Antihyperglycaemic And Antioxidant Activity Of Brassica Oleracea In Streptozotocin Diabetic Rats

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Introduction

Brassica or cruciferous vegetables are the "Wonder Kids" of the vegetable world. This includes cabbage, cauliflower, broccoli and kholrabi. They are known as Knol-Khol in Indian vernacular languages. These vegetables are rich in the antioxidant vitamins C, E and carotene and are good sources of dietary fibre. They also contain sulphoraphanes and other isothiocyanates, which are believed to stimulate the production of protective enzymes in the body[1,2].

Reactive oxygen species (ROS) are an important part of the defence mechanisms against infection, but excessive generation of free oxygen radicals may damage tissue[3]. The role of ROS in tissue damage in various human diseases such as cancer, ageing, neurodegenerative disease, diabetes and atherosclerosis has been recognized[4].

Brassica oleracea var. *gongylodes* (Brassicaceae) commonly known as Knol-Khol, kholrabi has similar composition as other Brassica vegetables[5]. Aqueous extract of Knol-Khol is reported to have antidiabetic activity in alloxan induced diabetic model[6]. In our preliminary studies a valuable finding was that the dose required to produce antihyperglycaemia with petroleum ether extract of Knol-Khol was very low when compared to the aqueous extract. Though the plant is traditionally used by South Indian tribal people in their culinary as a nutrient, the detailed information regarding Knol-Khol antihyperglycaemic and antioxidant activity is not scientifically documented. Therefore, the present study is designed to find out hypoglycaemic and antioxidant activity of petroleum ether extract of Knol-Khol in streptozotocin induced diabetic rats.

Materials and Methods

Plant material

Fresh stem of the plant was collected from the local market, Belgaum, Karnataka, India in the month of November and authenticated as *Brassica oleracea var. gongylodes* (Brassicaceae) by Dr.P.S.N.Rao, Botanical Survey of India, Pune and a voucher specimen has been deposited at BSI, Pune, India (AS-2).

Preparation of extract

The fresh stem (10 Kg) was cut into small pieces and dried under shade for about a week. The dried material was ground to coarse powder. This powder was then subjected to extraction with petroleum ether solvent (40-60°C) by maceration. The solvent was then evaporated at room temperature and dried mass was collected and weighed (4.217 g).

Animals

Healthy Albino rats (150-180gms) of either sex obtained from central animal house, Jawaharlal Nehru Medical College, Belgaum were used for the study. Ethical clearance was obtained from Institutional Animal Ethics Committee, K.L.E.S's College of Pharmacy, Belgaum. Animals were housed individually under standard laboratory conditions and fed with commercial pellet rodent diet and water *ad libitum* till the end of the experiment.

Chemicals

Streptozotocin was obtained from Hi-media Ltd., Mumbai. Glutathione standard and Glutathione reductase from Sigma Chemical Company, St Louis, MO, USA. Thiobarbituric acid, Meta phosphoric acid and all other chemicals were of AR grade.

Experimental diabetes

Diabetes was induced by 65 mg/kg of streptozotocin (STZ) administered i.p. in citrate buffer (pH 4.2) (Krishnakumar et al, 1999). After 7 days blood glucose levels were measured to confirm the induction of diabetes. Rats with glucose level above 200 mg/dL were selected as diabetic rats and were included in the experiment.

The dose of the petroleum ether extract was calculated taking into consideration that an average human being consumes 400 g of the stem per day. Thus the petroleum ether soluble fraction in this will be 0.168 g. This dose was then converted to an equivalent dose in rats using the dose conversion table[7].

The animals were divided into 4 groups of 6 animals each – normal control, diabetic control, diabetic rats treated with 15.12 mg/Kg of petroleum ether extract of Knol-Khol and diabetic rats treated with Pioglitazone (10 mg/kg. p.o. daily) for 60 days.

On 60^{th} day, blood glucose level and antioxidant enzymes levels were measured in blood samples collected by retro-orbital puncture under light ether anaesthesia.

Estimation of glucose

Blood glucose was measured by using commercial Glucomonitor kit (Pulsatum glucomonitor)[8].

Estimation of peroxidation product and antioxidant enzymes

The level of peroxidation product viz. Malondialdehyde (MDA) was measured in blood[9] where the reaction depends on the formation of a coloured complex between malondialdehyde (MDA) and thiobarbituric acid (TBA) having an absorption maximum at 532 nm.

Similarly the level of glutathione (GSH) content in blood was measured[10] where 5-5^1-Dithiobis 2-nitro benzoic acid (DTNB) is reduced by glutathione, forming highly coloured yellow anion. The optical density of this yellow substance was measured at 412 nm.

After estimating MDA and GSH level in blood the remaining blood was used for the preparation of RBC haemolysate[11]. Then the haemoglobin content of this haemolysate was determined[12]. Haemolysate was further used to check the activities of antioxidant enzymes.

Superoxide dismutase (SOD) activity was measured in haemolysate[13]. Epinephrine can be autooxidised to adrenochrome by superoxide radicals. The ability of SOD to inhibit the autooxidation of epinephrine to adrenochrome has been used as the basis for the assay of this enzyme.

Catalase (CAT) and glutathione peroxidase (GSHPxase) were measured in haemolysate[10] where the rate of decomposition of hydrogen peroxide by catalase was measured spectrophotometricaly at 230 nm and in case of glutathione peroxidase, the rate of reduction of oxidized glutathione by glutathione reductase was measured.

Histopathology

At the end of the study, animals from each group were sacrificed, pancreas excised and sent for histopathological examination. The staining was done using H&E stain.

Statistical analysis

Results were expressed as mean ± SEM and evaluated for statistical significance by ANOVA followed by Dunnet's 't' test. Values of $P< 0.05$ were considered to be statistically significant.

Results

Blood glucose

The hyperglycaemic animals showed significant ($F_{(3/20)} = 1104$, $P<0.001$) decrease in the blood glucose level on long term treatment for 60 days with Knol-Khol extract and Pioglitazone, when compared to diabetic control rats. (Table 1).

Lipid peroxide concentration

The concentration of malondialdehyde (MDA) was significantly (($F_{(3/20)} = 3266$, $P<0.001$) decreased in the Knol-Khol extract and Pioglitazone treated groups as compared to diabetic control. (Table 1).

Group	MDA (nmol/100ml)	GSH (mg/100ml)	Blood glucose (mg/100 ml)	SOD (IU/gm of Haemoglobin)	CAT (IU/gm of Haemoglobin)	GSHPxase (IU/mg of Haemoglobin)
I	11.56 ± 0.33	63.80 ± 0.84	82.84 ± 1.19	681.16 ± 4.84	8.96 ± 0.28	31.30 ± 0.68
II	171.84 ± 0.48#	41.16 ± 0.48#	400.34± 2.68#	539.34 ± 0.76#	4.52 ± 0.18#	17.68 ± 0.52#
III	134.50 ± 0.34*	57.00 ± 0.74	151.16± 7.78*	639.34 ± 1.04*	7.96 ± 0.04*	27.36 ± 0.98*
IV	130.30 ± 0.50*	60.16 ± 0.84*	112.50 ± 2.54*	664.16 ± 0.90*	8.12 ± 0.02*	29.65 ± 0.20*

Group – I : Non – diabetic rats.

Group – II: Streptozotocin induced diabetic control rats.

Group – III: Extract treated rats.

Group – IV: Pioglitazone treated rats.

Each value is the mean ± SEM of 6 rats.

# P <0.001 as compared to group-I (Control), * P < 0.001 as compared to group-II (Diabetic control)

(ANOVA followed by Dunnet's 't' test).

Table 1: Effect of Knol-Khol (Petroleum ether extract) on concentration of MDA, GSH, blood glucose and activities of antioxidant enzymes in streptozotocin induced diabetic rats

Glutathione content

The glutathione content was significantly ($F_{(3/20)}$ = 174.5, P<0.0001) increased in the Knol-Khol extract and Pioglitazone treated groups as compared to diabetic control (Table 1).

Antioxidant enzymes levels

The levels of superoxide dismutase, catalase and glutathione peroxidase was significantly ($F_{(3/20)}$ =118.3, P<0.0001, $F_{(3/20)}$ = 143.3, P<0.0001, $F_{(3/20)}$ = 95.03, P<0.0001 respectively) increased in Knol-Khol extract and Pioglitazone treated groups when compared to diabetic control (Table 1).

Histopathological studies

Section from the nondiabetic rats showed normal acini and islets while the section from diabetic control rats showed minute and reduced number of islets. Section from Knol-Khol extract treated diabetic rats showed good number of regenerating tiny islets, which could be comparable to that of nondiabetic rats (Fig 1,2,3,4).

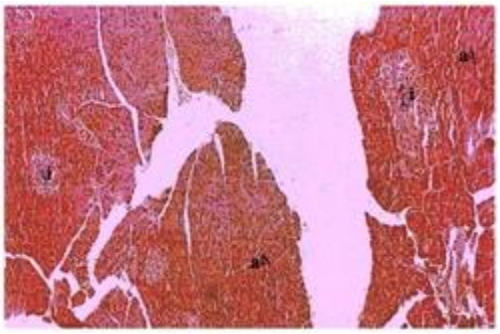

Figure 1: Section from pancreas of non-diabetic rat which shows normal acini (a) and islets (i) (H & E stain, 100 X).

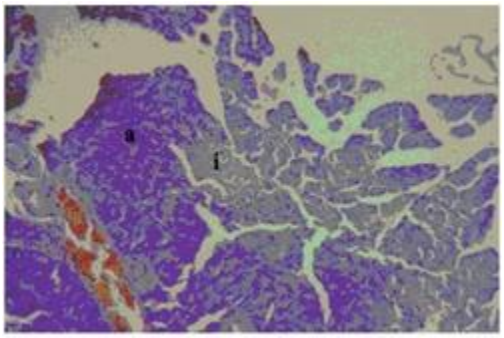

Figure 2: Section from pancreas of diabetic control rat which shows normal acini (a) and minute islets (i), which are scanty (H & E stain, 100 X).

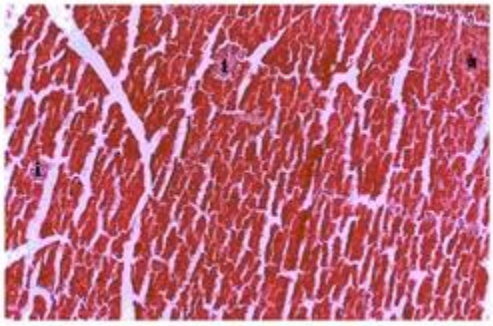

Figure 3: Section from pancreas of Brassica *oleracea* (petroleum ether extract) treated diabetic rat which shows normal acini (a) and islets (i) that are comparable to that of non-diabetic rat (H & E stain, 100 X).

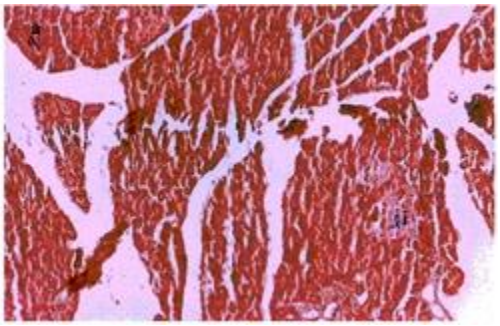

Figure 4: Section from pancreas of Pioglitazone (10 mg/Kg) treated diabetic rat which shows

normal acini (a) and islets (i) that are comparable to that of non-diabetic rat (H & E stain, 100 X).

Discussion

Diabetes mellitus and its complications are mainly due to impaired pancreatic, antioxidants function and lack curative treatment. Oxidative stress resulting from enhanced free radical formation and/or defects antioxidants defense caused severe tissue damage and may lead to number of diseases like coronary artery disease, atherosclerosis, cancer and diabetes. Increased oxidative stress in streptozotocin diabetic rats has been reported[3]. This oxidative stress is also implicated in the development of diabetic complications[14].

An elevated level of lipid peroxides in the plasma of streptozotocin diabetic rats and lipid peroxidation is one of the characteristic features of chronic diabetes[15]. The increased levels of thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), malondialdehyde and hydroperoxides are indices of lipid peroxidation[16].

In the present study we found that the levels of lipid peroxidation product viz. MDA was decreased significantly in diabetic rats given Knol-Khol extract as compared to diabetic control where the levels were significantly high. This indicates that Knol-Khol extract may inhibit lipid peroxidation and thereby oxidative damage to tissues and organs in diabetes.

Under *in vivo* condition, glutathione (GSH) acts as an antioxidant and its decrease is reported in diabetes mellitus[17]. In the present study we found that, treatment with Knol-Khol extract significantly increased the glutathione content in blood when compared to diabetic control rats where the levels were significantly decreased. This increased GSH content in blood of the rats treated with Knol-Khol extract may be one of the factors responsible for the inhibition of lipid peroxidation.

Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPxase) are the three major scavenging enzymes that remove the toxic free radicals *in vivo*[16].

The activities of SOD, CAT and glutathione peroxidase are low in diabetes mellitus[18]. Erythrocytes were probably affected more due to the higher vulnerability thus causing inhibition of erythrocyte SOD, CAT and GSHPxase activity in diabetic rats. Antidiabetic treatment neutralized oxidative stress and increased erythrocyte SOD, CAT and GSHPxase activities[19].

The activities of SOD, CAT and GSHPxase were significantly lowered in the erythrocytes of diabetic control rats. However the treatment with Knol-Khol extract significantly increased the SOD, CAT and GSHPxase activities in erythrocytes of diabetic rats. This indicates that treatment with Knol-Khol extract increases the antioxidant enzymes activities in STZ induced diabetic rats and thereby scavenges the toxic free radical which are responsible for tissue damage *in vivo*.

Increased oxidative stress in chronic diabetic state has also been reported. Further, supplementation with Vitamin C and Vitamin E are reported to protect STZ diabetic rats against

oxidative stress[3]. Treatment with Knol-Khol extract lowered oxidative stress in STZ diabetic rats by lowering glucose level in blood and by increasing the antioxidant enzyme activities. This activity of the extract in the present study is probably due to the presence of Vitamin E, carotene and other antioxidant constituents in it. Vitamin C may not play any role here as it is a water-soluble vitamin, which may not be isolated with petroleum ether. The reason for decrease in the blood glucose level in Knol-Khol extract treated group could be increased responsiveness of the tissues to insulin or increased release of insulin and possibly due to regeneration of islets of langerhans in the pancreas. In the present study, pioglitazone was used as reference drug to compare the activity of Knol-Khol. The result suggests that the antihyperglycaemic activity of Knol-Khol extract was almost comparable to that of the pioglitazone.

Conclusion

From these results, we conclude that petroleum ether extract of Knol-Khol regenerated the pancreas, lowered hyperglycemia and oxidative stress. Thus, it may be useful for the treatment of diabetes and associated complications. However, the present study strongly recommends clinical trials with this extract to establish its role in the treatment of diabetes and its complications, if not an alternative to insulin or oral antihyperglycaemic agent, at least as an adjuvant.

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