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Original Research

Evaluation of vegetables and fish oils for the attenuation of diabetes complications

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Abstract: The present study was accomplished to examine and compare the effect of specific antioxidant-rich oils on hyperglycemia, dyslipidemia, renal function markers and oxidative renal damage in diabetic rats for four weeks. Papaya (P), olive (O), fenugreek (Fe), bitter gourd (B) and fish (Fi) oils were used for this purpose. Streptozotocin (STZ) was injected intraperitoneally in a single dose to induce diabetes. All oils were given orally at a dose of 3g/kg for four weeks in respective group after induction of diabetes. After treatment with oils, blood was collected, and their kidneys were stored. The level of fasting blood glucose (FBG), glycated hemoglobin (HbA1c), total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) increased while amylase and high-density lipoprotein cholesterol (HDL-C) level decreased in the diabetic rats. These changes were augmented by fenugreek, bitter gourd and olive oils treatment. Diabetic rats showed elevated renal function markers in serum, including, serum creatinine (Scr), blood urea nitrogen (BUN) and alkaline phosphatase (ALP), which were restrained significantly by fenugreek and bitter gourd oil treatment. Moreover, fenugreek and bitter gourd oils treatment significantly modulated the level of thiobarbituric reactive substances (TBARS), malonaldehyde (MDA) and catalase (CAT) in the kidney of diabetic rats. The histopathological examination also showed the protective effect of these oils. The study suggests that vegetable oils are effective in reducing hyperglycemia, dyslipidemia and renal damage related to the side effects of diabetes. Thus they may have therapeutic value for preventing diabetes side effects and may be included in oil diet treatment synergically. Thus, our data suggest that oils as potent antidiabetic agent and beneficial in the control of diabetes-related abnormalities such as hyperglycemia, dyslipidemia and renal damage of STZ induced rat model of type 2 diabetes. Our study also supports the suggestion that synergistic possibilities exist concerning the use of these oils in the treatment of diabetes mellitus.

Key words: Fenugreek oil; Bitter gourd oil; Type 2 diabetes; Hyperglycemia; Dyslipidemia; Renal damage.

Introduction

Diabetes mellitus (DM) is the most severe, non-communicable metabolic disorder characterized by abnormalities in insulin secretion and insulin resistance of significant target tissues (1). The International Diabetes Federation (IDF) estimates that worldwide there are 285 million people with diabetes in 2010 and this will increase to 438 million by 2025 (54% increase) (2). DM is classified as Type I diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) on the etiological basis. T1DM is a metabolic disorder ascribed by hyperglycemia occurs due to a deficiency of circulating insulin levels or absent insulin. T2DM is very common among all diabetic form affecting ≈90% of the patients (3). It is linked with a variety of problems and disorders described by high blood glucose level resulting from a defect in insulin action, or secretion or both (1,4). Long term accumulation of blood glucose leads to tissue damage, with consequent often serious complications (5,6). DM often correlated with numbers of metabolic and physiologic complications including high blood pressure, high cholesterol level, dyslipidemia and cardiovascular disorder (7). Chronic complications of DM include retinopathy, cerebrovascular disease, coronary heart disease, nephropathy, neuropathy, and peripheral vascular disease (8). Diabetes imposes a significant economic burden on the individual, national healthcare system, and the economy (9,10,11).

Although insulin and other types of insulin analogs are currently available as therapeutic agents for the treatment of DM, there is growing research in plant-based remedies because these therapeutic agents has showed some harmful effects on body (12,13). So now, scientist have more interested to search a therapy which is plant-based as this would be more harmonious with our biological systems (14,15). Some oils, and in particular fenugreek oil, have been reported as being very potential in the management of T2DM (16,17). Fenugreek oil is quite helpful in diabetes. Although very little is known about the effect of fenugreek oil on type 2 diabetes, even though Fenugreek oil has potential to improve the glucose intolerance and can also restore hyperglycemia (16). It also has been claimed to stimulate the pancreatic beta cells and reduces damage. This action is contributed due to the presence of polyunsatu-

rated fatty acids (omega-3 fatty acid) and the presence of antioxidants in the oil (18). Moreover, fenugreek oil has been demonstrated as neuroprotective and immunomodulatory (16). Bitter gourd has been claimed to manage diabetes related problems, reducing respiratory infections such as pneumonia, helping to reduce inflammation and increasing immunity, increasing cancer-protection, treating skin and having antiviral, antibacterial properties and treating kidney stones (19,20). Further, several researchers have shown bitter gourd as a means of managing diabetes (21-23) and some researchers have demonstrated the use of bitter melon oil of managing diabetes (24-26). The mechanism of action can be ascribed due to the presence of two essential compounds called charatin and momordicin which play a crucial role in restoring blood glucose level (26). Due to its antioxidant property, bitter gourd can prevent all the disorders which are related to hypoglycemia. In comparison to other vegetable oils, there is the availability of enough research data into their efficacy in modulating the health problems generated by diabetes. Consequently, the present study deals with the evaluation of certain vegetable oils (papaya, olive, fenugreek, bitter gourd) and fish oil on hyperglycemia, dyslipidemia and renal function markers as well as on renal oxidative damage in diabetic rats.

Materials and Methods

Chemicals and reagents

Trichloroacetic acid (TCA), thiobarbituric acid (TBA) and Streptozotocin (STZ) were procured commercially. All other chemicals and reagents were of the analytical grade, supplied by S. Merck (India).

Animals

In the study, male Wistar rats (160–200g) were obtained from the Animal House of Jamia Hamdard, New Delhi (India). They were kept eight rats per cage at ambient temperature 25 ± 2°C and 45–55% relative humidity with 12 h light/dark cycles. Before the dietary manipulation, rats had free access to standard rodent pelleted diet (Hindustan Lever Ltd., Bombay, India) and water ad libitum. Approval to perform animal experimentation was obtained from Institutional Animal Ethics Committee registered under the Committee for the Purpose of Control and Supervision of Experimental Animals (173/CPCSEA) Chennai, India.

Development of animal model

Diabetes mellitus was induced in rats by single intraperitoneal injection of Streptozotocin (55 mg/kg BW). After three days of STZ injection , blood was drawn via the tail vein after 12 h overnight fasting. Blood glucose concentration was measured via a strip-based glucometer. The rats that maintained fasting blood glucose higher than 250 mg/dl were considered diabetic and selected for further studies.

Experimental design

In the experiment, rats were separated into seven groups. Rats were treated with fish oil, fenugreek oil, bitter gourd oil, olive oil, and papaya oil respectively (3 g/kg body weight) for four weeks after diabetes induction. Group I (control (C)) rats were fed normal diet. Group II (D) rats were given STZ (55 mg/kg body wt); intraperitoneally (IP); in citrate buffer; pH 4.5). Group III (DFi) rats were diabetic rats and given fish oil.Group IV (DFe) rats were diabetic rats and given fenugreek oil. Group V (DB) rats were diabetic rats and then supplemented with bitter gourd oil; group VI (DO) rats were diabetic rats and then supplemented with olive oil; group VII (DP) rats were diabetic rats and then supplemented with papaya oil.

Tissue preparation

After anesthesia, the blood was drawn and stored at 4^{o}C for further analysis. Rats were then sacrificed by cervical dislocation and their kidneys were dissected out immediately and kept with ice-cold saline. For biochemical test, kidneys tissues were homogenized with phosphate buffer containing protease inhibitors in a homogenizer. The homogenate was centrifuged to separate the nuclear debris and was used for estimation of malonaldehyde (MDA) and thiobarbituric reactive substances (TBARS). The supernatant was further centrifuged to get the post-mitochondrial supernatant, which was used for enzymatic assays.

Parameters analyzed

Serum glucose estimation

Serum glucose was estimated by the GOD/POD Method using a commercial diagnostic kit. from Crest Biosystems, Goa, India.

Glycosylated Hemoglobin (GHb) estimation

Glycosylated hemoglobin was estimated by the Ion Exchange Resin method using a commercial diagnostic kit.

Assay for amylase activity

Amylase activity was measured by street and close method using a commercial diagnostic kit.

Assay for dyslipidemia

Dyslipidemia (triglycerides (TG), total-cholesterol, and HDL cholesterol (HDL-C)) markers were determined using enzymatic kits. Serum Triglyceride was estimated by GPO-PAP, End Point Assay using a commercial diagnostic kit. LDL and VLDL Cholesterol were calculated using Friedewald's equation**.**

Renal function markers in serum

The following markers evaluated kidney damage during diabetes in serum: serum creatinine (Scr) concentration, alkaline phosphatase (ALP) activity and blood urea nitrogen (BUN) level. Scr, BUN, and ALP were determined using enzymatic kits supplied by Span diagnostic Limited, Surat, India.

Oxidative stress markers

TBARS content

The method of Utley et al. (27) was used to determine the rate of lipid peroxidation (LPO) with some alternation. Estimation was started with the transfer of homogenate into test tubes and then incubation at 37°C

in a metabolic shaker for one hour. Same volume of homogenate was transferred into a centrifuge tube, placed at 0 °C and referred as zero hour incubation. After one hour of incubation, chilled trichloroacetic acid (TCA) was added, followed by TBA (w/v) and then centrifugation was done for 15 min at 1000 × g. After that, the supernatant was pipetted-out to other test tubes and was placed in a water bath at 100°C for 10 min. The absorbance was measured at 535 nm using a spectrophotometer. The TBARS content was calculated by using a molar extinction coefficient of 1.56 x 105 M^{-1} cm^{-1} and expressed as nmol of TBARS formed min^{-1} mg^{-1} of protein.

Assay formalondialdehyde (MDA)

MDA which is the end product of LPO was estimated as defined by Ohkawa et al (28). Homogenate 0.1 ml was transferred to centrifuge tubes, follwed by 20% acetic acid (pH 3.5), 0.8% TBA and 8.1% sodium dodecyl sulfate. The mixture was heated at 100°C for one hour and then cooled with tap water followed by adding of n-butanol: pyridine (15:1%, v/v) and distilled water (1 ml). The mixture was kept on a shaker and then centrifuged for 10 min at 4000 g. The organic layer was pipetted, and absorbance was taken using a spectrophotometer at 532 nm. The quantity of MDA formed in each of the samples was expressed as nmol of MDA formed h^{-1} $mg^{-1}$of protein by using a molar extinction coefficient of 1.56×105 M^{-1} cm^{-1}.

Assay for CAT

Catalase activity was assayed by the method of Claiborne (29). Briefly, the assay mixture consisted of phosphate buffer, hydrogen peroxide (H_2O_2), and PMS in a total volume of 3.0 ml. Changes in absorbance were observed at 240 nm using a spectrophotometer. The catalase activity was expressed as nmol H_2O_2 consumed min^{-1} mg^{-1} protein.

Protein content

Lowry et al.method was used to estimate the protein content by using bovine serum albumin (BSA) as a standard (30).

Histological examinations

Kidneys preserved in 10% buffered formalin solution were used for histopathological examinations. After fixation in 10% buffered formalin solution, thin slices of kidney tissue containing cortex and medulla were dehydrated and postfixed in paraffin. At least four cross-sections of 3-4 µm thickness were taken from each kidney and then stained with Jones periodic acid-Schiff (PAS), Masson Trichrome (MT)and hematoxylin and eosin (H and E) respectively. Following two changes of xylene washes of 2 min each, tissue sections were mounted with DPX mountant. The slides were observed for bright field microscopic evaluation and microphotographs were taken using an Olympus BX50 microscope system (Olympus, Japan). The PAS and MT stains of kidney were used to assess the extent of mesangial expansion, glomerular lesions, the presence or absence of any collagen deposits, tubular atrophy and lymphocytic infiltration. The H and E stains of kidneys were used to assess the extent of damage to the glomerulus and basement membrane.

Statistical analysis

All results are expressed as Mean±SEM. The statistical analysis was done by using SPSS 16 software and applying the analysis of variance (ANOVA) followed by Tukey's test.The p-value less than 0.05 was considered as a statistically significant.

Results

Effect of oils on FBG level in the diabetic group

In the diabetic group, a significant ($p< 0.001$) increase in blood glucose level was observed as compared to the control (Table 1). Treatment with oils significantly ($p< 0.05$) decreased blood glucose level in the diabetic treated rats compared to the diabetic group. However, Bitter gourd oil treatment was found more effective but very close to fenugreek oil.

Effect of oils on HbA1c in the diabetic group

A significant ($p< 0.01$) elevation in glycated hemoglobin (HbA1c) level was also seen in the diabetic rats when compared to the control rats (Table 1). Oils administration in the DFe, DO and DB groups significantly ($p< 0.05$) decreased the HbA1c level when compared to other oils treated groups. There was more restoration in glycated hemoglobin (HbA1c) level in the DB treated group, when it is compared to other treated groups.

Effect of oils on amylase in the diabetic group

A significant ($p< 0.01$) decrease in amylase activity was seen in the diabetic rats when compared to the control rats (Table 1). Oils administration in the treated groups significantly ($p< 0.05$) increased the amylase level. More significant changes were observed in bitter gourd and fenugreek oils treated animal group.

Effect of oils on dyslipidemia in the diabetic group

The diabetic rats revealed a significant ($p< 0.01$) increase in TG, TC, VLDL-C and LDL-C levels while a significant ($p< 0.01$) decrease in HDL-C level in serum compared to the control group (Table 2). Administration of oils in the treated groups significantly ($p< 0.05$) mo-

Table 1. Effect of oils treatment on FBG, HbA1c and amylase in the STZ- induced diabetic rats

Parameters/ groups	Control (C)	Diabetic (D)	DFi	DFe	DB	DO	DP
FBG (mg/dl)	90.50±4.7	287.50±5.3$^{\#\#\#}$	240.25±5.6*	216.25±5.0*	213.00±5.3*	220.25±5.9*	226.50±4.9*
HbA1c (%)	5.38±0.24	11.10±0.34$^{\#\#}$	9.60±0.31*	8.99±0.35*	8.59±0.29*	8.67±0.32*	9.68±0.30*
Amylase (U/dl)	29.78±3.8	14.59±1.6$^{\#\#}$	18.95±2.3*	22.07±2.5*	22.54±1.9*	20.77±2.8*	18.47±3.0*

The data represented as the mean ±SEM. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$ P<0.001 diabetic (D) group vs. control (C) group. *P<0.05, ** P<0.01, *** P<0.001 DFi, DFe, DB, DO and DP group vs. diabetic (D) group.

Table 2. Effect of oils treatment on serum dyslipidemia in the STZ- induced diabetic rats.

Parameters/ groups	Control (C)	Diabetic (D)	D + Fi	D + Fe	D + B	D + O	D + P
TC (mg/dl)	151.49±3.3	267.19±4.4$^{\#\#}$	200.94±4.2*	187.39±3.9*	197.45±4.0*	180.47±3.8*	220.79±4.3*
TG (mg/dl)	121.76±2.2	209.49±3.8$^{\#\#}$	186.81±3.1*	158.99±2.1*	173.94±2.6*	160.15±3.0*	183.37±2.6*
HDL-C (mg/dl)	45.02±1.1	22.20±0.78$^{\#\#}$	26.22±0.89*	35.19±0.99*	38.47±0.88**	38.67±0.85**	28.76±0.97*
LDL-C (mg/dl)	82.12±2.4	203.09±3.9$^{\#\#\#}$	137.36±2.4*	120.40±1.8*	124.20±2.0*	109.77±1.9**	155.36±2.1*
VLDL-C (mg/dl)	24.35±0.93	41.90±1.6$^{\#}$	37.36±0.90*	31.80±0.80*	34.78±0.86*	32.03±0.90*	36.67±0.82*

Effect of oils treatment on TC, TG, LDL-C, HDL-C and VLDL in diabeticrats.The data represented as the mean ±SEM. $^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 diabetic (D) group vs. control (C) group. * P<0.05, ** P<0.01, *** P<0.001 DFi, DFe, DB, DO and DP group vs. diabetic (D) group.

Table 3. Effect of oils treatment on renal function markers (BUN, Scr and ALP) in serum of the STZ induced diabetic rats.

Parameters/groups	Control (C)	Diabetic (D)	DFi	DFe	DB	DO	DP
BUN (mg/dl)	30.95±2.0	69.17±4.2, $^{\#\#}$	60.44 ±3.9*	40.15±4.0**	43.05 ±3.7*	55.85 ±4.2*	58.38 ±3.8*
Scr (mg/dl)	0.67±0.06	1.74 ±0.09, $^{\#\#\#}$	1.25 ±0.07*	0.80 ±0.05***	0.78 ±0.04***	1.12 ±0.08*	0.90 ±0.07**
ALP (units/dl)	23.94±0.94	49.71±3.1, $^{\#\#}$	40.31±2.8*	36.21±1.8*	39.41±2.5*	39.33±2.4*	40.56±3.0*

The data represented as the mean ±SEM. The diabetic group showed a significant increase in renal function markers (BUN, Scr and ALP) in serum of the diabetic group compared to the control group. $^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 diabetic (D) group vs. control (C) group. * P<0.05, ** P<0.01, *** P<0.001 DFi, DFe, DB, DO and DP group vs. diabetic (D) group.

dulated all the alteration in lipid profile compared to the diabetic group. However, treatment with olive oil was found more efficient compared to other oils treatment, but fenugreek oil also augmented the changes efficiently when compared to the diabetic group.

Effect of oils on renal function markers in the diabetic group

Efficacy of oils on renal function markers (BUN, Scr and ALP) were estimated to determine renal functioning in the serum of the diabetic rats. BUN, Scr, and ALP were showing significant ($p< 0.01$) increment in the diabetic rats compared to the control rats. While treatment with oils showing significant ($p< 0.05$) decrement in these markers in the treated groups compared to the untreated diabetic group (Table 3). The maximum restoration was found for Scr in fenugreek and bitter gourd oils treated groups (DFe and DB, $p < 0.01$)).

Effect of oils on TBARS contents and MDA level in the diabetic group

The level of these parameters were significantly ($p<0.001$) increased in the diabetic rats compared to the control rats. TBARS and MDA content decreased significantly ($p<0.05$, $p<0.01$) in the diabetic group with oils treatment (Fig 1 & 2).

Effect of oils on CAT activity in the diabetic group

The activity of CAT was declined significantly ($p< 0.01$) in the diabetic rats compared to the control rats. Treatment with oils showing significant ($p< 0.05$) increment in the activity of this enzyme in the DFi, DFe, DB, DO and DP groups compared to the diabetic group. And more significant ($p< 0.05$) restoration was seen in DFe and DB treated groups.

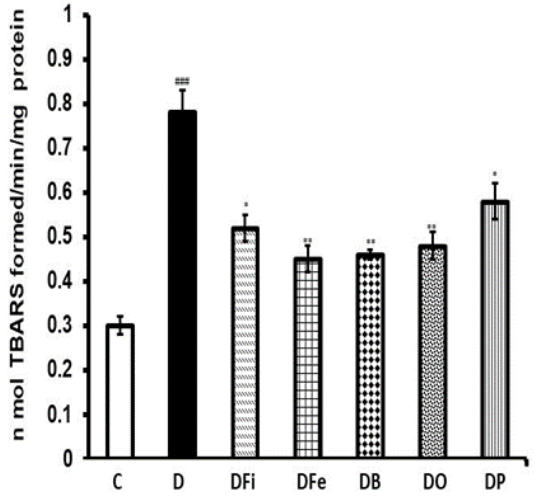

Figure 1. Effect of oils treatment on TBARS levels. The D group showed a significant increase in TBARS levels compared to the control group ($^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 diabetic (D) group vs. control (C) group).Oils treatment significantly decreased TBARS levels in the DFi, DFe, DB, DO and DP group compared to the D group (* P<0.05, ** P<0.01, *** P<0.001 DFi, DFe, DB, DO and DP group vs. diabetic (D) group).

Histological analysis of kidney

Histopathology with HE stained kidney (figure 4), control group showing normal renal parenchyma with normal glomerulus. No thickening of the basement

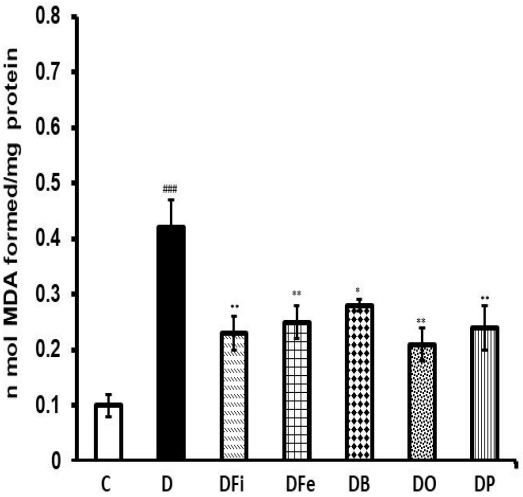

Figure 2. Effect of oils treatment on MDA level. The D group showed a significant increase in MDA level compared to the control group ($^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 diabetic (D) group vs. control (C) group).oils treatment significantly decreased MDA level in the DFi, DFe, DB, DO and DP group compared to the D group (* P<0.05, ** P<0.01, *** P<0.001 DFi, DFe, DB, DO and DP group vs. diabetic (D) group).

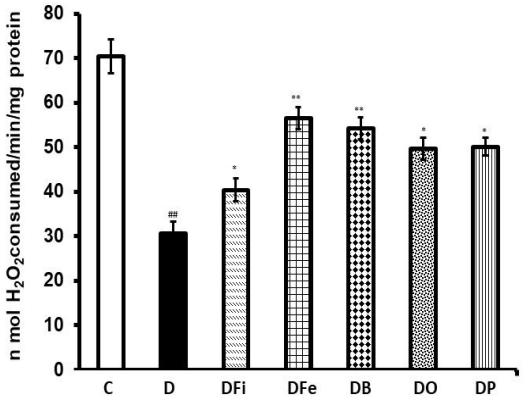

Figure 3. Effect of oils treatment on CAT activity. The diabetic (D) group showed a significant decrease in CAT activity compared to the control group ($^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 diabetic (D) group vs. control (C) group). Oils treatment significantly increased enzyme activity in the DFi, DFe, DB, DO and DP groups compared to the Dgroup group (* P<0.05, ** P<0.01, *** P<0.001 DFi, DFe, DB, DO and DP group vs. diabetic (D) group).

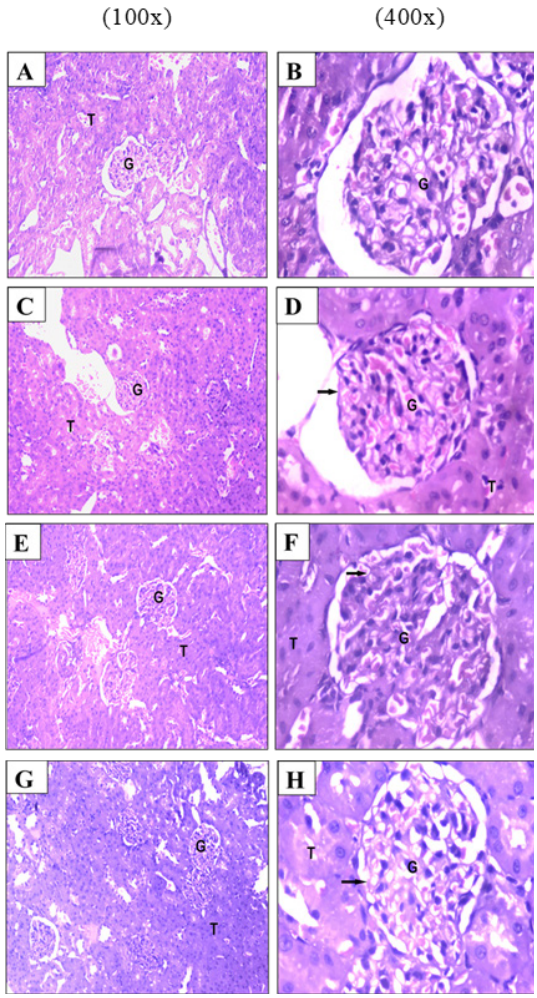

Figure 4. Representative images of HE stained kidney, showing effects of oil-treatment in diabetic nephropathy. (A) Control group animal was showing normal renal parenchyma. (B) Control group was showing a glomerulus with normal structure. No thickening of the basement membrane is seen. (C) Kidney section from Diabetic group animal showing normal renal parenchyma at this magnification.. (D) The same section showing a glomerulus with significant thickening of basement membrane (Arrow) seen.. (E) Kidney section from bitter gourd oil treatment showing normal renal parenchyma at this magnification. (F) Higher power photomicrograph from the samesection showing a glomerulus with minimal thickening of basement membrane (Arrow) seen. (G) Kidney section from fenugreek oil treatment group showing normal renal parenchyma at this magnification. (H) The same section showing a glomerulus with minimal thickening of basement membrane (Arrow) seen. Magnification is given at the top of the left and right panel.

membrane is seen. Kidney section from the diabetic group animal showing a glomerulus with significant thickening of basement membrane, while bitter gourd and fenugreek oil treatment showing normal renal parenchyma at this magnification and glomerulus with minimal thickening of the basement membrane. In the histological analysis of MT stained kidney, Control group stained for collagen deposits showing minimal collagen around the interstitial blood vessels. Kidney section from Diabetic group showing collagen deposits with a definite increase in collagen around the interstitial blood vessels. Kidney section from bitter gourd oil treatment group showing minimal collagen around the interstitial blood vessels, while fenugreek oil treatment group showed a definite increase in collagen around the interstitial blood vessels alongwith inflammatory cell infiltration in the interstitium. Results from PAS-stained kidney showed, a common histological representation of kidney showing glomerulus, with no increase in mesangium and a thin basement membrane in control group. Diabetic group showed glomerular hypertrophy withmesangial expansion. The confined areas of tubular atrophy showing thickening of the tubular basement membrane. Bitter gourd oil supplemented group showed glomerulus with no increase in mesangium and a thin basement membrane. However, fenugreek supplemented group, showing mild damage. The glomerulus shows mesangium expansion and thickening of basement membrane but comparatively lesser than the diabetic group.

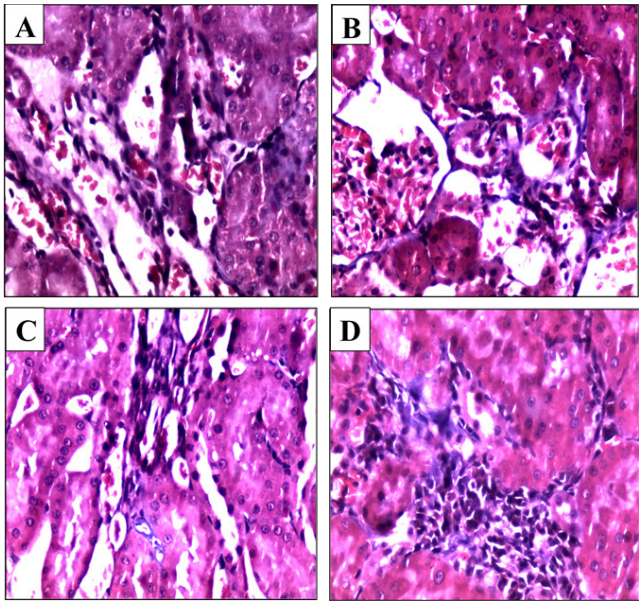

Figure 5. Representative images of MT stained kidney, showing effects of oil-treatment in diabetic nephropathy. (A) Control group stained for collagen deposits showing minimal collagen around the interstitial blood vessels. (B) Kidney section from Diabetic group stained for collagen deposits shows a definite increase in collagen around the interstitial blood vessels. (C) Kidney section from bitter gourd oil treatment group showing minimal collagen around the interstitial blood vessels. (D) Fenugreek oil treatment showed a definite increase in collagen around the interstitial blood vessels along with inflammatory cell infiltration in the interstitium. Collagen deposits are stained blue. (Masson Trichrome x 400).

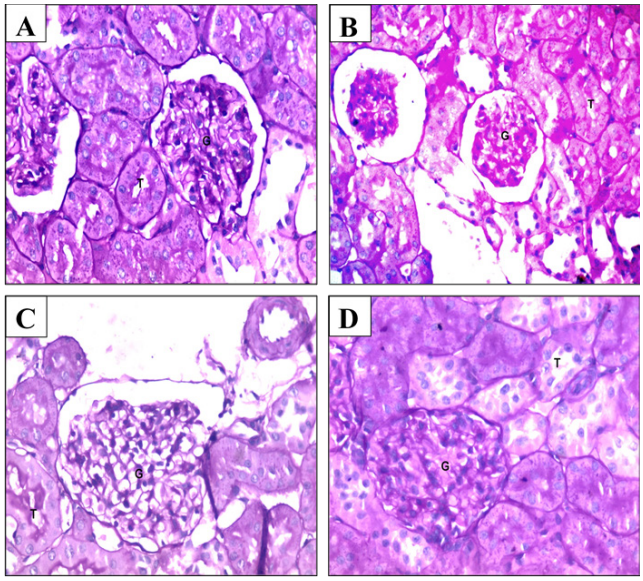

Figure 6. Representative images of PAS stained kidney, showing effects of oil-treatment in diabetic nephropathy. (A) A normal histological appearance of kidney showing glomerulus, with no increase in mesangium and a thin basement membrane. (B) Renal section of the diabetic group showing glomerular hypertrophy withmesangial expansion. The local areas of tubular atrophy showing thickening of tubular basement membrane and presence of proteinaceous casts in the lumen. (C) D + B supplemented group, glomerulus with no increase in mesangium and a thin basement membrane. (D) D + Fe supplemented group, showing mild damage. Most of the tubules are undamaged. The glomerulus shows mesangium expansion and thickening of basement membrane but comparatively lesser than the diabetic group. G = Glomerulus, T = Tubule (PAS x 400).

Discussion

This study showed that STZ-induced model in rats carried out alterations such as hyperglycemia, dyslipidemia followed by the incidence of renal damage. Moreover, we investigated that administration of papaya, olive, fenugreek, bitter gourd, and fish oils, by virtue ameliorated STZ-induced alterations in the rat model of diabetes.

Results showed that diabetic rats had elevated glucose levels and declined amylase level than of control rats. While treatment with oils lower the blood glucose level in the diabetic rats. Maximum reduction was found in fenugreek and bitter gourd oils treated groups. Antihyperglycemic effect of oils is dependent upon their antioxidant properties, increased insulin secretion, and facilitated carbohydrate digestion (18,24-26). Recent studies have also been showed to exert low sugar activity of oils through antioxidant properties (31,32). There is a direct relationship between hemoglobin level and diabetes. Measurement of HbA1c gave the longer view of glucose in blood and had been proved mostly helpful in observing the effectiveness of treatment in DM (33). In the present study, HbA1c level increased in the diabetic group when compared to control rats. Administration of oils to diabetic group restored the changes by its free radical scavenging property and thus decreased the level of HbA1c. Reduced blood glucose level might also aid to the lower level of glycated hemoglobin in oils treated diabetic group. Though bitter gourd oil administration improved hyperglycemia condition more than any other oils but further research needed to know its potentiality in the treatment of diabetes, however consuming bitter gourd oil may aid in diabetes due to the presence of two essential compounds (26).

It was noticed that hyperglycemia associated with a variety of changes in regulatory and metabolic processes, which leads to dyslipidemia in diabetic patients (34). Further, it was found, diabetic rats in the present study showed changes in lipid profile as indicated by the significantly increased level of serum TG, TC, VLDL-C, LDL-C and decreased HDL-C levels. While the administration of oils for four weeks significantly augmented all these changes by decreasing TG, TC, VLDL-C, LDL-C and increasing HDL-C levels in diabetic rats. The maximum restoration was seen in olive oil and fenugreek oil treated groups. Already researchers have shown that olive oil due to the presence of monounsaturated fatty acids and antioxidants, has demonstrable efficacy in protecting LDL from oxidation and in restoring the levels of dihomo-gamma-linolenic acid (20: 3, n – 6) in serum cholesterol esters, and phospholipids in type 2 diabetic subjects (35,36). Moreover, the protective effect of fenugreek oil is due to the presence of steroidal saponins that are converted in the alimentary canal to sapogenins which increase biliary cholesterol excretion, leading to decreased cholesterol levels (37). This finding suggested that this treatment strategy is highly beneficial in not only modulating but even restoring lipid abnormalities associated with diabetes side effects.

Further, recent evidence suggests that hyperglycemia

leads to morphological changes and eventually function alteration in the kidney (38,39). Already published data showed the increased level of BUN, Scr, and ALP during renal damage (40). The present study coincided with the previous report as BUN, Scr, and ALP levels were found increased in serum in the diabetic group (41,42). In contrast, the DFe and DB treated rats showed substantial restoration of these changes, thus demonstrating its ability to protect against diabetes-induced renal damage. Both oils demonstrated the positive effect in the recovery of renal impairment.

H_2O_2 is processed in the form of water and oxygen by the antioxidant enzyme, Catalase (CAT). The increase in $H_2O_2$and other oxyradicals due to hyperglycemia induces the peroxidation of polyunsaturated fatty acids. That causes the formation of markers of oxidative damage such as TBARS and MDA (43). Consistent with previous studies, (44,45) the present study noticed increased TBARS and MDA contents and a decreased antioxidant enzyme (CAT) in the kidney. While treatment with oils, more effectively fenugreek, and bitter gourd oil as a powerful antioxidant, significantly ameliorated all the oxidative damage evidenced by hyperglycemia in the kidney tissue of the rats.

All these results were further supported with the histological examination. Significant structural abnormalities were seen in the glomerulus and tubules of diabetic control rats. The perceived glomerular and tubular damage might occur due to hyperglycemia and oxidative stress in the glomerulus and tubular cells. The damage caused by these conditions decreased considerably by treatment with fenugreek and bitter gourd oils, indicating effective protection offered by these oils in DN.

Fenugreek oil was found effective due to the presence of omega-3 fatty acid and their antioxidants properties. The role of fenugreek in reducing blood glucose level and curing insulin resistance has been reported previously (46-48). The possible mechanism of action could be attributed via managing carbohydrate metabolism (49). Previous data showed that bitter gourd consist compounds named as oleanolic acid glycosides which have beneficial effect on glucose tolerance in type 2 diabetics, by restraining or curing insulin resistance (50). Further, a study has shown that bitter melon triterpenoids activate an enzyme, called AMPK which is responsible for transporting glucose from the blood into the cells, and also regulates metabolism (51). Improvement of hyperglycemia by bitter gourd has been ascribed by many possible mechanisms via inhibiting glucose absorption in the alimentary canal, enhancing glucose metabolism, increasing the glucose uptake by tissues, and raising insulin-like action and pancreatic beta-cell stimulation (42,52,53).

On the basis of present findings, it was suggested that the mechanism of action of oils is through their antioxidant property. Thus, our data indicated that oils as potent anti-diabetic agent and play adorable role in managing diabetes-related conditions such as hyperglycemia, dyslipidemia and renal damage in STZ-induced rat model of T2DM. Further research is warranted to know the underlying mechanism of oils that could attribted to prevent oxidation, accumulation of lipids and high blood glucose level associated with diabetes and whether these oils alone or in blend form can offer an alternate treatment for the patients of T2DM.

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Interest conflict

The authors declare that there are no conflicts of interest.

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