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Influence of Sunflower Oil Supplementation in Streptozotocin Induced Diabetic Rats.

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ABSTRACT

The effect of supplementing sunflower oil and the combined effect of sunflower oil and glibenclamide were studied for 60 days in STZ-induced diabetic rats. Diabetes was induced in adult male Wistar rats by intraperitoneal administration of streptozotocin (40 mg/kg b.wt). The control group had normal rats supplemented with ghee. All the groups were supplemented with 8% lipid containing isocaloric diet. Supplementation of sunflower oil significantly reduced blood glucose level when compared to diabetic rats. When sunflower oil and glibenclamide was given together there was a significant reduction in blood glucose level. When diabetic rats were fed with sunflower oil, serum insulin level was increased marginally which was evident by the better glycemic control. Sunflower oil supplementation restored deranged lipid metabolism of diabetic rats by decreasing TG, TC, LDL-cholesterol, VLDL-cholesterol levels and by increasing HDL-cholesterol indicated that supplementing sunflower oil had anti-hyperlipidemic effect. Sunflower oil supplementation exhibited significant improvement in antioxidant enzyme activities of CAT, SOD, GPx and GSH levels in liver, kidney and pancreas of diabetic rats. Histopathological studies on the liver, kidney and pancreas in diabetic rats showed degenerative changes. Supplementation of oil, oil combined with glibenclamide to diabetic rats was able to ameliorate the degenerative changes seen in the above tissues indicating their protective effect on the respective tissues. CAT- catalase, GPx- glutathione peroxidase, GSH- glutathione, HDL- high density lipoprotein, LDL- low density lipoprotein, SOD- superoxide dismutase, STZ- streptozotocin, TC- total cholesterol, TG- triglycerides, VLDL- very low density lipoprotein

Keywords: Blood glucose, Diabetes, Glibenclamide, Streptozotocin, Sunflower oil.

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INTRODUCTION

Diabetes mellitus is one of the most common noncommunicable diseases prevalent globally and there is substantial evidence that it is a modern epidemic in many developing and newly industrialized nations, thus posing a serious threat to be met within the 21st century (Pradeepa and Mohan, 2002).

Diabetes has been affecting lives for thousands of years.According to recent estimates (2017), the number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014, the global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014 (WHO). The prevalence of diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity(Sarah *et al.,* 2004).

While the management of diabetes mellitus includes diet, exercise, oral hypoglycemic agents and insulin, these strategies do not effectively cure diabetes or prevent the complications of diabetes mellitus (Plumbo, 2001). In recent years, traditional medicine using medicinal plants for treating and preventing various diseases including diabetes has gained much attention. Herbal treatments are the most popular form of traditional medicine due to lesser side effects and are highly lucrative in the international market. There is a growing interest in traditional medicines of plant origin due to several factors such as availability, affordable cost, safety and efficacy (Kamsiah*et al.,* 2014).

Vegetable oil is a common ingredient in the diet of majority of peopleacross the globe. Apart from providing energy as calories, oils provide lipid content and fat soluble vitamins to the body. One of the most common indispensable oil in south-indian cooking is Sunflower oil rich in 69% polyunsaturated and 20% monounsaturated, 11% saturated fatty acids lower LDL or “bad” cholesterol. It also prevents some oxidative stress related diseases. Daily dietary intake of fatty acids with different chain-length and degree of unsaturation can cause changes in the lipid composition of tissues. Thus, dietary fatty acid composition, to a large extent, determines the relative availability and storage of FAs in tissues (Hodson*et al.,* 2008), which in turn can affect membrane permeability and receptorsensitivity.

Recent research suggests that there is a reduction in glucose levels in diabetic rats treated with vegetable oils (Amina*et al.,* 2013). Hence, the present study was undertaken to investigate the effect of sunflower oil on the circulating blood glucose levels in streptozotocin induced diabetic rats. The study will also help to understand the combined effect of sunflower oil and glibenclamide, a hypoglycemic drug in experimentally induced diabetic rats.

MATERIALS AND METHOD

Chemicals

All chemicals used were of analytical grade. Streptozotocin (STZ) waspurchased from Sigma Chemicals Co., St. Louis, USA and glibenclamide (Daonil) from Apollo pharmacy. Chemicals for antioxidant assay were purchased from M/s Merck chemicals, Mumbai, India andthe diagnostic kits for the assay of serum parameters from M/s Agappe diagnostics, Ernakulum, Kerala, India. Insulin IRMA (Immuno Radio Metric Assay) kit was purchased from M/s Anand Brothers, Delhi, India.

Experimental animals

Male Wistar rats, weighing about 150-180 g were obtained from Laboratory Animal Medicine Unit, Madhavaram, Tamil Nadu Veterinary and Animal Sciences University, Chennai - 51, India. All animals were housed in cages with 12/12 hours light/dark cycle. The animals were fed *ad libitum*isocaloric experimental diet and water throughout the experimental period. The animals were acclimatized for three weeks prior to the start of the experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), MVC, Chennai - 7. Animal ethical guidelines and good laboratory practice guidelines were followed throughout the experimental period. In addition, all the precautions were taken to minimize pain and discomfort to the animals.

Feed profile

The experimental rats were fed with isocaloric mash type diets during the experimental period. The diets were formulated considering the energy and protein requirement of the rats. The formulated diets were prepared at Central Feed Technology Unit, Kattupakkam, Kanchipuram from where it was procured.Sunflower oil (SVS) was purchased from local market. Ghee was procured from Dairy plant, Madras Veterinary College, Chennai-07.

Experimental design

This study was conducted for sixty days. Forty male Wistar ratswith the body weight of 150 – 180 g were randomly divided into five groups, each consisting of eight animals. The mean body weight variation of rats was not exceeding 20 per cent in between the groups. Experimental animals were weighed at weekly intervals during the experimental period.

Group – I	Normal control (8% Ghee included diet)
Group – II	Diabetic control (8% Ghee included diet)
Group – III	Diabetic control+ glibenclamide (2.5mg/kg b.wt) (8% Ghee included diet)
Group – IV	Diabetic control (8% Sunflower oil included diet)
Group – V	Diabetic control (8% Sunflower oil included diet) + glibenclamide (2.5mg/kg b.wt)

Each group received one of five diets designed to support growth, with similar energy, protein and fat content. The diets had similar composition except for fat source, which consisted of 8% of ghee and sunflower oil. The rats were given *ad labium* access to food and water. The fat sources of the experimental diets consisted of ghee for group I, II and III; sunflower oil for group IV and V. In addition, group III and V were treated with glibenclamide at the dose rate of 2.5mg/kg b.wt along with respective diet.

Induction of diabetes mellitus

Before induction, the blood glucose level was assessed by ACCU CHEK Active glucometer to rule out spontaneous diabetes in the rats. Those animals, showing normal blood glucose levels of 80-110 mg/dL, were selected for the study. The selected animals were fasted overnight and a single intraperitoneal injection of a freshly prepared solution of STZ (40 mg/kg b.wt) in 0.1 M cold citrate buffer (pH 4.5) was given to induce diabetes. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dL on the third day (72 hours) after STZ injection. Treatment was started on the fourth day after STZ injection and the day was considered as first day of treatment and the treatment was continued for 60 days.

Collection of sample

Blood samples were collected by retro-orbital puncture at 0 and 60thdays of experiment. Serum was separated by centrifugation at 3000 rpm for 15 min and was used for the estimation of total protein, cholesterol, triacylglycerol, urea, creatinine, ALT, AST, calcium, phosphorous, sodium and potassium as per the protocol described in the standard kits (Agape diagnostics) using CECIL CE 2021 UV spectrophotometer. Animals were sacrificed at the end of the experiment, tissue samples of liver, kidney, pancreas, heart and aorta were collected for histopathological studies and liver, kidney and pancreas were collected for assay of antioxidant
Status.

Histopathology

After the tissues were fixed completely in 10 per cent formalin, all the collected tissue samples were embedded in paraffin. Serial sections were cut and stained with haematoxylin and eosin. The sections were examined under high power microscope (200X) and photomicrographs of tissues picture were taken.

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Statistical analysis

The results were expressed as mean ± S.E. All the data were analyzed by SPSS package version 20, one way analysis of variance followed by Duncan's test multiple comparison test (Snedecor and Cochran, 1994). A value of ($p< 0.05$) and ($p< 0.01$) were considered statistically significant.

RESULTS

Table 1 shows the effect of sunflower oil on body weight and blood glucose in normal and STZ-induced diabetic rats. A significant weight loss was observed in diabetic control groups. The weight loss was minimal in oil treated group,significant improvement of weight was observed in the group treated with standard drug. The effect was maximum when diabetic rats treated with combination of oil and drug. A drastic increase in blood glucose level was found in the diabetic control rats. A significant reduction in blood glucose level was found in the diabetic animals fed sunflower oil. The reduction was highly significant in drug treated group. The reduction was maximum when oil and drug given combinedly.

Table 1: Effect of sunflower oil in diet on blood glucose and body weight in normal and diabetic animals.

Groups	Body weight (grams)		Blood glucose level (mg/dL)	
	Before treatment (0 day)	After treatment (60 day)	Before treatment (0 day)	After treatment (60 day)
I	$179.00^{a} \pm 1.36$	$251.62^{a} \pm 3.78$	$92.00^{b} \pm 3.49$	$98.25^{e} \pm 2.31$
II	$179.25^{a} \pm 1.12$	$115.62^{d} \pm 2.54$	$505.50^{a} \pm 4.93$	$568.75^{a} \pm 3.84$
III	$178.75^{a} \pm 3.40$	$164.82^{c} \pm 2.23$	$504.75^{a} \pm 5.25$	$228.37^{c} \pm 2.81$
IV	$179.00^{a} \pm 1.25$	$160.50^{c} \pm 5.96$	$506.87^{a} \pm 6.04$	$367.25^{b} \pm 5.44$
V	$178.50^{a} \pm 1.12$	$198.75^{b} \pm 3.56$	$507.12^{a} \pm 4.93$	$167.75^{d} \pm 4.29$

Table 2 illustrates the effect of supplementation of oil on serum TG, TC, HDL-C, LDL-C and VLDL-C levels in normal and STZ-diabetic rats. In our study, diabetic rats had elevated levels of TG, TC, LDL-C and VLDL-C and decreased level of HDL-C when compared with normal rats. Diabetic rats fed with sunflower oil showed a significant decrease in the levels of serum TG, TC, LDL-C and VLDL-C, significant increase in HDL-C level, diabetic rats treated with oil and drug showed better improvement.

Table 2: Effect of sunflower oil in diet on TG, TC, VLDL-C, LDL-C and HDL-C in normal and diabetic animals

Groups	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
I	$113.40^{e} \pm 1.91$	$84.60^{e} \pm 1.07$	$42.80^{a} \pm 0.86$	$19.12^{e} \pm 1.57$	$22.68^{e} \pm 1.38$
II	$318.00^{a} \pm 2.791$	$64.20^{a} \pm 1.24$	$19.80^{c} \pm 0.37$	$80.80^{a} \pm 1.49$	$63.60^{a} \pm 1.42$
III	$157.00^{c} \pm 2.16$	$94.60^{d} \pm 2.67$	$39.80^{a} \pm 1.15$	$23.40^{d} \pm 3.66$	$31.40^{c} \pm 0.57$
IV	$223.60^{b} \pm 1.961$	$18.40^{b} \pm 1.07$	$27.40^{b} \pm 1.43$	$46.28^{b} \pm 2.15$	$44.72^{b} \pm 0.37$
V	$133.00^{d} \pm 2.66$	$105.44^{c} \pm 1.33$	$41.20^{a} \pm 0.86$	$37.60^{c} \pm 2.36$	$26.60^{d} \pm 0.53$

Table 3 shows the effect of sunflower oil on ALT, AST, creatinine and urea in the serum of normal and STZ-diabetic rats. AST, ALT level increased significantly was observed in diabetic rats when compared with normal rats. Significant decrease in ALT, AST were observed in diabetic rats treated with sunflower oil and also treated with drug, though it was more prominent in the combination of oil and drug treated group. Significant decrease in serum creatinine and urea level in sunflower oil supplemented rats, drug treated rats and also oil and drug combined rats but these levels were increased in STZ-diabetic rats.

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Table 3: Effect of sunflower oil in diet on total protein, albumin and globulin in normal and diabetic animals

Groups	Total protein	Albumin(g/dL)	Globulin(g/dL)	A/G ratio(g/dL)
I	7.08^{a}± 0.07	3.81^{a}± 0.06	3.27^{c}± 0.08	1.17^{a}± 0.05
II	6.15^{c}± 0.27	2.87^{d}± 0.09	3.27^{c}± 0.12	0.88^{d}± 0.07
III	6.91^{b}± 2.81	3.31^{c}± 0.10	3.60^{a} ± 0.11	0.92^{c}± 0.03
IV	6.97^{b}± 0.04	3.54^{b}± 0.07	3.44^{b}± 0.09	1.03^{b}± 0.02
V	7.14^{a}± 0.06	3.57^{b}± 0.04	3.56^{ab}± 0.07	1.00^{b}± 0.04

Table 4 illustrates the effect of supplementation of sunflower oil on total protein, albumin and globulin concentration and A/G ratio in normal and STZ-diabetic rats. A significant decrease in serum total protein and albumin concentration whereas marginal decrease in globulin level was observed in diabetic rats when compared with normal control rats. The levels were increased significantly when diabetic rats treated with oil, drug, oil and drug combinedly.

Table 4: Effect of sunflower oil in diet on AST, ALT, creatinine and urea in normal and diabetic animals

Groups	AST(U/L)	ALT(U/L)	Creatinine(mg/dL)	Urea(mg/dL)
I	32.20^{d}± 0.86	41.00^{e}± 0.94	0.53^{b}± 0.04	47.03^{d}± 3.52
II	58.60^{a}± 0.51	95.40^{a}± 1.33	2.00^{a}± 0.04	63.09^{a}± 2.51
III	35.40^{c} ± 1.20	59.60^{b}± 0.24	1.41^{c}± 0.07	51.33^{c}± 2.33
IV	51.80^{b}± 1.52	49.40^{c}± 0.92	1.54^{b} ± 0.03	55.67^{b}± 2.02
V	36.00^{c}± 1.78	47.04^{d}± 0.54	1.36^{d}± 0.04	47.08^{d}± 2.08

Table: 5 Effect of sunflower oil supplementation in liver antioxidants and lipid peroxidation levels

Groups	CAT	SOD	GPx	GSH	LPO
I	28.00^{a} ± 0.71	33.80^{b}± 0.86	13.60^{a}±0.92	47.20^{b}± 0.86	0.190^{e} ± 0.01
II	10.58^{d}± 0.68	17.00^{e}± 0.70	3.50^{d}± 0.64	24.00^{e}± 0.70	0.766^{b}± 0.29
III	21.00^{b}± 1.3	30.80^{c}± 0.66	8.80^{b}± 0.37	37.00^{c}± 0.71	0.242^{d}± 0.02
IV	15.60^{c}± 0.10	28.60^{d}± 0.67	8.00^{c}± 0.31	34.20^{d}± 0.58	1.210^{a}± 0.06
V	27.80^{a}± 0.37	38.80^{a}± 2.31	13.01^{a}± 0.44	53.00^{a}± 0.70	0.450^{c}± 0.02

Table: 6 Effect of sunflower oil supplementation in kidney antioxidants and lipid peroxidation levels

Groups	CAT	SOD	GPx	GSH	LPO
I	15.00a± 0.70	16.60a± 0.81	8.60b± 0.51	23.00b± 1.22	0.106e± 0.06
II	5.56e± 0.12	3.60e± 0.67	2.50e±0.64	6.60e± 0.67	0.704c± 0.12
III	11.24c ± 0.19	11.60c ± 0.97	7.20c± 0.37	18.60c± 0.50	0.262d± 0.00
IV	8.20d± 0.09	9.80d± 0.37	5.80d± 0.37	15.40d± 0.50	1.518a± 0.08
V	12.83b± 0.06	14.67b± 0.50	11.03a±0.47	25.80a± 1.01	0.888b± 0.01

Table: 7 Effect of sunflower oil supplementation in pancreatic antioxidants and lipid peroxidation levels

Groups	CAT	SOD	GPx	GSH	LPO
I	9.48^{a}± 0.18	18.40^{a}± 0.74	7.02^{b}± 0.70	12.60^{b}± 0.81	0.170^{e}± 0.02
II	1.60^{d}± 0.87	6.60^{e}± 0.67	3.50^{d}± 0.64	2.40^{e}± 0.50	0.706^{c}± 0.02
III	7.06^{b}± 0.49	14.40^{c}± 0.50	6.80^{b}±0.37	8.20^{c}± 0.37	0.254^{d}± 0.01
IV	5.62^{c} ± 0.08	11.60^{d}± 0.51	5.20^{c}± 0.37	7.70^{d}± 0.89	1.358^{a}± 0.02
V	7.16^{b}± 0.50	17.20^{b}± 0.66	10.20^{a}± 0.58	16.40^{a}± 0.74	0.756^{b}± 0.03

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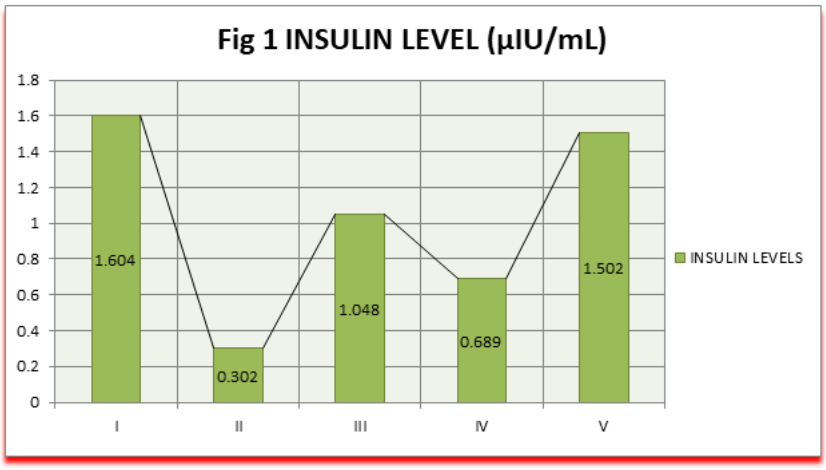
Fig 1 shows significant increase in serum insulin activity in oil, oil and drug treated diabetic rats compared with diabetic rats. Fig 2 shows Histopathological appearance of pancreas in normal, diabetic , oil and oil and drug treated groups.



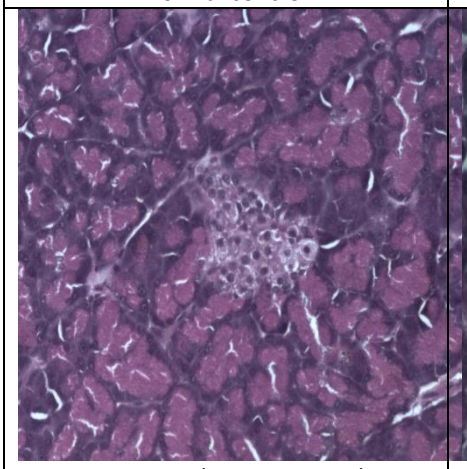
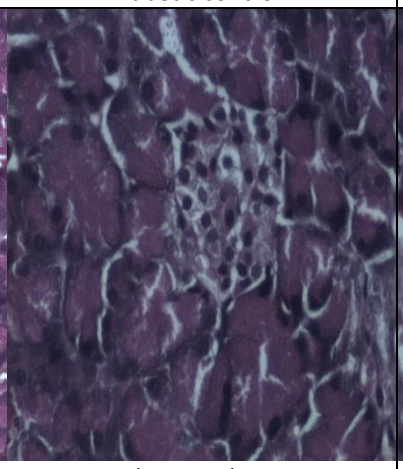
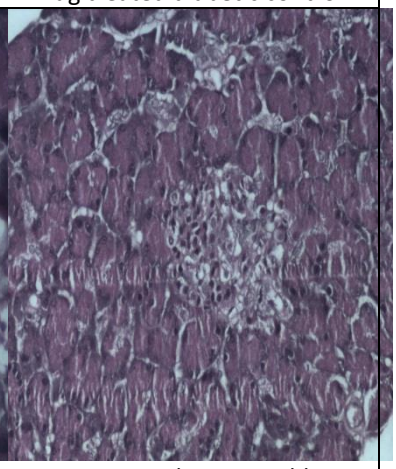
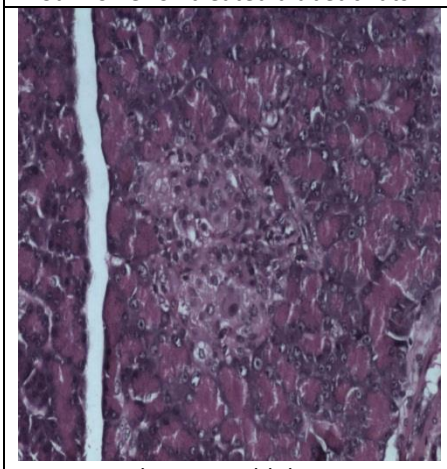
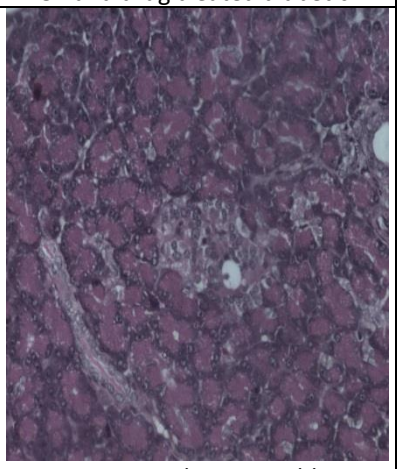
Normal control	Diabetic control	Drug treated diabetic control
		
Pancreas showing normal architecture	Pancreas showing degeneration and necrosis of islets of Langerhans	Pancreas showing mild degeneration of islets of Langerhans
Sunflower oil treated diabetic rats	Oil and drug treated diabetic	
		
Pancreas showing mild degeneration of islets of Langerhans	Pancreas showing mild degeneration of islets of Langerhans	

Fig 2: Histopathology of pancreas

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DISCUSSION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of different organ especially eyes, kidneys, nerves, heart and blood vessels (2).

STZ is a commonly employed compound for the induction of diabetes mellitus in experimental rats (51). It causes DNA strand breaks in pancreatic islets, stimulates nuclear poly (ADP- ribose) synthetase and thus depletes the intracellular NAD^+ and $NADP^+$ levels, which inhibits proinsulin synthesis and induces diabetes (54). It leads to significant decrease in serum insulin level in diabetic rats. The increase in the blood glucose levels observed when intraperitoneal administration of STZ is due to the destruction of pancreatic β-cells (9, 15).

Glibenclamide treated diabetic rats showed a highly significant decrease in the level of glucose during the entire study. Sulfonylurea, such as glibenclamide has been widely used to treat type 2 diabetic patients over a long period. The mechanism of action seems to be initiated by the linkage of drug molecules with surface receptor in the β-cell surface and subsequent reduction of conductance of the ATP sensitive K^+ channels. The reduced K^+ channels efflux determines membrane depolarization and influx of Ca^{2+} through Ca^{2+} channels that eventually determine insulin secretion (22). The drug also reduces fatty acid oxidation and therefore hyperglycemia by inhibiting carnitine palmitoyltransferase-I activity, which is required for the transport of long chain fatty acids from cytoplasm to mitochondria for oxidation. On the other hand treatment of diabetic rats with glibenclamide increases the insulin level when compared to STZ-diabetic rats. It stimulates insulin secretion and also reduces hepatic glucose production resulting in reduced blood glucose (41).

Significant decrease in the blood glucose level and significant increase in serum insulin level were observed in sunflower oil fed diabetic rats which may be due to presence of linoleic acid in oil. Fatty acids are identified as insulin secretion modulators depending on their chain length and degree of unsaturation (35). Thus linoleic acid, the major fatty acid found in sunflower oil may be involved in the modulation of pancreatic β-cell function. Other studies have demonstrated that linoleic acid reduced the voltage gated K^+ channel in rats β-cells through GPR40 and cAMP- protein kinase A system leading to an increase in intracellular Ca^{2+}concentration and insulin secretion. Similar data were also found *in vivo* in mice (8,56)in which dietary supplementation of conjugated linoleic acid and omega 3 polyunsaturated fatty acid augmented insulin secretion partly because of increased islet glucose oxidation. *In vitro* studies performed by 5, 32 on perfused mouse islets of Langerhans or isolated rat pancreas showed that the insulin release induced by a physiologic glucose concentration was increased by unsaturated fatty acids. The potential benefit of linoleic acid in preventing diabetes may also be related to its potential anti-inflammatory effects (53, 33). Diet high in unsaturated long chain n-6 and n-3 fatty acids may inhibit hepatic lipogenesis and stimulate hepatic fatty acid oxidation, which may improve hepatic insulin sensitivity (6). There was a significant improvement in the results of oral-glucose-tolerance tests in the group that consumed linoleic acid enriched diet (17). The decreased glucose level observed in sunflower oil fed diabetic rats may be attributed to increased insulin secretion, decreased gluconeogenesis and increased oxidation of glucose. Roche *etal.* (2014) have found that sunflower oil fed rats showed more numbers of B cells in the islets of Langerhans and higher insulin concentration in serum which agrees with our present finding. The effect of fatty acids on sulfonylureas may be varied. However, the effect of sunflower oil on glibenclamide activity was additive. Sunflower oil supplemented together with glibenclamide, the hypoglycemic effect was increased, which may be attributed to the presence of unsaturated fatty acids, which increase sensitivity for the binding and action of insulin and also due to the presence of glibenclamide.

The reduction in body weight observed in STZ-induced diabetic rat group may be due to muscle wasting and loss of tissue proteins upon induction of diabetes with STZ as reported by(20). As glibenclamide has been used for the treatment of type 2 diabetes, it controls the blood glucose, carbohydrate metabolism and it is also involved in the secretion of insulin. Hence, there is an increase in body weight (28). Sunflower oil fed diabetic rats had significant increase in body weight.It is postulated that presence of linoleic acid in sunflower oil modulates β-cell function thereby decreases glucose level, which minimizes weight loss in diabetic rats (8),hence the improvement in the body weight may be due to glycemic control by linoleic acid

which is abundant in sunflower oil. When the rat in addition to the oil feed diet was administered with glibenclamide, body weight increased significantly may be attributed to better glycemic control by the drug and linoleic acid present in sunflower oil (47).

From the result of this study, it was evident that there was significant increase in the concentration of TG, TC, LDL and VLDL, while there was low level of HDL in diabetic control group when compared to the control group. The increase in TG, TC, LDL and VLDL and decrease in HDL denotes an increase in the risk of cardiovascular diseases associated with diabetes mellitus. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats (29).

However, it was observed that there was a decrease in the concentration of TG, TC, LDL and VLDL, while there was an increase in the level of HDL in diabetes fed a sunflower oil diet when compared to diabetic control rats. Dietary fats differ in chain length and degree of unsaturation and this affects plasma lipoprotein constitution. Serum cholesterol level increases when dietary carbohydrates are replaced by saturated fatty acids and decreases when carbohydrates are replaced by polyunsaturated fatty acids. Increased PUFA is associated with hypocholesterolemic and hypotriacylglycerolemic condition.Numerous studies have demonstrated that those enriched in unsaturated fatty acids lower TC and TG (50, 27). These findings are agrees with our findings. Sunflower oil supplementation will be complementary to diabetic rats treated with antidiabetic drug, glibenclamide. A lowering of serum lipid level through dietary/drug therapy seems to be associated with the decrease in the risk of vascular diseases (4).

In our present study, due to hyperglycemia the ALT and AST activities significantly increased in diabetic rats when compared to normal control rats. Elevation in the levels of serum ALT and AST was considered as predictors of diabetes (12). A rise in ALT activity indicates the hepatocellular damage followed by cardiac tissue damage and is usually accompanied by a rise in AST activity (40). Increase in the activities of these enzymes in the serum of diabetic rats may be due to the leakage of these enzymes from the liver cytosol into blood stream as a consequence of the hepatic tissue damage (46). Treatment of diabetic rats with sunflower oil reduces the concentration of transaminases, which indicates protective role of oils on the liver tissue.Diabetic rats supplemented with sunflower oil along with drug showed significant decrease in ALT and AST level. This may be due to the synergistic effect of sunfloweroil and glibenclamide.

Moving on to serum urea and creatinine parameters, it is evident that hyperglycemia has profound negative effects on renal function, leading to significantly elevated levels of serum urea and creatinine in STZ-diabetic rats. The serum creatinine level indicates protein catabolism in the body. There is accelerated proteolysis in uncontrolled diabetes as a result of deranged glucagon-mediated regulation of cAMP formation in insulin deficiency condition, which is reflected by considerable increase in the serum creatinine levels (7). The elevated levels of serum urea and creatinine in diabetic rats are due to catabolism of the protein and nucleic acids (55). Treatment with glibenclamide had a significant decrease in urea and creatinine levels, which could be due to the better glycemic control and prevention of proteolysis, which prevents damages to the renal cells and improves its activity. In our study the observed elevated urea levels in diabetic rats were partially reduced by the treatment with sunflower oil, which suggest the proper regulation of carbohydrate and protein metabolism by the presence of oil. Treatment with vegetable oil and glibenclamide showed a significant reduction in urea and creatinine levels. As the combination effectively increases insulin secretion and inhibits proteolysis, which reduces glucose load on kidney and the production of reactive oxygen species that improves the renal function.

In our present study, a significant decline in total protein and albumin values were recorded in STZ-diabetic rats. Hypoalbuminemia and hypoproteinemia observed in diabetes is generally attributed to the presence of nephropathy and/or may be due to increased protein catabolism (38, 49). When diabetic rats treated with glibenclamide showed a significant increase in total protein and albumin level, which may be due to decreased protein degradation by the action of glibenclamide. As the drug controls hyperglycemia and improves carbohydrate metabolism, which prevents protein catabolism. In the treatment of diabetic rats with sunflower oil alone and combination of oil and glibenclamide showed a significant increase in the level of serum total protein and albumin. These findings suggest that treatment of diabetic rats with sunflower oil ameliorates induced toxicity. The effect was more pronounced in diabetic rats treated with sunflower oil along with glibenclamide.

Diabetes mellitus has been reported to generate reactive oxygen species (ROS). ROS, such as free hydroxyl radicals (•OH) and superoxide (O2•-), can cause lipid peroxidation (26). Membrane lipid peroxidation results in loss of PUFA, decreased membrane fluidity and loss of enzymes and receptor activity. The products of lipid peroxidation are capable of interacting with DNA and cause oxidative damage (23). In STZ-diabetic rats, a significant increase in LPO level was observed when compared to normal control rats, which could be due to increased generation of free radicals during hyperglycemia. There are several reports, which demonstrates the elevated levels of lipid peroxides, hydroperoxides and protein carbonyls in the hepatic tissues of experimental diabetic models (58, 39). Diabetic rats treated with showed a significant decrease in LPO level by decreasing glucose level thereby reducing the production of reactive oxygen species. Due to the presence of unsaturated fatty acids in sunflower oil that lead to increase in the level of LPO in oil fed diabetic rats. Diabetic rats supplemented with oil and drug combination, there was a significant reduction in LPO level, which may be attributed to the effect of glibenclamide, which improves glycemic control thereby reduces oxidative stress.

Antioxidant enzymes are critical part of cellular protection against reactive oxygen species and ultimately oxidative stress. Oxidative stress is determined by the balance between the generation of ROS such as superoxide anion (O_2^-) and the antioxidant defense systems. Antioxidant enzymes involved in the elimination of ROS include SOD, CAT and GPx. Since oxidative stress contributes significantly to the pathophysiology of diabetes, substances that suppress oxidative stress might be therapeutically beneficial. Studies have shown that exogenously administered antioxidants have protective effects on diabetes, thus providing insight into the relationship between free radicals and diabetes (19). In the present study, diabetic rats showed a decrease in the activities of SOD, CAT and GPx in tissues of liver, kidney and pancreas, which could be due to an increase in the utilisation of these enzymes for scavenging of free radicals. The decrease activity of enzyme is also due to increased glycation. Drug treated diabetic rats exhibited significant increase in SOD, CAT and GPx levels, which could be due to reduced free radicals production by the action of glibenclamide, as it reduces glucose and improves carbohydrate metabolism. Diabetic rats treated with sunflower oil increased the activity of SOD, CAT and GPx significantly, which could be attributed to better glycemic control. When oil and drug fed combinedly, there was a significant increase in SOD, CAT and GPx level, which may be attributed to synergistic effect of drug and fatty acids in vegetable oils on glycemic control. It may also be due to antioxidant components present in the oil.

GSH is a chief intracellular redox buffer that functions as a direct free radical scavenger, co substrate for GPx activity and cofactor for many enzymes. The maintenance of normal ratio of GSH to oxidized glutathione requires NADPH. The cellular demand for NADPH is obviously increased, when the level of GSH decreased and the level of oxidized glutathione increased. This necessitates an increase in glucose oxidation via the pentose phosphate cycle. During insulin deficiency the level of intracellular NADPH is declined because of defective glucose oxidation (24). The notable decline in the key cellular non-enzymatic antioxidant defense system extensively provokes the hepatocytes susceptibility to oxidative stress (13). In our present study, a significant decline in GSH level in diabetic rats, which may be due to hyperglycemia, increased free radical production and increased utilisation of glutathione. Glibenclamide treated diabetic rats showed a significant increase in GSH level, which may be due to antidiabetic effect of drug. Supplementation of sunflower oil has improved the antioxidant status owing to glycemic control, presence of antioxidants and some active principles present in the oils. Synergistic effect of vegetable oil and drug on the reduction of blood glucose followed by reduction in the oxidative stress recorded in oil and drug combination.

Histopathology

In the present study, normal architecture of pancreatic islets showing predominant beta cells was observed in pancreatic tissue of normal control rats. Degeneration, necrosis of islets and reduction in the number of islets of Langerhans were observed in pancreatic tissues of STZ-induced diabetic rats. These results agreed with the findings of 42, 52who reported that type-2 diabetic induce markedly abnormal changes in rat's islets. Sunflower oil supplementation and combined action of oil and glibenclamide showed only mild degeneration of islets cells, which indicate the protective nature of oil in STZ-induced diabetic rats.

CONCLUSION

Sunflower oil exhibited better glycemic control which may be due to increased insulin secretion. The effect was more pronounced when oil and drug given together. Vegetable oil supplementation to the diabetic

rats was able to restore partially the deranged lipid profile and there was an improvement in antioxidant status.

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