Indian Journal of Experimental Biology
Vol. 45, August 2007, pp. 696-701

Preliminary studies on antihyperglycemic effect of aqueous extract of *Brassica nigra* (L.) Koch in streptozotocin induced diabetic rats

Prachi Anand, Murali K Y, Vibha Tandon, Ramesh Chandra* & P S Murthy†

Dr B R Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110 007, India

Received 26 October 2006; revised 3 April 2007

In the streptozotocin induced diabetic rats treated separately with aqueous, ethanol, acetone and chloroform extracts of the seeds of *B. nigra*, the increase in serum glucose value between 0 and 1 hr of glucose tolerance test (GTT) was the least (29 mg/dl) in aqueous extract treated animals while it was 54, 44 and 44mg/dl with chloroform, acetone and ethanol extracts respectively. In further studies carried out with aqueous extract, the effective dose was found to be 200 mg/kg body weight in GTT. Administration of 200 mg/kg body weight of aqueous extract to diabetic animals daily once for one month brought down fasting serum glucose (FSG) levels while in the untreated group FSG remained at a higher value. In the treated animals the increase in glycosylated hemoglobin (HbA1c) and serum lipids was much less when compared with the levels in untreated diabetic controls. These findings suggest that further studies with the aqueous extract of *B. nigra* seeds on its antidiabetic activity would be useful.

Keywords: Antidiabetic activity, *Brassica nigra*, Diabetes mellitus, Glucose tolerance test

In view of the side effects reported with the use of insulin and oral hypoglycemic agents[1,2], medicinal plants and some active constituents isolated from them are preferred and even recommended by WHO for the treatment of diabetes mellitus[3-6]. In the present study the seeds of *Brassica nigra* (L.) Koch, (family *Brassicaceae* Mustard in English, Sarson in Hindi, Aavalu in Telugu) were chosen because these seeds have the advantage of being an edible spice. Mustard is reported to be useful for pulmonary congestion, arthritis, rheumatism and also as diuretic, emetic, rubefacient and stimulant. Seed decoctions or other liquid preparations were used for tumors of sinax, indurations of the liver and spleen, carcinoma, throat tumors, and imposthumes and seed flour as an antiseptic[7,8]. Preliminary studies on *Brassica juncea* showed a significant hypoglycemic effect in streptozotocin induced diabetic mice[9]. Although Srinivasan reported that *B. nigra* possesses hypoglycemic activity, the exact study mentioned in his review was on *Brassica juncea* and not on *B.nigra*[10]. Further, there are no detailed studies on *Brassica nigra*. The present study reports not only the antidiabetic effect but also antihyperlipidemic effect of aqueous extract of *Brassica nigra* seeds since hyperlipidemias are common complications in diabetes.

For correspondence:
Phone: *011-27666272; 011-27667151
†0120-2510141, 09910317375
e-mail:*acbrdu@hotmail.com
†psmurthy_2000@yahoo.com

Materials and Methods

Preparation of extracts — Dried seeds of *B.nigra* were purchased locally and were authenticated by Dr. Veena Aggarwal, Department of Botany, University of Delhi. Dried seeds (800 g) were crushed and divided into four equal parts. One part of the crushed seeds (200 g) was soaked overnight in 600 ml of either water or different organic solvents (chloroform, acetone and ethanol) at room temperature (about 25°-30°C). Next morning the extract was filtered over cotton and the filtrate was centrifuged at 10,000 rpm for 10 min at room temperature to remove any finely suspended material. The supernatant of organic solvents was further evaporated to dryness under reduced pressure in a rotary evaporator. Since concentration of water at room temperature takes long time, concentration of aqueous extract in rotary evaporator was done at 55°-60°C and then lyophilized. Yield of chloroform, acetone, ethanol and aqueous extracts obtained from the above preparation was 6.6, 4.8, 5.7and 5.18% (w/w), respectively.

Animals — Male Wistar rats (6-7 weeks old) weighing 150-200 g obtained from National Institute of Communicable Diseases, Delhi were used. The

animals were kept in the air-conditioned animal house of the Center, at 25°–30° C and at 45–55% RH and acclimatized with free access to food (Golden feed, Delhi, India) and water *ad libitum* for 1 week under 12 hr each of light and dark cycles. The animal studies were approved by Animal Ethical Committee of the Center.

Induction of experimental diabetes — Diabetes was induced as described earlier with a single i p injection of freshly prepared streptozotocin (STZ) in a dose of 50 mg/kg body weight dissolved in citrate buffer *p*H 4.5 to overnight fasted rats. Experiments on hyperglycemic animals were carried out one week after STZ injection and after ensuring stabilization of diabetes[11]. Only animals, with fasting serum glucose (FSG) of 240 mg/dl and above were used.

Effect of different solvent extracts on glucose tolerance in glucose tolerance test — Different solvent extracts of *B. nigra* were assessed by oral glucose tolerance test (GTT) based antidiabetic activity in STZ induced diabetic rats[12]. In this method the advantage is that the same group of animals served as their self control as indicated below. Four groups (groups I- IV) of six rats each (one for each solvent extract) were used in this experiment. The animals were fasted overnight and fasting blood samples were drawn from the tail vein. In order to obtain first the GTT pattern in untreated animals, empty gelatin capsules were fed to the animals after withdrawal of fasting blood samples. Again, after 90 min blood was drawn. This sample was taken as '0' hour value for GTT. The animals were given immediately aqueous glucose solution 2 g/kg of body weight orally and blood samples were drawn at 1, 2 and 3 hr after glucose administration to get the GTT pattern of the untreated diabetic animals (diabetic control). After a week, same animals were again fasted overnight to carry out GTT with drug. Fasting blood samples were drawn. Solvent extracts (200 mg/kg body weight) of *B. nigra* were administered orally to rats in gelatin capsules. Animals of groups I-IV received chloroform, acetone, ethanol and aqueous extracts respectively. After 90 min (this time was allowed for the extract or metabolites of the compounds in the extract to show their effect in the body), blood samples were drawn again. This served as '0 hr' blood sample of the treated diabetic rats in GTT. Then GTT was performed as mentioned above to get the glucose tolerance pattern of the same diabetic animals but after treatment with the extracts. In earlier experiments, when GTT was performed after one week in untreated diabetic animals, there was not much of difference in GTT patterns. So no separate group of untreated control animals for each extract was kept.

Effective dose (ED) determination of aqueous extract — Since aqueous extract was found most active, further studies were carried out with the aqueous extract. GTT was conducted as before with different doses of aqueous extract viz. 0, 100, 200, 300 and 400 mg/kg body weight. The animals in the group, which did not receive any drug (0 mg/kg body weight) served as untreated diabetic control.

Assessment of antidiabetic effect in rats — Effect of aqueous extract on fasting serum glucose (FSG) in experimental diabetes was assessed as follows. Four groups (I to IV) each of six rats were used. Group I served as normal healthy controls and group II as untreated diabetic animals and were given water during the experimental period as the vehicle used for drug administration was water. Group III diabetic rats were given effective dose (200 mg/kg body weight) of aqueous extract while group IV rats were given the standard drug glibenclamide (Sigma Chemical Co. USA; 0.20 g/kg body weight) orally once daily[13]. The drugs were given in the morning to animals (with free access to food) for one month. Fasting blood was collected in the beginning, 1, 2 and 3 weeks and after one month. Glycosylated hemoglobin (HbA1c) (in blood) and serum glucose (fasting serum glucose, FSG), total cholesterol (TC), triglycerides (TG), HDLC (high density lipoproteins cholesterol) and LDLC (low density lipoproteins cholesterol) were estimated in each sample. Urine glucose levels were also estimated at the same time intervals. FSG and parameters of lipid profile were estimated using kits from Ranbaxy Laboratories Ltd, New Delhi, India. Glycosylated hemoglobin level was estimated using kit from Erba Diagnostics, Germany. Urine glucose level was estimated using Uristix from Bayer Diagnostics Pvt ltd, Mumbai, India.

Assessment of hypoglycemic activity of active extract in normal healthy rat — It was intended to know the effect of aqueous extract of *B. nigra* on glucose tolerance pattern of normal healthy animals also. Initially water was given to overnight fasted rats and GTT was performed. After one week aqueous extract (200 mg/kg body weight) was given and after

90 min GTT was performed as described earlier for diabetic animals. Glucose was estimated in the blood samples collected after overnight fasting (FSG), 90 min after giving aqueous extract and 1, 2 and 3 hours after glucose administration in GTT.

Statistical analysis — The results were expressed as mean ± S.D. The statistical analysis involving two groups was performed by means of paired *t*-test, whereas analysis of variance (ANOVA) followed by Dunnett's multiple comparison test were used in order to compare more than two groups. All the data were processed with GraphPad Prism version 4.01 software.

$P < 0.05$ was considered significant and $P < 0.01$ was considered more significant.

Results

Antidiabetic activity of different solvent extracts of B. nigra seeds — Aqueous extract significantly ($P < 0.01$) reduced (Table 1) the rise in blood glucose from 0 to 1 hr after glucose administration when compared with the corresponding value of its control during GTT by 53.2 %. But chloroform, acetone and ethanol extracts produced a fall of 23.9, 27.8 and 39.7 % respectively when compared with their respective control values. So aqueous extract was found most active and was therefore used for further work.

Effective dose (ED) determination — The increase in serum glucose between 0 and 1 hr was the lowest with 200 mg/kg body weight of aqueous extract of *B.nigra* (66 mg/dl) which was 46.7% of the untreated

Table 1 — Antidiabetic activity of different solvent extracts of *B. nigra* seeds on glucose tolerance in streptozotocin induced diabetic rats during GTT

[Values are mean ± SD from 6 animals in each group]

Extract treated group		Serum glucose (mg/dl)					Increase in serum glucose (mg/dl) between 0 and 1 hr
		Fasting	0 hr	1 hr	2 hr	3 hr	
Chloroform	C	332±4.3	326±6.2	397±5.9	356±4.6	334±6.3	71
	T	314±6.5	289±5.8	343±6.3	296±5.4	284±7.1	54 (23.9%)[#]
Acetone	C	310±6.6	330±4.2	391±4.6	357±7.7	349±6.4	61
	T	352±5.8	326±5.6	370±5.4	347±7.6	343±5.2	44 (27.8%)[#]
Ethanol	C	298±5.9	315 ±5.6	388±6.1	343±7.0	294±5.7	73
	T	309±6.3	293±6.0	337±6.8*	312±5.3	282±4.8	44 (39.7%)[#]
Aqueous	C	303±6.5	311±5.9	373±4.8	332±5.6	310±7.7	62
	T	318±5.7	319±6.4	348±6.0**	282±7.2	248±8.1	29 (53.2%)[#]

P values: *< 0.05; **< 0.01 as compared with control values at the same time

C = Control, T = Treated

[#] Percent of the untreated control of the same group.

Table 2 — Effective dose (ED) determination of aqueous extract of *B. nigra* seeds in streptozotocin induced diabetic rats during GTT

[Values are mean ± SD from 6 animals in each group]

Group	Dose (mg/kg body weight)	Serum glucose (mg/dl) during GTT					Increase in serum glucose (mg/dl) between 0 and 1 hr
		Fasting	0 hr	1 hr	2 hr	3 hr	
1	0	273± 7.3	266±7.5	390±4.7	351±6.2	353±5.8	124
2	100	276±5.1	280±4.7	355±6.2*	312±7.4	320±7.2	75
3	200	264±6.6	270±6.3	336±4.6*	295±6.9	289±6.3	66
4	300	295±7.2	310±5.8	396±6.3*	366±5.7	373±8.1	86
5	400	261±6.5	252±6.3	322±4.7*	309±6.5	287±4.6	70

* $P < 0.01$ as compared with group 1 value at the same time

control group (124 mg/dl; Table 2). But 100, 300 and 400 mg/kg body weight of aqueous extract produced fall of 39.5, 30.6 and 43.5%, respectively when compared with the untreated controls. Thus, the effective dose was taken as 200 mg/kg body weight, which was used in subsequent experiments.

Effect of aqueous extract and glibenclamide on diabetic animals — The effect of aqueous extract (200 mg/kg body weight) of the seeds of *B.nigra* on the serum glucose, glycosylated hemoglobin (HbA1c) and urine glucose is shown in Table 3. Results in Table 4 show the effect of extract on the serum lipid profile in diabetic animals. Treatment for one month showed significant ($P < 0.01$) reduction of 52.6 and 56.3% of serum glucose with aqueous extract and glibenclamide respectively when compared with the values of untreated diabetic control animals. After the treatment, urine sugar was in trace (±) amounts and nil (-) respectively with aqueous extract and glibenclamide while in the untreated animals it was

Table 3 — Effect of treatment of diabetic rats with aqueous extract of *B. nigra* (200 mg/kg body weight) and glibenclamide (200m g/kg body weight) for one month on glucose and glycosylated hemoglobin levels

[Values are mean ± SD from 6 animals in each group]

Groups of animals	% of HbA1c (in blood)	Glucose levels (mg/dl) at different periods of treatment									
	After treatment	Before		1 week		2 weeks		3 weeks		1 month	
		Serum	Urine	Serum	Urine	Serum	Urine	Serum	Urine	Serum	Urine
Healthy control	4.8±0.2	89±7.7	-	91±10.1	-	94±9.2	-	93±12.1	-	92.6±14.2	-
Diabetic control	9.4±0.5	247±8.4	+ +	249±7.4	+ +	256±8.8	+ +	261±7.1	+ +	268±6.8	+ +
Extract treated[#]	6.6±0.3	254±7.9	+ +	198±9.6	+ +	153±4.3	+	135±5.6	±	127±9.4* 52.6 %**	±
Glibenclamide treated[#]	5.7±0.4	241±9.3	+ +	201±7.7	+ +	160±8.3	+	131±11.5	±	117±17.6* 56.3%**	-

*$P < 0.01$ when compared with the diabetic control values

[#] 200 mg/kg bw dose administered; ** the percentage reduction is of one month value when compared with corresponding diabetic control value

Table 4 — Effect of treatment with aqueous extract of *B. nigra* (200 mg/kg bw) and glibenclamide for one month on lipid profile in streptozotocin induced diabetic rats

[Values are mean ± SD from 6 animals in each group]

Groups of animals	Total cholesterol (mg/dl)			Triglycerides (mg/dl)		
	Before	15 days	1 month	Before	15 days	1 month
Healthy control	121±5.8	119±5.3	118±6.4	61±4.4	60±6.3	59.3±3.4
Diabetic control	225±9.7	248±11.3	266±11.2	139±10.1	145±9.5	157±12.4
Extract treated*	231±6.8	205±9.0	181±10.2	141±7.6	126±12.5	98±13.4
Glibenclamide treated*	229±8.4	197±7.7	176±8.7	137±8.5	119±7.9	95.6±9.8
	HDLC[#] (mg/dl)			LDLC[# #] (mg/dl)		
Healthy control	35.5±3.3	37.1±4.5	36.2±3.8	73.2±6.3	69.9±5.4	70±4.7
Diabetic control	47±2.4	47.9±5.9	50±8.2	150.2±5.4	171.1±2.3	184±1.9
Extract treated*	45.3±4.1	54±7.4	61±8.6	157.5±6.7	125.8±7.3	100±13.7
Glibenclamide treated*	44.7±3.5	59±3.8	68.2±2.3	156.9±7.4	114.2±5.9	88.6±11.9

*200 mg/kg body weight dose administered; [#]High density lipoproteins cholesterol, [##]Low density lipoproteins cholesterol

+ + (0.5 g/l). Thus glibenclamide was slightly more effective than the aqueous extract. A significant fall in serum TG (37.5%), TC (31.9%), LDLC (45.6%) and glycosylated hemoglobin (29.8%) in the blood/serum was observed in the aqueous extract of *B.nigra* treated group of animals when compared with untreated diabetic animals. In the glibenclamide treated animals the fall in TG, TC, LDLC and HbA1c was 39.1, 33.8, 52 and 39.3%, respectively. These values are almost similar to those in aqueous extract of *B.nigra* treated animals. There was an increase of 18.0 and 26.7% in HDL cholesterol in the extract and glibenclamide treated diabetic rats respectively.

Effect of aqueous extract on normal healthy rats — In order to know the effect of treatment with aqueous extract on healthy animals, 200 mg/kg body weight extract was given to overnight fasted healthy animals and the serum glucose levels during glucose tolerance test are shown in Table 5. The reduction in serum glucose at 1 hr during GTT was insignificant (10.9%) in the treated animals.

Discussion

In the preliminary screening studies, the effect of different solvent extracts of the seeds of *B.nigra* was assessed by using the glucose tolerance test developed by Babu *et.al*[12]. This GTT has the advantage that the same group of animals, serve as controls and after one week as treated ones. Chloroform, acetone, ethanol and aqueous extracts were effective in lowering serum glucose during GTT (Table 1) in rats with STZ induced diabetes. This points out that *B. nigra* has got water soluble as well as chloroform, acetone and ethanol soluble compounds with antidiabetic activity and that the water soluble compound is more active in improving GTT than the water insoluble compounds. Regarding the water insoluble active compounds further studies are required to know whether the chloroform, acetone and ethanol soluble compounds are the same or different. Studies with different doses (100-400 mg/kg body weight) of the aqueous extract (Table 2) indicated that 200 mg/kg body weight is the most effective dose. Therefore, further studies were carried out with aqueous extract at a dose of 200 mg/kg body weight daily once for one month in order to know its antidiabetic effect in diabetic rats. Aqueous extract of *B.nigra* showed improvement in all the parameters tested. It reduced serum glucose and HbA1c levels by 52.6 and 29.7%, respectively. Now a days fall in HbA1c is considered as a more reliable indicator of antidiabetic activity than the fall in blood glucose which can vary due to other factors also. It is well known that in diabetic animals with only partial or improper control, one of the complications is the elevation of blood lipid levels, which are seen in the present study also. Aqueous extract was effective in controlling the complication of altered lipids. The elevated levels of total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDLC) were brought down in the one month treatment period (Table 4). An interesting feature was that treatment with aqueous extract of *Brassica nigra* increased the high density lipoprotein cholesterol (HDLC) level, which is commonly considered as good cholesterol (Table 4). Normally a decrease is expected in HDLC in diabetes with lipid complications. But in the present study, there was increase in HDLC in untreated diabetic animals compared to that in healthy controls, which may look unexpected. The explanation is as follows: in the normal healthy controls TC was 118±6.4mg/dl (at one month) and HDLC was 30.6% of TC. But in the diabetic untreated animals the TC increased (266±11.2 mg/dl) and HDLC (50±8.2 mg/dl) was only 18.8% of TC. Thus, there was decrease in percent HDLC. Further studies on the mechanism of action of this extract and isolation of the active compounds in a pure state are in progress, which could lead to the development of antidiabetic drugs

Table 5 — Effect of aqueous extract of *B. nigra* (200 mg/kg body weight) on serum glucose values during GTT in normal healthy rats

[Values are mean ± SD from 6 animals in each group]

Group of animals	Serum glucose (mg/dl)					Increase in serum glucose (mg/dl) between 0 and 1 hr
	Fasting	0 hr	1hr	2hr	3hr	
Control	82±7.8	78±8.9	160±2.9	102±6.9	92±5.4	82
Aqueous extract treated	88±5.4	69±7.4	142±6.3	96±4.9	76±3.9	73 (10.9%*)

* Percent of the untreated control of the same group.

from this plant. The earlier study on *B.nigra* was of a preliminary nature on just one parameter, viz. GTT. But the present studies confirm the usefulness of the aqueous extract of *B. nigra* in STZ induced diabetes by lowering not only serum glucose and HbA1c but by reversing the lipid abnormalities. There are also reports of some plants showing reduction in blood glucose, HbA1c and lipids[4]. Since *B. nigra* is an edible spice, it is likely to be non toxic or less toxic.

Acknowledgement

PA and MKY are thankful to the UGC, New Delhi and ICMR, Delhi, India, respectively for senior research fellowships.

References

1 Holman R R & Turner R C, Oral agents and insulin in the treatment of NIDDM, in *Textbook of Diabetes* (Blackwell, Oxford) 1991, 467.

2 Kameswara Rao B, Giri R, Kesavalu M M & Appa Rao C, Herbal medicine: In the management of diabetes mellitus, *Manphar Vaidhya Patrika*, 1, (1997) 33.

3 World Health Organization Expert Committee on Diabetes Mellitus: Second Report on Diabetes Mellitus, (Geneva, World Health Organization) 1980, Tech. Rep. Ser. 646.

4 Shukla R, Sharma S B, Prabhu K M & Murthy P S, Medicinal plants for treatment of diabetes mellitus, *Indian J Clin Biochem*, 15 (2000) 169.

5 Grover J K, Yadav S & Vats V, Medicinal plants of India with anti-diabetic potential, *J Ethnopharmacol*, 81 (2002) 81.

6 Gupta A K, *Quality standards of Indian medicinal plants*. Vol. 1, (Indian Council of Medical Research, New Delhi) 2003, 262.

7 Walker, Winifred, *All the plants of the Bible* (Doubleday & Co. Inc. Garden City, N.Y) 1979.

8 Schery, Robert, *Plants for man*, (Prentice-Hall, Inc., Englewood Cliffs, New Jersey), 1972, 282.

9 Grover J K, Yadav S P & Vats V, Effect of feeding *Murraya koeingii* and *Brassica juncea* diet in kidney functions and glucose levels in streptozotocin diabetic mice, *J Ethnopharmacol*, 85 (2003) 1.

10 Srinivasan K, Plant foods in the management of diabetes mellitus: Spices as beneficial antidiabetic food adjuncts, *Int J Food Sci Nutrition*, 56 (2005) 399.

11 Murali Y K, Ramesh Chandra & Murthy P S, Antihyperglycemic effect of water extract of dry fruits of *Terminalia chebula* in experimental diabetes mellitus, *Indian J Clin Biochem*, 19 (2004) 202.

12 Babu B V, Radha Moorty, Pugazhenthi S, Prabhu K M & Murthy P S, Alloxan recovered rabbits as animal model for screening the hypoglycemic activity of compounds, *Indian J Biochem Bio*, 25 (1988) 714.

13 Tatiana Schoenfelder, Thayse Marcon Cirimbelli & Vanilde Citadini-Zanette, Acute effect of *Trema micrantha* (Ulmaceae) on serum glucose levels in normal and diabetic rats, *J Ethnopharmacol*, 107 (2006) 456.

14 Moorthy R, Prabhu K M & Murthy P S, Studies on the isolation and effect of orally active hypoglycemic principle from the seed of fenugreek (*Trigonella foenum-graecum*), *Diabetes Bull*, 9 (1989) 69.