ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITY OF Brassica oleracea LEAF IN ALBINO RATS

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ABSTRACT

To evaluate the anti diabetic activities of methanolic Leaf and Stem extracts of Brassica oleracea in alloxan-induced diabetic rats. Group I served as Normal untreated rats. Group II served as Normal rats were administered with Brassica oleracea L. extract Group III served as Diabetic control Group IV served as Diabetic rats were administered with Brassica oleracea L. extract 500mg/kg body weight in aqueous solution daily for 30 days. Group V served as Diabetic rats were administered red glibenclamide 600μg/kg body weight in aqueous solution daily for 30 days. The results of the study indicates that Brassica oleracea extract significantly (P<0.05) reduced the blood sugar level. The Leaf extract significantly reduced the levels of Serum total cholesterol, triglycerides, LDL- and VLDL- cholesterol in alloxan induced diabetic rats. Further confirmed in histopathological observations. The study shows that the ethanolic leaf extract of Brassica oleracea possess potent antidiabetic activity.


1. INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The total number of people with diabetes is projected to increase from 171 million in 2000 to 366 million in 2030 (Wild S et al., 2004). Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin (Prasad Sk et al.,2009). Diabetes mellitus, one of the most common endocrine metabolic disorders has caused significant morbidity and mortality due to micro vascular (retinopathy, neuropathy and nephropathy) and macro vascular (heart attack, stroke and peripheral vascular disease) complications (Patel DK et al., 2011). .

The treatment of diabetes mellitus is considered as the main global problem and successful treatment has yet to be discovered. Even though insulin therapy and oral hypoglycemic agents are the first line of treatment for the diabetes mellitus these have some side effects and fail to significantly alter the course of diabetic complications. Currently available oral therapies for treatment of diabetes mellitus are sulfonylureas, biguanides, glucosidase inhibitors and glinides, which can be used alone or combined with other drugs to achieve better effect. Many of these oral antidiabetic agents have a number of serious adverse effects, thus, the management of diabetes without any side effects is still a challenge.
A vast range of these natural products and medicinal plants, including crude extracts and isolated compounds from plants can be used to regulate carbohydrate metabolism and prevent chemical induced diabetes mellitus. In the recent decades, these have been vastly used in management of diabetic due to presence of several components with different antidiabetic and anti-oxidant effects on glucose metabolism and fat oxidation. World health organization (WHO) has recommended the evolution of traditional plant treatment for diabetes as they are effective, non toxic, with less or no side effects and are considered to be excellent candidates for oral therapy (Saroj Arora et al., 2003). Traditionally, a number of plants have been used in various herbal preparations in the management of diabetes and only a few of them have been proven scientifically (Jia Q et al., 2009). More than 800 plants have been studied for their antidiabetic potentials (Noor A et al., 2008; Daisy P et al., 2007) among thousands of plants used in various regions of the world. The medicinal value of the chosen plant Brassica oleracea has not been extensively worked out.

Brassica oleracea is known as an aquatic fern belongs to the family of Brassicaceae. Leaves are sweet, cooling and stomachic, anti scorbatic, emollient, constipating diuretic anthelmintic and cardiotonic. They are useful in abdominal disorders pruritus, skin diseases, diarrhoea, strangury, intestinal worms, cough bronchial asthma, fever, warts, urorrea, haemorrhoids, gout and vitiated conditions of pitta and vata (Prajapati ND et al., 2006). Brassica oleracea L. extract has also prevented oxidative stress induced in livers and brains of animals exposed to paraquat (Igarashi K et al., 2000) and N-methyl-D-aspartate (Lee et al., 2002). On the basis of traditional uses in the present study was to investigate the antidiabetic activity of aqueous extract of Brassica oleracea in alloxan induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Animals

Male albino rats of Wistar strain approximately weighing 150-180g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2°C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided ad libitum. They were acclimatized to the environment for one week prior to experimental use.

2.2 Collection, identification and preparation of plant material

The fresh plant of Brassica oleracea L. was collected from Thanjavur, Tamil Nadu, and India. The plant was identified and the voucher specimen has been kept in our laboratory for future reference. The leaves were sliced into small pieces and dried under shade for about a week. The dried material was ground to coarse powder and passed through a 40 mesh sieve, and kept in a well closed container for further extraction.

2.3 Preparation of plant extract

500g of dried, powdered plant material were extracted successively with ethanol using soxhelt apparatus. The residual extract was suspended in water for overnight and filtered. The filtrate was dried and was stored at 4 °C until used. A known volume of the residual extract is suspended in distilled water and was orally administrated to the animals during the experimental period.

2.4 Preparation of aqueous extract

The dried and powdered plant was Soxhelt extracted with distilled water for 72 h. The extract was slowly evaporated to dryness using a Rotary evaporator at 40 °C to afford dry residues which was stored at 4 °C until use.

2.6 Induction of diabetes mellitus

The method described by Pari and Satheesh et al., 2004 was adopted. In the experiment a total of 30 rats (18 diabetic surviving rats and 12 normal rats) were used. The rats were divided into 5 groups (6 rats / group) after the induction of alloxan-diabetes.

3. Experimental design

3.1 Route of administration

500mg/kg of body weight in aqueous solution daily for 30 days. The suspension was fed orally by an infant feeding catheter 3mm size attached to syringe. The catheter was inserted into the gastric region of the experimental albino rats and fixed volume of 500mg/kg body weight in a aqueous solution was discharged slowly with adequate care into each animal. Group I served as Normal untreated rats. Group II served as Normal rats were administered with Brassica oleracea L. extract. Group III served as Diabetic control Group IV served as Diabetic rats were administered with Brassica oleracea L. extract. Group V served as Diabetic rats were administered glibenclamide 600μg/kg body weight in aqueous solution daily for 30 days (Pari L et al., 1999).
3.2 Collection of blood and preparation of serum sample
At the end of the experimental period, the animals were anaesthetized using chloroform vapor prior to dissection. Blood was collected by cardiac puncture into serum separator tubes. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000 rpm for 10 minutes and then the serum (supernatant) was isolated and stored at refrigerated until required for analysis.

3.3 Histological studies
The liver and pancreas were fixed in 10% normal saline for 72 h after which the tissues were sliced to a thickness of 2.1mm each. These were dehydrated using alcohol of graded concentration. They were further treated with paraffin wax and cast into blocks sections of the tissues were cut on a microtome to 5 μm. These were later attached to a slide and dried. The samples slides were viewed on a photographic microscope to find out histological changes.

3.4 Biochemical Estimations
Urine sugar was detected by the method of Benedict SR .,1985 . Blood glucose level was estimated by the method of Sasaki T et al., 1972. Plasma insulin was estimated using RIA assay kit (for rats) supplied by Linco Research Inc. (Stat Diagnostics, Mumbai). Haemoglobin was estimated by the cyan methaemoglobin method of Drabkin and Austin , 1932. Glycosylated Haemoglobin was estimated by the method of Nasay and Pattabiraman (Nayak SS et al., 1981) . Triglycerides in plasma were estimated by the method of Rice EW, 1970 . Plasma and tissue cholesterol content was estimated by the method of Parekh and Jung , 1970. Free fatty acid content was determined by the method by Hron and Menahan , 1981. Lipoproteins were fractionated by dual precipitation techniques ( Burstein M et al.,1972). Acid phosphatase was assayed by the method of King J , 1959. Alkaline phosphatase was assayed by the method of King J ,1959. SGOT and SGPT activity were determined by the method of Reitman and Frankel , 1957. Protein was estimated by the method of Lowry OH , 1951.

3.5 Statistical analysis
Values were expressed as mean □ SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons. The results were statistically analyzed and p< 0.05 was considered to be significant.

4. RESULTS
4.1 Diabetic markers
Diabetic rats increased glucose, glycosylated hemoglobin, cholesterol, triglycerides, free fatty acids VLDL and HDL cholesterol while insulin and hemoglobin content was decreased. The aqueous leaf extract of Brassica oleracea showed significant decreased in serum glucose level at 500 mg/kg bw (p<0.05) and increased in Insulin and hemoglobin level (p<0.05) (Table. 1). The extracts of leaf Brassica oleracea showed significantly reduced levels of Total cholesterol, free fatty acids, triglycerides, LDL and VLDL (p<0.05) (Table. 1). It also showed significant increased in HDL level (p<0.05).

4.2 Liver markers
The activity of SGOT, SGPT, ALP, ACP and protein in the serum of control and experimental animals is presented in Table 3. It is observed that administration of alloxan induced diabetic rats produced a significant increase in the activities of all the marker enzymes, the increase being two-fold for SGOT, SGPT, ALP, ACP and decreased protein content, when compared to control rats. Treatment with Brassica oleracea leaf treated animals significantly restored the activities of these enzymes to normalcy (Table 2).

4.3 Histopathological observation
4.3.1 Pancrease
Group I and II rats shows the normal architecture of pancrease (Plate 1A and B). A diabetic rat shows the atrophy, islet cell, diffuse, cell disintegration (Decrease in both the number of islets and the number of cells per islet present). Disorganization of the cells in the islet present) (Plate 1C). Brassica oleracea leaf and standard treated rats shows the near normal structure of pancrease (Plate 1D - E).

4.3.2 Liver
Normal control rats and Brassica oleracea leaf alone treated rats shows normal hepatocytes with well brought out nuclei and cytoplasm (Plate 2 A and B). Alloxan treatment elicited severe injury of liver and shows degenerated parenchymatous cells with severe necrosis dilation of sinusoids and loss of concentric arrangement of the hepatocytes around the central vein (Plate 2 C). Liver of diabetic rats treated with leaf (500mg/kg bw) extract of Brassica oleracea and glibenclamide (600μg /kg bw) restored the arrangement of hepatocytes with nearly normal appearance and minimal necrosis and appeared well brought out nuclei and cytoplasm (Plate D – E).
Plate 1 Histopathological changes in pancreas of control and experimental rats

Plate 1A. Normal pancreatic islet cells

Plate 1B. Normal rats treated with *Brassica oleracea* L. extract showing normal islets cells

Plate 1C. Diabetic control rats showing fatty infiltration and shrinkage of islets cells

Plate 1D. Diabetic rats treated with *Brassica oleracea* L. extract showing reduced fatty infiltration and normal islets cells

Plate 1E. Diabetic rats treated with glibenclamide showing no fatty changes and normal pancreatic islets

Plate 2 Histopathological changes in liver of control and experimental rats

Plate 2A. Normal rat liver hepatocytes

Plate 2B. Normal rats treated with *Brassica oleracea* L. extract showing normal hepatocytes

Plate 2C. Diabetic control rats showing a portal tract with dilated and congested vein, associated with fatty infiltration

Plate 2D. Diabetic rats treated with *Brassica oleracea* L. extract showing reduced fatty infiltration and architecture of the hepatic lobule that appears more or less like control

Plate 2E. Diabetic rats treated with glibenclamide showing architecture of the hepatic lobule showing no fatty change
5. DISCUSSION

Pancreas is the primary organ involved in sensing the organism’s dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose insulin is secreted (Edem et al., 2009). Blood sugar level increased in alloxan treated rats (Table 1), since alloxan causes a massive reduction in insulin release by the distribution of the beta cells of the islets of Langerhans (Goldner M et al., 1981). Hyperglycemia the primary clinical administration of diabetes mellitus is associated with the development of micro and macro vascular diabetic complications (Brownlee M et al., 1981). Over production of glucose by Means of excessive hepatic glycogenolysis and glyconeogenesis is one of the fundamental bases of hyperglycemia in diabetes mellitus (Latner A et al., 1988). In the oral administration of Brassica oleracea L. Plant extract and glibenclamide significantly decrease in blood glucose level in diabetic rats. The anti diabetic effect of Brassica oleracea L. extract could be linked to more than one mechanism. The possible mechanism includes the stimulation of beta cells and subsequent release of insulin activation of the insulin receptors. In this context, a number of other plants have also been reported to have anti hyperglycemic and insulin release stimulatory effect (Kaleem M et al., 2006; Pari L et al., 1999; Prince PS et al., 1998).

Insulin deficiency leads to various metabolic alterations in the animals wise increased blood glucose, increased cholesterol, increased levels of alkaline phosphatase and transaminase (Shanmugasundaram KR et al., 1983; Mushsin aydin et al., 2012; Felig Evans JL et al., 2003; Shanmugasundaram KR et al., 1981). In this respect, the mode of action of these extracts is similar to those reported for extracts of Gymnema Silvestr (shanmugasundaram KR et al., 1981), Momordica Charantia (Cakici et al., 1994) and other plant extracts (Maroo J et al., 2002; Sekar DS et al., 2005; Reyes BS et al., 2006; Nerendhira Kannan RT et al., 2006) In the diabetic rats had decreased level of insulin but the Brassica oleracea L. plant treated rats showed increased level of insulin (Table 1). Insulinogenic activity with the treatment of some medicinal plants was reported (Karunanayake EH et al., 1984).

Hemoglobin is an important component in RBC, decides the oxygen carrying capacity of blood. World Health Organisation (WHO) indicate a worldwide anemia prevalence of about 30% with higher rates in developing countries The Specific activities of the enzymatic antioxidants like superoxide dismutase and catalase are expressed in terms of the level of hemoglobin concentration in the body. (Ravan R et al., 2007) found that the hemoglobin levels were significantly lower in the diabetics than in the non-diabetic groups. According to WHO 2005, diagnosis of anemia is confirmed if the hemoglobin value is less than 12g/dl for adult men and women. The normal blood hemoglobin level is between 14-16g/dl for males and 13-15g/dl for females. Glycosylated haemoglobin is produced by glycosylation of haemoglobin is formed progressively and irreversibly over a period of time and is stable over the life span of the red blood cells. It is unaffected by diet, insulin or exercise. Therefore, glycosylated haemoglobin can be used as an excellent marker of overall glycemic control. Since it is formed slowly and it does not dissociate easily, it reflects the real blood glucose level (Bunn HF et al., 1981; Allen DW et al., 1964) In this study, the diabetic rats had elevated levels of glycosylated haemoglobin (Table 1). Therefore the significant decrease in the level of glycosylated haemoglobin in alloxan induced diabetic rats.

The total haemoglobin level was found to be decreased in diabetic animals (Table 1). During diabetes mellitus, the excess glucose present in the blood leads to glycation of tissue proteins. Oral administration of Brassica oleracea L. extracts to diabetic rats significantly increased the level of haemoglobin and this might be due to the decreased level of blood glucose. The haemoglobin abnormal is associated with the resolution in red cell life span (Allen DW, 1964). A similar result was observed in Leucas Lavandulaefolia (Chandrasekar KS et al., 2009).

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The increased levels of insulin in Brassica oleracea extracts treated diabetic rats may be due to the activation of remnant β-cells in the pancreas, which was in accordance with the observed histological observations (Plate 1). Present finding is in agreement with Subramaniam et al., 2012 Ayoola et al., 2009 and Sharma and Garg, 2009 studies. This study also corroborated with Jaya and Anuradha
possible mechanism by which *Brassica oleracea* leaf brings about its hypoglycaemic action may be by potentiating of the insulin effect on plasma by increasing either the pancreatic secretion of insulin from β-cells of islets of Langerhans or its release from the bound form or insulin mimetic action (Staney Mainzen Prince P et al., 1998). In this context, a number of other plants species have been observed to possess antihyperglycemic activity and an insulin-release stimulatory effect (Shimizu S et al., 2001; Xie JT et al., 2003).

The most commonly observed lipid abnormalities in diabetes are hyper triglyceridemia and hyper cholesterolemia (Shepherd J, 2005) and such an elevation represents a risk factor for coronary heart disease (Al-shamaony et al., 1994). Lowering of serum lipids levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease (Shirwaikar A et al., 2005). The liver is the only organ that can catabolize and excrete quantitatively important amounts of cholesterol. Lipids play a vital role in the pathogen of diabetes mellitus. Abnormalities in lipid profile are one of the most complications in diabetes mellitus such as hyper triglyceridemia and hyper cholesterolemia (Rhoads GG et al., 1976). Changes in the concentrations of the lipid with diabetes mellitus contribute the development of vascular disease (Nikkila et al 1973; Howard BV et al., 1978). Fatty acids important components of cell membranes are eico saniod precursors and are therefore required for both the structure and function of every cell in the body (Rajasekaran et al., 2006).

Hypolipidemic potential of various plant extracts has already been reported (Pinent M et al., 2008; Aguliar FA et al., 2005). This lipid lowering effect of the extract may be due to the action of these phytochemical compounds either independently or in carbonization. Hypercholesterolemia and hyper triglyceridemia have been reported to occur in alloxan diabetic rats (Sharma S et al., 1996; Pushparaj P et al., 2000). Furthermore, it has been reported that the increase in glucose levels in alloxan-induced diabetic rats is associated with dyslipidaemia characterized by elevated serum triglycerides and total cholesterol levels (Table 2).

Alterations in secretion of insulin and glucagon also profoundly affect lipid, ketone, and protein metabolism. At concentrations below those required to stimulate glucose uptake, insulin inhibits the hormone-sensitive lipase in adipose tissue and thus inhibits the hydrolysis of triglyceride stored in the adipocyte. This counteracts the lipolytic action of catecholamines, cortisol, and growth hormone and reduces the concentrations of glycerol (a substrate for gluconeogenesis) and free fatty acids (a substrate for production of ketone bodies and a necessary fuel for gluconeogenesis). These actions of insulin are deficient in the diabetic patient, leading to increased gluconeogenesis and ketogenesis. Insulin also enhances the transcription of lipoprotein lipase in the capillary endothelium. This enzyme hydrolyzes triglycerides present in very low density lipoproteins (VLDL) and chylomicrons, resulting in release of intermediate-density lipoprotein (IDL) particles. The IDL particles are converted by the liver to the more cholesterol-rich low-density lipoproteins (LDL). Thus, in the untreated or undertreated diabetic patient, hypertriglyceridemia and hypercholesterolemia often occur. In addition, deficiency of insulin may be associated with increased production of VLDL.

In the adipose tissue of diabetic individuals, the effect of the decrease in insulin and increase in glucagon results in inhibition of lipogenesis and inactivation of lipoprotein lipase, and activation of hormone-sensitive lipase. This leads to release of increased amounts of glycerol (a substrate for gluconeogenesis in the liver) and free fatty acids, which are used by skeletal muscle and liver as their preferred metabolic fuels, so sparing glucose (Davis L et al., 2001). The improvement of blood glucose level induced by most hypoglycaemic treatments is associated with a reduction of serum triglycerides and total cholesterol (Dhanabal M et al., 2001; Saravanan G et al., 2006).

The hepatic and cardiac tissues release AST and ALT and the elevation of plasma concentrations of these enzymes is an indicator of hepatic and cardiac damage (Crook MA, 2006). Serum transaminases (AST and ALT) and alkaline phosphatase have long been considered as sensitive indicator of hepatic injury (Molander DW et al., 1955). Injury to the hepatocytes alters their transport functions and membrane permeability, leading to the leakage of enzymes from their cells (Krishnamohan G et al., 2007). This leakage causes an increase in levels of serum ALT, AST and ALP. In the present work indicated that the treated rats showed a significant elevating tendency in the serum ALT, AST, ALP (Table 3). These similar results reported other plants by Ethan et al., 2003. The measurement of enzymatic activities of phosphates such as acid phosphates (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changed in their activities are indicative of tissue damage by toxicants (Singh SN et al., 2001). ALP is present in all tissues of the body, especially in cell membrane and the levels are high in the liver, kidney, bone and placenta.
In our study, the activity of serum ALP and ACP increased in diabetic rats as compared with control rate (Table 3). Thus the decreased activity of serum enzymes is due to the regenerational of beta cells in pancreas brought by the administration with the crude extracts of *Brassica oleracea* L.

In diabetes mellitus, a variety of proteins are subjected to non enzymatic glycation and this is thought to contribute to the long term complications of the disease (Vlassara H et al., 1981). The level of serum total proteins were found to be decreased in diabetic rats may be ascribed to i) decreased amino acid uptake ii) greatly decreased concentration of variety of essential amino acids. iii) Increased conversion rate of glycogenic amino acids to carbon dioxide and water iv) reduction in protein synthesis secondary to a decreased amount and availability of mRNA ( Ahmed RG et al., 2005). Insulin is a physiological factor, which plays an important role in the maintenance of protein balance. Since it not only stimulates the uptake of amino acids and protein synthesis but also inhibits protein degradation. Due to insulin deficiency, protein content is decreased by proteolysis (Vats V Yada SP et al., 2003). There is a reduced level in total protein content blood in alloxan induced diabetic rats in the present study. *Brassica oleracea* L. significantly increased serum proteins.

5.1 Histopathological observation

The present study revealed that the immediate action of alloxan induced diabetes by destroying β-cells even at a single dose of 120 mg/kg body weight. The ultra structure of alloxan diabetic pancreas showed considerable reduction in the islet langerhans and depleted islets (Gholamali AT et al., 2005). The diabetic rats showed pancreatic islet regeneration. The regenerative effect of the pancreatic cells by *Brassica oleracea* L. extracts via exocrine cells of pancreas may enlighten the positive effect of these agents on the production of insulin.

The degenerative changes in the histology of liver and pancreas brought about by alloxan administration are similar to earlier observations. Histologically, liver section of alloxan induced diabetic rats showed marked structural alterations in the liver as a result of absence of insulin. The major alteration was periportal fatty infiltration, necrosis of hepatocytes. This damage is partially reversed by the *Brassica oleracea* L. extract treatment and the similar result were observed by *Gymnema sylvestre* therapy in alloxan induced diabetic rabbits by Shamugasundaram et al., 1983 and *Vinca rosea* extract in alloxan- induced diabetic rats by Ghosh et al., 2001.

6. CONCLUSION

The present study demonstrated that the *Brassica oleracea* leaf extract possess antidiabetic and hypo lipidemic effect revealed by decreased serum lipid levels, restored glucose and insulin and therefore attributes to therapeutic value of *Brassica oleracea* leaf extract to combat the diabetic condition in rats as compared to stem. The potential antidiabetic activity of *Brassica oleracea* leaf extract due to the phytochemicals.

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Conflict of interest statement

We declared that we have no conflict of interest.

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