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Hypoglycemic effect of *Brassica juncea* (seeds) on streptozotocin induced diabetic male albino rat

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ABSTRACT

Objective: To evaluate the hypoglycemic effect of *Brassica juncea* (seeds) on streptozotocin induced diabetic male albino rats. **Methods:** Hypoglycemic activity of *Brassica juncea* (seeds) aqueous extract at a dose of 250, 350 and 450 mg/kg body weight was evaluated. Adult male Swiss albino rats of six numbers in each group was undertaken for study and evaluated. **Results:** The serum insulin levels were recorded a significant depletion in all groups, short term as well as long term diabetic animals, when compared to that of normal animals. 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. **Conclusions:** The aqueous seed extract of *Brassica juncea* has potent hypoglycemic activity in male albino rat.

1. Introduction

Diabetes mellitus (DM) is a chronic and major endocrine disorder caused by inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is a growing health problem in most countries and its incidence is considered to be high (4%-5%) all over the world[1]. Chronic hyperglycemia causes complications linked to diabetes, such as heart disease, retinopathy, kidney disease, and neuropathy. It is also a common cause of chronic morbidity and disability among the working population in the world. Several drugs, such as sulfonylureas, metformin, and a-glucosidase inhibitors, are used presently to reduce the hyperglycemia. In spite of the use of many hypoglycemic agents, diabetes and its linked complications are still an important medical problem[2]. Excessive oxidative stress has been implicated in the pathology and complications of diabetes mellitus[3, 4]. The increased blood glucose levels in diabetes produce superoxide anions, which generate hydroxyl radicals via Haber-Weiss reaction, resulting in peroxidation of membrane lipids and protein glycation. This leads to oxidative damage to cell membranes. These radicals further damage other important biomolecules including carbohydrates, proteins and DNA[5]. Streptozotocin (STZ) selectively destroy β-cells of pancreas by generating excess ROS and carbonium ion (CH_3^+) leading to DNA breaks by alkylating DNA bases. The N nitroso-N methyl urea portion of the molecule exhibits diabetogenic activity. Glucose may act as carrier for this cytotoxic group[6]. Plants have been commonly used to treat diabetes since ancient times and have served as an exemplary source of medicine[7]. We have recently reviewed the Indian plants that may have an antidiabetic potential[8]. The treatment of DM in clinical practice has been confined to use of oral hypoglycemic agents and insulin, the former being reported to be endowed with characteristic profiles of serious side effects[9]. This leads to increasing demand for herbal products with antidiabetic factor but little side effects. A large number of plants have been recognized to be effective in the treatment of DM[10]. *Brassica juncea* L. (Cruciferae) (*B. juncea*)is a traditional medicinal plant, which has been reported to be anodyne, apertif, diuretic, emetic, rubefacient, and stimulant. Indian mustard is a folk remedy for arthritis, footache, lumbago, and rheumatism. Its seed is used for tumors in China, root used as a galactagogue in

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Africa[11]. However, systematic studies on the indigenous medicinal plants that have been used in the treatment of DM are scanty. In the present study folklore medicinal plant *B. juncea* has been selected for the hypoglycemic study.

2. Materials and methods

2.1. Plant material

The seeds of *B. juncea* were freshly collected in and around Vellore District, Tamilnadu, India. The leaves were cleaned and shade dried at room temperature and authenticated. A voucher specimen (No: VCV/5/2010) is kept at the Department of Botany, Voorhees College, Vellore -632 001, Tamilnadu, India.

2.2. Plant extracts preparation

100 g powdered seed of the plant were taken and mixed with 500 mL of distilled water and magnetically stirred in a container overnight at room temperature. The residue was removed by filtration and the aqueous extracts were concentrated under vacuum to get solid yield of 10%. The plant extract was administered to animals in aqueous solution.

2.3. Animals

Adult male albino rats of Wistar strain weighing around 180-200 g were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of (25±2) ℃ and 55%-65% relative humidity. A (12±1) hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions. They were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore. India) and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

2.4. Experimental induction of diabetes

Diabetes was induced in the overnight fasted animals by a single intra peritoneal injection of freshly prepared solution of streptozotocin (STZ) (Sigma,USA) 35 mg/kg body weight in 0.1M cold citrate buffer pH 4.5[12-14]. The animals were allowed to drink 5% glucose solution to overcome the drug-induced hypoglycemia. The control rats were injected with citrate buffer alone as placebo. The animals were considered diabetic if the blood glucose values were >250 mg/dL on the third day after STZ injection.

2.5. Experimental design

The studies were conducted in the four groups of animals. Group I: Normal rats; Group II: Diabetic (STZ induced) control rats; Group III: Short term (ST): Diabetic animals kept for 7days; Group IV: Long term (LT): Diabetic animals kept for 25 days.

The diabetic animals of both groups (ST and LT) were fed with seed extract in increasing dosages of 250 mg, 350 mg, and 450 mg/kg body weight to assess therapeutic effect of the extracts. Separate batches were maintained in each group for each dose level.

Plasma insulin was estimated using RIA assay kit for rats supplied by Ljico Research inc. (Stat Diagnostics, Mumbai).

The results were expressed in mean±standard deviation. Statistical analysis was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS).

3. Result

To study the antidiabetic effect of the aqueous seed extract, diabetes was induced into the male albino rats by the intraperitoneal injection of streptozotocin. After 48 hrs of injection of STZ to normal rats diabetes was evidenced. The blood glucose levels were significantly elevated(+343 %) in the STZ injected rats when compared with that of normal (Placebo), therefore considered as diabetic animal (Control). The seed extract of *B. juncea* that was fed to the diabetic animals recorded significant lower blood glucose level of 291, 185 and 103 mg/dL at I hr, II hr, and IV hr of time intervals respectively. However the percentage of decrement was elevated at IV hr after extract was fed to the diabetic animals.

Studies were designed to assess the impact of aqueous seed extract of *B. juncea* on serum insulin levels in STZ induced diabetic male albino rats. The studies were conducted in two groups of STZ-induced diabetic animals. Group-I was short term (7 days-diabetic animals). Group-II long term (25 days-diabetic animals). The animals of both groups were fed with plant extracts in increasing doses *i.e.* 250 mg, 350 mg, 450 mg/kg body weight to assess the insulin augmenting effect in the diabetic animals.

The serum insulin levels were recorded a significant depletion in both groups, short term as well as long term diabetic animals, when compared to that of normal animals. The serum insulin, significant augmenting effect of the seed extract was recorded in the both short term as well as long term groups and also recorded as dose dependant of the extract (Table 1).

Table 1

Hypoglycemic effect of aqueous seed extract of folklore medicinal plant, *B. juncea*. Serum insulin levels (μ/mL).

Term	Normal (N)	Diabetic control	Experiment groups (mg/kg body wt)		
			250	350	450
Short Term	17.27±1.67	12.10±1.30	13.13±1.40*	15.30±1.08*	16.26±1.40*
Long term	17.27±1.67	7.32±1.21	8.61±0.83*	8.80±1.10*	9.17±0.92*

Data are expressed as Mean±SD of 6 individual observations. Statistical significance * $P<0.001$.

4. Discussion

The aqueous seed extract of the folklore medicinally valued plant *B. juncea* clearly envisaged hypoglycemic effect by depleting the serum glucose levels in STZ-induced diabetic animals. The blood glucose levels were monitored at three time intervals, I hr, II hr, and IV hr. The hypoglycemic effect was recorded as highest at the IV interval. This might be due to the time taken for the intestinal absorption of the aqueous seed extract of *B. juncea*. Earlier works[15,16] carried to assess the effect of various edible plant products has also shown that plants have a potential to treat diabetes and its complications. Among these we have shown that *Fenugreek* (Methi), *Murraya koeingii* (Curry Patta), *B. juncea* (Rai), *Momordica charantia* (Karela) and *Eugenia jambolana* (Jamun) which are used as spices in India have been found to possess hypoglycemic activity in experimental as well as clinical studies[17,18]. Further, the hypoglycemic effect of the seed extract of *B. juncea* was attributed to stimulation of glycogen synthesis leading to increase in hepatic glycogen content and suppression of glycogen phosphorylase and other gluconeogenic enzymes[19].

Further investigation was carried out to assess the hypoglycemic effect of the above plant extract on serum insulin levels in STZ-induced diabetic animals. It was correlated and assessed that higher serum glucose levels were due to lower insulin levels in the diabetic animals, which might be due to lower secretion of insulin from the beta- cells of islets langerhans.

In the present study the plant extract augmented the serum insulin levels suggesting an improved state of availability of serum insulin to control blood sugar. This might be due to higher secretion of the hormone in seed extract fed animals. The present study showed that insulin serum augmenting effect was recorded highest at the dose of 450 mg/kg body weight suggesting that the serum insulin effect of the seed extract is dose dependant. However, the therapeutic effect of the seed extract was recorded higher in short term group animals when compared to that of long term group. This might be due to the inability of the beta cells to recoup from the STZ effect in the long term animals. The insulin augmenting effect of the *B. juncea* aqueous seed extract was significantly higher in short term STZ-induced diabetic animals when compared to the long term group. This suggests that the beta cells of langerhans regenerating effect of the *B. juncea* seed extract was higher in the short term group. In conclusion, our results showed that the oral administration of the aqueous seed extract of *B. juncea* has a beneficial effect to diabetic rat. The study revealed that a potent drug with hypoglycemic and insulin augmenting effect may be formulated from the aqueous seed extract of *B. juncea*.

Conflict of interest statement

We declare that we have no conflict of interest.

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