STUDY OF BLOOD GLUCOSE AND SGPT LOWERING EFFECTS OF 
DAPHNE MUCRONATA ROYLE IN INDUCED DIABETIC RABBITS

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Abstract

This study was carried out to study Anti-diabetic effect of the plant Daphne mucronata extract in alloxan induced diabetic rabbits. To the first fifteen healthy rabbits taken and divided them into five groups as each group contains three numbers of rabbits. Group one kept in normal condition means no induction or treatment observed. Group second was given the diabetic control dose in quantity (12mg/kg). Group three also treated with the dose rate (125mg/kg) the D. Mucronata (methanol) extract. Similarly group no four was treat with the subject extract in the concentration of (225mg/kg). And the last fifth group of rabbits had the dosage of extract (325mg/kg). This mechanism of entrance was orally ingestion and the treatment duration was eight hours, after each of the next two hours from the start the blood samples were collected, serum separated and the glucose level determined by the method known by Kit method. After all the observations we reached to an impressive conclusion that the D. Mucronata (methanol) extract has significant potential to reduce blood glucose level and also recognized to be effective to reduce the blood SGPT enzyme level, so it can be suggested that this plant has such antidiabetic and SGPT reduction effect.

Keywords: Diabetes mellitus D. mucronata., Alloxan monohydrate, glucose, SGPT
Introduction

*Daphne mucronata* Royle is a shrub present mostly along the river banks at around 800-3000 meters in altitude. In regions trans-indus, Kaghan, Hazara and poonch etc. its large distribution is in Pakistan, especially in western Pakistan and north western areas of Pakistan. In trans-indus from Garhwal west side to Muree at 1-3000 meter from the sea level. Also it is distributed in Afghanistan, Iran, south Europe and northern Africa. So it is distributed throughout the world.

This plant is evergreen shrub type and its height is approximately 2.5 meters. Its branches are small and pale green in color. The leaves are poisonous, that’s why the goats and other cattle avoid eat them as food. And bark can be used for healing diseases related to bones and also for washing hairs to strengthen them. The fruit of this plant is edible and used for dying of leather. “Charcol” the unit of gunpowder is made from this wood.

Its younger branches are densely covered with short matted wooly hairs. The leaves are alternate and 3-5.8 cm in length, 0-4.1 cm in width, elliptic to lanceolate, mucronate, less often rounded at the apex, coriaceous (tough but pliable), and sessile as well.

Flowers are white in color, sub sessile and terminal bunches. The length of Corolla tube is 6-8 mm, covered with densely matted wooly hairs, expanded at the basal portion, 4-lobed rounded like an egg, edge is broader at the end having the length of 4mm. Stamens are spread and are eight in number, two parts (seriate), 4 antisepalous and sub sessile. Ovary egg shaped, 2.5 mm long covered with fine soft hairs. Style absent. Stigma is downright enlarged at the tip. Berry sub glubose, orange in color and 10 mm in length, covered with soft tiny hair like structures.

**Part used:** Any of the plant part may be used for medicinal purposes, while the leaves contain more active chemical constituents as compared to any other part.
Materials and methods

Selection of plant:

*Daphne mucronata* plant is found in lower Chitral, Chitral town and nearby surrounding areas of Chitral KPK.

Collection of plant:

This plant is was collected from village Golain district and tehsil Chitral KPK.

Drying of plant:

Firstly the plant leaves were collected and washed with fresh water. The washed plant leaves in shade in between two newspapers for minimum 21 days for the purpose to dry. And they were little exposed to the sunlight time to time for the prevention from fungal attack.

Grinding of plant parts:

The dried plant leaves were grinded with the grinding machine from the local market In a way firstly the machine washed with tab water then dried and the work done.

Extraction from plant:

In amount 2250 gm. of the dried plant powder was mixed with 1650 ml of methanol very carefully. The methanol totally removed and the filtrate shifted to the tube. The rotary evaporator applied to gain the residue so the dry accumulated received in the flask. This process again applied thirdly and finally repeated the procedure to gain chemical constituents in valuable amount. After these all the received extract chemical kept at room temperature for more dryness.

Animal selection for experiment:

The rabbits (*Oryctolagus cuniculus*) selected for the experimental purpose to apply the chemical constituents on them. 20 rabbits purchased from the local market supplier, then shifted them to the house having wider grassy lawn, then to the University of Chitral in experimental lab. Provided them sufficient food and water in times.

Grouping the Rabbits:

After half of the day randomly selected 15 rabbits from them and divided into five groups following the principal that each group had same weighted rabbits. So in this way each group had three numbers of rabbits.
**Diabetes mellitus induction:**

Alloxan monohydrate is used to affect the rabbits with diabetes mellitus.

**Drug administration:**

Group A was kept in normal condition means no any medications applied on them.

Group B was medicated with Glucophage with the rate of 12 mg/kg body weight accordance.

Group C was given the plant methanolic extract with the dose of 125 mg/kg weight of body.

Group D was treated with the rate of the dose extract 225 mg/kg weighted rabbits.

Group E was given the extract with the dosage of 325 mg/kg body weight.

**Blood samples collection:**

Samples of blood collected from each and every group according to the following principal;

After 2, 4, 6 and 8 hours of duration blood samples were collected.

**Serum isolation:**

Analyzed all the samples of the blood of the rabbit groups. And the serum was separated and collected in the falcon tubes. And these were centrifuged for 6 to 9 minutes at the speed of 4000 rounds per minute. And analyzed by the machine “blood chemistry analyzer” (1400, Italy) and “Scherzo double beam ultraviolet spectrophotometer” Japan) for different biochemical measurements.

**RESULTS**

Group B was given Glucophage (Glibenclamide) for regular 8 hours at the interim of zero hrs, 2 hrs, 4hrs, 6 hrs and 8 hrs. At the last of the process of treatment, the glucose and SGPT level of Group B was recorded as 185 mg/dl and 26 IU/L respectively.

Group C was kept on *Daphne mucronata* plant’s extract at dosage of 125 mg/dl for continuous 8 hrs at the interim of zero hrs, 2hrs, 4hrs, 6hrs and 8 hrs. At the last of the process of treatment, the glucose and SGPT level of Group C was recorded as 315 mg/dl and 53 IU/L respectively.
Group D was treated with plant extract of *Daphne mucronata* at dosage of 225 mg/kg for continuous 8 hrs at the interim of zero hrs, 2 hrs, 4hrs, 6 hrs and 8 hrs. At the last of the process of treatment, the glucose level and SGPT of Group D was recorded as 233 mg/dl and 40 IU/L respectively.

Group E was treated with plant extract at the dose rate of 325 mg/kg for regular 8 hrs. It was given at the interval of zero hrs, 2 hrs, 4 hrs, 6 hrs and 8 hrs. At the last of the process of treatment, the glucose and SGPT level of Group E was recorded as 143 mg/dl and 33 IU/L respectively.
Discussion

When induced the alloxan monohydrate to the rabbits, this chemical destroys the pancreatic beta cells. Hence the level of the glucose rise in the serum And results to have decreased serum insulin level due to the permanent destruction of pancreas. So this observed that when the recipe applied that the regeneration of destroyed cells occurred. And after approximately 72 hours of the period this process was at its maximum level. Hence some of the beta cells became active and begin to releasing insulin, thus the serum glucose level decreased in induced diabetic rabbits and the serum lipid level becomes abnormal due to the moment of fatty acid from the fat depots. This stabilization of blood glucose level reveals that phytoestrogens and human in Daphne may exert their role in the maintenance. In diabetic condition the patient may face the abnormal kidney disorders and have the blood high urea level, so the treated group found to reduction in urea level. Apart from that the main antioxidant in the body uric acid its level decreased due to the diabetes affection, the applied extract proved that it also have the ability to normalize the uric acid level, so the oxidative stress goes to its lower level result of rise in the uric acid level. Diabetes effected rabbits have the high concentration of the enzyme SGPT level in the blood, so the extract works acts against it and decrease its level and the is also the main focusing point in this research. During diabetic condition body weight loses occurs due to different abnormal processes like lipolysis, proteolysis and loss of the fluid from the body so on of the ability of the property of this extract is the weight rehabilitation in the treated groups and the body metabolism becomes accurate again after treatment. All the results are giving message that the D. mucronata methanol extract have effective anti-diabetic activity in dosage etiquette application.

A deciduous plant named ‘Madhuca longifolia’’ which is common to possess anti-diabetic, anti-microbial, analgesic, anti-ulcer and anti-inflammatory. The objective of this reading is to explore the constructive and helpful property of M. longifolia aqueous folio get against DFC-induced female Wister albino rats’ renal toxicity. In this path thirty female Wister albino rats were on bad terms into five groups on the footing of their credence as all band together had six rats and the
drugs administrated explicitly on a piece creature group. After the medicine period, the rats were sacrificed for the evaluation of the big changes in enzyme markers of kidney, antioxidant behavior in renal handkerchief plasma and homogenate, histopathology of kidney and expression levels of proteins. The cytokines like TNF-α, IL-1β and IL-6 were considered through the techniques of ELISA and the levels of NF-κB, Caspase-3 and COX-2 were restrained by the skill named western blotting method (WBT). In chat *M. longifolia* was experimental to demonstrate a outdo consequence in stabilizing the toxicity caused by diclofenac chemical. The sizeable outcomes of the aqueous sheet dig up from *M. longifolia* was owing to its ability for the reinstallation of renal event by restoring preventing cellular reimbursement and antioxidants (S JP1 and Evan., 2018).
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Table 1: Blood glucose level (mg/kg) in alloxan induced diabetic rabbits.

|                          | 0 hr Reading | 2 hr Reading | 4 hr Reading | 6 hr Reading | 8 hr Reading |
|--------------------------|--------------|--------------|--------------|--------------|--------------|
| Untreated Group          | 89.66        | 96.33        | 95.33        | 92           | 95           |
| Diabetic Control + Glibenclimide (12 mg/kg) | 288          | 288.33       | 265          | 207.33       | 185.66       |
| Diabetic + Methanolic Extract (125 mg/kg) | 378          | 342.66       | 312.66       | 305.33       | 315          |
| Diabetic + Methanolic Extract (225mg/kg)    | 303.33       | 292          | 266.66       | 255          | 233.33       |
| Diabetic + Methanolic Extract (325 mg/kg)    | 198.66       | 190.33       | 181          | 179.66       | 143          |
Figure-1. Blood glucose level of Rabbits

Table 2: Showing blood SGPT (IU/L) level of rabbits

|                          | 0 hr Reading | 2 hr Reading | 4 hr Reading | 6 hr Reading | 8 hr Reading |
|--------------------------|--------------|--------------|--------------|--------------|--------------|
| Untreated Group          | 18           | 16           | 17           | 20           | 18           |
| Diabetic Control + Glibenclimide (12 mg/kg) | 49           | 32           | 32           | 28           | 26           |
| Diabetic + Methanolic Extract (125 mg/kg)  | 56           | 59           | 54           | 51           | 53           |
| Diabetic + Methanolic Extract (225 mg/kg)  | 46           | 46           | 44           | 41           | 40           |
| Diabetic + Methanolic Extract (325 mg/kg) | 39           | 41           | 33           | 33           | 33           |
Figure 2: Showing the SGPT (IU/L) level of rabbits.