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Hypoglycemic and Hypolipidemic Activity of *Vitis vinifera* Leaf Extract in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

The aim of the study is to analyse the hypoglycemic and hypolipidemic effect of oral administration of methnolic extract of *Vitis vinifera* (MEVV) on Streptozotocin (STZ) induced diabetic rats. Oral administration of methnolic extract of *Vitis vinifera* (MEVV) to diabetic rats for 30 days significantly reduced the levels of blood glucose and lipids. The purpose of the study was to investigate the effect of oral administration (400 mg/kg) of the methnolic extract of *Vitis vinifera* which active ingredients act as hypogycemic and hypolipidemic. The methnolic extract of *Vitis vinifera* (MEVV) supplementation is useful in controlling the blood glucose level, improves the plasma insulin, lipid metabolism and is beneficial in preventing diabetic complications in experimental diabetic rats; therefore, it could be useful for prevention or early treatment of diabetes mellitus. It also warrants further investigation to isolate and identify the hypogycemic and hypolipidemic principles in this plant so as to elucidate their mode of action.

KEYWORDS

Vitis vinifera, Antioxidant, Blood Glucose, Diabetes mellitus, Free radicals, Oxidative stress

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INTRODUCTION

Diabetes mellitus is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels. Treatment with sulphonylureas and biguanides are associated with side effects[2]. However, for a number of reasons, complementary medicine has grown in popularity in recent years. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine were used commonly in India. Many indigenous Indian medicinal plants have been found to be useful to successfully managediabetes and some of them have been tested and their active ingredients isolated[3]. The World Health Organisation has also recommended the evaluation of the plants for their effectiveness and conditions where we lack safe modern drugs[4]. In recent years, much attention has been focused on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications[5]. Free radicals are continuously produced in the body as a result of normal metabolic processes and interaction with environmental stimuli. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems that has increased free radical production or reduced activity of antioxidant defences or both. Implication of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free-radical generation but also due to non-enzymatic protein glycosylation auto-oxidation of glucose, impaired glutathione metabolism, alteration in antioxidant enzymes and formation of lipid peroxides[6]. Many of the complications of diabetes mellitus, including retinopathy and atherosclerotic vascular disease, the leading cause of mortality in diabetes mellitus, have been linked to oxidative stress, and antioxidants have been considered as treatments[7] . *V. vinifera* a large deciduous climber, climbing by means of intermittent, leaves opposed, large, often bifid tendrils, cultivated in many part of India[8] . From the different parts of this plant, in particular from the fruits, several preparations that are used in folk medicine have been derived. Among the most interesting constituents responsible for the therapeutical properties of the plant, procyanidins (other names: procyanidolic oligomers or OPC, leucoanthocyanins, condensed tannins, pycnogenols) received particular attention and are used for the

treatment of microcirculatory disorders. More recently, procyanidins have been demonstrated to be among the most interesting antioxidant agents from Plant Kingdom, and are considered for the preventive therapy of chronic degenerative diseases and the modulation of skin unattractiveness linked to the aging process**.** It is also used as a nervine tonic[9]. The chemical analysis has shown the presence of procyanidins, anthrocyanins, flavanoids, hydroxylcinnamic acid derivatives, triterpenes, sterols, tannins, polysaccharides, monosaccharides,and non alkaloid nitrogen containing compounds[10]. The objective of this investigation was to ascertain the scientific basis for its use in the treatment of diabetes mellitus. Therefore, this study was designed to investigate the protective effect of *Vitis vinifera* on lowering the blood glucose level, tissues lipid peroxides and enzymic antioxidants in STZ-induced diabetic rats.

MATERIALS AND METHODS

Drugs and chemical used: STZ was purchased from Sigma Chemical Co St. Louis, MO, USA. All the other chemicals used were of analytical grade and purchased from commercial sources. Other chemicals used for extraction purpose and phytochemical tests were of laboratory grade.

Collection and authentication of plants: The leaves of the plant were collected from the Balaji Nursery, Jagatpura, Jaipur district, Rajasthan State, India in the month of March 2009. The identity of the collected plant was confirmed by Dr. / Mr. P. J. Parmar, Joint Director of Botanical Survey of India (BSI), Jodhpur (Rajasthan, India) the herbarium of the plants was deposited in the BSI against voucher specimen NO. JNU/JPR/PC/JS-1.

Preparation of plant extract: The leaves of plant were washed, shade dried and powdered. The powdered material was defatted with petroleum ether and extracted with methanol by cold maceration process. The extract was concentrated for further studies at reduced pressure and temperature in a rotary evaporator. Methanolic extract was tested for presence of secondary metabolites by different phytochemical tests.

Experimental animals: Swiss Albino mice (25-30 gm) and healthy Wistar albino rats of either sex (150-200 gm) were taken for study. They were housed in polypropylene cages in air- conditioned area at 25±2 ºC with 12/12 h light/dark cycle. All animals had free access to standard pellet diet (Mahavir industries,

Delhi) and clean water *ad libitum*. The norms for Good Laboratory Practice (GLP) were followed for care of laboratory animals. The present studies were duly approved by IAEC (Institutional Animal Ethical Committee clearance) 002/2009/IAEC/JNU.

Acute toxicity test:[11] Acute oral toxicity study for the test extract of the plant was carried out using OECD/OCED guideline 425. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity. Healthy, young adult albino Wistar rats of either sex (200 -250 g) were used for this study. Animals should be fasted prior to dosing (food but not water should be withheld Overnight). The fasted body weight of each animal is determined and the dose is calculated according to the body weight.

Antidiabetic activity:[12-13] The animals fasted overnight and diabetes was induced by a single intraperitoneal injection of freshly-prepared STZ (30 mg/kg body weight of rats) in 0.1 M citrate buffer (pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 200 mg/dL on the third day after the STZ injection. The treatment was started on the fourth day after the STZ injection and this was considered the first day of treatment. The treatment was continued for 30 days.

The rats were divided into four groups comprising eight animals in each group as follows:

Group 1: Control Group rats were administered only buffer.

Group 2: Diabetic controls Group (STZ 30 mg/kg) body weight of rats.

Group 3: Diabetic rats treated with *vitis vinifera* (MEVV) (400 mg/kg body weight of rats/day) for 30 days.

Group 4: Diabetic rats treated with protamine-zinc insulin *i.p*. injection (6 units/kg body weight of rats/day)

Vehicle of MEVV and insulin were administered once daily for 30 days from the induction. Blood was drawn from tip of tail and blood glucose level was estimated on 0, 7th, 15th, and 30th day of experiment with the help of glucometer (one touch Ultra Johnson and Johnson Ltd.) using strip method on 30th day. Blood sample was taken by orintal sinus bleeding mehod for measuring serum cholesterol

and TG level using an auto analyser (CHEM 400). Fresh urine was collected and glucose and ketone in urine were checked using keto diastix strip on 0 to 30th day of experiment.

All the grouped data were statistically evaluated using the Statistical Package for Social Sciences (SPSS) Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant differences test. p-values of less than 0.05 were considered to indicate statistical significance. Al the results were expressed as mean ± standard deviation (SD) for eight animals in each group.

screening revealed the presence of flavonoids, saponins and carbohydrate, tannins and phenolic compound in methanolic extract.

Acute toxicity test: Acute toxicity studies revealed that *V. vinifera* extract did not produce any toxic symptoms when administered (2000mg/kg) orally to rats.

Antidiabetic activity: A significant increase in the level of blood glucose and a decrease in body weight were observed in diabetic rats when compared to control rats. Administration of *V. vinifera* and insulin to diabetic rats significantly decreased the level of blood glucose and increased body weight gain to near control level [Table1].

Table 1 Effect of MEVV for 30 day on blood glucose level in STZ – induced diabetic rats

Groups	**Blood glucose (mg/dl) in day**				**Change in body weight (g)**
	0	7	15	30	
Normal control	82.0 ± 4.6	84.2 ± 2.6	89 ± 3.6	32.4 ± 2.3	32.4± 2.3
Diabetic control	273.8 ± 6.0	278.3 ± 7.0	279.5 ± 5.7	269.8 ± 6.4	-34.1 ± 2.0*
Diabetic rats+ MEVV(400mg/kg)	279.5± 5.7*	259.5 ± 3.9*	194.5 ± 4.7*	119.5 ± 2.57*	21.6 ± 1.3*
Diabeticrats+insulin	269.5 ± 3.4*	249.5 ± 5.76*	179.5 ± 6.7*	112.5 ± 3.17*	20.0 ± 1.1*

Values are given as mean ± SD for groups of 8 animals each. Values are statistically significant at *p<0.05. Diabetic rats were compared with control rats; MEVV treated diabetic rats were compared with diabetic rats; insulin-treated diabetic rats were compared with diabetic rats.

RESULTS

Phytochemical screening: Phytochemical

Serum lipid profile: The effect of MEVV and insulin in the untreated diabetic rats serum levels of cholesterol and TG were significantly increased [Table 2]. The effect of the standard drug (insulin) on serum TG and cholesterol in the diabetic rats were comparable to those of the herbal extract. Total cholesterol and TG were significantly elevated in diabetic group in comparison to control group. Administration of MEVV for 30th day significantly reduced the serum levels of cholesterol and TG in comparison to diabetic control [table 2].

Urine glucose and ketone

Table 2 Effect of MEVV for 30 days on serum lipid profile in STZ- induced diabetic rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Normal control	88.0 ± 7.6	74.6 ± 5.16
Diabetic control	185.4 ± 8.3	165.4 ± 6.13
Diabetic rats + MEVV	98.7 ± 9.0*	87.7 ± 4.3*
Diabetic rats + insulin	81.1 ± 3.69*	79.0 ± 7.6 *

Values are given as mean ± SD for groups of 8 animals each. Values are statistical y significant at *p<0.05. Diabetic rats were compared with control rats; MEVV treated diabetic rats were compared with diabetic rats; insulin-treated diabetic rats were compared with diabetic rats.

Table 3 Effect of MEVV for 30 day on urine glucose and ketone in STZ – induced diabetic rats

Groups	0 day		30 day	
	Glucose	Ketone	Glucose	Ketone
Normal control	-	-	-	-
Diabetic control	+++	trace	+++	trace
Diabetic rats + MEVV	+++	trace	-	-
Diabetic rats + insulin	+++	trace	-	-

+++ = presence of glucose, -- shown absence of gluccose and ketone

Urine analysis on day 0 showed the presence of glucose (+++) in entire group, except normal control. However, on 30th day glucose and ketone traces were absent in MEVV and insulin treated groups while they were present in diabetic control group [Table 3].

DISCUSSION

Various types of plants are used to treat diabetes. These studies have identified that compounds such as polysaccharides[14] flavonoids terpenoids, tannins and steroids are responsible for antidiabetic effect[15]. STZ a beta cytotoxin, induces diabetes mellitus by damaging the insulin secreting beta cells of the pancreas, resulting in decreased endogenous insulin release, STZ administered rats become hyperglycaemic in a short period of time, followed by hepatic glucose overproduction. Administration of STZ (30 mg/kg) effectively induces diabetes mellitus in normal rats. The antihyperglycaemia effect of MEVV includes the stimulation of beta cells and/or subsequent release of insulin and / or activation of the insulin receptors[16]. The possible mechanism of action of MEVV could be correlated with promoting insulin secretion by closure of K^+ATP channels, membrane depolarization and stimulation of Ca^+ influx, an intial key step in insulin secretion[17]. Traditional plant remedies have been used for centuries in the treatment of diabetes but only a few have been scientifically evaluted[18]. Diabetes is associated with hyperlipidemia. It is well known that insulin activates enzyme lipoprotein lipase which hydrolyzes triglyceride under normal condition. Destruction of beta cells leads to depletion of plasma insulin, which results hyperlipidemia. The significant control of

plasma lipid levels suggests that the MEVV may produce its action by improving insulin secretion[19]. Diabetogenic agents significantly increase the cholesterol and TG level. The impairment of insulin secretion results in enhanced metabolism of lipids from adipose tissue to the plasma. Further it has been reported that diabetic rats treated with insulin shows normalised lipid levels[20] .Thus the results indicate the MEVV shows insulin like actionby virtue of its lipid lowering levels. STZ induced diabetes is characterized by severe loss in body weight and this was also seen in the presence study. MEVV and insulin administration controlled this loss in body weight. However, it did not normalize the body weight completely as it remained lesser than normal control rats. The decrease body weight observed in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source[21]. The most significant findings of this study is that the MEVV has shown beneficial effect not only on blood glucose, but also on glucose and ketone levels of urine in STZ induced diabetic rats. Urine analysis on 0 day showed the presence of glucose and traces of ketone in the entire group except normal control. However, on 30th days glucose and ketone traces were absent in MEVV and insulin treated groups. While they were present in diabetic control. The results obtained from this study are quite promising and comparable with insulin a standard drug use to treat DM. The observation confirm that methanolic extract of the leaf of the plant has antidiabetic activity and is also involved in correction of altered biological parameters. It also warrants further investigation to isolate and identify the hypoglycemic principles in this plant so as to elucidate their mode of action.

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