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Original Research Article

HYPOGLYCAEMIC ACTIVITY OF DIFFERENT FRACTIONS OF *BERBERIS ARISTATA* ROOT-BARK IN NORMAL AND ALLOXAN DIABETIC RABBITS

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ABSTRACT

A number of plants including *Berberis aristata* roots are considered effective as antidiabetic agents in ethnomedical practices. In this study effects of certain isolated fractions from root-bark of *Berberis aristata* on blood glucose levels were determined in normal and alloxan-diabetic rabbits. In normal rabbits, ethanolic fraction in 0.5, 1.0 and 1.5 g/kg produced significant decrease ($P<0.05$ or 0.001) at 8 and 12 hrs. Its acidified-basified fraction showed significant lowering of blood sugar at 2, 4, 8 and 12 hrs. The chloroform: methanol fraction also produced significant hypoglycaemic effect at these intervals. In alloxan-diabetic rabbits, ethanolic fraction in doses similar to normal animals produced significant decrease in blood glucose at 2, 4, 8 and 12 hrs. Also, acidified-basified fraction of the root-bark in 100 and 125mg/kg doses produced significant decrease in the levels at 2, 4 and 8 hrs. In these animals, its chloroform: methanol fraction (4 and 5mg/kg) produced significant effect at 8 and 12 hrs, while at the dose 6mg/kg body weight was found to be significant decrease blood glucose level at 2 and 4 hrs and highly significant decrease blood glucose level at 8 and 12 hrs interval. Gliclazide in 500mg/kg produced significant decrease blood glucose level at 2, 4, 8 and 12 hrs in normal rabbits only but not in alloxan-diabetics. Thus our data showed that the test fractions of *B. aristata* root-bark produce significant hypoglycaemia in both, normal and diabetic rabbits. These appear to be more potent hypoglycaemic than even gliclazide. In addition, it appears that active ingredients of this plant act by producing an organotropic effect on pancreatic β-cells, which results in increased release of insulin from the islets of Langerhans in the rabbits. These natural products also possess some insulin-like activity in alloxan-diabetic rabbits as alloxan has been reported to be a specific β-cytotoxic drug by complexing with the metal ions in the islets. However, further investigations are still needed to elucidate the mechanism of hypoglycaemic effect and to isolate the exact active principles of the *B. aristata* roots.

Keywords: Hypoglycaemia; *Berberis aristata;* alloxan; diabetes

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INTRODUCTION

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants and a number of medicinal plants, traditionally used for over 1000 years are present in herbal preparations of traditional health care systems (Said, 1969). World Health Organization has listed 21,000 plants which are used for medicinal purposes around the world (Modak *et al*., 2007). Momoh *et al.* (2011) have validated the use of *Costus afer* as a hypoglycaemic plant in native medicine. These effects have been attributed to its phytochemical constituents. *Costus afer* could be a promising plant for the development of anti-diabetic drugs.

Akhtar *et al.,* (1992) have described hypoglycaemic activity of a number of medicinal plants and have reported that some reduced blood sugar levels only in normal rabbits, whereas others caused hypoglycaemia in alloxan-diabetic rabbits. In addition some plants produced this effect in both normal and diabetic rabbits. Alarcon-Aguilara *et al* (1998) have examined 28 medicinal plants for hypoglycaemic activity but only eight showed this activity. In addition, Punitha *et al.,* (2006) have observed that oral administration of berberine to normal and diabetic rats for 12 days resulted in significant changes in serum lipid profiles, thiobarbituric acid reactive substance, glycosylated haemoglobin and liver glycogen levels. They have also found a significant increase in both enzymatic and non-enzymatic antioxidants. Simultaneously, berberine produced a significant increase in glycolytic enzymes whilst a decrease in gluconeogenic enzymes in diabetic rats Serum creatinine and urea levels also declined significantly. Earlier, Sung *et al.* (2004) made activity-guided fractionation studies on *P. japonicum* which led to isolation and characterization of coumarin and cyclitol which produce significant inhibition of postprandial hyperglycemia. Kaleem *et al* (2006) have reported that oral administration of *A. squamosa* aqueous extract to diabetic rats for 30 days significantly reduced levels of blood glucose, lipids and lipid peroxidation but increased the activities of plasma insulin and antioxidant enzymes, like catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase. Oyedemi *et al* (2011) have recently reported that aqueous extract of *Leonotis* leonurus leaves possesses antihyperglycemic potential and thus could support ethnotherapeutic usage of this plant.

Berberis aristata is a well known medicinal plant that has been used in Asian traditional medicines for various diseases including diabetes (Nadkarni, 1964; Said, 1969). In the present investigation, activity-oriented studies were carried out in the light of previous research works in an effort to pinpoint fraction of *B. aristata* root-bark which possess active hypoglycaemic principle(s). Thus hypoglycaemic effects were studied in both normal and alloxan-diabetic rabbits and compared with a standard oral hypoglycaemic drug, gliclazide.

MATERIALS AND METHODS

Plant material

The roots of *B. aristata,* locally known as Sumlu were collected from hills of Rawalakot (Azad Kashmir).The root were washed with fresh water and the bark were separated and dried under the shade. After drying the bark were powdered with China grinder. The powdered material was stored in well closed cellophane bags at 4ċ in the refrigerator.

Chemicals and drugs

Ethanol (extra pure), methanol (extra pure), acetic acid, ammonia, chloroform, distilled water, petroleum ether, gliclazide, alloxan monohydrate, gum-tragacanth, Neutral aluminum oxide were of purest grade and were obtained from standard manufacturers.

Preparation of ethanolic fraction of *Berberis aristata* root bark

Dried and powdered root-bark of *B. aristata* was put in Soxhlet apparatus. Then the apparatus were put on heating mantle and ethanol was used as solvent for extraction in the rounded bottom flask. The extraction was carried out for 10 hrs and the extracts were dried and the solvent evaporated.

Preparation of acidified-basified fraction of *B. aristata* extract

Powdered root-bark of *B. aristata* was extracted with hot ethanol. The extract was evaporated to a brown residue and acidified with 20% acetic acid. Acidic aqueous solution was then extracted exhaustively with chloroform which was then basified with 20% ammonium hydroxide solution to pH 9 and extracted with chloroform to obtain tertiary bases. The chloroform layer was separated and evaporated to a brown gum which consisted of free alkaloids.

Preparation of chromatographic chloroform-methanol fraction

The acidified basified alkaloidal fraction was chromatographed on a neutral aluminum oxide column. The column was packed in petroleum ether and different solvents were run as petroleum ether, petroleum ether chloroform, chloroform methanol and finally with methanol. The fraction eluted with chloroform methanol give a small quantity (1 g) of a brown colour mass was obtained.

Animals used

Healthy male and female albino rabbits, weighing; 1000-1200 g were used in these experiments. The animals were kept under observation for one week before experimentation under usual managemental conditions in the animal-room of the Faculty of Pharmacy, University of Sargodha. They were fed on green fodder and a standard rabbit-feed and tap water was supplied *ad libitum.*

Preparation of drug solutions

150 mg of alloxan monohydrate was weighed by using an weighing balance, dissolved in a 3 ml of distilled water and prepared as fresh solution before injection to the rabbits. Weighed 500 mg of gliclazide with an electronic balance dissolved in 5ml of distilled water and prepared a fresh solution just before administration to animals.

RESULTS

Standard curve for blood glucose determination

Standard curve for glucose estimation was drawn by plotting absorbance on ordinate against glucose concentration as abscissa and was found to be linear up to 300 mg /100 ml of glucose. The glucose values above 300mg/ml were therefore re-determined after dilution of sample.

Effect of different fractions of *B. aristata* root bark on blood glucose level in normal rabbits.

Blood glucose levels ± SEM of control and drug treated animals after oral administration of different fractions of *B. aristata* root-bark at various time intervals are given in Table 1. It is clear that blood glucose levels of control group administrated with 15 ml of gum tragacanth was not affected at 2, 4, 8, 12, 24 hrs intervals Fig. 1 also shows that blood glucose levels of animals treated with 500 mg/kg dose of the ethanolic extract of *B. aristata* root-bark at zero hr was 91.67 ± 0.99 mg/100ml which was a significantly ($P<0.05$) decreased at 4 and 8 hrs from zero hr but the levels at 2 and 24 hrs were non-significant ($p > 0.05$). The blood glucose levels of animals treated orally with 1g/kg of ethanolic extract *B. aristata* root-bark were significantly ($p < 0.05$) decreased at 2, 4, 8 and 12 hrs but glucose level at 24 hrs became non-significantly($p > 0.05$) different from zero hr. Table 1 also shows that treatment with 1.5g/kg dose of ethanolic extract *B. aristata* root-bark also caused significant ($P < 0.05$) decrease in glucose levels at 2 and 4 hrs but highly significantly ($P<0.001$) decreased at 8 and 12 hrs after its administration. The blood glucose level at 24 hours intervals of drug was also lower than at zero hour but difference was found to statistically non-significant ($p > 0.05$).

Effects of acidified-basified fraction of *B. aristata* root-bark on blood glucose level in normal rabbits

Table2 shows that blood glucose levels of animals treated orally with 100mg/kg dose of acidified-basified fraction of *B. aristata* root-bark were highly significantly ($P<0.001$) decreased at 4 hrs after its administration. The levels at 2, 8, 12 and 24 hrs were not significantly different from zero level ($p > 0.05$). The oral treatment with 125mg/kg dose of acidified-basified fraction of *B. aristata* root-barks at zero hour also caused significant ($p <0.05$) decrease in glucose levels at 2, 4, 8 and 12 hrs but the level at 24 hrs was non-significant ($p >0.05$). Table 2 also shows that blood glucose levels after treatment with 150g/kg dose of acidified-basified fraction *B. aristata* root-bark were significantly($p< 0.05$) decreased at 2, 4, 8 and12 hrs but difference was non-significant at 24 hrs.

Effects of chloroform-methanol fraction of *B. aristata* root-bark on blood glucose level in normal rabbits

Table3 shows that blood glucose levels of animals treated orally with 4 mg/kg dose of chloroform-methanol fraction of *B. aristata* root-bark was significantly ($P<0.05$) decreased at 2, 4 and 12 hrs and highly significantly ($P< 0.001$) decreased at 8 hrs interval. However, it was non-significantly different at 24hrs.

Table 1: Mean blood glucose level of normal rabbits (mg/dl ± SEM) at various time intervals after orally treatment of 2% gum tragacanth solution and ethanolic fraction of *B. aristata* at the doses of 500 mg/kg and 1 and 1.5 g/kg body weight in 20 ml of 2% gum tragacanth aqueous solution.

Time intervals (Hrs)	Group I treated with 2% gum tragacanth aqueous solution (20 ml)	Group IIA treated with ethanolic fraction of *B. aristata* (500mg/kg)	Group IIB treated with ethanolic fraction of *B. aristata* (1 g/kg)	Group IIC treated with ethanolic fraction of *B. aristata* (1.5g/kg)
0	91.67±0.80	91.67±0.99	92±1.18	90.83±0.94
2	91.67±0. 95 ^{NS}	90.±0.73 ^{NS}	88.50±1.18*	85±2.58*
4	92.00±0.89 ^{NS}	86.67±0.76*	85.50±1.34*	79.50±2.39*
8	91.67±0.61 ^{NS}	86.33±0.76*	83.67±1.58*	72.67±1.63**
12	91.50±0.76 ^{NS}	90.50±0.43^{NS}	88.00±1.34*	74.17±2.44**
24	91.50±0.67 ^{NS}	91.33±0.49^{NS}	92.33±0.56^{NS}	92.67±1.14^{NS}

*= Significant decrease as compared to zero hour level (P<0. 05)
**= Highly significant decrease as compared to zero hour level (P < 0. 001)
^{NS}= Non-significant decrease as compared to zero hour level (P > 0.05)

Table 2: Mean blood glucose level of normal rabbits (mg/dL ± SEM) at various time intervals after oral treatment of 2% gum tragacanth solution and acidified-basified fraction of *B. aristata* root-bark in 100,125 and 150mg/kg body weight doses orally.

Time interval (Hrs)	Group I treated with 2% gum tragacanth aqueous solution	Group IIIA treated with acidified basified fraction of *B. aristata* (100mg/kg)	Group IIIB treated with acidified basified fraction of *B. aristata* (I25 mg/kg)	Group IIIC treated with acidified basified fraction of *B. aristata* (150mg/kg)
0	91.67±0.80	91.67±0.88	90.83±1.14	91.50±0.92
2	91.67±0.95 ^{NS}	89.33±1.52 NS	86.00±2.57*	81.17±3.72*
4	92.00±0.89 ^{NS}	86.17±1.08**	79.83±3.92*	69.83±5.15*
8	91.67±0.61 ^{NS}	87.83±1.80 ^{NS}	73.67±3.30*	65.17±4.92*
12	91.50±0.76 ^{NS}	91.00±0.93^{NS}	77.00±2.98*	64.33±5.21*
24	91.50±0.67 ^{NS}	91.33±0.42 ^{NS}	92.33±1.45^{NS}	90.50±1.02^{NS}

*= Significant decrease as compared to zero hour level (P < 0.05)
**= Highly significant decrease as compared to zero hour level (P < 0.001)
^{NS}= Non-significant decrease as compared to zero hour level (P > 0.05)

Table 3: Mean blood glucose levels of normal rabbits in mg/100ml ± SEM at various time intervals after oral treatment of chloroform-methanol fraction of *B. aristata* root-bark (4, 5 and 6 mg/kg body weight) and 500mg/kg of gliclazide.

Time interval (Hrs)	Group V treated with gliclazide (500 mg/kg)	Group IVA treated with chloroform-methanol fraction of *B. aristata* (4mg/kg)	Group IVB treated with chloroform-methanol fraction of *B. aristata* (5mg/kg)	Group IVC treated with chloroform-methanol fraction of *B. aristata* (6mg/kg)
0	90.33±0.80	91.17±1.01	89.67±0.56	92.67±0.99
2	86.33±1.71*	87.00±1.10*	80.00±2.96*	83.67±2.70*
4	83.83±1.40*	77.00±2.64*	68.67±4.53*	69.33±5.03*
8	84.33±1.91*	70.33±3.25**	64.00±1.18**	59.33±0.67**
12	86.33±2.08^{NS}	87±1.37*	80.50±1.34**	59.17±0.79**
24	89.33±0.75^{NS}	91.33±0.61^{NS}	90.17±0.40^{NS}	92.67±0.88 ^{NS}

*= Significant decrease as compared to zero hr level ($P < 0.05$)
**= Highly significant decrease as compared to zero hr level ($P < 0.001$)
^{NS}= Non-significant decrease as compared to zero hr level ($P > 0.05$)

Table 4: Mean blood glucose Level of diabetic rabbits in (mg/dL ± SEM) at various time intervals after oral administration of 2% gum tragacanth solution and ethanolic extract of *B. aristata* root-bark in 500mg/kg 1.0g and 1.5g/kg doses.

Time interval (Hours)	Group VI treated with 2% gum sol.	Group VIA treated with ethanolic extract of *B. aristata* (500mg/kg)	Group VIB treated with ethanolic extract of *B. aristata* (1 g/kg)	Group VIC treated with ethanolic extract of *B. aristata* (1.5g/kg)
0	271.67±3.96	271.67±3.96	272.83±2.75	277.17±2.48
2	272.33±4.06 ^{NS}	267.17±3.50*	271.17±2.83^{NS}	274.83±2.71*
4	272.33±3.82 ^{NS}	263.00±4.11**	261.50±2.78**	263.67±3.31**
8	272.83±4.30 ^{NS}	259.00±2.41*	259.00±2.41**	258.83±2.57**
12	272.16±4.24 ^{NS}	256.33±2.78*	256.33±2.78**	260.83±4.39**
24	271.83±3.96 ^{NS}	273.33±2.63^{NS}	273.33±2.63^{NS}	276.33±2.35^{NS}

*= Significant decrease as compared to zero hour level ($P < 0.05$)
**= Highly significant decrease as compared to zero hour level ($P < 0.001$)
NS= Non-significant decrease as compared to zero hour level ($P > 0.05$)

Table 5: Mean blood glucose level of diabetic rabbits in mg/dL ± SEM at various time intervals after oral treatment of 2% gum tragacanth solution and acidified-basified fraction of *B. aristata* root-bark in 100, 125 and 150 mg/kg doses.

Time interval (Hours)	Group VI treated with 2% gum sol.	Group VIIIA treated with acidified-basified fraction of *B. aristata* (100mg/kg)	Group VIIIB treated with acidified-basified fraction of *B. aristata* (I25mg/kg)	Group VIIIC treated with acidified-basified fraction of *B. aristata* (150mg/kg)
0	271.67±3.96	274.83±2.06	269.17±3.17	273.17±2.76
2	272.33±4.06^{NS}	272.67±2.65^{NS}	265.50±2.49*	271.33±4.79^{NS}
4	272.33±3.82^{NS}	268.33±2.78*	261.67±2.33*	260.83±3.07**
8	272.83±4.30*	268.50±2.40*	261.83±2.59**	255.17±3.24**
12	272.16±4.24^{NS}	270.33±2.06**	262.67±2.70**	255.00±3.85**
24	271.83±3.96^{NS}	282.00±3.89^{NS}	269.67±2.75^{NS}	272.83±1.49^{NS}

*= Significant decrease from zero hour level (P < 0.05)
**= Highly significant decrease from zero hour level (P< 0.001)
NS= Non-significant decrease from zero hour level (P > 0.05)

Table 6: Mean blood glucose level of diabetic rabbits in mg/dl ± SEM at various time intervals after oral treatment of 2% gum tragacanth solution and chloroform-methanol fraction of *B. aristata* root-bark in 4, 5 and 6 mg/kg doses.

Time interval (Hrs)	Group VI treated with 2% gum sol (20 ml)	Group IXA treated with chloroform-methanol fraction of *B. aristata* (4mg/kg)	Group IXB treated with chloroform-methanol fraction of *B. aristata* (5mg/kg)	Group IXC treated with chloroform-methanol fraction of *B. aristata* (6mg/kg)
0	271.67±3.96	266.17±3.57	276.00±4.13	274.83±5.54
2	272.33±4.06^{NS}	262.17±4.71^{NS}	274.00±4.65^{NS}	266.50±5.97*
4	272.33±3.82^{NS}	257.50±3.58*	267.33±3.86*	260.50±5.01*
8	272.83±4.30^{NS}	258±3.76*	265.17±3.57*	254.83±4.90**
12	272.16±4.24^{NS}	260.83±2.87^{NS}	269.83±4.81^{NS}	257.83±2.90*
24	271.83±3.96^{NS}	265.17±3.03^{NS}	277.67±3.19^{NS}	270.50±4.79^{NS}

*= Significant decrease from zero hour level (P < 0.05)
**= Highly significant decrease from zero hour level (P<0.001)
^{NS}= Non-significant decrease as compared to zero hour level (P > 0.05)

Administration of 5mg/kg of the chloroform-methanol fraction also significantly decreased glucose level at 2 and 4 hrs, highly significantly at 8 and 12 hrs but non-significant at 24 hrs. The 6mg/kg dose of chloroform-methanol fraction produced a significant decrease in blood glucose level at 2 and 4 hour and highly significant decrease at 8 and 12 hrs but at 24 hrs interval difference was non-significant from 0 hr.

Effects of gliclazide 500 mg/ kg body weight orally on blood glucose level in normal rabbits

Treatment with 500 mg/kg of gliclazide produced highly significant ($P<0.001$) reduction in blood glucose levels at 2, 4 and 8 hrs, only significantly ($P < 0.05$) at 12 hrs but after 24 hrs the level was non-significantly different from zero hr (Tab 3).

Effect of gum and ethanolic fraction of *B. aristata* root-bark on blood glucose level in alloxan-diabetic rabbits

Table 4 shows that aqueous gum solution did not reduce blood glucose levels at all time intervals ($P >0.05$). The animals treated with 500mg /kg ethanolic extract were found to have reduced at 2, 8 and 12 hrs significantly ($P<0.05$) and highly significantly ($P< 0.001$) at 4 hrs. The 1.0g /kg dose decreased the blood glucose at 4, 8, 12 hrs highly significantly while at 2 and 24 hrs the values were not significantly different from zero hr (Table 4). The animals treated with 1.5g /kg of the ethanolic extract had reduced glucose level at 2 hr significantly and at 4, 8 and 12 hrs highly significantly while at 24 hrs they became non-significant ($P > 0.05$) from zero hr.

Effect of acidified-basified fraction of *B. aristata* root-bark on blood glucose level of alloxan-diabetic rabbits

Treatment with 100 mg /kg of acidified-basified fraction of *B. aristata* root-bark decreased blood glucose levels at 4 and 8 hrs significantly ($p< 0.05$), at 12 hrs highly significantly ($p < 0.001$) and at 24 hrs non- significantly different from zero hr level. Similarly, with 125 mg /kg dose of acidified-basified fraction , blood sugar levels at 2 and 4 hrs were found to be significantly reduced and highly significantly at 8 and 12 hrs but became not different from zero hrs at 24 hrs. The 150 mg /kg dose of the fraction decreased glucose levels at 4, 8 and 12 hrs highly significantly while at 2 and 24hrs they were not different from zero hr level.

Effect of chloroform-methanol fraction of *B. aristata* root-bark on blood glucose levels of alloxan-diabetic rabbits

Oral treatment with 4 mg /kg chloroform-methanol fraction of *B. aristata* root-bark was found to produce hypoglycaemia at 4 and 8 hrs significantly while at 2, 12 and 24 hrs blood glucose levels were non-significantly different from zero hr. However, with 5 mg /kg dose this fraction decreased the levels at 4 and 8 hrs significantly while at 2, 12 and 4 hrs values were non-significant different from zero hr. Oral administration of 6 mg /kg dose of chloroform-methanol fraction produced significant effect at 2, 4, and 12 hrs and highly significantly at 12 hrs which became non-significant from zero hr at 24 hrs interval.

DISCUSSION

Diabetes mellitus is mainly due to absolute or relative lack of insulin. Insulin promotes the transfer of glucose from tissue fluid into body tissues. Thus diabetes usually occurs when B-cells

are unable to produce insulin adjusted individually and depending upon the severity of symptoms, it may involve either the use of orally active hypoglycaemic agents or one or more injections of insulin daily (Goth., 1985). Insulin is mostly obtained from animal pancreas and is a replacement therapy. It does not completely cure or prevent the disease. Stimulation of the pancreatic B-cell to produce more insulin and enhance the activity of hepatic enzymes so that glycogen deposition is increased e.g., sulphonylureas have been reported to act in this way (Goth., 1985). Obviously, these oral hypoglycaemic drugs are of no value in the treatment of sever diabetes of any type and in insulin dependent diabetes mellitus (IDDM) as their islets have already lost all potential ability to secrete insulin. Therefore, still today search for more effective and safer antidiabetic agents continues to be an area of active research. Diabetes mellitus is a leading chronic endocrine disease and is an important cause of morbidity and mortality all over the globe. It is generally believed that the incidence of diabetes in society is alarmingly increasing, whether in Pakistan or any other developed country.

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulphonylureas, biguanides, a-glucosidase inhibitors, and glinides, which

are used as monotherapy or in combination to achieve better glycaemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects; thus, managing

diabetes without any side effects is still a challenge (Jung *et al*., 2006).Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation. A considerable number of hypoglycaemic plants and herbs are known through folklore but their introduction into modern therapy waits pharmacological testing by modern methods. The discovery that the substance other than insulin can be used as effective antidiabetic drugs has stimulated several workers to search for compounds of similar action and nature in plant kingdom.

Berberis aristata is a well known medicinal plant that has been used in traditional medicine for various diseases including diabetes mellitus. There were many claims of its antidiabetic effects in literature but those were not studied and confirmed experimentally, before. The study was conducted to evaluate the hypoglycaemic effect of dried and powdered root of *Berberis aristata* and its extract in water and methanol on blood glucose levels. *Berberis aristata* roots contain some potent orally effective hypoglycaemic chemical principle(s) which possess insulin triggering and insulin-like activities (Akhtar *et al.* 2008). However, its active principles or active fractions were not studied. Thus in the present work, investigations were carried out, in the light of previous research works to evaluate the hypoglycaemic activity in other medicinal plants, to confirm the hypoglycaemic activity of *B. aristata* root bark in both normal and alloxan-diabetic rabbits. For comparison, effects of a standard oral hypoglycaemic drug gliclazide were also observed in these rabbits.

The result obtained showed that 2% gum tragacanth aqueous solution used as a vehicle in these experiments did not produce any significant ($p>0.05$) in the blood glucose of normal as well as alloxan-treated diabetic rabbits (Table 1). This finding is in agreement with others (Akhtar *et al*., 1985). The administration of ethanolic extract of *B. aristata* root bark to the normal rabbits, at dose level of 1 g/kg body weight produced significant ($p<0.05$) decrease in blood glucose level at 4 and 8 hour interval, after oral administration of the drug. The drug at the dose level of 1.5 g/kg

body weight also produced significant decrease blood glucose level at 2, 4, 8 and 12 hour intervals. The higher dose of 2 g/kg body weight ethanolic extract of the *B. aristata* root bark produced significant decrease blood glucose level at 2 and 4 hour while highly significant ($p<0.001$) decrease in the blood glucose level at 8 and 12 hour intervals.

The administration of acidified basified fraction to the normal rabbits, at dose level of 100 mg/kg produced significant ($p<0.05$) decrease blood glucose level at 2 hour interval and produced highly significant ($p<0.001$) decrease of blood glucose levels at 4 hour interval. The drug at the dose level of 125 and 150 mg/kg produced significant decrease of blood glucose levels at 2, 4, 8 and 12 hours. The administration of chromatographically produced chloroform- methanol fraction of *B. aristata* root-bark to normal rabbits at the dose level of 4 mg/kg body weight produced significant decrease of blood glucose levels at 2, 4 and 12 hour intervals and highly significant decrease blood glucose level at 8 hour interval. The fraction at the dose level of 5 mg/kg produced significant decrease of blood glucose levels at 2 and 4 hours and produced highly significant decrease blood glucose level at 8 and 12 hour interval. The higher dose at the level of 6 mg/kg body weight produced significant ($p<0.05$) decrease blood glucose level at 2, 4 and 12 hour interval while highly significant ($p<0.001$) decrease blood glucose level at 8 hour interval. For comparison of hypoglycaemic activity, gliclazide (a standard oral hypoglycaemic sulfonylurea drug) was administered orally in 500mg/kg dose to normal rabbits produced significant decrease blood glucose levels at 2, 4, 8 and 12 hours while it had no effect on alloxan-diabetic rabbits due to the absence of insulin secreting beta-cells..

In an effort to further explore the possible mechanism of hypoglycaemic action of different fractions of *B. aristata* root bark, it was also administered to the alloxan- treated diabetic rabbits. It is clear from our data that in the diabetic rabbits too, oral administration of ethanolic extract of the *B. aristata* root bark in 1, 1.5 and 2 g/kg produced significant ($p<0.05$ or 0.001) decrease in blood glucose levels at various time intervals. Similarly, administration of acidified basified fraction to alloxan diabetic rabbits in doses of 100, 125 and 150 mg/kg body weight produced significant ($p<0.05$ and 0.001) decrease in blood glucose levels at 4 and 8 and 12 hours. Moreover, administration of chloroform-methanol fraction at the dose of 4, 5 and 6 mg/kg produced significant ($p<0.05$ and 0.001) decrease in blood glucose level at 4 and 8 hour intervals. However, gliclazide (500 mg/kg) could not produce any significant hypoglycaemic effect in the alloxan-diabetic rabbits as it can secrete insulin from active beta cells only.

Alloxan is well known to cause selective beta cytotoxicity and alloxan diabetic animals show usual clinical significance of human diabetes, i.e. hyperglycaemia, glycouria, polydipsia, polyuria, polyphagia, loss of body weight and acidosis (Reurup, 1970). It has been reported that single intravenous injection of 150 mg /kg-body weight of alloxan to rabbits is 100% effective in killing their β-cells (Butt, 1962; Laurence and Bacharach, 1964). Thus this dose of alloxan already known to produce deficiency of the β-cells was selected for these experiments which increased the blood glucose level of the rabbits to about 3-4 times of their normal levels. In addition, a drug like biguanides produce hypoglycaemia is by the increase of glycolysis and decrease of gluconeogenesis in the liver. In addition, these drugs are reported to decrease intestinal glucose absorption and to increase uptake of glucose in the muscles (Larner and Haynes, 1975). However, it has been demonstrated that the biguanides including metformin do

not produce hypoglycaemia in the normal subjects because in them the increase in peripheral glucose utilization it compensated by an increase in hepatic glucose output (Goth, 1985).

Thus, it is conceivable that the fractions of *B. aristata* root-bark tested in these studies contain some hypoglycaemic principle(s) which act by stimulating the release of insulin and also possess insulin-like action. The *B. aristata* root bark do not seem to act like biguanides as it has decreased the blood glucose level in both normal as well as diabetic rabbits. It has become evident that different fractions of *B. aristata* root-bark do exert significant and consistent hypoglycaemic effect in normal and alloxan-induced diabetic rabbits. These results also suggest that the active principle(s) responsible for hypoglycaemic action of *B. aristata* root bark are perhaps due to stimulation of insulin release from the pancreatic β-cells in normal animals and in alloxan-induced diabetic animals, the hypoglycaemic effects could be due to their direct insulin-like effect. However, further studies should be essentially conducted to elucidate exact mechanism(s) of hypoglycaemic action of fractions isolated *Berberis aristata* root-bark and to establish their real efficacy and safety for further clinical use in diabetic patients.

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