Brassica oleracea var. acephala of kashmir as a promising candidate for improvement of lipid profile associated with hyperglycemia through in vivo study in male albino rats

Shabnum Shafi, Sunny Dhiman, and Gunjan Mukherjee
University Institute of Biotechnology, Chandigarh University, Gharuan, Mohali, Punjab 140413

Abstract. Diabetes mellitus commonly considered as clandestine killer is a metabolic disorder that affects people all over the world and is a silent killer. A variety of treatments are available, including insulin and oral anti-diabetic medications for regulating blood sugar levels in the body but every treatment option has certain unfavorable side effects that might cause abnormalities with the macro and microvascular systems in the human body.

Kale (Brassica oleracea var. acephala) from the brassica family is gaining popularity due to its phytochemical content and ability to prevent many ailments, notably those brought on by oxidative stress. The current study assessed Brassica oleracea var. acephala of the UT of Kashmir locally known as khanyari as it is loaded with various bioactive substances including total phenols, flavonoids, and glucosinolates that impart antioxidant and antidiabetic properties. Kale (K-29) showed impressive results in normalizing the blood glucose levels (102.30mg/dl) as well as improving the lipid profile such as cholesterol (76.22mg/dl), HDL (36.4mg/dl), LDL (36.1mg/dl), VLDL (21.2mg/dl) and triglycerides (85.40mg/dl) that is usually accompanied with hyperglycemia. The treatment of K-29 also showed improvisation in the various hepatic parameters such as S.G.P.T (91.14), S.G.O.T (41.18), Alkaline phosphatase (102.32U/L), Albumin (6.12g/dl), S. bilirubin direct (0.6mg/dl), S. bilirubin indirect (0.43mg/dl).

1 Introduction

During the previous couple of decades, there has been a growing interest in the general public with respect to food and its components on overall human health. Several consumers are in agreement with the fact that food provides energy and satisfy the hunger, also prevents the onset of various oxidative stress-related diseases [1]. In healthy diets, eating green vegetables are highly recommended. As far as green leafy vegetables are concerned, they are vital for human health, especially in terms of their bioactive nutrient molecules. Scientific outcomes claimed that adequate intake of green vegetables provides numerous positive outcomes such as reduced risks of some chronic diseases, decreased overall mortality, and increases life expectancy [2]. Approximately 10,000 plant species are being used as vegetables worldwide [3]. Among the various green leafy vegetables cultivated for human consumption mostly
belong to the family Brassicaceae, often known as Cruciferae. As a group, they are bursting with numerous health benefits and have high nutritional values. A diet rich in crucifers can significantly help to meet our daily Recommended Daily Allowance (RDA) that a human body requires to function at its best. This class of vegetables has got attention for several decades for lowering threat of various kinds of deep-seated illness such as cardiovascular diseases, carcinoma, diabetes, etc. [4].

Since the time immemorial, the utilization of plants and its derivatives were reported with potent application in treatment of diabetes [5]. Herbal plants showcase much greater significance in Diabetes mellitus (DM) management especially in majority of the developing nations, wherein the resources appear to be seemingly meagre. The Diabetes disease poses to be one of the existing metabolic disorders and a silent killer with a global prevalence in almost every country. From a global survey, the DM prevalence in India ranks to be highest in terms of the overall global reports of diabetics. Reasons for frequent global rise of this disorder includes sedentary lifestyle, consumption of energy-rich diet, obesity and life span [6]. The prevalence of DM is forecasted to be 422-425 million adults globally [7], and if the essential and suitable actions are not implemented today, it is projected that the number may climb to roughly 629 million (48 per cent increase) by the year 2045. In low-income nations like India, where healthcare is already scarce, diabetes poses a significant problem. Uncontrolled hyperglycemia due to glucose metabolism dysregulation characterizes this endocrinological disorder [8] which collapse pancreatic beta cells to produce insulin, or a cell damage to react with insulin in the blood [9] [10].

Multiple complications are a frequent result of this condition, with a continual and simultaneous rise in mortality index [11], despite the availability of a variety of pharmacological medicines such as insulin or oral hypoglycemic agents to regulate several features of this disease. Therefore, there is a pressing need to find and create some new therapies or treatments that are considerably more effective and potent in combating diabetes. Regarding diabetes treatment in particular, many plants have a extensive history of usage in traditional medical practices, suggesting that phyto-therapy may hold significant promise. Despite the fact that some plants offered antidiabetic activity [12], additional studies in this area are necessary before drawing any firm conclusions. Several efforts have been considered to manage and understand the DM as the disease and its impact which tends to worsen gradually. Despite the widespread availability of many pharmacological options for treating this illness, remedies based on medicinal plants remain popular. Plant products are in great demand since they are readily available, inexpensive, and have few negative side effects [13].

Recent studies have shown promising results for the treatment of diabetes-related postprandial hyperglycemia by using plant extracts that serve as natural inhibitors of carbohydrate- metabolizing enzymes. In this regard, the traditional Kashmiri vegetable kale (Brassicoleracea L var. acephala) with strong antioxidant nature, remains largely untapped, despite its potential to combat a wide range of degenerative disorders. Carotenoids, alkaloids, sterols, phenolic chemicals, and flavonoids are only some of the secondary metabolites found in abundance in the brassica groups and are accountable for imparting its bioactive and pharmacological effects. Furthermore, kale contains a wealth of pharmacological bioactive chemicals glucosinolates, including isothiocyanates, thiocyanates, nitriles, and epithionitrioles [14], [15], [16].

Antioxidant, anti-cancerogenic, anti-diabetic, and protection against cardiovascular and gastrointestinal system damage are few important biological properties linked with kale [[17]]. Both the anti-diabetic potential of this crop and the precise mechanism through which it exerts that potential remain poorly characterized. In Diabetes there is obstacle in the digestive system with weak metabolism and it has been surveyed that about 5% of world population are suffering from this disease and it takes much more lives when compared to AIDS globally. A huge population belongs to the nations like Bangladesh, India, USA, China,
Japan, Indonesia, Pakistan, Brazil, Russia and Italy are the victims of diabetes [18]. India is a country called Diabetes Capital of the world as every ¼ diabetic patient is an Indian with highly affected people being from urban population. Among them 25-26.5% people are in the first stage of diabetes i.e. prediabetic stage and population of 2.5-8% suffer from diabetes. There are many medicines and drugs available for reducing the hyperglycemia but every medicine has its own side effects. As it is a challenge for the whole medical community, it is must to search for a new compound/drug to get over from diabetes. When comparing to synthetic ones, plant-based medicines or herbal formulations are less toxic with lower side effects [19]. It can be observed that compilation and documentation of ethno ecological knowledge are being a top priority, as there is an increase in demand of herbal medicines. People who have lived in close proximity to plants for a long time may have insightful observations to share, and incorporating traditional knowledge is becoming increasingly important in many regions of the world [20]. Indian Ayurvedic Medicine Particularly the Ayurvedic and Unani systems advocate the use of natural medications to treat diabetes. It is important to gather all the information regarding the medicinal plants which were used by ethnic communities to get gradually shifted to traditional and natural medicine globally [21]. Some of the therapeutic molecules like minerals, alkaloids, vitamins, glycosides and volatile oils are synthesized and accumulated by a plant in its many parts such as rhizome, leaves, fruits and seeds [22]. By exploiting such plant parts, it will be helpful for improving the country’s economy and most importantly it provide good health care [23].

Brassicaceae and its family is made of 4060 species and 372 genera which comprises of Brassica oleracea, Brassica rapa and Brassica napus, Vitamins, glucosinolates, phenolic compounds, anthocyanins, carotenoids and nutrients that are abundant in Brassicaceae vegetables [24]. They are rich in ascorbic acid and phenolic compounds, both of which are powerful antioxidants. Hence, they may be helpful in preserving the vandalization done by reactive oxygen species (ROS) in human body. Kale (Brassica oleracea) is the class of vegetables that has got attention for several decades for minimizing the possibility of various kinds of chronic illness such as cardiovascular diseases, cancer, diabetes, etc. The Ayurvedic and Unani medical systems of India both make claimed that diabetes can with natural remedies. Kale's hypoglycemic impact has been researched extensively, both in animal models of diabetes and in people with type 1 and type 2 diabetes in humans. Numerous phytochemicals, including sterols, alkaloids, cardiac glycosides, and terpenoids, may be found in kale. It has been found that saponins have antidiabetic and hypocholesterolemic effects. Kale has a high concentration of plant phytochemicals like polyphenols, flavonoids, carotenes, glucosinolates, lutein, and zeaxanthin, and is boosted in vitamins (especially C and E), minerals (including Fe, Zn, and Mn), macronutrients (including Ca and Mg), dietary fiber, and glutamine (an amino acid with anti-inflammatory properties). Hyperglycemia can be managed with the use of Brassica oleracea because of its possible antidiabetic effects, which include correcting —Fasting blood glucose (FBG), oral glucose tolerance, glycated hemoglobin, insulin, and Hemoglobin levels [25-26]. Postprandial rises in plasma glucose levels are reduced by 7 percent after a single 7 g serving of kale, and a dosage of up to 14 g is safe. The B. oleracea var. acephala have been attributed for treating various kinds of illness such as Diabetes, weakness in bones, ophthalmologic complications. Brassica oleracea (var. capitata) minimize the risk of diabetic vascular problems and improve blood rheology in STZ-induced diabetic rats by preventing the loss of pancreatic β-cells, hyperglycemia, and the development of glycated hemoglobin [27].

2. Methodology:
2.1 Experimental animals

For the experiment, 24 adult male rats (180-200g) were obtained from IIIM, Jammu India (Institutional ethical clearance issued, Reg.No.1809/GO/ReBi-S/Re-L/15/CPCSEA). The animals were kept in a controlled environment with a standard temperature and humidity level and an alternated 12-hour light and dark cycle in the animal house of SKUAST-Kashmir’s faculty of veterinary science. The animals received unlimited access to food and water. The care and management of the animals was carried out in accordance with the standards established by the Committee for the Control and Supervision of Experiments on Animals (CPCSEA).

2.2 Experimental design

Twenty-four streptozotocin (STZ)-diabetic rats were subjected to a 21-day study of the effects of aqueous extract of Kale. They were randomly divided into four experimental groups (group1, group2, group3, group4 with six rats taken in each group. The six non-diabetic rats in the normal control group (group 1) and the STZ-induced diabetic rats in the diabetic control group (group 2) were given distilled water (1 ml) orally once daily. In the positive control group (group 3), the STZ-induced diabetic rats were given the standard drug glibenclamide (1 mg/kg.bw) orally. Members of Group 4 were given aqueous extract of kale at a daily dose of 200mg per kg of body weight for 21 days. On days 0, 7, and 21 of therapy, —blood glucose, serum insulin, and body weight were examined across all six groups. All of the animals in groups had their weights taken on a top-loading electronic weighing scale (Docbell, India). Snipping the tail provided the blood samples required for regular blood glucose/serum insulin measurement. On day 21, after all four groups of animals had been fasted for 3 hours, they were killed via cervical dislocation. Each sample of blood taken from heart of the rat was spun at 4,000 rpm for 10 minutes in a small, portable clinical centrifuge.

The 24 Wistar albino rats into four groups is given as:

Group I: Normal control
Group II: Diabetic/positive control (STZ)
Group III: Diabetic treated with Glibenclamide
Group IV: Diabetic group was treated with extracts of Kale (Khanyari)

2.3 Induction of diabetes

After dividing the rats into normal (n = 6) and diabetic cases (n = 6), the diabetic group will receive an injection of freshly prepared streptozotocin (50 mg per kg) in 0.1M citrate buffer (pH 4.5), which will cause diabetes. Rats with non-fasting plasma glucose levels more than 300 mg/dl after receiving STZ injections are deemed diabetic and are randomly allocated into three groups.

Oral glucose tolerance test (OGTT)

Rats given starch alone or in conjunction with plant extract were subjected to an oral glucose tolerance test, which revealed the influence on glucose levels at several time points. Blood will be drawn from the tail veins of normal (number of rats equal to 8) and two treatment groups of rats (n = 24) that have fasted overnight (no of rats equal to 8 for each). Blood samples will be taken from rats in the normal group at 15, 30, 60, 90, and 120 minutes after they consume starch, and their blood sugar levels will be evaluated using a commercial glucometer. The identical procedure will be used in the treated groups, except that the extract will be administered orally (at a dose of 1.5 g per kg) before the glucose load.
2.4 Estimation of serum triglycerides and lipid profile

Enzymatic determination of total cholesterol and triglyceride levels in blood samples was performed using a semi-autoanalyzer (RMS, India) and kits purchased from Agappe Diagnostic Ltd., India.

2.5 Determination of various parameters of liver

The hepatic parameters for the blood samples taken from various rat groups after the period of 21 days which were given different treatments were analyzed according to instructions of manufacturer of kit.

2.6 Determination of liver glycogen content

Hydrolysis of the frozen liver tissue (50 mg) was performed using 30% KOH(2ml) in a boiling water bath (100°C) for 15 minutes. 2.4 ml of 95% ethanol was added to cold liver hydrolysate. After an overnight incubation at 4°C, the mixture was centrifuged at 3000 rpm for 15 minutes. After 10 minutes of inverted drainage, the supernatant was discarded and the glycogen pellet was collected. After adding 5 ml of anthrone reagent (0.05 percent anthrone, 1 percent thiourea, 72 percent (v/v) H$_2$SO$_4$) and placing the test tubes in cold water, the pellet was liquefied in 1 ml of distilled water with vigorous shaking. The reaction mixture was heated to 100 ºc in a boiling water bath for 15 minutes before being cooled under running water. The Hitachi U-1800 Spectrophotometer (USA) was used to measure the absorbance at 620 nm of each reaction combination. Glucose was used as standard for the quantification of glycogen content.

Glycogen content(mg/g) = DU/DSX0.1vol of extract/g of tissue x100x0.9

Where DU=optical density of unknown
DS=optical density of standard
0.9=conversion factor for converting glucose value to glycogen value

2.7 Statistical analysis

The statistical analysis has been employed using CRD with the help of ANOVA test to analyze the variance using LSD. The comprehensive statistical package SPSS (Version 20) for windows has been implemented or the study.

3 Results

In this study the Kale was given to STZ-induced diabetic Male wistar albino rats for its hypoglycemic index. The lipid profile, various enzymes of liver, antioxidant enzymes were monitored.
3.1 Acute toxicity testing

The selected genotype was scrutinized for any possible toxicity. For this wistar albino rats were fed with different doses (100mg/kg, 500mg/kg, 1000mg/kg) of k-29. There was no sign of toxicity or mortality because kale itself is loaded with antioxidant and can be considered as a natural herb.

3.2 Oral glucose tolerance test

In glucose-loaded animals, dose-dependent and substantial reduction in blood sugar levels were seen after brassica oleracea treatment. At dose of 50, 100, 200, and 400 mg per kg of body weight, it reduced the amount of sugar in the blood by 13.9, 30.6, and 47.9%, respectively. Glibenclamide, a frequently used antihyperglycemic pharmaceuticals, reduced blood glucose levels by 48%. Thus, the ability of plant extract to decrease the blood sugar was approximately similar to that of glibenclamise at the maximum dose of 200mg/kg.

3.3 Effect of kale extract on blood glucose levels in streptozotocin-induced diabetic rats

The figure clearly indicates that administration of STZ to all groups caused an increase in fasting BGLs within 3 days. In the diabetic control group, the BGL was raised (230 mg/dl) throughout the 21 days of study group. Experimental outcomes showed that the conc. of Brassica oleracea extract impacts in a dose dependent way. However, giving kale extract orally to diabetic rats was able to cause significant reduction in the raised BGLs upto 102.3 mg/dl during the experiment. The results obtained were very close to the levels seen in rats which were given standard drug Fig.2.
Fig. 2. Effect of kale on glucose levels of rats in various groups. Values are represented as Mean ± SD

4 Effect of k-29 extract on lipid profile

4.1 Effect of K-29 on serum cholesterol in streptozotocin-induced diabetic rats

The Fig. 3. clearly displayed the administration of STZ to albino rats increased the serum cholesterol levels significantly time variant during 21 days of experimental period. It was seen that the kale extract given orally at the dose of 200 mg/kg b.w to the diabetic rats significantly decreased the levels of cholesterol up to 76.22 mg/dl. The results obtained were close to those observed by giving the standard drug.

Fig. 3. Effect of kale on Cholesterol levels. Values is represented as Mean ± SD
4.2 Effect of k-29 extract on HDL in streptozotocin- induced diabetic rats

The diabetic rats treated with streptozotocin increased the HDL levels drastically. The delivery of K-29, an extract from Brassica oleracea, caused HDL levels to decline as shown in fig 4. Untreated diabetic rats' HDL levels were found to be much higher than those of normal rats. HDL levels dropped significantly, reaching a maximum impact of 36.4 mg/dl in the group receiving 200 mg/kg of Brassica oleracea extract.

![Fig 4. Effect of kale on HDL levels. Values is represented as Mean ± SD](image)

4.3 Effect of k-29 extract on LDL in streptozotocin- induced diabetic rats

The figure clearly displayed the administration of STZ to albino rats increased the serum LDL levels significantly in a time dependent manner during 21 days of experimental period. It was seen that the kale extract given orally at the dose of 200mg/kg b.w to the diabetic rats notably decreased the levels of cholesterol upto 36.1 mg/dl. The results obtained were close to those observed by giving the standard drug as shown in fig. 5.
4.4 Effect of k-29 extract on VLDL in streptozotocin-induced diabetic rats

The figure clearly displayed the administration of STZ to albino rats increased the serum VLDL levels notably in a time dependent manner during 21 days of experimental period. It was seen that the kale extract given orally at the dose of 200mg/kg b.w to the diabetic rats significantly decreased the levels of VLDL upto 21.2mg/dl as shown in fig 6. The results obtained were close to those observed by giving the standard drug.
4.5 Effect of k-29 extract on serum triglycerides in streptozotocin-induced diabetic rats

The figure clearly displayed the administration of STZ to albino rats increased the serum triglycerides remarkably in a time dependent manner during 21 days of experimental period. It was seen that the kale extract given orally at the dose of 200mg/kg b.w to the diabetic rats significantly decreased the levels of serum triglycerides upto 85.40 mg/dl as shown in fig 7. The results obtained were close to those observed by giving the standard drug.

![Effect of kale on Triglycerides](image)

**Fig.7.** Effect of kale on Triglycerides. Values are represented as Mean ± SD

4.6 Effect on hepatic parameters in streptozotocin-induced diabetic rats

The effect of kale extract on liver enzymes of albino wistar rats was analyzed. The level of serum bilirubin direct and indirect, serum glutamic-pyruvic transaminase (S.G.P.T), Serum Glutamic Oxaloacetic Transaminase (SGOT), S. Alkaline Phosphatase and albumin was assessed. The outcomes were compared with the standard drug Glibenclamide (100 mg/kg)

The k-29 showed 91.14µl of S.G.O.T compared to standard drug that exhibited the level of S.G.O.T of 93.16µl(fig 8). Figure 9 displayed the level of S.G.P.T (U/L) in various rat groups. S.G.P.T should be low for best outcomes, and the findings clearly show that it is lower in rats administered with k-29 extract compared to normal control and diabetic group. The k-29 dosage of 200 mg/kg showed S.G.P.T levels as 41.18(U/L) comparable to those of glibenclamide that is 40.55 (U/L). The level of alkaline phosphatase seemed to decrease when rats were administered with k-29 that is 102.32(u/l) at dosage of 200mg/kg(fig 10). Figure 11 shows the level of S.Bilirubin Direct. The results obtained after the rats were fed with k-29 at the dosage of 200mg/kg were 0.6mg/dl that were comparable to those of glibenclamide that showed the level of S.bilirubin Direct as 0.48mg/dl. Figure 12 shows the level of albumin. Oral treatment of the k-29 to rats at a dosage of 200 mg/kg lowered albumin levels to 6.12(g/dl) compared common medication glibenclamide that showed the level of albumin as 7.52(g/dl). Figure 13 shows the level of S.Bilirubin indirect. The level was lowered after therapy with k-29 extract which showed the level of S.Bilirubin indirect as 0.34(mg/dl) compared to those of glibenclamide that gave results as 0.48mg/dl.
4.7 Effect of Kale extract on liver glycogen content in streptozotocin-induced diabetic rats:

The lower glycogen content in the liver of diabetic rats in the current investigation reflected the diminished ability of the liver to synthesise glycogen in diabetes. It was discovered that some of the STZ-induced diabetic control group rats' liver samples contained much less hepatic glycogen than expected. However, the lowered glycogen content was dramatically raised in a dose-dependent manner after treatment with the aqueous kale extract. Further research revealed that within 21 days of the trial period, daily oral dosage of 200 mg/kg.bw of the extract nearly restored and normalised the lowered glycogen levels. As shown in fig 14. The recovered levels were 6.1 mmol/L, which were almost identical to the treatment with glibinclimide (6.2 mmol/L).
Type I diabetes is characterized by the degradation of insulin-secreting beta cells in the pancreas due to an autoimmune response, while type II diabetes is characterized by the development of insulin resistance in body cells. More than 150 million individuals worldwide are living with Type II diabetes right now, 90% of whom are adults. This is an extremely high number. Although there are a lot of pharmacological treatments, such as insulin or oral hypoglycemic agents, accessible to regulate various elements of this condition, many complications are a typical result of this disease, and the death index is rising steadily alongside it. Therefore, there is a pressing need to discover and create some cutting-edge diabetic therapies or medications with much improved efficacy and potency. Numerous plants have a long history of usage in traditional medical practices, particularly for the management of diabetes, suggesting that phyto-therapy may hold considerable promise in this area. From the dawn of time, humans have relied on edible plants, particularly green leafy vegetables, as a significant source of nutrients that have an impact on their health and wellbeing. Vegetables, which are rich in phytonutrients including minerals, vitamins, dietary fibre, and phytochemicals, make up a large percentage of the average human diet in many regions of the world [28]. Phytochemicals included in vegetables are powerful antioxidants that may reduce the risk of chronic illnesses by eliminating free radicals, detoxifying carcinogens, and influencing the processes involved in the reprogramming of tumors cells [29]. In fact, atherosclerosis and lipid abnormalities are the leading causes of cardiovascular illness in people with diabetes. Lipoprotein lipase is an enzyme responsible for hydrolyzing triglycerides in a healthy metabolic state in response to insulin. Research suggests that the ideal treatment of diabetes, in addition to glycemic control, should have a favorable effect on lipid profiles. There are many reports that suggest supplementation of kale leaves has beneficial effects on the lipid profile of streptozotocin-induced diabetic rats. Treatment of diabetic rats with kale extract significantly controlled and reduced the increased levels of serum lipids (triglyceride and cholesterol) in a dose-dependent manner, as compared to the untreated diabetic control group. The possible mechanism of kale's lipid-lowering effect will be due to insulin-mediated activation of lipoprotein lipase or the inhibition of cholesterol biosynthetic pathway. Our findings are consistent with those of a previous study in which supplementation with Brassica species reduced blood glucose and lipid profile (total cholesterol, triglycerides, low density lipoprotein cholesterol, and very low-density
lipoprotein cholesterol‖) in males who were not insulin-dependent diabetics. The liver (the master gland) is crucial in the control of postprandial hyperglycemia because it is a significant location for endogenous glucose synthesis via gluconeogenesis or glycogenolysis. Hyperglycemia is linked to diabetes and is caused by increased endogenous glucose synthesis caused by impaired pancreatic function and/or decreased glucose clearance. Reduced glycogen content in the liver of STZ-induced diabetic rats is reflective of their impaired capacity of the liver to synthesize glycogen as compared to the normal group. Impairment of glucose storage capacity is one of the major symptoms in diabetes, along with defective glucose uptake of the cells, and is attributed to the lack of glycogen synthase activity, the rate-limiting enzyme in glycogenesis. However, the liver glycogen content of diabetic rats was dramatically enhanced after 21 days of daily oral administration of the k-29 extract, with the maximum amount seen in the liver of rats provided with 200 mg/k.bw of the kale extract. The results showed that kale extract treatment boosted hepatic glycogen content to a level similar to that of normal rats and was closer to that of Glibinclamide-treated diabetic rats. The results strongly show that therapy with 200mg/kg.bw kale extract was significantly more successful in normalizing the poor glycogen storage of the diabetic group compared to the standard medication (glibinclamide).

Conclusion

The anti-diabetic and antioxidant effects of the many kale genotypes of the Kashmir valley have not been evaluated, despite their widespread usage than any other plant component in the treatment of a wide variety of ailments. Similarly, not all genotypes have been examined for secondary metabolites such phenolic compounds, alkaloids, and steroids, nor has a link been established between these metabolites and anti-diabetic ability. Therefore, the primary goal of the present study was to make use of the health-enhancing properties and anti-diabetic potential of kale

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