



Antidiabetic and Hepatoprotective Activity of Seed Extract of Brassica Nigra Seed on Alloxan induced Model

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Abstract : The present study investigated the hepatoprotective and antidiabetic effect of BRASSICA NIGRA in alloxan induced rats. Antidiabetic activity of the aqueous extracts of B. nigra seeds (AEBN) was studied in alloxan induced diabetic rats. The effect of extract on fasting blood glucose, body weight, lipid profile, and total protein were analyzed. Metformin was used as standard reference drug.

Aqueous extract B. nigra seeds (AEBN) showed highly significant blood glucose lowering effect. After diabetic rats treated with AEBN(400 mg/kg) for 21 days, there were a significant decrease in fasting blood glucose, total cholesterol and total significant increase in body weight, serum total protein levels as compared to untreated diabetic rats.

The results of the experiments showed that AEBN seeds might be useful for management of hepatic damage, diabetes mellitus and other abnormalities associated with this metabolic disorder. The present study might support the traditional use of B. Nigra for diabetes mellitus treatment.

Key words : *Brassica nigra*, diabetes mellitus, alloxan and hepatoprotection.

Introduction

The word diabetes aroused from latin word diabetes which comes from diabenin from ancient Greek. It is classified as Madhumeha or honey urine by Indian physicians. Prameha is the term also used for diabetes which means watering. Diabetes is a complex and multivariuous group of disorder of metabolism causing excessive thirst and production of large volume of urine. Deficiency of secretion of antidiuretic hormone causes diabetes insipidus which is due to inflammatory and neoplastic lesions of the hypothalamo- hypophyseal axis, destruction of neurohypophysis due to surgery, radiation, head injury and lastly are idiopathic cases.

Diabetes mellitus is a heterogenous metabolic disorder having disturbance in metabolism of carbohydrate, fat and protein, glycosuria, hyperlipaemia, negative nitrogen and sometimes ketonaemia.

The detoxification and metabolization of drug takes place in liver which regulates many metabolic functions. Distortion of these metabolic functions are the indications of hepatic injury. Multiple parameters represents proper functioning of liver. Damaged hepatic cells releases some useful enzymes and metabolites in the blood indicating the possibility of liver damage.

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Materials and Methods

Collection of drugs and chemicals

The seeds of *Brassica nigra* were collected from the local market, Bareilly. The drugs and chemicals used in the study were Metformin (USV pharmaceuticals), alloxan, silymarin, carbon tetrachloride (CCl₄), diagnostic kits (Arkray). All the chemicals used in the study were of analytical grade.

Rats : 150-250gm.

Instrument : Blood Analyzer (Matrix healthcare Pvt. Ltd., Model-MX300).

Identification and Authentication

The plant material was identified and authenticated by the department of plant sciences, M.J.P. Rohilkhand university, Bareilly, India. A voucher specimen has been preserved at our laboratory for future reference.

Extraction

The seeds were dried in shade at room temperature, grinded to a fine powder and stored in a well closed container. Aqueous extract of powder prepared by dissolving 200g of powdered sample – *Brassica nigra* seed in 600ml of distilled water. The mixture was heated on a hot plate with continuous stirring at 30-40°C for 20min. Then the aqueous extract was filtered through cotton and used for qualitative and phytonutrient analysis (Harborne, 1984 and Kokate *et al.*, 1995). The solvent was completely removed under reduced pressure.

Phytochemical screening of extract

For preliminary phytochemical analysis the aqueous extract was subjected to qualitative chemical tests for the detection:

1. Test for carbohydrate

Molisch's test : A few drops of alcoholic alpha naphthol solution and a few drops of concentrated Sulphuric acid along the sides of the test tube was added to the aqueous extract. Positive result gives purple or violet colored ring at the junction.

Fehlings test : An equal amount of Fehlings A and B solution was added to the aqueous extract, heated the tubes in a boiling water bath. Brick red precipitation of cuprous oxide is formed, indicating the presence of sugar.

Benedicts test : Benedicts reagent was added to the aqueous extract, the tubes were heated in a boiling water bath. Red precipitation indicates a positive result.

2. Test for alkaloids

Wagners test : Few drops of Wagner's reagent (dilute Iodine solution) was added to aqueous extract solution, formation of reddish-brown precipitate indicated the positive response

Hagers test : 2 ml of aq. extract was allowed to react with 0.2 ml of dilute hydrochloric acid and 0.1 ml Hager's reagent. A yellowish precipitate suggested the presence of alkaloid.

3. Test for steroids

Liebermann-Burchard test : To the aq. extract 2ml of chloroform, 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid was added. Bluish red to a cherry red color in chloroform layer showed positive results.

Salwoski test : Few drops of chloroform and concentrated sulphuric acid was added to the aq. extract the bluish red to cherry red color indicated the presence of steroids.

4. Test for Glycosides

Legal test : To the extract added pyridine and sodium nitroprusside. Positive result showed pink red color.

Baljet test : To the extract added picric acid. Appearance of orange color signified positive result.

5. Test for saponins

Foaming test : Foams produces when the aq.extract was mixed with water.

6. Test for tannin and phenolic compounds

Ferric chloride test : 5 ml of aq.extract solution was allowed to react with 1 ml of 5% ferric chloride solution. Greenish black coloration indicated the presence of tannins.

Potassium dichromate test : 5 ml of the aq.extract was treated with 1 ml of 10% aqueous potassium dichromate solution. Formation of yellowish brown precipitate suggested the presence of tannins.

Gelatin test : 1% gelatin solution containing 10% sodium chloride was added to the aq.extract. Appearance of white precipitation confirms the presence of tannin.

7. Test for protein and amino acids

Biuret test : 4% sodium hydroxide, and a few drops of 15% copper sulphate was added to the aq.extract . Appearance of purple color confirms the presence of protein.

Ninhydrin test : Bluish violet color forms when a solution of ninhydrin and aq.extract mixture was heated.

Heat test : Protein coagulation showed a positive result when aq.extract was heated on a boiling water bath.

8. Test for fixed oil

Copper sulphate test : Blue colour formed when the extract was mixed with 1ml of 1% copper sulphate and 10% sodium hydroxide.

Maintenance of animals and approval of protocol

Healthy albino wistar rats (150-250g) were procured from disease free small animal house, Indian veterinary research institute (IVRI), Bareilly(U.P.). They were maintained in an air-conditioned experimental room at 12 hour light and dark cycles. The animals were randomized into experimental and control groups and were housed in a polypropylene cage. Standard pellets were used as a basal diet during the experimental period. The control and experimental animals were provided with purified drinking water ad libitum. The animals were maintained in accordance with the "CPCSEA guidelines for laboratory animal facility" (Committee for the Purpose of Control and Supervision of Experiments on Animals) and the approval number is (CPCSEA Regd. No. 1884/GO/Re//S/16/CPCSEA, dated 29/04/2016). Before starting the experiment the animals were carefully marked on different parts of their body, which was later used as identification mark for a particular animal, so that the response of a particular rat prior to and after the administration could be noted separately.

Induction of diabetes to test animals

The selected rats were weighed, marked for individual identification and fast for overnight. The alloxan monohydrates at the rate of 150 mg/kg body weight were administered subcutaneously (S.C.) for making the alloxan induced diabetic rat model. Blood glucose level of these rats were estimated 72 hr after alloxan administration, diabetes was confirmed by blood samples collected from the tip of the tail using a blood glucometer (Accusure , Taiwan). Animals with blood glucose level equal or more than 200 mg/dl were declared diabetic and were used in entire experimental group.

Induction of hepatic damage/injury to test animals

Hepatic injury was produced due to diabetes.

Acute oral toxicity studies

The acute toxicity study was carried on albino mice as per the guidelines No:423 given by the Organization for Economic Co-operations and Development, Paris[OECD 423, 2001]. *Brassica nigra* in the increasing dose range of 100,200,500,1000,2000,5000 mg/kg body weight oral gavage administration different group of rats comprised of 6 rats in each group. Animals were kept under close observation for 4 hours after administering the fraction for behavior, neurological and autonomic profile and then observed for any change in the general behavior and /or physical activities, mortality were recorded within 72 hours. Acute toxicity was determined according to the method of Lorke [19].

Experimental design

Six groups of rats, six rats in each received the following treatment schedule.

Group I: Normal control (distilled water)

Group II: Alloxan treated control (150 mg/kg body weight i.p)

Group III: Alloxan (150 mg/kg body weight i.p) + *brassica nigra* (seed extract at the dose of 400 mg/kg b.w).

Group IV: Alloxan (150 mg/kg body weight i.p) + Metformin(at the dose of 500 mg /kg b.w).

Group V: Carbon tetrachloride (ccl₄) treated control(1 ml/kg p.o.).

Group VI: Carbon tetrachloride (ccl₄)(1ml/kg p.o.) + silymarin(50mg/kg B.W.).

Brassica nigra seed extract and D.W. were administrated by Gavage's method. Group I serve as normal control, which received D.W. for 21days. Group II to Group IV diabetic control rats (which previously received alloxan) are given fixed dose *brassica nigra* seed extract (400 mg/kgb.w) for 21consecutive days.

Assessment of antidiabetic activity

Effect of aqueous extract on fasting serum glucose (FSG) in experimental diabetes was assessed as follows, six groups (I to VI) each of six rats were used. The drugs were given to animals (with free access to food) for twenty one days. Fasting blood was collected in the beginning and after 7, 14 and 21 days. Serum glucose (fasting serum glucose, FSG) total cholesterol, total bilirubin and total protein were estimated in each sample using kits.

Assessment of hypoglycemic activity of active extract in normal healthy rat

It was initiated to know the effect of aqueous extract of *Brassica 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 (400mg/kg body weight) was given and after 90 min. GTT was performed. 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.

Assessment of hepatoprotective activity

Replenishing a known quantity of fresh food daily at 8.00 a.m. and thereby measuring the food intake of the previous day and carried out measurement of daily food consumption. Body weight of rats was recorded weekly to assess percentage of weight gain of each animal. Animals were kept starved overnight on the last day. On the next day, after recording the weight of each animal, they were euthanized by decapitation under ether anesthesia, by the method of retro orbital plexus blood was collected in sterile centrifuge tubes and allowed to clot. The liver was dissected out immediately, rinsed with ice cold phosphate buffer and homogenized with 5% formalin solution. The liver homogenate was used for antidiabetic and hepatoprotective studies and a part of the tissue was processed for histological examination. Serum was separated from the collected blood by centrifugation and subjected to various biochemical estimations like serum glucose, total bilirubin, total protein, total cholesterol determine the functional state of the liver.. Liver slices fixed for 12 h in Bouine's solution were processed for paraffin embedding following standard micro techniques. Liver section of 5 μ m stained with alum hematoxylin and eosin was observed microscopically for histopathological changes.

Result

Table – 1. Qualitative analysis of phytochemicals in *Brassica nigra* seeds

S.No	Name of the test	Results
1	Test for carbohydrate a) Molisch's test b) Fehlings test	+++ +++
2	Test for alkaloids a) Wagners test b) Hagers test	--- +++
3	Test for steroids and sterols a) Libermann - Burchard test b) Salwoski test	++ ++
4	Test for Glycosides a) Legal test b) Baljet test	++ +
5	Test for saponins Saponin test	+
6	Test for tannin and phenolic compounds a) Ferric chloride test b) Potassium dichromate test c) Gelatin test	+++ ++ +
7	Test for protein and amino acids a) Biuret test b) Ninhydrin test c) Heat test	+ +++ +
8	Test for fixed oil a) Copper sulphate test	+++

+++ - Strong, ++ Moderate, + -low

Table 2- Serum Glucose Level Pre and after Treatment

Fasting Blood Sugar	Control	Diabetic Control	<i>Brassica Nigra</i> Extract	Metformin
Pre-Diabetes	84.167±2.762	82.5±2.643	90.667±1.892	81.833±3.027
48 Hrs After Diabetization	77.167±2.688	216.833±5.947	189.167±2.372	183.5±2.778
End of I Week (After 7 Days)	83.333±2.813	215.167±4.262	167.333±2.848	92.667±1.498
End of II Week (After 14 Days)	77.667±2.290	215.333±3.930	165.833±2.535	93.333±3.383
End of III Week (After 21 Days)	77.5±1.784	215.167±1.966	155±1.966	90.833±2.786

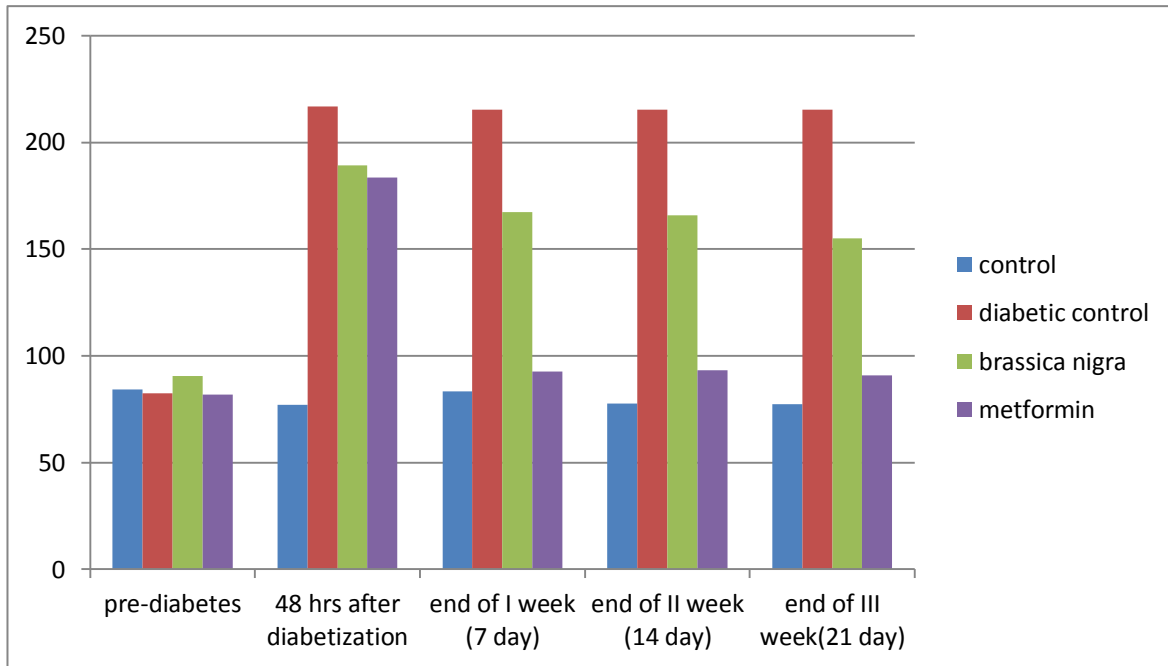


Fig-1 Comparison of serum glucose level among the groups.

Table 3-- Levels of Total Protein and Total Bilirubin in Blood Serum

Parameters	Control	Hepatic Control	<i>Brassica Nigra</i>	Silymarin
Total Protein	8.002±0.316	6.428±0.427	7.073±0.193	6.597±0.198
Total Bilirubin	0.563±0.088	0.938±0.040	0.833±0.017	0.059±0.023

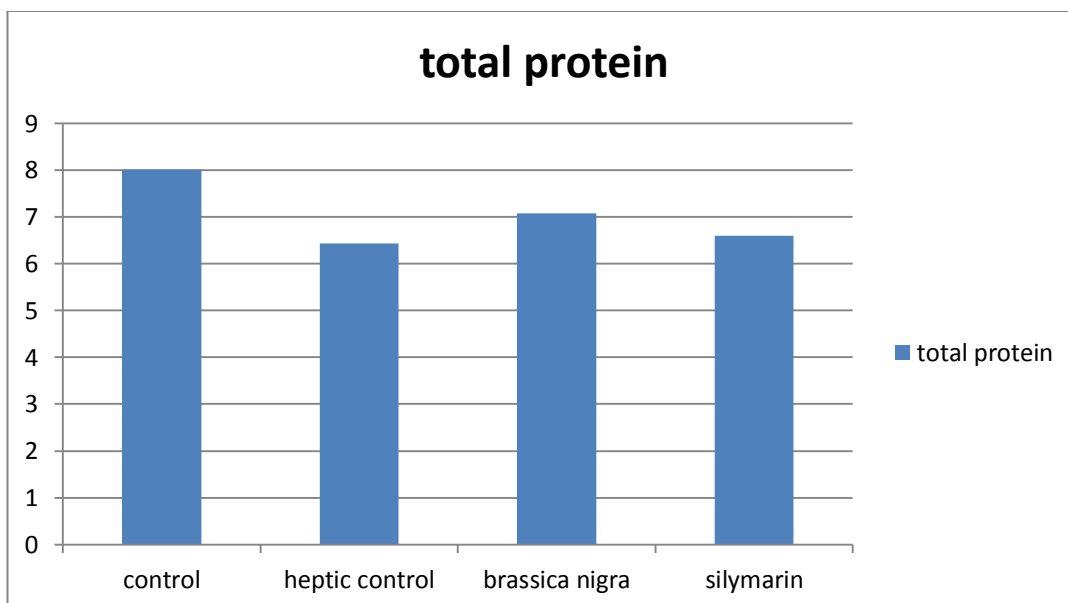


Fig 2 Comparison of levels of total protein among the groups.

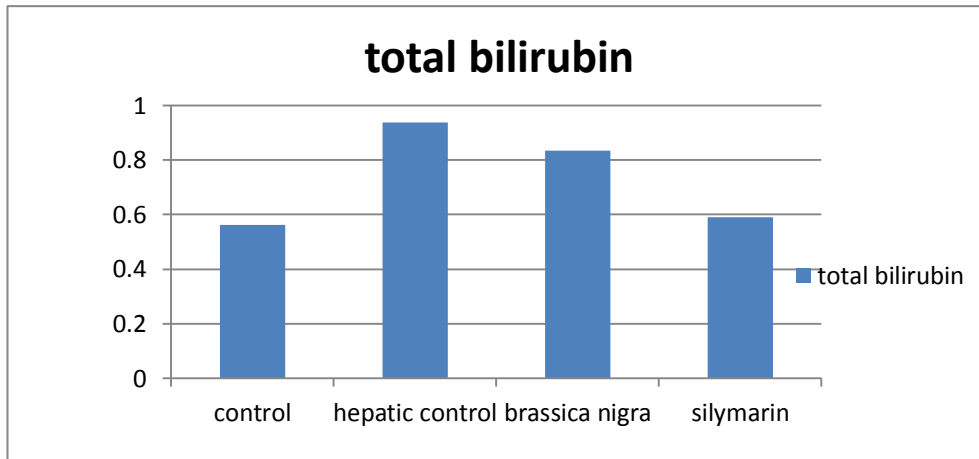


Fig 3 comparison of levels of total bilirubin among the groups

Table 4 – Levels of Total Cholesterol in Blood Serum

Parameters	Control	Diabetic Control	Hepatic Control	Brassica Nigra	Metformin	Silymarin
Total Cholesterol	198±1.713	240±3.24	216.7±2.14	237.7±3.373	192.5±2.062	203.7±1.453

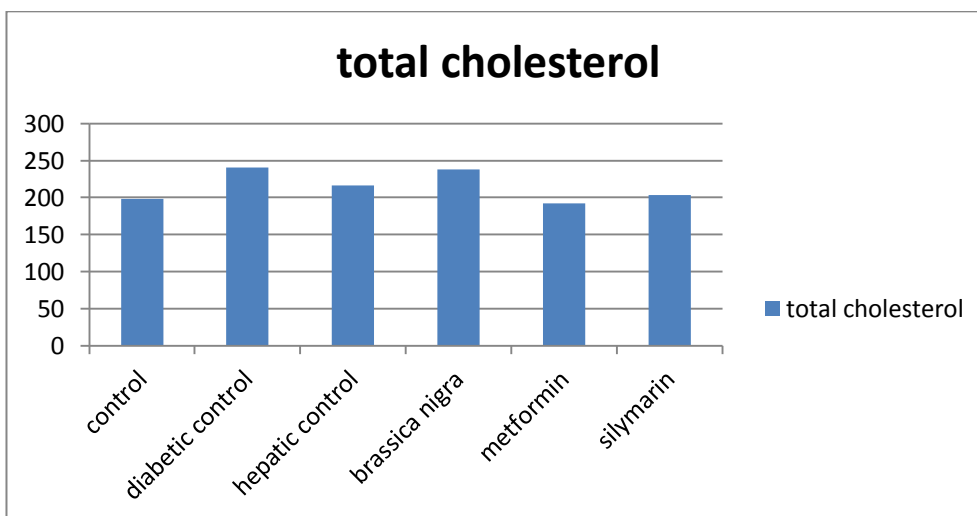


Fig 4 comparison of levels of total cholesterol among different groups

Table 5 – Effects on body weight as compared to normal :

Control	Diabetic control	Hepatic control	B.N.	Metformin	silymarin
202.2 ± 6.274	160 ± 6.191	183.3 ± 5.578	167 ± 5.416	194.2 ± 5.231	193.3 ± 4.41

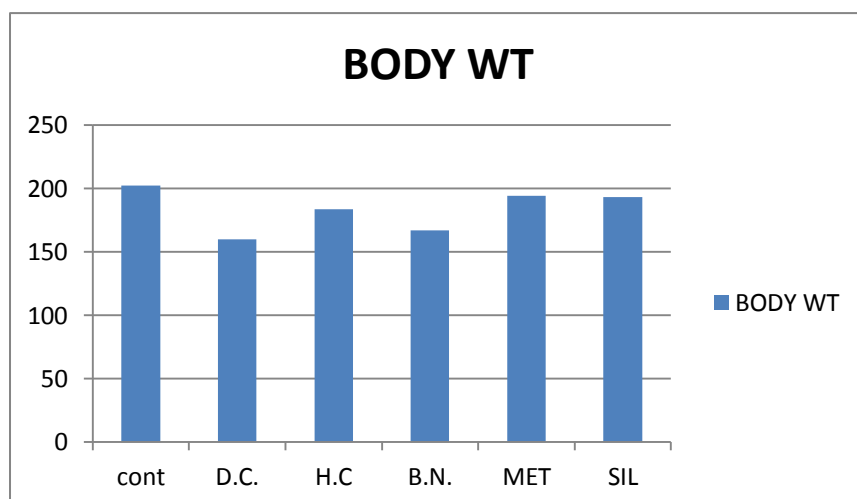


Fig 5 Effect on body weight among different group

Effect of plant aqueous extracts on body weight

Rats administered *Brassica nigra* at the dose of 400mg/kg body weight showed an increase in the body weight as compared to control groups. Administration of *Brassica nigra* to diabetic rats showed a significant change in body weight and it was increased as compared to control groups.

Effect of plant aqueous extract on serum protein contents

Data represented an increase in total serum protein. Treatment with aqueous extract of *Brassica nigra* at the dose of 400mg/kg decreased the serum total protein but it was observed that there is no significant effect in treated rats.

Effect of plant aqueous extract on serum bilirubin

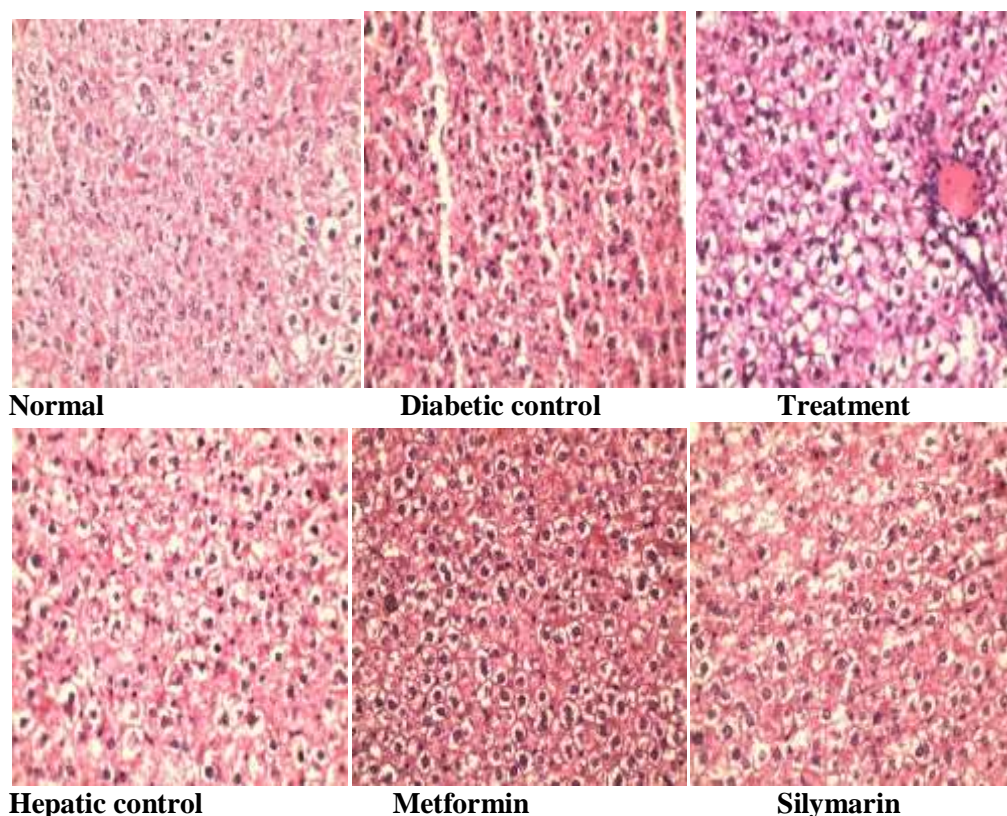
An increase in the total bilirubin was observed. Administration of aqueous extract of *Brassica nigra* significantly decreased the serum total bilirubin but showed no significant effect.

Effect of plant aqueous extract on serum glucose

It was observed that aqueous extract of *Brassica nigra* administered at the dose of 400mg/kg significantly ($P < 0.0001$) decreased the level of serum glucose in treatment group.

Effect of plant aqueous extract on lipid profile

There was a significant increase in the levels of total cholesterol in diabetic rats as compared to normal control. The administration of *Brassica nigra* at the dose of 400mg/kg reversed the level of cholesterol not significantly.



Discussion

The present investigation revealed the antidiabetic and hepatoprotective activity of the aqueous extract of the seed of *brassica nigra* Linn. in alloxan- induced diabetic model.

It was observed that there was significantly increased in the fasting blood glucose (FBG), total cholesterol (TC), serum biochemical parameters (total protein and total bilirubin) levels in the alloxan induced diabetic rats when compared to normal control group. The treatment with aqueous extract of *brassica nigra* at dose of 400 mg/kg by orally continue for 21 days significantly normalized elevated blood glucose level, body weight and restored serum and liver biochemical parameters towards normal values in treated group when compared to diabetic control group. An increase in blood glucose level was observed in normal glucose loaded rats than AEBN treated rats during oral glucose tolerance test (OGTT).

Chemically, alloxan (2,4,5,6- tetraoxypyrimidine) is an oxygenated pyrimidine derivative. It is a well- known diabetogenic agent that is used to induce type 1 diabetes in experimental animals. Induction of diabetes with alloxan is associated with a characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins (Swanston et al., 1990). Diabetic rats treated with AEBN (aqueous extract of *brassica nigra*) showed significant improvement in body weight. Hence AEBN exhibited a marked effect in controlling the loss of body weight of diabetic rats. Alloxan results elevation of triglycerides and total cholesterol.

AEBN significantly reduced serum total cholesterol in alloxan induced diabetic rats. Elevation of serum biochemical parameters such as total cholesterol, total protein and total bilirubin was observed in diabetic rats indicating impaired liver function, which was obviously due to hepatocellular necrosis. It has been reported that liver necrosis occurred in alloxan-induced diabetic rats (Ohaeri,2001). Therefore, increase in the activities of total protein, total cholesterol and total bilirubin gives an indication on the hepatotoxic effect of alloxan. Twenty-one days of treatment with AEBN restored all the above mentioned serum hepatic biochemical parameters toward the normal values in a dose-dependent manner, thereby alleviating liver damage caused by alloxan-induced diabetes.

Summary and Conclusion

- The above findings revealed that the aqueous extract of the seeds of *B. Nigra* possesses potent antidiabetic and hepatoprotective activities.
- It can be useful in the treatment of lipid abnormalities associated with diabetes mellitus.
- It can be also useful for maintaining the disturbed biological parameters associated with hepatic damage.
- However, the exact mechanism of the antidiabetic and hepatoprotective effects of Brassica nigra extract is unknown. Hence, future studies are required to study its mechanism of action.

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