was collected on the glucometer strip and the glucose level was checked.

After 72h the mice with BGL above 150mg/dl of blood glucose were selected for further treatment and were divided into five groups of six mice in each group and were labeled as group 2, 3, 4, 5 and 6. While group 1 mice were receive no alloxan monohydrate. Details of the group were given below Group 1: Normal group (which receive no treatment

even it wouldn't receive alloxan monohydrate) and served with standard diet pellets and fresh water. Group 2: Positive control group, which receive 5mg/kg glibenclamide (a standard drug use locally for diabetic treatment) to reduce the diabetic effect and receive standard diet pellets and fresh water. Group 3: Negative control group (which receive alloxan monohydrate to make them diabetic and receive no treatment) served with standard diet pellets and fresh water. Group 4: Methanolic plant extract (receive 500mg/kg *Pistachia khinjuk* extract dose) and entertained with standard diet pellets and fresh water. Group 5: Methanolic plant extract (receive 250 mg/kg *Pistachia khinjuk* extract dose) and entertained with standard diet pellets and fresh water. Group 6: Methanolic plant extract (receive 500 mg/kg *Pistachia khinjuk* (wax dose) and entertained with standard diet pellets and fresh water. All the mice were marked with different markers for identification.

## 2.6 Collection of blood

After administration, the mice were hold in Stainless steel mice net holder and after holding mice in the net holder, the tail was puncture with the help of glucometer puncture and 0.2ml of blood were collected on the glucometer strip by tail tipping method and run on the glucometer to check the blood glucose level. Similarly same samples of blood were collected from the tail before and after drug administration after 24h. After the collection of blood from the tail, the tail was clean and rubbed with ethyl alcohol with the help of cotton to avoid any kind of infection.

## 3 Statistical Analysis

All the results are described by using  $\pm$ SEM of mean blood glucose level and all the readings are compared on behalf of its grouping pattern by analyzing through graphical view.

## 4 Results

### 4.1 Acute toxicity studies

Acute toxicity (LD50) of *Pistachiakhinjuk* extract was determined to be 4243mg/kg while the acute toxicity (LD50) of *Pistachiakhinjuk*wax was determined to be 2450mg/kg. details of acute toxicity is given in the table below:

**Table 1.** Acute toxicity index of *Pistachi akhinjuk* Extract.

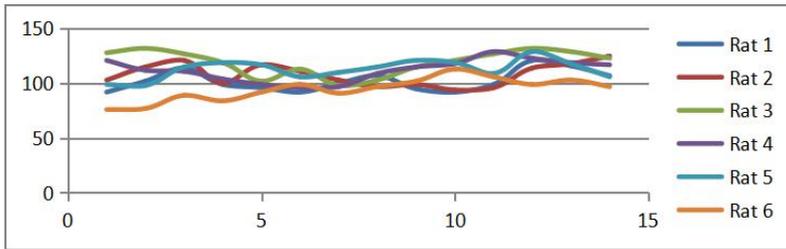
Group	Extract dose mg/kg	Mortality
1	10	0 out of 2
	100	0 out of 2
	500	0 out of 2
2	1000	0 out of 2
	3000	1 out of 2
	6000	2 out of 2

Acute toxicity (LD50)= $\sqrt{\text{lower dose that cause death} \times \text{highest dose that cause death}}$

Acute toxicity (LD50) for *Pistachia khinjuk* extract =  $\sqrt{(3000 \times 6000)} = 4243\text{mg/kg}$

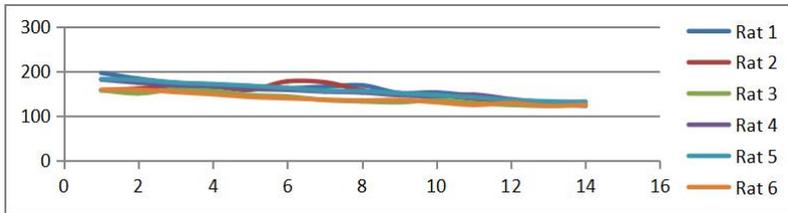
### 4.2 Hypoglycemic screening

The results of antidiabetic screening of *Pistachia khinjuk* extract is: Group 1 served as normal group and not receives any alloxan monohydrate and treatment so its blood glucose level of 14 days is explained in the graph of Figure 1.



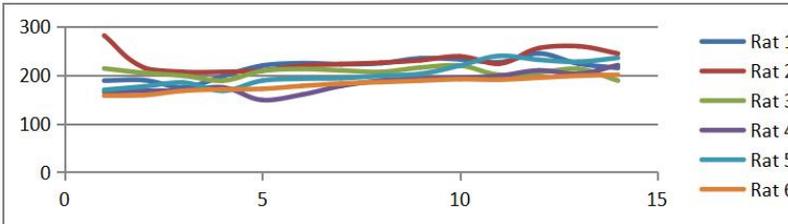
**Fig. 1.** Glucose level of Group 1 (Normal group).

Group 2 served as positive control group after inducing alloxan monohydrate and receives standard drug (glibenclamide 5mg/kg) and treatment so its blood glucose level of 14 days is explained in the graph of figure 2.



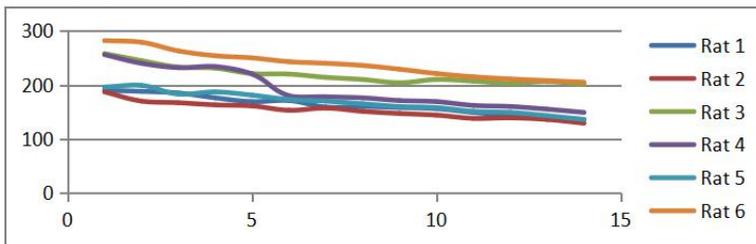
**Fig. 2.** Glucose level of Group 2 (Glibenclamide 5mg/kg)

Group 3 served as negative control group after inducing alloxan monohydrate and receives no treatment so its blood glucose level of 14 days is explained in the graph of figure 3.



**Fig. 3.** Glucose level of Group 3 (Negative control group)

Group 4 served as positive control group after inducing alloxan monohydrate and receives *Pistachia khinjuk* extract (500mg/kg), so its blood glucose level of 14 days is explained in the graph of figure 4.



**Fig. 4.** Glucose level of Group 4 (*Pistachia khinjuk* extract 500mg/kg).

Group 5 served as positive control group after inducing alloxan monohydrate and receives *Pistachia khinjuk* extract (250mg/kg), so its blood glucose level of 14 days is explained in the graph of figure 5.

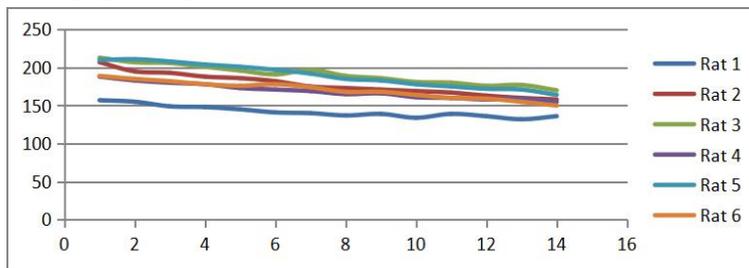


Fig. 5. Glucose level of Group 5 (*Pistachia khinjuk* 250mg/kg).

## 5 Discussion

*Pistachia khinjuk* is also considered as medicinal plant because it showed many biological agents that are effective. In literature *Pistachia khinjuk* showed antioxidant, anti-inflammatory, antipyretic, antileishmanial, antimicrobial, antitumor, antiasthmatic and antiviral properties, while other *Pistachia* species showed hyperglycemic properties that's why we conduct hyperglycemic activity of *Pistachia khinjuk*. So, on behalf of its biological properties *Pistachia khinjuk* could be able to reduce diabetic constituent and reduce blood glucose level. Group 1 was considered as normal group and not induced diabetes, so they show normal behavior and have no specific difference were shown in their blood glucose level which is between 78-135mg/dl. Group 2 was served with standard dose (glibenclamide 5mg/kg) used for diabetic treatment for 14 days. Glibenclamide shows significant difference in blood glucose level and act as antidiabetic drug. The ratio of blood glucose level during the treatment of 14 days was 190-130mg/dl. Group 3 was treated as negative control group and receives no treatment after receiving diabetes, so their blood glucose level was increased with the passage of time and their ratio is between 158-239mg/dl. Group 4 was treated with *Pistachia khinjuk* extract 500mg/kg after inducing alloxan monohydrate. In that case their blood glucose level was decreased significantly and their ratio is between 256-129mg/dl. Mice 6 has highest blood glucose level 282 mg/dl at start of the treatment which decreases to 205mg/dl while mice 3 had highest blood glucose level 258mg/dl and decreases to 201mg/dl. So it is cleared that 500mg/kg dose of *Pistachia khinjuk* extract show antidiabetic activity. Group 5 was treated with *Pistachia khinjuk* extract 250mg/kg after inducing alloxan monohydrate. All the mice in this group show minor decrease in their blood glucose level and their ratio is in between 213-136mg/dl. If we see rat 1, its initial blood glucose level was 157mg/dl which reduce to 136mg/dl. While same case is with rat 3, its initial blood glucose level was 213mg/dl which reduces to 170mg/dl and rat 6 had 189mg/dl and reduced to 150mg/dl. As we described group 6 which was treated with 500mg/kg of *Pistachia khinjuk*'s extract for 14 days showed that it significantly increased blood glucose level in all the mice of this group. That means wax of *Pistachia khinjuk* can increase the blood glucose level of diabetic patients. This study shows that daily intake of 250mg/kg or 500mg/kg *Pistachia khinjuk* extract dose for 14 days reduce blood glucose level into almost normal level in the alloxan monohydrate induced mice. The reason behind hyperglycemic activity of *Pistachia khinjuk* is not found that how it works but on behalf of literature review, every plant treats blood glucose differently with different way of mechanism for example action of  $\beta$  cells, enhancing insulin activity, absorption of carbohydrates or effect the quality of  $\beta$ -cells. All these factors may change blood glucose level. In this study results shows that *Pistachia khinjuk* extract

has the ability to maintain the blood glucose level to a normal range. Further research on this plant (*Pistachia khinjuk*) is required by phytochemical isolation and characterization.

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