A Study on Insulin Levels and the Expression of *Glut 4* in Streptozotocin (STZ) Induced Diabetic Rats Treated with Mustard Oil Diet

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Abstract The study was undertaken to evaluate the therapeutic effect of mustard oil incorporated diet in streptozotocin (STZ)-induced type 1 diabetic rats. Dietary composition has shown to play a significant role in improving insulin sensitivity. Various authors have reported the hypoglycemic effect of mustard oil in experimentally induced diabetic rats. In the present study, reverse transcriptase polymerase chain reaction (RT-PCR) was done to analyze the *Glut 4* expression in STZ induced diabetic rats as it is a key player in glucose homeostasis. The effect of mustard oil on serum biochemical parameter and insulin levels was also studied. Twenty-four male Wistar rats were randomly divided into three different groups with each containing eight animals. The first, second and third groups were control, diabetic control and treatment group with mustard oil respectively. All the rats in respective groups were fed for 60 days with iso-caloric mash diet containing 8% lipid. Diabetes was induced by intra-peritoneal administration of STZ (40 mg/kg body weight). A highly significant reduction in blood glucose level, with an increase in insulin activity was observed in mustard oil-treated diabetic rats when compared to control group indicating anti-hyperglycemic activity of mustard oil. Mustard oil-treated diabetic rats showed increased expression of *Glut 4* in muscle tissue when compared to

diabetic control. A significant reduction in the levels of triacylglycerols, total cholesterol, VLDL and LDL and raised plasma HDL were noticed in mustard oil-treated diabetic rats when compared to diabetic control rats. Histopathological studies revealed a mild regeneration of β cells of pancreas in mustard oil-treated diabetic rats. The results from our investigation suggest that mustard oil elicits hypoglycemic effect by increased insulin activity and up-regulation of *Glut 4* gene expression in muscle tissue of STZ-induced diabetic rats.

Keywords *Glut 4* · Hypoglycemia · Mustard oil · Fatty acid analysis · Wistar rat · RT-PCR

Introduction

Diabetes mellitus (DM) is an endocrine and metabolic disorder, that occurs due to defects in insulin secretion, insulin action or both resulting in hyperglycemia with disturbances in the metabolism of carbohydrates, proteins and fats [1]. Management of diabetes by improving glycemic control is one of the most important therapeutic approaches for preventing diabetic complications [2]. Chronic hyperglycemia during the diabetes mellitus leads to biochemical changes and tissue destruction. Numerous researches are going on in developing of oral anti-hyperglycemic drugs; but the success rate is very nominal with individual therapy, therefore combination therapy is followed with different diet charts [3]. There are many anti-hyperglycemic drugs available in the market with serious side effects such as liver damage, loss of body weight, impaired vision etc. Therefore, investigations are conducted with herbal drugs to reduce glucose level without damaging other organs and causing fewer environment

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damages. WHO also supports the development of herbal drugs [4]. These negative impacts on the body have been reduced by antioxidants presents in plants and essential oil. Vegetable oils are being in use as therapeutic agents for years without any significant adverse health effects [2]. A type of ancient Indian ayurvedic treatment for diabetes includes “Snehana”, a practice where oils were applied externally or administered internally [5]. Incorporation of vegetable oils like sunflower oil [6], *Nigella sativa* oil [7], olive oil [8], sesame oil and mustard oil [9] has shown beneficial effects in patients with diabetes. Though the beneficial effects have been demonstrated, the molecular mechanisms behind these effects have not yet been fully understood. Diets rich in unsaturated fatty acids have proven to elicit better glycemic control. Mustard oil is rich in unsaturated fatty acids particularly oleic, linoleic and linolenic fatty acids. Hence, mustard oil was chosen as a preliminary choice to investigate molecular mechanisms behind reduction of blood glucose. Diets incorporated with variety of fatty acids are of special interest as they influence the glucose metabolism by altering cell membrane function, enzyme activity, insulin signaling and gene expression [10]. There are 13 GLUT isoforms identified in human beings with distinct tissue specificities and kinetic properties [11]. The principal glucose transporter protein that mediates the uptake of glucose is glucose transporter 4 (GLUT 4), which plays a key role in regulating body glucose homeostasis. Glucose transport into muscle cell is also regulated by the concentration of GLUT 4 on the cell surface [12]. Incorporation of fish oil, rice bran oil and other oils in the diet induces the expression of *Glut 4* in rat muscle [13]. In our previous unpublished works, we have observed a reduction in blood glucose levels when different vegetable oils were incorporated in the diet. Hence, the present study was undertaken as a pilot study to check whether mustard oil (*Brassica juncea* oil) has any influence on glucose levels and on *Glut 4*. Though many isoforms of glucose transporters are playing role in maintaining glucose homeostasis, we had chosen GLUT-4, as it is an major insulin responsive glucose transporter in skeletal muscle. The following study was a preliminary step for the investigation of molecular mechanism involved in reduction of blood glucose level with the use of mustard oil. A further detailed study of the expression of various genes related to diabetes during administration of mustard oil was done at our laboratory, which will be published later. This study was mainly focused to investigate the effect of mustard oil in reduction of blood glucose level, circulating insulin levels and expression of *Glut 4* in muscle tissue.

Materials and Methods

Chemicals and Animals

Analytical grade streptozotocin (STZ) was purchased from Sigma Chemicals Co., St. Louis, USA. The diagnostic kits for the assay of glucose, total protein, albumin, cholesterol, HDL cholesterol, triacylglycerol, urea, creatinine, alanine aminotransferase, aspartate aminotransferase, calcium, phosphorus, sodium and potassium were purchased from M/s Agappe diagnostics, Ernakulum, Kerala, India. Insulin IRMA (Immuno Radio Metric Assay) kit was purchased from M/s Anand Brothers, Delhi, India. Mustard oil (Fortune, trade name) was purchased from local market. Ghee was procured from Dairy plant, Madras Veterinary College, Chennai-07. Wistar albino rats (weighing about 150–200 g) were procured from Laboratory Animal Medicine Unit, TANUVAS, Chennai-51.

Experimental Animals

Male Wistar rats of 5 weeks of age, were housed in polypropylene cages on clean corncob beddings and were maintained under a controlled temperature of $20 \pm 2^\circ\text{C}$ (12 h light:dark cycles) and were fed ad-libitum supply of feed and purified water. Rats were acclimatized for 2 weeks prior to the start of treatment. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), Madras Veterinary College, Chennai-07 and animals were taken care according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Experimental Diets

The experimental rats were fed with ad-libitum, isocaloric mash diets during the experimental period. The diets were formulated considering the energy and protein requirement of the rats. The formulated diets were prepared and procured from Central Feed Technology Unit, Katupakkam, Kanchipuram. The diets had similar composition with an exception of fat source, which consisted of 8% of ghee for group II which was diabetic control and mustard oil for Group III which was diabetic treatment.

Induction of Diabetes

The selected animals of Group II and III were fasted overnight and a single intraperitoneal injection of a freshly prepared solution of STZ (40 mg/kg b.wt) dissolved in 0.1 M cold citrate buffer (pH 4.5) was administered to induce diabetes. The development of diabetes was

confirmed by measuring blood glucose concentration after 72 h of injection with STZ. The rats with a blood glucose level above 250 mg/dl were considered diabetic and selected for the experiment. The fourth day, after STZ injection was considered as the day one of treatment and the experiment was continued for 60 days [14].

Experimental Design

Twenty-four male Wistar rats were randomly selected and divided into three groups with eight animals per group. Experiment was conducted for a period of 60 days. The mean body weight variation of rats was not more than 20% in between the groups.

Group I: Normal control

Group II: Diabetic control

Group III: Diabetic + mustard oil diet

At the end of the experiment, all the animals were sacrificed as per the guidelines of IAEC at the end of the experiment. Blood samples were collected on the day of sacrifice. Serum was separated by centrifugation at 3000 rpm for 15 min and was used for the estimation of glucose, cholesterol, triacylglycerol and HDL-cholesterol as per the protocol described in the standard kits using CECIL CE 2021 UV spectrophotometer. Insulin was estimated in the serum using Immuno Radio Metric Assay (IRMA) kit. Muscle tissue was collected in RNase lather solution and stored at -20°C for expression study. Tissue samples of the pancreas were collected in 10% formalin for histopathological studies [15–18].

Estimation of Fatty Acid Composition in Serum and Muscle Using Gas Chromatography (GC)

Estimation of fatty acid composition in serum and muscle was done according to Folch et al. [19]. In brief, 4 g of sample was triturated with 10–20 ml of chloroform–methanol mixture (for 2 min), kept stable for 6–8 h and then filtered through Whatman no. 42 filter paper, rinsed with 5 ml folch solution. To the filtrate 25% of 0.88% sodium chloride solution was added and kept overnight. Later chloroform layer was collected. Vacuum evaporation of chloroform at 45°C was done and 30 ml of alcoholic KOH (10%) was added and kept at dark overnight. Saponification was done under reflux for 1 h. Content cooled and transferred to separating funnel. 30 ml of distilled water and 50 ml of hexane were added for phase separation. Lower phase was collected, to which 15 ml of HCl and 50 ml of hexane were added and allowed to separate in a separating funnel. The hexane (the upper layer) was collected and step was repeated with 30 ml of distilled water and 50 ml of hexane. Finally, the hexane

was evaporated to dryness. 1 ml of methanol and 3 ml of acetyl chloride methanol mixture were added to dried hexane layer and heated at $85\text{--}90^{\circ}\text{C}$ for an hour. The test tubes were cooled and transferred to centrifuge tubes with addition of 8 ml of 0.88% sodium chloride and 3 ml of n-hexane and centrifuged at 2000 rpm for 10 min. Top hexane layer was collected, sodium sulphate was added to remove moisture and filtered through $0.22\text{ }\mu\text{m}$ membrane filter. The fatty acid methyl esters were analyzed using gas chromatography with flame ionization detector with SPTM-2380 capillary column.

Real Time PCR

Total RNA was extracted from muscle tissue using TRIZOL[®]LS reagent as per manufacturer's instructions (Sigma Aldrich, USA). cDNA synthesis (reverse transcription) was performed using random hexamers with the initial concentration of 500 ng of total RNA isolated from each tissue sample using the high capacity cDNA synthesis kit as per manufacturer's instructions. Oligonucleotide primers for the *Glut 4* were designed using primer designing bioinformatics software tool . PRIMER 3 PLUS. For endogenous control β -actin gene, published primers were used [20]. Real-time PCR was carried out in Eppendorf gradient 4 Mastercycler[®] (Eppendorf, Germany). Forward and reverse primer sequence for rat β actin gene were 5'TTGCTGACAGGATGCAGAAG3' and 5'ACATCTGCTGGAAGGTGGAC3' respectively, while forward and reverse primers for *Glut 4* were 5'TTCATCTTCA CCTTCCTA3' and 5'CCTAAGTATTCAAGTTCTGT3' respectively.

Relative Quantification of *Glut 4*

The Ct values were recorded for *Glut 4* gene and the internal control gene, β -actin. Melt curve analysis was carried out to check the specificity of the products. The relative quantification and expression pattern of the target gene at different intervals were analyzed keeping β -actin as endogenous control. Statistical analysis was performed separately and Δ Ct method was used to analyze the relative expression. The results were expressed as averages and standard deviations.

Statistical Analysis

Results were expressed as mean $\pm$ SE, for eight rats in each group. Data were analyzed using SPSS package version 20, where one-way analysis of variance followed by Duncan's test multiple comparison tests were performed. A value of ($p < 0.05$) and ($p < 0.01$) was considered statistically significant and highly significant respectively.

Results

Changes in Body Weight, Serum Glucose Level, Liver Glycogen and Insulin Concentration of Experimental Rats

A dose of 40 mg/kg body weight of STZ administered diabetic control and diabetic treatment rats, developed diabetes mellitus due to destruction of pancreatic β cells. The comparison of body weight, serum glucose level, liver glycogen level and insulin concentrations among the three groups of experimental rats is given in the Table 1.

STZ induced diabetic control rats showed a highly significant ($p < 0.01$) decrease in body weight, insulin concentration and liver glycogen level and a highly significant ($p < 0.01$) rise in blood glucose level when compared to normal control rats. Mustard oil treated diabetic treatment group showed a decrease in blood glucose level and highly significant increase ($p < 0.01$) in the liver glycogen level, body weight and insulin concentration when compared to diabetic control group.

Changes in the Lipid Profile of Experimental Rats

Diabetes mellitus also produces several changes in the lipid profile. Comparison of various parameters of the lipid profile among the three groups of experimental rats is listed in Table 2.

STZ induced diabetic control group displayed a highly significant ($p < 0.01$) increase in triacylglycerol, total cholesterol, VLDL-cholesterol, and LDL-cholesterol value when compared to normal control group. In contrast highly significant decrease ($p < 0.01$) of HDL cholesterol was observed in diabetic control group against the normal control group.

Diabetic treatment group with mustard oil incorporated diet, exhibited highly significant ($p < 0.01$) decrease in triacylglycerol, total cholesterol, VLDL-cholesterol, and LDL-cholesterol value when compared to the diabetic control group. While HDL cholesterol level of the diabetic

treatment group showed a highly significant increase on comparison with that of the diabetic control group.

Changes in the Fatty Acid Profile of Serum and Muscle

The levels of different fatty acids in serum and muscle among various experimental groups are mentioned in Table 3. There was a significant increase in the levels of oleic acid and linolenic acid in the muscle of diabetes treatment rats when compared to diabetic control. There was a significant increase in linoleic acid levels in both serum and muscle of diabetes treatment rats when compared to diabetic control.

Histopathological Changes of Pancreas Observed Among the Experimental Groups

On histopathological examination, pancreas of diabetic treatment group revealed mild regeneration of beta cells while diabetic control rats displayed vascular degeneration in islets of langerhans (Fig. 1).

Changes in the Expression of *Glut 4*

The relative quantification of *Glut 4* among different groups is displayed in Fig. 2. Relative expression of *Glut 4* using β -actin gene as an endogenous control for normalization revealed that the *Glut 4* was slightly down-regulated about – 1.362-fold in the diabetic control group when compared to normal control. *Glut 4* was up regulated about 8.5-fold in mustard oil-treated diabetic group when compared to diabetic control.

Discussion

Diabetes mellitus is an endocrine and metabolic disorder, which occurs either due to insulin resistance or reduction in insulin production. The disease progresses with reduced uptake of glucose from the blood into liver, muscle and

Table 1 Changes in the body weight, serum glucose level, liver glycogen level and insulin concentration among experimental groups of rats

Groups	Body weight (g)	Serum glucose (mg/dl)	Insulin (μ U/ml)	Liver glycogen (mg of glucose/g of tissue)
Group I	292.00 ^c $\pm$ 9.03	87.80 ^a $\pm$ 0.78	1.58 ^c $\pm$ 0.04	21.81 ^c $\pm$ 0.32
Group II	183.75 ^a $\pm$ 3.89	552.76 ^b $\pm$ 5.37	0.47 ^a $\pm$ 0.04	5.40 ^a $\pm$ 0.13
Group III	221.75 ^b $\pm$ 7.23	356.94 ^c $\pm$ 4.23	0.82 ^b $\pm$ 0.03	12.60 ^b $\pm$ 0.14

Values are mean $\pm$ SE for eight rats in each group. $p < 0.01$ is highly significant. Means bearing different superscripts in a column differ significantly between groups

Table 2 Changes in the serum triacylglycerol, total cholesterol, VLDL-cholesterol, LDL-cholesterol and HDL-cholesterol levels in control and experimental groups of rats

Groups	Triacylglycerol (mg/dl)	Total cholesterol (mg/dl)	VLDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)
Group I	110.53 ^a ± 2.64	85.80 ^a ± 0.26	23.21 ^a ± 0.33	21.88 ^a ± 0.21	42.16 ^c ± 0.41
Group II	280.26 ^c ± 3.68	153.91 ^c ± 0.84	56.93 ^c ± 0.41	65.96 ^c ± 0.32	21.48 ^a ± 0.44
Group III	145.68 ^b ± 0.64	105.25 ^b ± 0.72	29.49 ^b ± 0.41	32.37 ^b ± 0.54	34.47 ^b ± 0.47

Values are mean ± SE for eight rats in each group. $p < 0.01$ is highly significant. Means bearing different superscripts in a column differ significantly between groups

Table 3 Fatty acid profile of serum and muscle

Fatty acid%	Serum			Muscle		
	Group I	Group II	Group III	Group I	Group II	Group III
Palmitic (C _{16:0})	32.34 ± 0.83 ^b	29.31 ± 1.25 ^b	25.33 ± 0.89 ^a	25.82 ± 1.37 ^b	26.68 ± 0.59 ^b	19.78 ± 0.53 ^a
Stearic (C _{18:0})	8.97 ± 0.59 ^a	8.88 ± 0.36 ^a	10.29 ± 0.18 ^b	6.71 ± 0.27 ^a	9.60 ± 0.34 ^b	5.74 ± 0.35 ^a
Oleic (C _{18:1})	30.42 ± 0.77 ^a	34.29 ± 0.80 ^b	29.46 ± 0.79 ^a	36.13 ± 0.74 ^b	32.54 ± 1.03 ^a	34.54 ± 0.96 ^{ab}
Linoleic (C _{18:2})	9.44 ± 0.49 ^a	10.70 ± 0.46 ^a	12.57 ± 0.51 ^b	18.50 ± 1.01 ^a	19.65 ± 1.47 ^a	25.97 ± 0.96 ^b
Linolenic (C _{18:3})	1.09 ± 0.14 ^a	1.31 ± 0.04 ^b	1.26 ± 0.03 ^{ab}	0.46 ± 0.05 ^a	0.65 ± 0.03 ^a	2.59 ± 0.13 ^b
Arachidic (C _{20:0})	1.59 ± 0.87 ^a	1.30 ± 0.124 ^a	1.50 ± 0.07 ^a	0.14 ± 0.01 ^a	0.19 ± 0.01 ^b	0.72 ± 0.01 ^c

Values are mean ± SE for eight rats in each group. $p < 0.01$ is highly significant. Means bearing different superscripts in a column differ significantly between groups

Fig. 1 Histopathological changes of pancreas

adipose tissue leading to hyperglycemia as well as disturbance in glucose metabolism.

Administration of STZ causes acute degeneration and progressive loss of pancreatic β cells, hence reduced serum insulin concentration [21]. The diabetogenic effect observed in rats may also be due to increased glycogenolysis and gluconeogenesis [22].

The body weight of the diabetic control group showed a highly significant decrease when compared to normal control group. Reduction in body weight in STZ induced

diabetic rats may be due muscle wasting and loss of tissue proteins [23] possibly to excessive metabolism of fats and proteins for gluconeogenesis. The reduction of glucose uptake by the tissues like liver, muscle and adipose would lead to hypoglycemia at the intracellular compartment, which will stimulate glycogenolysis and gluconeogenesis. Impairment of protein synthesis and deposition is noticed because proteins are metabolized at a faster rate for gluconeogenesis. Thus, enhanced muscle wasting and loss of tissue proteins accentuates the weight loss. There was a

Fig. 2 Relative quantification of *Glut 4*

significant increase in body weight of mustard oil-treated diabetic rats, which may be related to the increased level of insulin. Insulin has an anabolic effect on protein metabolism and stimulates protein synthesis and reduces protein degradation [24]. Another important action of insulin is the suppression of adipose tissue lipolysis, which may result in an inhibition of muscular tissue proteolysis and lipolysis [25]. Hence reduced proteolysis and lipolysis may have increased the body weight in the mustard oil-treated diabetic rats.

In mustard oil-treated diabetic rats there was a reduction in blood glucose level when compared to diabetic control rats. Similarly blood glucose lowering effect was noticed, which was related to both pancreatic (enhancement of insulin secretion) and extra-pancreatic (peripheral utilisation of glucose) activity when sesamum oil was administered [26]. Increased insulin levels in the mustard oil treated diabetic group may be attributed to the mild regeneration of β cells of the pancreas which was observed in the histopathological examination (Fig. 1), but the exact molecular mechanism of the regeneration is yet to be ascertained. Diabetic control group showed vascular degeneration of islet cells of Langerhans, which was induced by STZ administration. This indicates the protective role of the oil on the pancreas. The treatment group of rats also showed an increase in liver glycogen content. Some authors have suggested that 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 [9]. The increased

glycogen content of liver may be due to increased glycogen synthesis in liver and reduced utilization of glucose by peripheral tissues which is due to increased insulin secretion and action.

Diabetic control group of rats showed highly significant increase in serum triacylglycerols, total cholesterol, VLDL-cholesterol and LDL-cholesterol when compared to the normal control group. This can be attributed to the loss of protective activity of insulin against lipolysis on adipose tissue. The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease [27]. Contrary, the HDL-cholesterol level was less in diabetic control group when compared to normal control group. In mustard oil-treated diabetic rats there was a decrease in serum triacylglycerols, total cholesterol, VLDL-cholesterol and LDL-cholesterol with greater elevation in HDL-cholesterol levels as compared to diabetic control group of rats. Similar findings have been reported that mustard oil increases plasma insulin concentration in diabetic rats, which may result in inhibition of lipolysis and decreased plasma triglyceride and cholesterol levels [28].

Fatty acid composition of membranes is also a factor that may influence the action of insulin in skeletal muscle. Dietary fat induced alteration in membrane composition has been shown to influence the function of membrane associated proteins [29]. The dietary fat quality mainly affects the cell membrane functions such as membrane fluidity, ion permeability, insulin receptor binding/affinity and glucose transporters interaction with second messengers [30, 31]. Thus, incorporation of a diet with high

Table 4 Fatty acid composition of ghee and different types of edible oils

Fatty acids%	Sunflower oil (n = 5) Mean $\pm$ SD	Soybean oil (n = 3) Mean $\pm$ SD	Mustard oil (n = 5) Mean $\pm$ SD	Palm oil (n = 3) Mean $\pm$ SD	Coconut oil (n = 6) Mean $\pm$ SD	Ghee	Rice bran oil
Caprylic (C _{8:0})	–	–	–	–	6.21 $\pm$ 0.34	1.2	–
Capric (C _{10:0})	–	–	–	–	6.15 $\pm$ 0.21	2.9	–
Lauric (C _{12:0})	–	–	–	–	51.02 $\pm$ 0.71	3.1	–
Myristic (C _{14:0})	–	–	–	1.23 $\pm$ 0.28	18.94 $\pm$ 0.63	11.2	0.2
Palmitic (C _{16:0})	6.52 $\pm$ 1.75	14.04 $\pm$ 0.62	4.51 $\pm$ 3.83	41.78 $\pm$ 1.27	8.62 $\pm$ 0.50	30.6	15
Stearic (C _{18:0})	1.98 $\pm$ 1.44	4.07 $\pm$ 0.29	2.78 $\pm$ 0.59	3.39 $\pm$ 0.65	1.94 $\pm$ 0.17	13.8	1.9
Oleic (C _{18:1})	45.39 $\pm$ 18.77	23.27 $\pm$ 2.43	38.21 $\pm$ 21.88	41.90 $\pm$ 1.20	5.84 $\pm$ 0.50	28.7	42.5
Linoleic (C _{18:2})	46.02 $\pm$ 16.75	52.18 $\pm$ 2.64	25.31 $\pm$ 5.74	11.03 $\pm$.02	1.28 $\pm$ 0.18	–	39.1
Linolenic (C _{18:3})	0.12 $\pm$ 0.09	5.63 $\pm$ 3.48	11.30 $\pm$ 6.09	–	–	–	1.1
Arachidic (C _{20:0})	–	–	10.86 $\pm$ 3.29	–	–	–	0.5
Erucic (C _{22:1})	–	–	11.35 $\pm$ 13.83	–	–	0.2	–

unsaturated fatty acid content can result in an increase in the unsaturated fatty acid composition of membrane. Several studies have shown that edible vegetable oils especially those rich in unsaturated fatty acids exhibit a significant role in improving insulin sensitivity to glucose thus reducing the risk of diabetes and its complications. When the fatty acid compositions of several oils and ghee were compared (Table 4) [32], mustard oil is found to be rich in unsaturated fatty acids especially oleic, linoleic, linolenic, and erucic acid. But, the role of erucic acid in reducing blood glucose level is yet to be ascertained. Our study showed an increase in the level of unsaturated fatty acids namely oleic, linoleic, and linolenic acid in the muscle tissue, when compared to diabetic control, thus confirming the incorporation of these beneficial fatty acids into the membrane when mustard oil is supplemented in the diet.

There was a significant downregulation in the expression of *Glut 4* in diabetic control when compared to normal control. GLUT 4 protein and mRNA levels have been shown to decrease in skeletal muscle of several rodents of both genetically and experimentally induced diabetes [33–35]. As the *Glut 4* expression depends upon the level of insulin, it is plausible to relate the downregulation to the experimentally induced diabetic rats with less or no insulin. There was a significant upregulation in the expression of *Glut 4* in mustard oil treated diabetic rats when compared to diabetic control. This may be due to increased plasma insulin concentration by mustard oil supplementation. In skeletal muscle, glucose is taken up through facilitated diffusion by two transporters, GLUT 1 and GLUT 4.

GLUT 4 proteins are found in an intracellular organelle in the basal state from where it can be rapidly translocated to the plasma membrane by insulin or exercise, whereas GLUT 1 transporter is generally considered to be insulin independent [33, 36]. Both insulin and non-insulin dependent diabetes mellitus decrease muscle glucose utilization due to defective expression and/or regulation of GLUT 4 translocation. GLUT4 is responsible for facilitating glucose transport into the cells in response to insulin, and therefore, is considered as a vital regulator of entire body glucose homeostasis [10]. Thus, incorporation of mustard oil in diet brings about alterations in the membrane fatty acid composition, leading to increased insulin secretion and sensitivity to insulin action thereby lowering blood glucose levels. Further studies are required to decipher the molecular mechanisms underlying the increased insulin secretion when mustard oil is incorporated in the diet.

Conclusions

The potentiality of mustard oil in reducing blood glucose level by increasing insulin secretion by up-regulating the expression of *Glut 4* laid new pathway of combination therapy with synthetic drugs. Mustard oil in our diet might not only reduce blood glucose levels but also reduce other complication associated with tissue damages or biochemical changes.

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Compliance with Ethical Standards

Conflict of interest On behalf of all authors, the corresponding author declares that there is no conflict of interest.

Ethical approval On behalf of all authors, the corresponding author declares that all procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Ethical Committee (IAEC), Madras Veterinary College, Chennai-600007 with IAEC approval number 1679/DFBS/B/2015. The animals were taken care according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Informed consent On behalf of all authors, the corresponding author declares that no human subjects were included in this study hence the need does not arise.

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