Indian Journal of Experimental Biology
Vol. 52, June 2014, pp. 613-622

Antidepressant-like effects of *Brassica juncea* L. leaves in diabetic rodents

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Received 2 January 2013; revised 30 January 2014

The objective of the study was to evaluate for antidepressant like activity of a methanolic extract of *B. juncea* leaves (BJ 100, 200, and 400 mg/kg/day, po), and Imipramine (15 mg/kg/day, po) in alloxan monohydrate (120 mg/kg, ip) induced diabetic and nondiabetic rodents, using behavioural despair, learned helplessness, and tail suspension tests for antidepressants and locomotor activity test for quantifying the behavioural effects of treatments. In addition, effects of BJ treatments on brain levels of norepinephrine, serotonin and dopamine were also estimated. Enhanced depressive states, and motility were observed in diabetic animals. Antidepressant and motor function depressing effects of BJ were apparent in all behavioural tests in diabetic rats and mice only. Decreased contents of dopamine, norepinephrine and serotonin in brain of diabetic rats were also dose dependently compensated by repeated daily BJ treatments. However, brain dopamine level of BJ treated normal rats was higher than that in control nondiabetic. The results suggest that BJ could be a nutritional alternative for combating exaggerated depression commonly associated with diabetes.

Keywords: Ayurveda, *Brassica juncea*, Depression, Diabetes mellitus, Monoamines

Appropriate use of fruits and vegetables for controlling hyperglycemia and other diabetes associated metabolic disorders are now widely recommended for the prevention of diabetes. However, little concentrated efforts have been made to evaluate their potential uses for combating psychopathological comorbidities often encountered in diabetic patients. During recent past, numerous preclinical reports dealing with antidepressants like activities of plants have appeared[1], and plant polyphenolics and other phytochemical with antioxidative properties have been postulated to be their bioactive constituents involved in the antidepressant and as antidiabetic activities[2-4]. Diabetes mellitus is accompanied by numerous structural, biochemical and behavioural alterations of the central nervous system[5]. Most prominent neurochemical alterations were observed in alloxan induced diabetes mellitus in rat brain monoaminergic transmission[6]. Several co-morbid conditions including depression and anxiety have been described in rodent models of diabetes[7]. Prevalence of depression in diabetic subjects is higher as compared to normal population[8]. *Brassica juncea* L. is a polyphenols enriched edible plant. Diverse medicinal uses of its different parts have been known to Ayurvedic health care practitioners since centuries[9]. Therapeutic potentials of various extracts and bioactive secondary metabolites of *B. juncea* leaves and seeds have been reported[10,11]. *B. juncea* has antidiabetic, antioxidant, antimicrobial, antiatherogenic and astrocyte developing activity[12-17]. The information that an isorhamnetin glycoside could be a quantitatively major secondary metabolite of *B. juncea* leaves responsible for antidiabetic activity in animal models triggered the interest in evaluating its potential metal health benefits[18].

Isorhamnetin is a secondary plant metabolite of many plants, and *B. juncea* (mustard) has higher isorhamnetin contents than many other edible plant species[19]. Since diabetes is the spreading epidemic of the 21st century, and antidiabetic as well antidepressant efficacy of isorhamnetin are known[12,20], it was of interest to test whether an isorhamnetin containing extract from the edible leaves of *B. juncea* could be used for treatment of depressive disorders often encountered in diabetic patients.

Materials and Methods

Plant material and extraction—*B. juncea* leaves were collected from a local agricultural area in

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Varanasi (UP, India), and were botanically authenticated by Prof. N. K. Dubey in Herbarium of Department of Botany, Banaras Hindu University as *B. juncea* (Linn.) species Czern & Coss family Brassicaseae (Voucher specimen number: Dubey-12/Nov/2009). The leaves were dried at room temperature and powdered. The leaf extract used in this study was prepared by exhaustive soxhlet extraction of the powder (800 g) with 2 L of aqueous methanol (90%) for 3 h. Solvent was evaporated, and the extract was dried in vacuum at 40 °C. Calculated yield of the extract (BJ) was 11% by weight of the dried leaves.

Extract characterization—A well validated HPLC method revealed that BJ contains 0.37% kaemferol and 0.29% isorhamnetin[21]. Briefly, a Shimadzu LC 2010HT HPLC system equipped with a quaternary pump, UV detector, degasser and an auto sampler with "Lab solution" software was used. Mobile phase was acetonitrile: phosphate buffer (*p*H 2.5-2.8). Column was C18-ODS (octadecyl silane) 5 µ size, 250 x 4.6 mm, wavelength: 280 nm, flow rate: 1.5 mL/min, injection volume: 20 µL, and run time was 40 min. All reference standards used for HPLC analysis were purchased from Sigma, India. Using this method no rutin, quercetin, naringin, luteolin apigenin, could be detect in BJ. Using a slightly modified spectrophotometric method total sinapic acid contents of the extract was found to be 5%[22]. Briefly, 2, 5, 7, 10 mL of standard preparation and 5 and 7 mL of sample preparation were pipetted out to different 100 mL volumetric flasks. Water (40 mL) was added to each then 5 mL of Folin- Ciocalteu reagent and 10 mL saturated solution of Na_2CO_3 solutions were added. Solutions were kept at room temperature for 30 min. Volume was made up to 100 mL with water and kept them for 20 more min and absorbance was recorded in a UV/Vis spectrophotometer at 750 nm. Calculation of the contents of sinapic acid was done by using calibration curve. The HPLC chromatogram of standard mixture and extract is depicted in Figs. 1 A and B respectively.

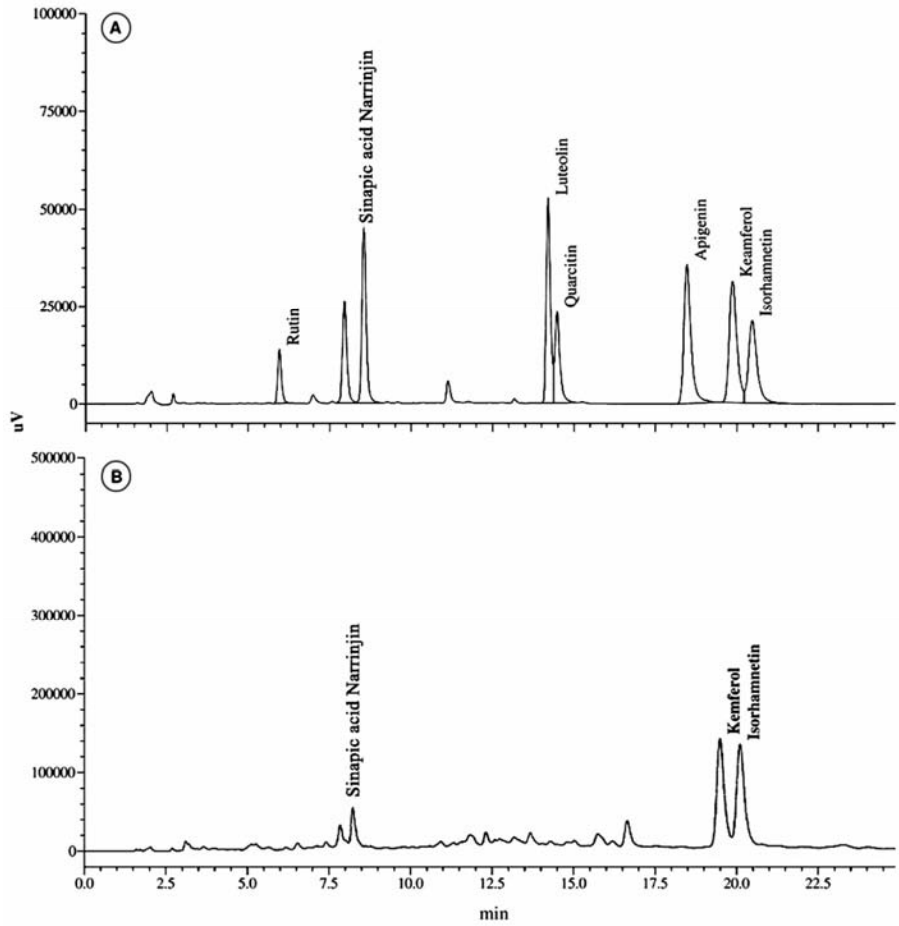

Fig. 1—HPLC chromatogram of (A) polyphenols standard, and (B) a hydrolyzed sample of the methanolic *B. juncea* leaf extract

Animals—Adult Charles foster rats (160 ± 20 g) and albino mice (20 ± 5 g) of 3 to 4 months old age were obtained from Central Animal House of Institute of Medical Sciences, Banaras Hindu University, Varanasi (Registration Number: 542/AB/CPCSEA). They were housed in groups of six in polypropylene cages at an ambient temperature of 25 ± 1 °C and 45-55% RH, with a 12:12 h light/dark cycle. Except when stated otherwise, they were always provided with commercial food pellets and water *ad libitum*. All animals were acclimatized to laboratory conditions for at least one week before using them for the experiments. Principles of laboratory animal care (NIH publication number 85-23, revised 1985) guidelines were followed, and prior approval for animal experimental work from the Central Animal Ethical Committee of the University was obtained (approval no.Dean/10-11/283, dated 19.10.2010).

Diabetes induction—Diabetes was induced in overnight fasted rats and mice by a single ip injection of alloxan monohydrate (120 mg/kg body weight) in normal saline. One hour after injection of alloxan all animal were provided with commercial food pellets and water. Hyperglycemia was confirmed by fasting blood glucose level measurement on the 3rd and 7th day after the alloxan injection. Mortality rates in different batches of alloxan treated animals were between 10 to 15%. Preselected diabetic animals with hyperglycemia on 7th day (fasting blood glucose levels > 250 mg/dL) were used for further studies.

Animal grouping and drug administration—Nine groups of 6 animals each (3 males and 3 females in each group) were used for the experiments[23,24]. Treatments of the diabetic groups started on the 7th day after alloxan challenge (day 1 of treatment). Suspensions of BJ, or of imipramine (Sun Pharmaceutical Industries Ltd., Mumbai, India), in 0.3% carboxymethylcellulose (CMC) were orally administered once daily for 10 consecutive days. The treatment groups were: Gr. I: nondiabetic control (CMC treated), Gr. II: nondiabetic treated with BJ (100 mg/kg/day); Gr. III: nondiabetic treated with BJ (200 mg/kg/day); Gr. IV: nondiabetic treated with BJ (400 mg/kg/day); Gr. V: diabetic control (CMC treated); Gr. VI: diabetic treated with BJ (100 mg/kg/day); Gr. VII: diabetic treated with BJ (200 mg/kg/day); Gr. VIII: diabetic treated with BJ (400 mg/kg/day); and Gr. IX: diabetic treated with the antidepressant drug imipramine (15 mg/kg/day). The experimental details are shown in Table 1.

Blood glucose estimation—After overnight fasting, blood sample (~0.5 mL) from rats was collected from the retro-orbital venous plexus under light ether anaesthesia using a glass capillary tube on the 10th treatment day as well as before the start of the treatments. Fasting blood plasma glucose levels were quantified by using a glucose oxidase-peroxidase

Table 1—Experimental details

Treatment groups	Duration of administration of drugs	Behavioural tests	Blood withdrawal
Gr. I: Nondiabetic control (CMC treated, po) Gr. II: Nondiabetic treated with BJ (100 mg/kg/day, po) Gr. III: Nondiabetic treated with BJ (200 mg/kg/day, po) Gr. IV: Nondiabetic treated with BJ (400 mg/kg/day, po) Gr. V: Diabetic control (CMC treated, po) Gr. VI: Diabetic treated with BJ (100 mg/kg/day, po) Gr. VII: Diabetic treated with BJ (200 mg/kg/day, po) Gr. VIII: Diabetic treated with BJ (400 mg/kg/day, po) Gr. IX: Diabetic treated with imipramine (15 mg/kg/day, po)	Single ip injection of alloxan was administered on day 1, and diabetes was confirmed by blood glucose level estimation on 3rd and 7th day after the alloxan injection. Animals with blood glucose level 250 mg/dl or more were included in the study. From 7th day onwards, drug treatments were given for 10 consecutive days (day 1 to day 10 of drugs treatment).	In a separate set of experiment, following behaviour tests were performed: 1. Rat behavioural despair test was performed on day 10 of drug treatments. 2. Rat learned helplessness test was performed on day 8, 9 and 10 of drug treatments. 3. Mice tail suspension test was performed on day 10 of drug treatments. 4. Rat locomotor activity was performed on day 10 of drug treatments.	Blood was withdrawn on 3rd and 7th day after the alloxan injection on 1st day and on day 10th of drugs treatment.

Each group consisted of 6 animals. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparison test. GraphPad Prism 5 and 6 were used for statistical analysis

(GOD-POD) method (Beacon Diagnostic Pvt. Ltd., Navasari- Glucose Kit). For mice, the fasting blood glucose levels were estimated by using one touch glucometer immediately after tail pinching with a sterile lancet (Dr. Morepen®; Model: Gluco One- BG- 03; with tail pricking sterile lancet and test strips code no. 35).

Rat behavioural despair test—The method of Willner[25] was followed. In short, a rat was individually placed in a cylinder (45 × 20 cm) containing 38 cm water (25 ± 2 ºC), so that it could not touch the bottom of the cylinder with its hind limb or tail, or climb over the edge of the chamber. Two swim sessions were conducted, an initial 15 min pre-test on day 9, followed by a 5 min test, 24 h later (on day 10). The period of immobility (remained floating in water without struggling and making only those movements necessary to keep its head above water) during 5 min test period was recorded.

Rat learned helplessness test—The experimental procedure has been described elsewhere in details[23]. In short, the two parts of the procedure were:

(a) Inescapable shock pretreatment*:* One hour after oral treatments on the 7th day, electric foot shocks were delivered in 20 x 10 x 10 cm plexiglass chamber with cover, and a floor made of steel grids for delivering shocks. A constant current shocker delivering 60 scrambled, randomized inescapable shocks (15 sec duration, 0.8 mA, every min) was used.

(b) Conditioned avoidance training*:* Avoidance training were initiated 24 h after inescapable shock pretreatment in a jumping box. The jumping box were divided into two equal chambers (27 x 29 x 25 cm) by a plexiglass partition with a gate providing access to the adjacent compartment through a 14 x 17 cm space. Animals were placed singly in one of the chambers of jumping box and were allowed to habituate to the test environment for 5 min (for the first session only) and then were subjected to 30 avoidance trials (inter-trial intervals being 30 sec). During the first 3 sec of each trial, a light signal (conditioned stimulus) was presented, allowing the animals to avoid shocks. If a response does not occur within this period, a 0.8 mA shock (3 sec duration) (unconditioned stimulus) was applied via the grid floor. In case no escape response occurs within this period, shock and light conditioned stimulus were terminated. Avoidance sessions performed for 3 consecutive days (days 8-10), and the number of escape failures, referred as no crossing response during shock delivery, were recorded.

Mice tail suspension test—The method of Chermat *et al.*[26] was followed. A mouse was hung by tail, 50 cm above the floor by adhesive tapes placed approximately 1 cm from the tip of the tail on a wire in an upside down posture so that its nostrils just touch the water surface in a container. After initial vigorous movements, the mouse assumes an immobile posture and the period of immobility during a 5 min observation period were noted.

Spontaneous locomotor activity—After 60 min of the last (10th) doses of BJ (100, 200 and 400 mg/kg, po), or of imipramine (15 mg/kg, po), rats were subjected to spontaneous locomotor activity test in photoactometer (Techno Electronics, India). Each rat was allowed for a period of 5 min in a square closed field arena (30×30×30 cm) equipped with 6 photocells in the outer wall for spontaneous locomotion immediately after placing the animals in the photoactometer[27]. Number of photocell beams interruption (locomotor activity) was recorded by means of a 6 digits resettable counter.

Estimation of brain monoamine levels—After behavioural study in learned helplessness test, the rats were sacrificed by spinal cord dislocation. Brain was removed and kept into ice-cold isotonic saline for a few seconds, and then were stored in deep freezer at -80 °C till use. Assays of dopamine, norepinephrine and serotonin were done by the spectrofluorometric method described by Welch and Welch[28].

Statistical analysis—Mean ± SE of mean (n=6) were calculated for the observed values in each experimental group. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparison test. GraphPad Prism 5 and 6 were used for statistical analysis. A P-value <0.05 was considered as statistically significant.

Results

Body weight—As compared to nondiabetic animals mean body weights of diabetic rats and mice was significantly decreased during 10 day treatment period (Table 2). Mean losses in body weights of BJ (100, 200 and 400 mg/kg/day) treated diabetic rats were significantly lesser in magnitude than that observed in the diabetic control rats [F (4, 25) = 140.7, $P < 0.05$]. However, this observed effect of the extract was not dose dependant. In diabetic mice, mean loss in body weights of BJ (100 mg/kg/day) treated animals were significantly lower than in the

corresponding control group. After higher BJ doses (200 and 400 mg/kg/day) the diabetic mice actually gained some weight during the treatment period [F (4, 25) = 13.94, $P < 0.05$]. No significant effects of BJ treatments on body weight increases of nondiabetic animals during the experimental period were observed (unpublished data).

Fasting blood glucose level—Fasting blood glucose level of rats and mice challenged with alloxan was significantly increased compared to the nondiabetic control animals (Table 3). In the diabetic control rats and mice, blood glucose level remained elevated till the 10th day of the experiment. Unlike in nondiabetic animals (unpublished data), BJ treatments significantly decreased fasting blood glucose levels in diabetic ones. This observed effect of the extract was dose dependant in both rats [F (4, 25) = 467.2, $P < 0.05$] and mice [F (4, 25) = 320.4, $P < 0.05$] as compared to respective diabetic control.

Behavioural despair test—Mean immobility period of the vehicle treated diabetic rat group was significantly higher than that of the nondiabetic one. Significant dose dependant reduction of immobility by BJ treatments were observed in diabetic rats. Observed effect of the lowest BJ dose tested (100 mg/kg/day) was statistically highly significant, which increased only slightly after its higher doses [F (8, 45) = 23.16, $P < 0.05$]. Quantitatively, the observed effect of the highest dose of BJ treated group (400 mg/kg/day) was of the same order of magnitude, as that observed for imipramine (15 mg/kg/day) in diabetic rats. However, no antidepressants like effects of BJ treatments were observed in nondiabetic animals (Fig. 2a).

Learned helplessness test—In comparison to the corresponding values of the nondiabetic control group on days 8, 9 and 10, significant increases in escape failure response, and decrease in avoidance response were observed in diabetic control rats. Qualitatively, the observed significant and dose dependant effects of BJ treatments in diabetic rats were analogous to those observed after treatment with imipramine. As shown in Table 4, the escape failures were decreased significantly on day 8 [F (5, 30) = 7.66, $P < 0.05$], day 9 [F (5, 30) = 29.45, $P < 0.05$] and day 10 [F (5, 30) = 36.10, $P < 0.05$] as well as avoidance responses were increased significantly on day 8 [F (5, 30) = 7.27, $P < 0.05$], day 9 [F (5, 30) = 10.58, $P < 0.05$] and day 10 [F (5, 30) = 30.26, $P < 0.05$] compared to diabetic control rats. In this model the antidepressant like efficacy of the highest dose of BJ tested (400 mg/kg/day) was somewhat lower than that of 15 mg/kg/day imipramine (Table 4). BJ treatments did not significantly alter these values in nondiabetic rats (unpublished data).

Tail suspension test—Immobility period of the diabetic control group was higher than that of the nondiabetic control one, and BJ treatments did not have

Table 2—Effect of *B. juncea* leaf extract on body weight of rodents

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

Treatment groups	Body weight of rats (g)		Change in body weight of rats (g)	Body weight of mice (g)		Change in body weight of mice (g)
	Initial (Day 0)	Final (Day 10th)		Initial (Day 0)	Final (Day 10th)	
Gr. I: Nondiabetic control (0.3% CMC)	156.33 ± 3.01	172.17 ± 3.37	+8.83 ± 0.40	18.33 ± 0.42	21.66 ± 0.66	+3.33 ± 0.76
Gr. V: Diabetic control (0.3% CMC)	162.17 ± 3.14	148.33 ± 2.74*	-13.83 ± 0.79*	19.66 ± 0.66	14.50 ± 0.42*	-5.16 ± 1.07*
Gr. VI: Diabetic + 100 mg/kg BJ	164.83 ± 2.89	157.33 ± 2.57	-7.50 ± 0.61¥	20.50 ± 0.67	18.66 ± 0.33¥	-1.16 ± 0.70¥
Gr. VII: Diabetic + 200 mg/kg BJ	160.25 ± 3.12	153.13 ± 2.89	-7.12 ± 0.76¥	20.75 ± 0.72	22.10 ± 0.48¥	+1.35 ± 0.65¥
Gr. VIII: Diabetic + 400 mg/kg BJ	158.17 ± 3.75	149.67 ± 4.16	-6.66 ± 0.88¥	20.83 ± 0.60	22.33 ± 0.98¥	+1.50 ± 1.11¥

P values: *<0.05 *vs*. nondiabetic control; ¥<0.05 *vs*. diabetic control

Table 3—Effect of *B. juncea* leaf extract on fasting blood glucose level of rodents

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

Treatment groups	Fasting blood glucose level in rats (mg/dL)		Change in fasting blood glucose level in rats (mg/dL)	Fasting blood glucose level in mice (mg/dL)		Change in fasting blood glucose level in mice (mg/dL)
	Initial (Day 0)	Final (Day10th)		Initial (Day 0)	Final (Day10th)	
Gr. I: Nondiabetic control (0.3% CMC)	82.16 ± 0.98	87.50 ± 1.47	+6.16 ± 0.30	79.83 ± 1.66	85.66 ± 1.30	+5.16 ± 0.30
Gr. V: Diabetic control (0.3% CMC)	288.83 ± 3.77*	310.33 ± 4.40*	+21.50 ± 2.55*	289.33 ± 4.66*	308.17 ± 4.65*	+18.83± 3.00*
Gr. VI: Diabetic + 100 mg/kg BJ	287.83 ± 6.70	194.17 ± 6.36¥	-93.66 ± 2.65¥	285.83 ± 6.74	196.17 ± 6.73¥	-91.50± 2.64¥
Gr. VII: Diabetic + 200 mg/kg BJ	285.56 ± 4.89	182.67 ± 5.89¥	-102.89 ± 2.56¥	287.45 ± 6.89	187.30 ± 4.56¥	-100.15 ± 2.58¥
Gr. VIII: Diabetic + 400 mg/kg BJ	283.83 ± 5.38	146.67 ± 2.4¥	-137.17 ± 5.76¥	278.50 ± 5.53	144.83 ± 2.94¥	-133.67 ± 7.03¥

P values: *<0.05 *vs*. nondiabetic control; ¥<0.05 *vs*. diabetic control

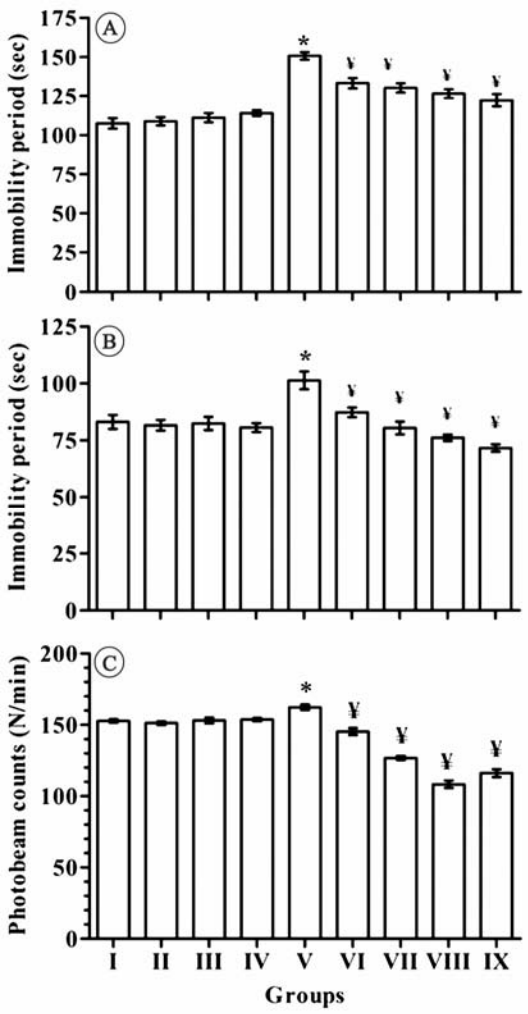

Fig. 2—Effect of *B. juncea* leaf extract on (a) behavioural despair test, (b) tail suspension test, and (c) locomotor activity of nondiabetic and diabetic rats. *P* values: *<0.05 *vs*. nondiabetic control; ¥<0.05 *vs*. diabetic control. Gr. I=nondiabetic control 0.3% CMC, Gr. II=nondiabetic+100 mg/kg BJ; Gr. III=nondiabetic+200 mg/kg BJ; Gr. IV= nondiabetic + 400 mg/kg BJ; Gr. V=diabetic control 0.3% CMC; Gr. VI=diabetic+ 100 mg/kg BJ; Gr. VII=diabetic+200 mg/kg BJ; Gr. VIII=diabetic+400 mg/kg BJ; and Gr. IX=diabetic+15 mg/kg imipramine

any effects in nondiabetic animals (Fig. 2b). However,dose dependant antidepressant like efficacy of BJ in diabetic rats was again observed in this test as well [F (8, 45) = 10.37, $P < 0.05$]. Again, quantitatively the efficacy of 400 mg/kg/day doses of BJ was somewhat lower than that of 15 mg/kg/day of imipramine.

Spontaneous locomotor activity—Unlike nondiabetic rats, significant inhibitory effect [F (8, 45) = 97.03, $P < 0.05$] of 10 daily oral 100, 200 and 400 mg/kg doses of BJ, 15 mg/kg/day of imipramine on locomotor activity was observed(Fig. 2c). The values of the diabetic controls were marginally but significantly higher than the nondiabetic control. This test was conducted to verify the possibility that motor activity stimulating effects of the extract is involved or not in the observed antidepressant like efficacy in diabetic animals.

Brain monoamines—Concentrations of serotonin (5-HT) and norepinephrine (NE) in BJ treated nondiabetic rats were not significantly different from the CMC treated nondiabetic controls. However, slight but significant dose dependant effects of BJ treatments on brain dopamine (DA) levels in nondiabetic animals were observed (Fig. 3). Mean brain concentrations of all the three monoamines quantified were significantly lower in CMC treated diabetic group than those in the corresponding nondiabetic one. Such reductions were not as severe in the BJ treated diabetic groups. Observed effects of BJ treatment on monoamines level viz. 5-HT [F (8, 45) = 30.58, $P < 0.05$], NE [F (8, 45) = 15.46, $P < 0.05$] and DA [F (8, 45) = 27.12, $P < 0.05$] in diabetic rats always increased with its increasing doses and qualitatively comparable to those of the standard drug imipramine.

Table 4—Effect of *B. juncea* leaf extract in learned helplessness test in rats

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

Treatment groups	Escape failures (N)			Avoidance response (N)		
	(Day 8th)	(Day 9th)	(Day 10th)	(Day 8th)	(Day 9th)	(Day 10th)
Gr. I: Nondiabetic control (0.3% CMC)	17.66 ± 0.49	17.83 ± 0.65	18.16 ± 0.47	4.66 ± 0.21	5.00 ± 0.36	4.83 ± 0.40
Gr. V: Diabetic control (0.3% CMC)	21.33 ± 0.91*	22.00 ± 0.57*	22.50 ± 0.92*	2.50 ± 0.42*	2.33 ± 0.21*	1.83 ± 0.16*
Gr. VI: Diabetic + 100 mg/kg BJ	18.00 ± 0.63¥	16.33 ± 0.49¥	14.16 ± 1.16¥	4.33 ± 0.33¥	4.50 ± 0.22¥	4.66 ± 0.21¥
Gr. VII: Diabetic + 200 mg/kg BJ	17.71 ± 0.48¥	14.59 ± 0.75¥	12.54 ± 0.76¥	4.52 ± 0.35¥	5.15 ± 0.46¥	5.46 ± 0.25¥
Gr. VIII: Diabetic + 400 mg/kg BJ	17.16 ± 0.40¥	13.00 ± 0.81¥	11.66 ± 0.55¥	4.83 ± 0.47¥	5.50 ± 0.34¥	6.50 ± 0.56¥
Gr. IX: Diabetic + 15 mg/kg Imipramine	16.50 ± 0.61¥	12.66 ± 0.55¥	10.50 ± 0.42¥	5.33 ± 0.33¥	5.66 ± 0.55¥	7.33 ± 0.33¥

P values: *<0.05 *vs*. nondiabetic control; ¥<0.05 *vs*. diabetic control. N= number

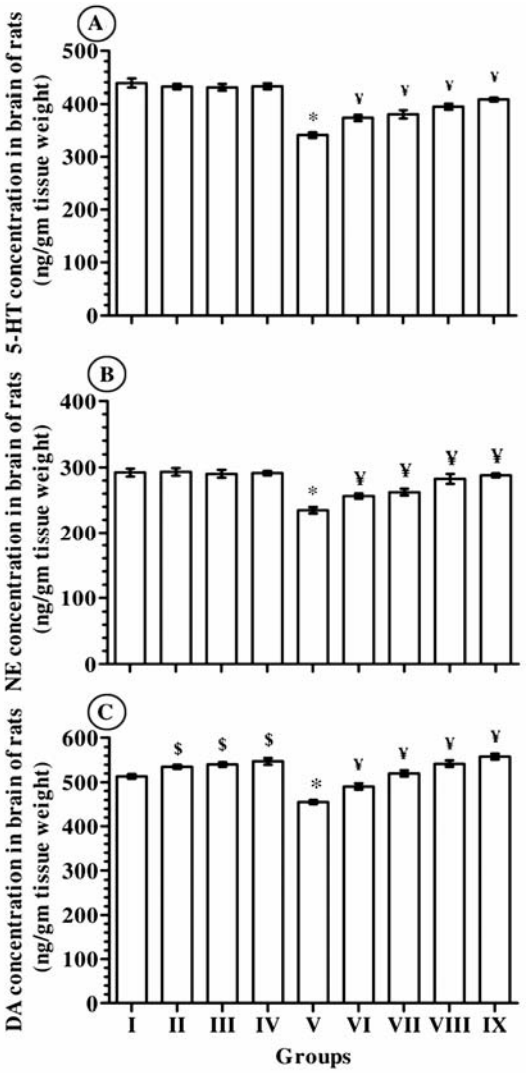

Fig. 3—Effect of *B. juncea* leaf extract on brain (A) 5-HT, (B) NE, and (C) DA level in nondiabetic and diabetic rats. *P* values: *<0.05, and $<0.05 *vs*. nondiabetic control; ¥<0.05 *vs*. diabetic control. Gr. I=nondiabetic control 0.3% CMC, Gr. II=nondiabetic+100 mg/kg BJ; Gr. III=nondiabetic+200 mg/kg BJ; Gr. IV=nondiabetic+400 mg/kg BJ; Gr. V=diabetic control 0.3% CMC; Gr. VI=diabetic+100 mg/kg BJ; Gr. VII=diabetic+200 mg/kg BJ; Gr. VIII=diabetic+400 mg/kg BJ; and Gr. IX=diabetic+15 mg/kg imipramine

Discussion

Diabetic patients are more prone to depressive disorders, and that coexistence of diabetes and depression is an increased mortality risk[29,30]. Alloxan induced diabetes is a well known animal model of type 1 diabetes[31], and exaggerated state of depression is also observed in alloxan-diabetic animals[32]. Observations reported in this communication reveal that BJ not only reduced alloxan induced hyperglycemia and body weight losses, but is also capable of affording protection against exaggerated depressive behaviour in alloxan-diabetic rodents. However, unlike conventionally known antidepressants or pure isorhamnetin[20], no effects of BJ treatments in nondiabetic animals were observed in any of the three behavioural models of depression used. It was interesting to note also that BJ treatments had no effects on the locomotor activity (ambulatory movements) in nondiabetic animals, and that its dose dependant suppressing effects were observed in diabetic animals only. Since the locomotor activity of the diabetic animals were also significantly higher than that of the nondiabetic ones, it seems reasonable to assume that alterations in glucose metabolism in diabetic animals are involved in all behavioural alterations observed in alloxan diabetes animals, and that the observed beneficial effects of BJ treatments in diabetic animals are due to its modulating effects on biological mechanisms and processes controlling both glucose metabolism and depressive states.

The possibility that central monoaminergic system could be involved in the mode of action of the extract is indicated by its observed effects on the brain monoamine levels. Reduction of brain levels of all the three quantified monoamines in diabetic rats was less severe in BJ treated animals, and this effect of the extract increased with its increasing doses. Most antidepressant drugs exerted their action by elevating synaptic 5-HT, NE and DA concentrations[33]. The results also revealed that BJ dose dependently restored the decreased 5-HT, NE and DA levels in diabetic rats. A study using postmortem brain tissue has demonstrated evidence of neurochemical disruption of serotonergic and noradrenergic neurons of depressed suicide victims[34], and similar disruption of serotonergic and noradrenergic chemistry has also been observed in rat model of depression, which can be normalized by antidepressant drugs treatment[35]. BJ probably act through restoration of neurotransmitters by increasing 5-HT and NE level at synaptic area of these monoaminergic neurons in diabetic rats. However, BJ treatment dose dependently and specifically increased brain dopamine level in nondiabetic rats. This finding could indicate that BJ primarily modulates brain dopamine synthesis and metabolism without any direct modulating effects on the synthesis and metabolism of norepinephrine and serotonin. Since central role of brain dopamine in regulating glucose metabolism and diabetes associate mental health problems are becoming increasingly

apparent[36], efforts are now being made to test whether BJ treatments could also have other behavioural effects known to be controlled by central dopaminergic system[37]. Observations made to-date during such efforts revealed that BJ possess a broad spectrum of therapeutically interesting psychopharmacological activity not only in diabetic but also in nondiabetic animals[38,39].

Diets rich in vegetables and mustard oil (oil obtained from *B. juncea* seeds) could contribute to lower risk of ischemic heart disease burden in India[40]. However, due to cultural and socioeconomic reasons a vast majority of population in India cannot either afford mustard oil for cooking their daily food, or do not like its taste. Since *B. juncea* leaves (mustard green) could be a cheaper agricultural byproduct of the large Indian mustard oil industries, and well accepted by its consumers as vegetables and salad, it could be a more realistic and affordable alternative of healthy vegetable even for its economically less privileged population. In view of the fact that India has been designated as "diabetes capital" of the world[41], the observations that *B. juncea* leaves possesses antidiabetic potentials with beneficial effects on diabetes associated psychopathologies could be of special therapeutic interest. During the course of the studies a few reports dealing with beneficial effects of *B. juncea* leaf extracts against diabetes associated cataract, or diverse other pathologies, have appeared[42-44], and all of them emphasize that antioxidative property of *B. juncea* leaf extracts could be involved in their observed bioactivities in animal models. If such would indeed be the case for BJ as well, its observed behavioural and neurochemical alterations in diabetic animals could also be explained by its antioxidative properties.

It cannot be ignored though, that BJ dose dependently increased brain dopamine levels in nondiabetic as well as diabetic animals (Fig. 3), whereas its antidepressant like efficacy observed only diabetic animals reached a plateau after its intermediate dose tested (Table 4 and Figs. 2a and b). Thus, it seems reasonable to assume that repeated daily administrations of BJ primarily modulates the brain dopaminergic system, and that its observed antidepressant like activity in diabetic animals is a consequence of the abnormal balance of the central monoaminergic system involved in behavioural processes. It is well recognized that that central dopaminergic system regulates numerous cognitive processes involved in obesity[45], and that obesity not only leads to diabetes but also to broad spectrum of comorbid mental health conditions for which no universally acceptable preventive or curative measures are yet available[46]. Efforts to identify the bioactive constituents of BJ involved in the observed elevated brain dopamine contents in nondiabetic rats could not only be useful for clarifying the neurochemical mechanisms involved in its behavioural effects, but also for combating diabesity by appropriate adjustments of diets. Moreover, since polyphenolics and other known bioactive components of *B. juncea* are also present in numerous other vegetables and fruits, observations made during such efforts could as well be useful for identifying other edibles that could more rationally enrich the modern dietary recommendations for diabetic patients.

Conclusion

Antidepressant like efficacy of methanolic extract of *B. juncea* leaf can be observed in hyperglycemic animals only. Compensations of monoaminergic deficits in diabetic animals caused by the tested extract could be involved in its observed behavioural effects. These observations, taken together with the present knowledge on bioactive secondary metabolites of other fruits and vegetables, strongly suggest that some other such edibles could also have potential benefits against mental health problems commonly encountered in diabetic patients.

Acknowledgement

Technical support from Natural Remedies Pvt. Ltd., Bangalore, in analytical standardization of *B. juncea* extract is acknowledged. AKT thanks the University Grants Commission, New Delhi, India for financial assistance.

Conflict of interest

The authors declare that they have no conflict of interest.

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