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Evaluation of Antidiabetic Activity of *Prunus Amygdalus Batsch* in Streptozotocin Induced Diabetic Mice.

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ABSTRACT

Prunus amygdalus Batsch (Family – Rosaceae) is widely used in traditional system of medicine to treat diabetes in India. The ethanolic extract (250 & 500 mg/kg) of leaf, flower and seed were taken up undertaken to evaluate the antidiabetic activity against normal and streptozotocin induced diabetic mice. Oral administration of extract for 21 days resulted in significant reduction in blood glucose level. Chronic effect of the extract on serum biochemistry were also studied and it was found that serum cholesterol, triglyceride, creatinine, urea, alkaline phosphatase levels were decreased significantly by all the extracts and Glibenclamide but HDL levels and total proteins were found to be increased after treatment. Thus, this studies shows that P. amygdalus has antidiabetic action and the extract should further be subjected to bioactivity guided drug discovery to isolate a lead compound responsible for this activity.

Keywords: Prunus amygdalus, Rosaceae, Diabetes, Streptozotocin, Mice, Traditional medicine.

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INTRODUCTION

Diabetes mellitus is the most common endocrine disorder more than 150 million people are suffering from it worldwide and it is likely to increase to 300 million by the year 2025. More than one fifth of them are Indians and the International Diabetes Federation, declared India "Diabetic capital of the World". Synthetic antidiabetic agents can produce serious side effects and they are not suitable for use during pregnancy. In view of the adverse effects associated with the synthetic drugs and considering natural medicine safer, cheaper and effective, traditional antidiabetic plants can be explored. Furthermore after the recommendation made by WHO on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become more important.

Prunus amygdalus Batsch var amara, Family Rosaceae, commonly known as Bitter almond is emollient, demulcent and laxative, and are used as sedative in coughs. Bitter almonds are described by Hakims as attenuent, astringent, lithontriptic and diuretic. Root is discutient and alternative.

MATERIALS AND METHODS

Chemicals

Streptozotocin was purchased from Hi Media, India. Total cholesterol (TC), serum high density lipoprotein(HDL), serum creatinine(SC), serum urea(SU), serum alkaline phosphate (SAP) and triglyceride(TG) were assayed using standard kits from Erba Diagnostics Mannheim Gambh, Germany and blood glucose level was measured using Elegance glucose meter (CT-X10) of Convergent Technologies, Germany.

Plant material

The leaves, flowers and seeds were collected in the month of July. The materials were cleaned thoroughly with distilled water to remove any type of contamination. Washed leaves, flowers and seeds were air dried in shade.

Preparation of the extract

The plant parts were separately powdered in a grinder and passed through sieve. 500g of each powder was filled in the Soxhlet apparatus for extraction. The whole assembly of the Soxhlet apparatus was set up and first defatted by petroleum ether(60-80 C) for 72h.After complete defatting, the drug powders were dried at room temperatures and extracted with ethanol for 48h. The alcoholic extracts of the different parts were dried at 45 C in rotary evaporator to produce a semisolid mass and stored in airtight container in refrigerator below 10 C.

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Phytochemical screening

The freshly prepared crude extracts were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts was perfomed using the following reagents and chemicals: Alkaloids with Dragendorff's reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce stable form and steroids with Libermann –Burchard reagent. These were identified by characteristic colour changes using standard procedures.

Animals

Albino mice of either sex, weighing about 30-35g were used in the experiments. Animals were maintained under standard environmental condition i.e. ambient temperature of 22 ± 2 ^{0}C and at 45-55% relative humidity for 12h, each of dark and light cycle and fed with a standard pellet mice diet.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Albino mice (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after that the extracts were administered orally at the dose level of 5mg/kg body weight

By intragastric tube and observed for 14 days. If mortality was observed in 2 – 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 300 and 2000 mg/kg body weight.

Streptozotocin induced diabetes

Mice were made diabetic by a single intraperitoneal injection of streptozotocin (150mg/kg i.p.) in sterile saline. Twelve days after streptozotocin injection, mice with blood glucose level > 140mg/dl were separated and used for the study.

Experimental design

Animals were fasted overnight and were divided into nine groups of six each. Group 1 served as a normal healthy control. Group 2 served as untreated diabetic control. Group 3 and 4 received leaf extracts 250 mg/kg and 500 mg/kg per body weight respectively. Group 5 and 6 received Flower extracts 250 mg/kg and 500 mg/kg per body weight respectively. Group 7 and 8 received seed extract 250 mg/kg and 500 mg/kg per body weight respectively. Group 9 received Glibenclamide (10 mg/kg B.W.). Blood samples were collected on 0, 5, 10, and 15 day

after oral administration and blood glucose level were measured using blood glucose test strips with elegance glucometer (Frankenberg, Germany).

Biochemical assay

After blood glucose estimation on day 15, whole blood was collected by cardiac puncture under mild ether anesthesia from mice. Serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase, HDL and total protein level were also evaluated in normal and streptozotocin induced diabetic mice. Total cholesterol and triglyceride were determined. Serum urea and creatinine were assayed by method of Tomas. Total protein and alkaline phosphatase were assayed by the method of Wilkinson et al and HDL cholesterol level was also measured.

Stastical analysis

All values were expressed as mean ± standard error of the mean (SEM). And comparison between the groups were made by analysis of variance (ANOVA) followed by student t – test. A value of $p<0.001$ was considered significant.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical analysis of crude extract revealed the presence of flavonoids, steroid, volatile oil and saponins.

Acute toxicity studies

These studies showed no mortality up to the dose of 2000 mg/kg body weight. So, the extracts are safe for long term administration.

Table 1 – Long term effects of P. amygdalous extracts on the blood glucose levels in normal and diabetic mice.

S. No.	Groups and doses (mg/kg., b.w.)				
		Initial day	Day 5	Day 10	Day 15
1	Normal control	72.25 ± 0.89	73.5 ± 1.0	73.75 ± 0.86	75.5 ± 1.58
2	Diabetic control	185 ± 2.88	186.2 ± 1.7	189.25 ± 1.25	192.5 ± 1.73
3	Leaf extract (250)	192 ± 2.6	151 ± 1.5	110.6 ± 3.0	80.6 ± 1.8
4	Leaf extract (500)	190.3 ± 4.98	148 ± 1.1	108 ± 3.4	77.6 ± 1.4
5	Flower extract (250)	197.3 ± 4.3	157.6 ± 5.2	129.6 ± 3.2	89.3 ± 2.5
6	Flower extract (500)	194 ± 2.8	148.3 ± 3.1	116.6 ± 5.3	84.6 ± 1.8
7	Seed extract (250)	185.3 ± 4.2	154 ± 1.1	134.6 ± 2.4	88.6 ± 1.2
8	Seed extract (500)	186.3 ± 4.3	146.3 ± 5.3	121.3 ± 1.4	80.3 ± 0.9
9	Glibenclamide	194.75 ± 2.84	156.5 ± 5.95	110.5 ± 5.24	75.73 ± 4.5

Anti diabetic activity

The effect of the treatment with all extracts and glibenclamide on blood glucose concentration in normal first aid and diabetic mice after treatment are shown in a table 1. at the end of experiment (15th day) blood glucose level was 80.6 ± 1.8 and 77.6 ± 1.4 mg/dl in the diabetic mice treated with 250 and 500 mg/kg b.w. of the leaves extract respectively. Whereas flower and seed extracts at a dose of 500 mg/kg b.w. also showed significant reduction ($p<0.001$) in a blood glucose level on the diabetic mice at 15th day of the study.

Body weight

Furthermore, daily treatment of all extracts for two week led to dose dependent fall in blood glucose level. Maximum effect seems to rich after 14th day of treatment and remains constant their after. Normal healthy control was found to be stable in their bodyweight but diabetic mice show reduction in bodyweight. In this study, the decrease of body weights were significantly dimished ($p<0.01$) by the extracts treatments after 14 days of treatments (Table 2).

Table 2

S. No.	Groups and doses (mg/kg., b.w.)				
		Initial day	Day 5	Day 10	Day 15
1	Normal control	27.3 ± 1.9	27.9± 1.0	31.1 ± 3.8	28.5 ± 1.1
2	Diabetic control	30.3 ± 1.2	28.5 ± 3.2	27.9 ± 3.2	26.2 ± 2.4
3	Leaf extract (250)	31.1± 1.9	29.2 ± 0.6	28.3 ± 2.7	30.7 ± 1.7
4	Leaf extract (500)	29.8 ± 2.1	27.1 ± 2.4	26.1 ± 3.0	28.3 ± 1.4
5	Flower extract (250)	28.6 ± 1.7	26.7 ± 2.0	25.4 ± 2.4	28.5 ± 2.4
6	Flower extract (500)	29.4± 1.9	27.9 ± 1.2	26.0 ± 2.6	28.3 ± 1.2
7	Seed extract (250)	31.7 ± 2.5	30.4 ± 1.7	28.7 ± 1.9	30.6 ± 2.1
8	Seed extract (500)	32.6 ± 1.4	30.8 ± 1.9	28.6 ± 1.4	30.7 ± 0.9
9	Glibenclamide	26.27 ± 1.80	27.7 ± 2.06	29.89 ± 2.25	30.46 ± 1.91

Biochemical estimation

Serum cholesterol, triglyceride, creatinine, urea, alkaline phosphatase levels were decreased significantly by all extracts of P. amygdalous and Glibenclamide but HDL levels and total proteins were found to be increased after treatment. (Table 3). The extract exhibited anti diabetic property in STZ induced diabetic mice, as evident from blood glucose levels. The activity may be due to presence of flavonoids.

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Table 3

Sr No	Groups and Doses Mg/kg b.w.	Cholesterol	Trigly cerides	Creatinine	Urea	Alkaline Phosphatase	HDL Chole Sterol	Total Proteins (g/dl)
1	Normalcontrol	148 ± 4.9	84.3 ± 4.6	0.6 ± 0.4	21.0 ± 0.4	116.2 ± 3.9	34.1 ± 1.8	8.3 ± 1.7
2	Diabeticcontrol	259 ± 13.7	256.1±11.4	1.8 ± 0.1	79.4 ± 2.7	364.2 ± 5.7	28.1 ± 1.4	3.9 ± 5.1
3	LeafExtract(250)	154.7 ± 5.9	138.4 ± 2.1	0.67 ± 0.1	37.2 ± 1.7	156 ± 10.1	47.2 ± 1.7	4.7 ± 0.2
4	Leafextract(500)	148.4 ± 1.9	142.1 ± 1.9	0.64 ± 0.3	39.5 ± 2.2	151 ± 4.3	51.9 ± 2.2	4.9 ± 1.6
5	Flowerextract(250)	154.2 ± 6.3	132.3 ± 1.7	0.64 ± 0.4	38.5 ± 1.7	162.5 ± 9.1	52.3 ± 1.7	5 ± 2
6	Flowerextract(500)	150.7 ± 4.7	130.5 ± 1.7	0.52 ± 0.4	32.7 ± 1.4	151.6 ± 8.7	57.9 ± 1.5	5.4 ± 1.7
7	Seed extract (250)	160.4 ± 4.6	134.4 ± 1.4	0.59 ± 0.1	33.6 ± 2.1	154.4 ± 3.7	54.2 ± 1.9	5.7 ± 0.9
8	Seed extract (500)	146.1 ± 9.5	146.8 ± 2.4	0.67 ± 0.1	36.7 ± 1.9	147.8 ± 5.2	58.6 ± 1.1	4.6 ± 1.1
9	Glibenclamide	120.1 ± 5.7	102.6 ± 6.5	0.42 ± 0.0	30 ± 3.2	110 ± 3.9	64.5 ± 1.9	8.4 ± 1.4

CONCLUSION

The results revealed that P. amygdalous extract posses significant antihyperglycaemic activity in STZ induced diabetic mice. Further studies are necessary to elucidate in detail the mechanism of action of medicinal plant at the cellular and molecular levels. This extracts also showed improvement in parameter like bodyweight, lipid profile and other biochemical parameter.

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