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Original Article

Bioactivity of methanolic extract of *Brassica juncea* in animal model of diabetes mellitus

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ABSTRACT

Objective: To study the bioactivity of methanolic extract of *Brassica juncea* on animal model of diabetes mellitus along with its effect on diabetic and metabolic parameters.
Methods: Diabetes mellitus was induced in rats by injecting streptozotocin (60 mg/kg) intraperitonealy. Blood glucose was measured on day 3 by GOD-POD method to confirm the diabetes mellitus. Rats having fasting blood glucose > 250 mg/dL were further selected for study and they were divided into four groups, control, control + streptozotocin, streptozotocin + metformin (75 mg/kg) and streptozotocin+ extract of *B. juncea* (450 mg/kg). Each group consisted of six rats of either sex. Metformin and experimental extract were administered for 21 d. Triglyceride, cholesterol level were measured on day 21 by commercially available kit. Blood glucose was measured on days 7 and 21. Anti-oxidant potential was assessed by estimating extent of lipid peroxidation (LPO) by malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD) and glutathione (GSH) in liver, kidney, pancreas, muscle tissues on day 21. Unpaired and paired student's *t*-test was applied for statistical analysis.
Results: The extract of *B. juncea* showed significant decrease in blood glucose level on day 21. The treatment group showed significant difference in oxidative stress by increasing SOD and GSH and decreasing LPO and NO activity on day 21. The treatment did not show statistically significant difference of cholesterol, and triglycerides level on day 21.
Conclusion: The study showed anti-hyperglycemic and anti-oxidative properties of methanolic extract of *B. juncea*.
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1. Introduction

Diabetes mellitus (DM) is a disorder of glucose and lipid metabolism and hyperglycemia is considered to be its defining feature. DM remains among topmost non-communicable diseases and many developing and developed countries are facing its disaster. Recent statistics indicate massive increase in prevalence of diabetes affecting more than 382 million people in 2013 which may progress to 592 million people by the year 2035 (Forouhi & Wareham, 2014). In India, the situation is quite same and more than 60 million people suffering from diabetes (Kaveeshwar & Cornwall, 2014). Classically diabetes is divided into Type 1 and Type 2 DM characterized by a deficiency of insulin and tissue resistance to insulin respectively. More than 90% of cases of DM belong to the Type 2 variety (Wu, Ding, Tanaka & Zhang, 2014) and about 5% cases of DM belong to monogenic forms (Yang & Chan, 2016). DM leads to increase in co-morbidities by microvascular changes in different organ systems. There are numerous drugs which are usually used in the management of DM but successful management of complications remains a problem.

Decreased anti-oxidant defense enzymatic pathways along with increased level of reactive oxygen species (ROS) coupled with poorly controlled DM result in numerous secondary changes of DM (Matough et al., 2012). Important ROS like superoxide, hydrogen peroxide, hydroxyl radicals are produced through many intermediary metabolic processes and are disposed by highly efficient anti-oxidant mechanisms so ROS are not accumulated. Main anti-oxidant enzymes are superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), and reduced glutathione (GSH). Deficiency in the action of these enzymes results in accumulation of free radicals which lead to lipid peroxidation and damage to cells. The role of free radicals and oxidative stress in pathogenesis of DM is already established (Ullah, Khan & Khan, 2016). Free radicals cause lipid peroxidation resulting in membrane damage and leaking of intracellular components like lysosomal enzymes responsible for tissue injury. They also cause cross-linking of proteins,

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lipids and nucleic acids resulting in damage to cellular contents (Birben, Sahiner, Sackesen, Erzurum & Kalayci, 2012).

Current management of DM focuses on the reduction of blood glucose level. These drugs do not affect much patho-physiology of DM. We are in need of molecules which are helpful in correcting pathology of DM. Plants can be source of agents which can offer benefits in underlying pathology of DM. Many plants have shown significant anti-hyperglycemic potential in animal models of DM (Patel, Prasad, Kumar & Hemalatha, 2012).

Brassica Juncea (L.) Czern. et Coss., also known as Indian mustard, is a traditional medicinal plant belonging to Brassiaceae family and considered to be having activity against arthritis and rheumatism. In China, the seeds are used as antitumor and roots of *B. juncea* are used as galactogogue in Africa (Thirumalai, Therasa, Elumalai & David, 2011). In Korea, the seeds are used for abscesses while in China leaves are used for bladder inflammation or haemorrhage (Parthiban, Anand, Vishnupriya & Mathiazhagan, 2015). *B. juncea* possesses numerous varieties of active phytochemicals including glycosides, flavonoids, phenolic compounds, sterols, triterpene alcohols, glucosinolates (GLSs), proteins and carbohydrates (Parikh & Khanna, 2014). Systemic studies elucidating effects of *B. juncea* on diabetic, metabolic and anti-oxidant markers are less, in the present study medicinal plant *B. juncea* is selected for evaluation of its hypoglycemic and anti-oxidative properties.

Streptozotocin (STZ) is one of chemical used to induce DM. Both Type 1 and Type 2 DM can be induced by STZ (Arulmozhi, Veeranjaneyulu & Bodhanka, 2004). STZ is transported to beta cell by GLUT2 (Eleazu et al., 2013). STZ causes alkylation of DNA and it also an NO donor explaining its cytotoxic effects (Nahdi, John & Raza, 2017) resulting in damage to beta cells of pancreas.

2. Materials and methods

2.1. Plant material and extract preparation

The dried aerial part of *B. juncea* was procured from local market of Varanasi and identified by the department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University. The seed of *B. juncea* of 500 g was crushed into small pieces and prepared by adding sufficient amount of methanol in a glass jar for 72 h and then filtered off. The methanolic extract of *B. juncea* was vacuum dried and stored at −20 °C until further use. The yield of the extract was 22.86 g.

2.2. Animals

Inbred Charles–Foster (CF) albino rats (150−200 g) of either sex were obtained from the central animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi. They were kept in the departmental animal house at (26 ± 2) °C with relative humidity of 44%−56%, light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 18−24 h before the experiment though water was allowed ad libitum. Approval from the Institutional Animal Ethical Committee was taken prior to the experimental work.

2.3. Drug treatment

A total of 32 rats were obtained from central animal house and 24 rats of either sex were chosen randomly for study with six rats per group. Diabetes was induced in adult rats weighing between (150 and 200) g by a single intra-peritoneal injection of streptozocin (STZ, 60 mg/kg) dissolved in citrate buffer (pH 4.5) and fed normally thereafter (Deeds et al., 2011). On day 3 the fasting plasma glucose was assessed to validate the model. Details of groups were as following: Group A-Control: The control rats were received citrate buffer only as per their body weight. Group B-Streptozotocin with no treatment: In this group streptozotocin was given and followed for 21 d without any other treatment. Group C-Standard: This group was received metformin (75 mg/kg) suspended in 1% carboxymethyl cellulose (CMC) in distilled water. Group D-Treatment Group: This group was received extract of *B. juncea* (450 mg/kg) orally once daily for 21 d.

2.4. Glycemic study

Fasting plasma glucose levels were estimated on day 7 and day 21 after extract administration. Blood samples were collected from the retro-orbital plexus. Rats showing fasting plasma glucose over 250 mg/dL under fasting conditions were used for the study. Plasma glucose was estimated by GOD-POD method.

2.5. Estimation of triglycerides and cholesterol

Blood samples were collected from the retro-orbital plexus of the rat and serum was separated by centrifugation at 3000 r/min for 3 min. Triglycerides level was estimated in the unhemolysed serum by (GPO-PAP method) using triglycerides test kit (Coral Clinical System). Total cholesterol levels were estimated in the unhemolysed serum by (CHOD-PAP Method) using Cholesterol Kit (Span Diagnostics Pvt. Ltd. India).

2.6. Estimation of free radicals and anti-oxidants

Test and standard drugs were given orally daily for 21 d and on the day of the experiment the animals were then sacrificed and various parameters was calculated in tissue and serum as described earlier. Liver, pancreas, kidney and muscle tissue were homogenized (5%) in ice cold 0.9% saline with a Potter–Elvehjem glass homogenizer for 30 s. The homogenate was then centrifuged at 800 r/min for 10 min followed by centrifugation of the supernatant at 12 000 r/min for 15 min and the obtained supernatant was used for the following estimations.

Antioxidants viz. superoxide dismutase SOD (nmol/g wet tissue) (Sairam, Priyambada, Arya, & Goel, 2003), and reduced glutathione GSH (nmol/g wet tissue) (Kumar, Joshi, Prabha, Dorababu, & Goel, 2006) and free radicals viz. extent of lipid peroxidation LPO by estimating MDA (nmol/g wet tissue) (Sairam et al., 2003) and nitric oxide NO (μmol/g wet tissue) (Miranda, Espey & Wink, 2001) were estimated in homogenate.

2.7. Statistical analysis

The statistical analysis was carried out using paired and unpaired *t*-test. The values are represented as mean ± SD. $P < 0.05$ was considered significant.

3. Results

3.1. Glycemic study

After streptozotocin administration, on day 3 the blood glucose level was markedly increased [(274 ± 11.9) mg/dL] as compare to control [(99.7 ± 17.7) mg/dL]. Group D showed a decrease in blood glucose level by 3.2% at day 7 and 61.5% at day 21. Group C showed a decrease in blood glucose level by 50.27% at day 21 (Table 1). The extract of *B. juncea* showed statically significant decrease in blood glucose level at day 21 ($P = 0.013$). At day 7, plant was showing slight decrease in blood glucose level which was not statistically significant.

Table 1
Effect on blood glucose level.

Groups	Blood glucose level/(mg·dL^{-1})			
	Day 0	Day 3	Day 7	Day 21
A	96.50 ± 18.97	99.66 ± 17.66	97.66 ± 6.74	97.16 ± 6.73
B	104.66 ± 12.83	274.00 ± 11.93	279.83 ± 12.62	285.66 ± 11.20
C	93.33 ± 16.23	277.83 ± 9.08	202.50 ± 3.78	138.16 ± 6.31
D	93.5 ± 13.76	312 ± 129.6	302.50 ± 127.7	120.00 ± 7.3

3.2. Lipid profile study

On day 21, blood cholesterol and triglycerides level were assessed. In Group B blood cholesterol and triglyceride level were higher than group A which was control group indicating deranged lipid profile. The triglycerides and cholesterol level of group D were comparable to group B, and *P* value was 0.07 and 0.61, respectively. This showed no significant benefit in triglycerides and cholesterol level (Table 2).

Table 2
Effect on plasma triglycerides and cholesterols level on day 21.

Groups	Parameters	
	Triglycerides/(mg·dL^{-1})	Cholesterols/(mg·dL^{-1})
A	74.33 ± 1.87	108 ± 6.72
B	97.5 ± 4.92	142.6 ± 10.63
C	81.83 ± 5.7	117.5 ± 8.96
D	90.3 ± 5.5	148.6 ± 40.8

3.3. Study of anti-oxidant parameters

On day 21, anti-oxidants i.e., glutathione (GSH) and super-oxide dismutase (SOD) level were assessed in four different tissues homogenate i.e., liver, kidney, pancreas, and muscle. Both are parts of inherent defense mechanism against oxidative stress. In group B, the levels of both anti-oxidants were less on day 21 compared to group A in all tissue samples showing decreased defense against oxidative stress. The GSH level was significantly high in group D as compared to group B in tissue sample of muscle having *P* value of 0.01 (Table 3).

The SOD level was significantly higher in group D as compared to group B in tissue sample of kidney and pancreas having *P* value of 0.02 and 0.03, respectively (Table 3).

On day 21, markers of oxidative stress i.e., extent of lipid peroxidation (LPO) and nitric oxide (NO) level were assessed in four different tissues homogenate (liver, kidney, pancreas, and muscle). Both are indicators of oxidative stress. In group B, the levels of both were high on day 21 compared to group A in all tissue samples showing increase in oxidative stress.

The NO level was significantly low in group D as compared to group B in tissue sample of kidney and pancreas having *P* value 0.014 and 0.02, respectively (Table 4).

The extent of LPO level was significantly low in group D as compared to group B in tissue sample of liver, kidney and muscle having *P* value of 0.01, 0.03 and 0.024, respectively (Table 4).

4. Discussion

In the present study, diabetes in rats was induced by streptozotocin and we selected the plant *B. juncea* commonly known as Indian Mustard. The study includes the status of various metabolic and diabetic parameters in streptozotocin-induced diabetic rats. Many medicinal plants can be considered as adjuvant for the treatment of diabetes. The present study was therefore intended to examine the effect of methanolic dried seeds of *B. juncea* in streptozocin-induced diabetic rats, using metformin as a reference drug.

Diabetes and hyperlipidemia are two major life threatening metabolic disorders often encountered in obese patients with sedentary lifestyles. Pharmacological actions of *B. juncea* strongly suggest their therapeutic potential against many diseases. However, no definitive role of phyto-constituents involved in their potential therapeutic effect is established. This is not only because of the diverse types of extracts and experimental designs were used in different studies, but also due to the fact that none of the animal models used to date for such studies truly represent the complex pathologies involved in metabolic disorders, and depend on the experimental conditions used. A recent report (Thirumalai et al., 2011) describes dose dependent (250, 350, and 450 mg/kg/d) beneficial effects of an aqueous mustard seed extract against hyperglycemia and insulin deficiency in streptozotocin induced diabetic rats, whereas in one study no such hypoglycemic effect of the seeds was observed in similar diabetes model (Grover, Yadav & Vats, 2002). In a study, *B. juncea* has significantly prevented the development of insulin resistance in rats with high fructose diet. These results indicated that *B. juncea* may have role in the management of pre-diabetic state of insulin resistance (Yadav, Vats, Ammini & Grover, 2004).

The result agrees with the findings of other authors who also reported a significant reduction of blood glucose level in rats using seed extracts of *B. juncea* (Thirumalai et al., 2011). A significant dosage dependent augmenting effect of the seed extract on the serum insulin was recorded in both short term as well as long term groups. In light of the present results, our work indicates that methanolic extracts of *B. juncea* have good anti-diabetic activity. *B. juncea* caused the reduction in glucose levels in moderate diabetes but not in severely diabetic rats (Thirumalai et al., 2011), so it can be concluded that antihyperglycemic activity of *B. juncea* requires presence of the β-cells which can release insulin.

B. juncea seeds likely prevented the damage to β-cells of islets in the pancreas by its anti-oxidant effect. This is an interesting finding and suggests the likelihood of *B. juncea* having anti-oxidant and free radical scavenger activities (Grover et al., 2002). *B. juncea* have been shown to have anti-oxidant property (Zou, Kim, Kim, Choi & Chung, 2002; Kim et al., 2003). Our study showed significant improvement in anti-oxidant parameters i.e. increase in GSH & SOD while decrease in NO, LPO in various tissue homogenate

Table 3
Effect on glutathione and superoxide dismutase level on day 21.

Groups	Tissue homogenate glutathione/(μmol·g^{-1})				Tissue homogenate superoxide dismutase/(units·g^{-1})			
	Liver	Kidney	Pancreas	Muscle	Liver	Kidney	Pancreas	Muscle
A	217.5± 27.02	196 ± 11.11	216.5 ± 16.63	187.33 ± 9.77	232 ± 6.75	202.83 ± 5.84	199.33 ± 7.04	198.33 ± 8.21
B	196 ± 20.89	158.66 ± 26.25	179.16 ± 16.63	132.33 ± 21.33	197.5 ± 7.23	167.5 ± 7.44	157.33 ± 14.06	164 ± 8.69
C	218.66 ± 28.73	187.5 ± 12.38	220.16 ± 19.27	190 ± 12.21	216.5 ± 4.08	184.66 ± 9.09	190.16 ± 9.19	187.5 ± 9.37
D	209.5±23.95	182 ± 12.4	193.3 ± 12.6	165.8 ± 10.50	205.3 ± 5.6	180.16 ± 7.99	181.5 ± 5.85	173 ± 7.23
P – value	0.137	0.09	0.13	0.01*	0.064	0.02*	0.03*	0.08

Table 4
Effect on nitric oxide and lipid peroxidation on day 21

Groups	Tissue homogenate nitric oxide/(μmol·g^{-1})				Tissue homogenate lipid peroxidation/(nmol·g^{-1})			
	Liver	Kidney	Pancreas	Muscle	Liver	Kidney	Pancreas	Muscle
A	42.16 ± 7.6	54.7 ±1.31	49.41 ± 4.5	45.33 ± 2.9	5.7 ± 0.91	7 ± 0.50	7.15 ± 0.70	7.61 ± 0.66
B	54.6 ± 6.9	58.08 ± 1.31	54.91 ± 3.05	49.66 ± 3.5	7.5 ± 0.53	10.4 ± 0.72	8.71 ± 0.94	9.65 ± 1.02
C	39.66 ± 3.2	56.78 ± 2.79	50.33 ± 3.1	46.91 ± 2.9	6.4 ± 0.73	8.33 ± 0.56	7.5 ± 0.89	8.41 ±1.22
D	45.3 ± 3.3	55.7 ± 1.4	50.2 ± 2.8	47.1 ± 3.2	6.6 ± 0.43	9.1 ± 0.82	7.7 ± 0.70	8.21 ± 0.8
P – value	0.1	0.014*	0.02*	0.24	0.01*	0.03*	0.07	0.024*

(muscle, kidney, liver, and pancreas) after 21 days of administration of extract of *B. juncea*.

5. Conclusion

In light of the present results, our work indicates that methanolic extracts of *B. juncea* have good anti-hyperglycemic activity on long term use. Our study showed significant difference in antioxidant parameters i.e., increased GSH & SOD while decreased NO, LPO in various tissue homogenate (muscle, kidney, liver, and pancreas) after 21 days of administration of extract of *B. juncea* indicating its anti-oxidant potential. Further studies must be performed to elucidate mechanism of decrease in insulin resistance with additional parameters.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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