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RESEARCH ARTICLE

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The effects of Fenugreek, Nigella and Termis seeds on lipid profile in diabetic albino rats

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ABSTRACT

Dyslipidemia, an established risk factor for coronary heart diseases (CHD), is strikingly common in patients with type 2 diabetes mellitus (T2DM). This study investigated the effects of some antidiabetic herbs used in Saudi Arabia on lipid profile in diabetic rats. Six experimental groups of adult male Albino rats each consists of twenty five rats. The first group was considered as a control group. The rest of groups were affected by induction of diabetes by Alloxan. The second group received no treatment. The third group was treated with the aqueous extract of the mixture contains an equal ratio of Fenugreek, Nigella and Termis seeds. The fourth group was treated with the aqueous extract of Nigella seeds, the fifth group was treated with the aqueous extract of Fenugreek seeds and the sixth one was treated with the aqueous extract of Termis seeds. After four weeks of treatment, biochemical parameters were performed, including: fasting blood glucose (FBG), serum insulin (SI), and lipid profile. Pancreatic samples were obtained for microscopic and quantitative evaluation. The usage of the mixture or each plant alone improves the dyslipidemia present in diabetic rats. Also, correct the glucose and insulin level. Microscopically, all treated groups showed a significant increase in β-cell number as compared to non-treated group. The aqueous extract of the mixture of the studied herbs is useful in reducing the hyperglycemia by increasing insulin level and regenerating β-cell of the pancreas. The aqueous extract of the mixture and Fenugreek are the most powerful in amelioration of lipid abnormalities present in diabetic rats.

Key words: Diabetes, Alloxan, Antidiabetic plants, Dyslipidemia, Pancreas

INTRODUCTION

Diabetes mellitus (DM) is possibly the world's fastest growing metabolic disease [1]. Patients with T2DM have a higher incidence of hypertension, dyslipidemia, and obesity, which contribute to their high risk of CHD [2]. Disturbance of lipid metabolism appears to be an early event in the development of T2DM, potentially preceding the disease by several years [3]. The spectrum of dyslipidemia in DM can include all the various types of dyslipidemia identified in the general population; however, the characteristic features are a high plasma triglyceride (TG) concentration, increased concentration of small density lipoprotein (LDL) particles and low high density lipoprotein (HDL) concentration which are attributed mostly to insulin resistance (IR) and insulin deficiency [4]. The main cause of diabetic dyslipidemia is the increased free fatty acid release from IR fat cells into the liver in the presence

of adequate glycogen stores promotes TG production, which in turn stimulates the secretion very low density lipoprotein cholesterol (VLDL) [5].

The increased number of VLDL cholesterol particles and increased plasma TG levels decrease the level of HDL and increase the concentration of small, dense LDL particles via several processes: VLDL transported TG is exchanged for HDL transported cholesteryl ester through the action of the cholesteryl ester transfer protein (CETP), which results in increased both atherogenic cholesterol-rich VLDL remnant particles and TG rich cholesterol depleted HDL particles [6]. Many plants have been investigated for their beneficial use in DM and their active principles have been reported to possess pancreatic beta cells re-generation, enhance glucose uptake by adipose or muscle tissues, inhibit glucose absorption from the intestine and glucose production from the liver, and antagonize IR [7]. The literature survey revealed that Nigella (Nigella sativa) has been shown to produce multi-systemic beneficial actions including hypocholestermic, hypoglycemic and antioxidant [8]. Oral administration of the ethanolic extract of Nigella seeds to streptozotocin induced diabetic rats for 30 days reduced the elevated levels of blood glucose and improved altered levels of lipid peroxidation products because of its antioxidant effects [9]. Fenugreek (Trigonella foenum-gracaeum) belongs to the family Fabaceae and is used in many parts of the world for the treatment of diabetes. Fenugreek seeds have been shown to possess hypoglycemic, hypolipidemic, and antioxidant effects [10]. Various hypotheses about the mechanism of the hypoglycemic activity of Fenugreek have been postulated, including delayed gastric emptying, increase the plasma insulin level and an agonist effect on insulin receptors [11, 12].

The presence of proteins and fiber (galactomannan) in Fenugreek seeds might exert a lipid lowering effect [13] and may form a viscous gel in the intestine and inhibit glucose and lipid absorption [14]. Termis seeds (Lupinus termis or Lupine) are a medicinal plant with potential value in the management of diabetes. In white mice, the extracts of seeds of the Termis were associated with increased tolerance to an oral glucose administration [15]. Some studies observed a beneficial influence of lupin protein on blood cholesterol concentrations and also partially in blood pressure [16]. Our previous work [17] revealed that, the usage of theses plants corrected the glucose and insulin level in the Alloxan induced diabetic rats and may have a cardiometabolic protective effect. Based on the above mentioned findings, This study was designed to examine the effects of the aqueous extracts of a mixture containing equal ratio of Nigella, Fenugreek and Termis and of each of these plants individually on lipid profile in diabetic rats.

MATERIALS AND METHODS

The study was approved by the Research Ethics Committees, College of Medicine, Taif University, KSA.

Plant material

The dried seeds of Nigella, Fenugreek and Termis purchased from a local market in Taif, KSA in Jan., 2012. A voucher specimen of each plant was kept in the Department of Pharmacognosy, College of Pharmacy, Taif University, KSA.

Preparation of plant extract

Exact weight (50 gm) of the dried powdered seeds of Nigella, Fenugreek and Termis and a mixture of equal ratio of the powdered seeds (16.66 g Nigella + 16.66 g Fenugreek + 16.66 g Termis) were separately extracted with 100 ml distilled water each, by the decoction method at temperature 100 °C for five minutes (boiling time). Each extract was separately filtered through cotton followed by re-filtration through filter paper. The obtained aqueous extract of each sample was concentrated under vacuum using a rotary evaporator to about 10 ml each followed by complete drying by lyophilization method using a freeze-dryer to afford dried extracts as follow: Nigella (5.56 gm), Fenugreek (6.48 gm), Termis (5.23 gm) and the mixture (5.04 gm). All dried extracts were kept on -40°C till used for this study. The obtained dried aqueous extracts of Nigella, Fenugreek, Termis seeds and the mixture, each were suspended in 0.5% carboxymethylcellulose sodium (CMC) solution immediately before use and was given separately by the oral route of administration to male albino diabetic rats in a dose 100 mg/kg body weight [18].

Animals' material

One hundred fifty adult male albino rats 10-12 weeks age with body weight ranging between 180-200 gm obtained from the Laboratory Animal Unit, King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, KSA. All experiments were taken place in the research laboratories, College of Medicine, Taif University, KSA. Animals were housed in a clean rodent cage, in a room with relative humidity not less than 30% and not exceeding 70%, and room temperature 22 °C - 30 °C, with artificial lighting with a sequence being 12 hours light and 12 hours dark.

Animals were fed on conventional laboratory animal diet for rats with an unlimited supply of drinking water. Animals were randomly selected, marked to permit group identification. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institute of Health as well as the guidelines of the Animal Welfare Act.

Induction of DM was done in 125 rats by giving a subcutaneous injection of Alloxan solution 120 mg/kg (powder from BDH chemical LTD, England), dissolved in acetate buffer (pH 5.5) prepared immediately before use. After an overnight fast, then 48 hours later, FBG level was determined according to glucose-oxidase method using kits from Diamond Diagnostic. Rats with a blood glucose level ranging from 180 to 250 were considered diabetic.

Plant extracts administration: Aqueous extracts of Nigella, Fenugreek, Termis seeds and a mixture of equal ratio of the powdered seeds, each with 0.5% CMC was separately given by oral gavage to diabetic rats in a dose 100 mg/kg body weight daily for four weeks.

The experiment was carried out on six groups; each group contains 25 rats as following:

Group I (Control group): normal rats were given a SC saline solution (0.01 ml/100 gm body eight).
The remaining one hundred diabetic rats were classified as follows:
Group II: was considered a diabetic group without receiving any treatment, only CMC.
Group III: were treated with the aqueous extract of the mixture of equal ratio of the seeds (100 mg/kg of body weight).
Group IV: were treated with the aqueous extract of Nigella seeds (100 mg/kg of body weight).
Group V: were treated with the aqueous extract of Fenugreek seeds (100 mg/kg of body weight).
Group VI: were treated with the aqueous extract of Termis seeds (100 mg/kg of body weight).

After 4 weeks, all animals fasted overnight, and then weighted, blood samples were obtained and then rats sacrificed. The animal is made comfortable in a restrainer while maintaining the temperature around at 24 to 27°C. If the vein is not visible, the tail is dipped into warm water (40°C). Local anesthetic cream applied on the surface of the tail 30 min before the experiment. A 23G needle is inserted into the blood vessel and blood is collected using a capillary tube or a syringe with a needle. After completing blood collection, pressure and silver nitrate ointment is applied to stop the bleeding. Blood samples centrifuged at 4000 xg for 10 min at 4°C and supernatant kept on -70°C for further biochemical measurements. Pancreas removed, then homogenizes separately, in 2, 5, 20 ml ice cold PBS, respectively.

Biochemical assays: Biochemical studies were done to assess the following biochemical parameters: FBG, serum SI, serum TG, serum total cholesterol (TC), HDL and LDL levels.

Histological studies: The samples of pancreas were fixed at 10% neutral buffered formal saline at pH 7.0 for 5 days. Dehydrated in ascending grades of alcohol and cleared in Benzol. Samples from each group were embedded in paraffin with a melting point between 55 °C and 56 °C for 4 hours and then paraffin blocks were prepared. Paraffin sections were made at 5 µm and stained with Hematoxylin and Eosin for demonstrating any histological changes. Modified Aldehyde Fuchsin [19] for detecting different cells of islets of Langerhans was done. Then sections were examined under the Microscope. Image analysis system (Leica DMDFC 299, Germany) was used for determination alpha, beta and delta cell number and diameters in the islet of Langerhans.

Statistical analysis
Data were analyzed using SPSS version 20 $X^{2.}$ Fisher exact tests were applied to observe an association between qualitative variables. Quantitative variables were expressed in means ± SDs. The comparison of quantitative data was performed by independent t-test or Mann Whitney test according to the normality of distribution for independent variables consisting of two groups and by ANOVA and Kruskal Wallis test according to the normality of distribution for independent variables consisting of more than two groups. Statistical significance was set at 0.05 levels [20]. A p-value of < 0.05 was considered as statistically significant.

RESULTS

Tables (1, 2), revealed that the serum glucose level raised to about 266 mg/dl (p= 0.008) and serum insulin level fallen to about 21 U/L (p= 0.008) with significantly increase levels of TC, TG and LDL and a significant decrease in HDL level in diabetic rats. After the 4th week of treatment, glucose concentrations (mg/dl) were 127 ± 1.58, 131.2

± 4.43 , 131.2 ± 1.92 and 134.8 ± 0.83 respectively. The glucose lowering effect occurs in all treated groups with a more marked decrease in the rats treated by mixture of herbs ($p<0.001$). Also, insulin was statistically increased in all treated groups compared with non-treated group ($p< 0.001$). The rise was more marked in rats treated by mixture of herbs ($p<0.001$). The levels of TC. TG and LDL were significantly decreased when compared with the diabetic group ($p< 0.001$). The lowering effect was more in mixture and Fenugreek groups ($p<0.001$). HDL showed non-significant increase among all treated groups. Histological study of pancreas in treated and non-treated groups of rats were shown (tables 3, 4 and figures 1). Induction of diabetes by Alloxan in rats had no effect on alpha and delta cells (number, cellular and nuclear diameters). However, a significant decrease in β-cell number and increase in β-cell diameter as well as nuclear diameters was found in the diabetic group as compared to control group ($P <0.001$). All treated groups showed a significant increase in β-cell number and decrease in β-cell diameter as well as nuclear diameters compared to non-treated group ($p<0.001$). However, the effect on β-cell number was more marked in rats treated with a mixture of herbs ($p<0.001$). No significant differences between all treated groups as regard effects on β-cell and nuclear diameter.

Table (1) Comparison between control and diabetic groups as regard metabolic profile

Parameters	Control (N=25)	Diabetic untreated group (N=25)	P
	mean ± SD	mean ± SD	
FBG mg/dl	136.8 ± 1.9	266.4 ± 0.89	0.0001
SI ng/ml	41.2 ± 0.83	20.2± 1.4	0.0001
TC mg/dl	143.4 ± 2.2	222.1± 1.1	0.0001
TG mg/dl	130.2±3.01	166.8±4.54	0.0001
LDL-C mg/dl	69.3 ± 2.8	125.9 ± 2.05	0.0001
HDL-C mg/dl	47.2 ± 0.75	44.1 ± 1.4	0.0001

**Fisher's Exact*

** p-value < 0.05 was considered as statistically significant.*

Table (2) Comparison between diabetic groups as regard metabolic profile

Parameters	Diabetic untreated group (N=25)	Mixture treated group (N=25)	Nigella treated group (N=25)	Fenugreek treated group (N=25)	Termis treated group (N=25)	P
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	
FBG mg/dl	266.4 ± 0.89	127 ± 1.58	131.2 ± 4.43	131.2 ± 1.92	134.8 ± 0.8	0.001
SI ng/ml	20.2± 1.4	45.2 ± 3.3	32.4 ± 3.2	38.4 ± 1.5	34.6 ± 2.3	0.001
TC mg/dl	222.1± 1.1	141.4 ± 0.79	191.1 ± 2.7	140.6 ± 1.3	190.5 ± 2.7	0.001
TG mg/dl	166.8±4.54	138.5± 3.6	147.7±1.7	136.3±2.4	157.8±3.5	0.0001
	NS between Mixture and Foenugreek treated groups					
LDL mg/dl	125.9 ± 2.05	68 ± 0.92	114.8 ± 1.6	67.8 ± 2.05	72.2 ± 1.5	0.001
HDL mg/dl	44.1 ± 1.4	45.8 ± 0.93	45.3 ± 2.4	45.2 ± 3	45 ± 2.05	0.294

**ANOVA test*

** p-value < 0.05 was considered as statistically significant.*

Table (3) Comparison between control and diabetic groups as regard to pancreatic cells

Parameters		Control Group (N=25)	Diabetic untreated group (N=25)	P
		mean ± SD	mean ± SD	
Alfa cells	Number	2.83±0.64	2.43±1.04	0.01
	Nuclear Diameter	1.34±0.43	1.50±0.42	0.169
	Cell Diameter	3.03±0.60	3.48±0.78	0.016
Beta cells	Number	32.66±13.94	10.80±5.14	<0.001
	Nuclear Diameter	1.58±0.35	2.39±0.48	<0.001
	Cell Diameter	3.25±0.29	4.49±0.74	<0.001
Delta cells	Number	4.3±1.6	4.40±1.84	0.970
	Nuclear Diameter	2.01±0.47	2.01±0.56	0.929
	Cell Diameter	4.45±1.36	4.33±0.62	0.482

**Fisher's Exact*

** p-value < 0.05 was considered as statistically significant.*

Fig (1): A photomicrograph of a section in the pancreas of a diabetic rat 30 day of herbal mixture showing decreased signs of vacuolation in β-cell with normal islets (Hx& E X 400)

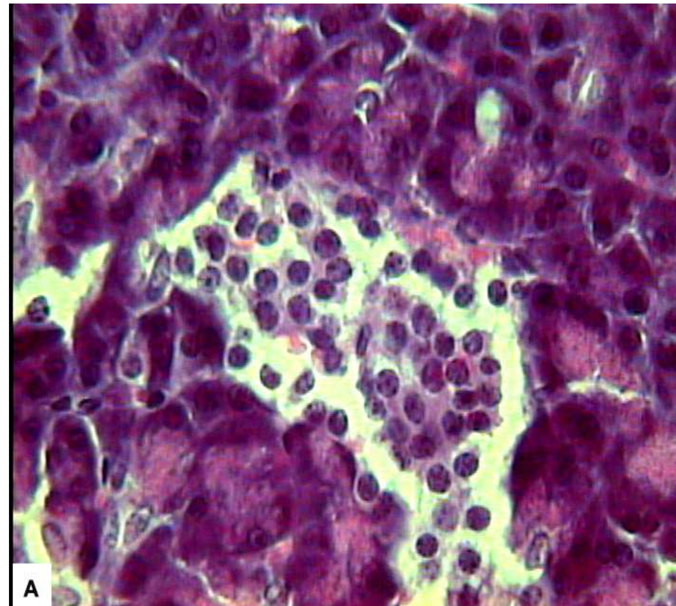

Fig (2): A photomicrograph of a section in the pancreas of a diabetic rat 30 day of Nigella Sativa ingested rat illustrating vacuolated β-cell, deeply stained nuclei, and with normal islets (Hx& E X 400)

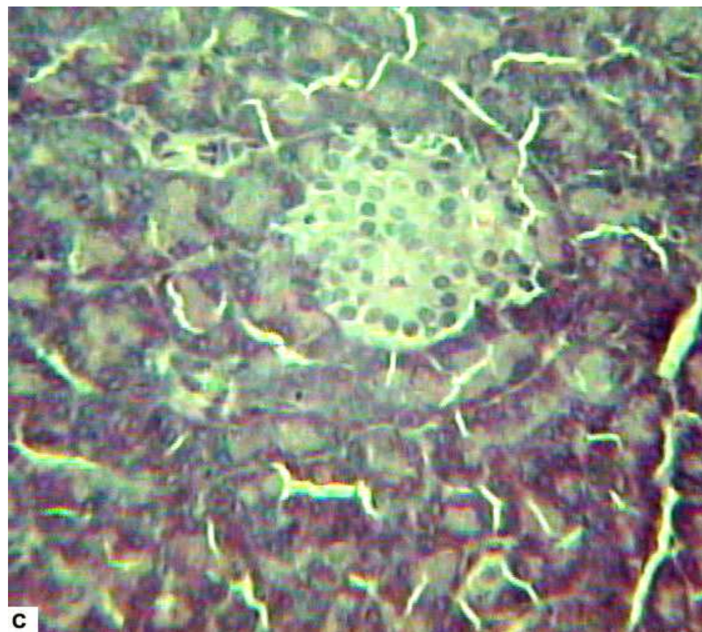

Fig (3): A photomicrograph of a section in the pancreas of a diabetic rat 30 day of Terms seeds ingested rat showing vacuolated and degenerated β-cell. Small islet having cellularity and poor vascularity (Hx& E X 400)

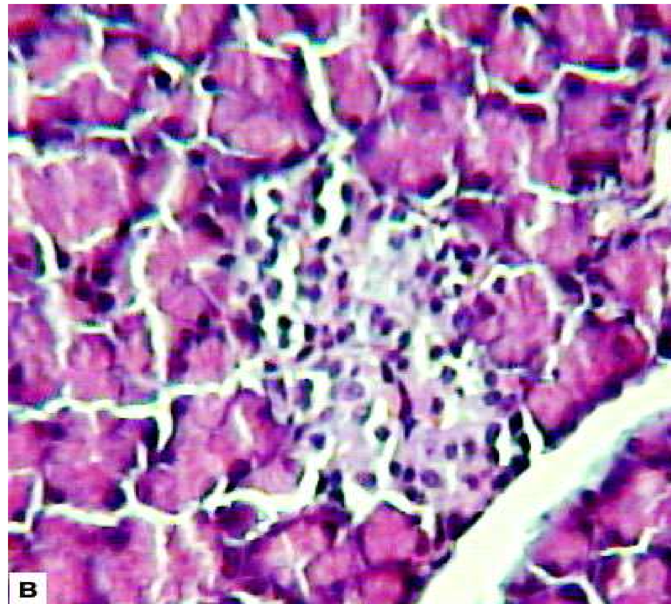

Fig (4): A photomicrograph of a section in the pancreas of a diabetic rat 30 day of Foenugreek ingested rat showing less vacuolated β-cells, and within normal islets (Hx& E X 400)

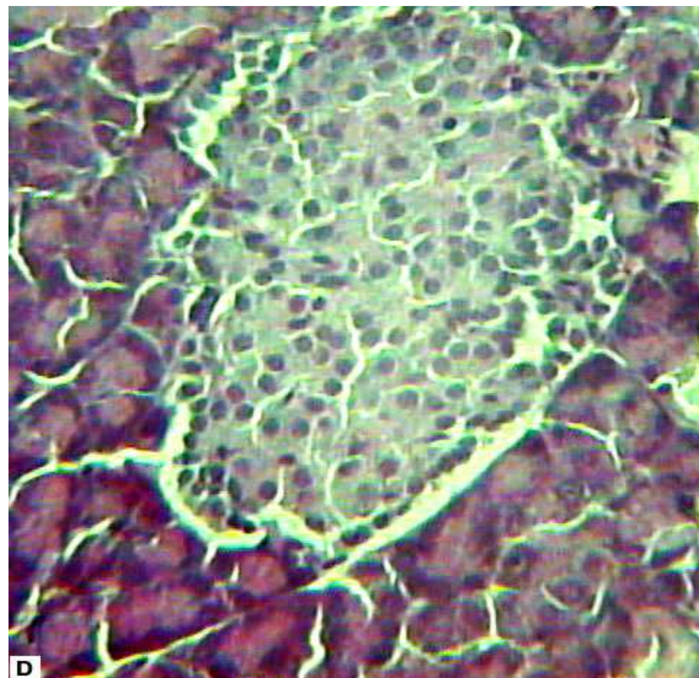

Table (4) Comparison between diabetic groups as regard different pancreatic cells after treatment

		Diabetic untreated rats (N=25)	Mixture treated group (N=25)	Nigella treated group (N=25)	Fenugreek treated group (N=25)	Terms treated group (N=25)
Alfa cells	Number	2.43±1.04	2.40±0.89	2.93±1.74	2.93±1.74	2.80±1.49
	Nuclear Diameter	1.50±0.42	1.27±0.31	1.25±0.28	1.25±0.28	1.36±0.39
	Cell Diameter	3.48±0.78	2.88±0.55	2.96±0.68	2.96±0.68	3.15±0.73
Beta cells	Number	10.80±5.14	34.12±16**	25.90±13.70*	25.90±13.70*	26±10.94*
	Nuclear Diameter	2.39±0.48*	1.32±0.17*	1.31±0.27*	1.31±0.27*	1.33±0.21*
	Cell Diameter	4.49±0.74	3.02±0.46*	2.70±0.38*	2.70±0.38*	3.06±0.50*
Delta cells	Number	4.40±1.84	3.40±1.10	3.46±0.89	3.43±1.04	3.40±1.16
	Nuclear Diameter	2.01±0.56	1.90±0.63	1.73±0.77	1.74±0.70	1.83±0.68
	Cell Diameter	4.33±0.62	3.66±1.06	3.34±0.64	3.33±0.67	3.03±0.46

**ANOVA test*

** p-value < 0.05 was considered as statistically significant.*

DISCUSSION

The present study was carried out to determine the effects of aqueous extracts of some antidiabetic medicinal herbs individually and as a mixture on lipid profile in rats with T2DM. Our results revealed a significant increase in the levels of TC, TG and LDL and a significant decrease in HDL level in diabetic rats. Dyslipidemia affecting almost 50% of patients with T2DM. An increase in the concentration of TC and LDL, and a decrease in HDL are associated with a raised risk of CHD [21]. TC, TG and LDL levels were significantly decreased in the treated groups compared to non-treated one ($p< 0.001$). The lowering effect was marked in mixture and Fenugreek groups ($p<0.001$). HDL showed non-significant increase among all treated groups. This may attributed to their stimulation to the most aspects of carbohydrate metabolism, including rapid cell glucose uptake, enhanced gluconeogenesis, increased absorption rate from the gastrointestinal tract and increased insulin secretion [22]. Severe hyperglycemia in diabetic rats in the present work can be considered as a direct reflex to the marked hypoinsulinemia caused by the selective destructive cytotoxic effect of Alloxan on the β-cells of the pancreas [23]. Our results revealed glucose lowering effect in all treated groups after the 4th week of treatment which is more marked in the mixture treated group by ($p<0.001$). Also, insulin was statistically increased in all treated groups compared with the diabetic group ($p< 0.001$). The rise was more marked in rats treated with herb mixture ($p< 0.001$).

Abdel Moneim et al. [24] reported that the hypoglycemic effect of Nigella may be attributed to an increase in the Islet numbers and to its effect on the time-course of glucose reabsorption from the intestine. On the other hand, the treated groups showed a significant increase in β-cell number and decrease in their diameters as well a nuclear diameter. These plants may have a stimulatory effect on the division of β-cells, block the diabetogenic action of Alloxan and restore insulin production [25]. Augusti and Sheela, 1996, [26] mentioned that some plants exert their effect on β-cell through both protection of the already present β-cell due to their antioxidant effect and through stimulation of the β-cell to release insulin. Akash et al. [27] mentioned that some medicinal herbs may have antioxidant effects especially Nigella. Marles et al. [15] suggested that, the hypoglycemic effect of some medicinal plants could be attributed to factors other than stimulation of insulin release only, e.g. Their effect on the number and /or affinity of insulin receptors on target cells and the post-receptors of these cells.

Zahida et al. [28] found that Nigella decreased cholesterol 14.58%, LDL 23%, triglycerides 15.16% and VLDL 15.16%, whereas HDL increased 3.18%, significantly ($P<0.05$) after six months of treatment and concluded that Nigella has ability to reduce the lipid profile which is a major risk factor for coronary artery disease in cardiac patients. Ghazi Mahmood et al. [29] stated that, Nigella is more effective than Gemfibrozil in hyperlipidemia and it can be used as an add on drug therapy in patients of metabolic syndrome with poor glycemic control. The most important action of Nigella that may be responsible for its beneficial effect in metabolic syndrome is its insulin sensitizing action [12]. Similarly the effects of Nigella seeds on the blood levels of cholesterol, triglycerides, HDL and LDL in white albino rats were studied by Ali et al., (2003) [30] a total of 200 rats, 150 experimental and 50 controlled were included in the study. Six doses of Nigella sativa were used (50, 100, 200, 300, 400 and 500 mg/day/200g rat). The dose was given for five durations: 1, 4, 7, 10, and 14 days. Generally, all doses of Nigella sativa produced significant reduction in the blood level of all studied parameters. There was no linear dose or time dependent effect of Nigella sativa on these parameters. The effect of Nigella sativa started after 4 days and continued some swings, for the rest of the duration.

The effective dose of Nigella sativa seemed to lie between 100-400 mg/kg. The carbonyl fraction of the seed extract has got excellent choleretic activity which may affect the body total lipid concentration and help prevent atherosclerosis [28]. Sirtori et al. (2004), [31] also showed that lupin protein isolates were able to reduce plasma total, VLDL and LDL cholesterol concentrations in rats. This effect was associated with the stimulation of LDL receptors by a well-defined protein component of the lupin seeds, as demonstrated by in vitro studies. Yoshie-Stark and Wa¨sche (2004), [32] also showed that the application of lupin-isolated protein had the capacity to bind bile acids to nearly the same extent as cholestyramine. Similarly, Martins et al. (2004, 2005), [33] found a hypocholesterolemic effect of Termis and stated that this effect was mainly the consequence of a marked decrease in the intestinal absorption of cholesterol probably modulated by bile acid reabsorption and a higher content of dietary phytosterols. Bähr et al. (2013), [34] found that a modest amount comprising 25.0 g/d of additionally consumed Termis is capable of lowering total and LDL cholesterol concentrations as well as the LDL:HDL cholesterol ratio from baseline to week 4, primarily in subjects with higher hypercholesterolemia. Proteins, galactomannan, and polyphenols from Fenugreek seeds have been reported to regulate dyslipidemia in obese and diabetic rodents. The presence of these phytoconstituents effectively inhibits fat accumulation and ameliorate dyslipidemia in obese rats, which is due to the improvement in glucose and lipid metabolism, increased insulin sensitivity, improve antioxidant defense, and downregulation of lipogenic enzymes [35].

CONCLUSION

The aqueous extract of the mixture of the studied herbs (Nigella, Fenugreek and Termis) is useful in reducing the hyperglycemia by increasing insulin level and regenerating β-cell of the pancreas. The aqueous extract of the mixture of the studied herbs and Fenugreek is the most powerful in amelioration of lipid abnormalities present in diabetic rats either through control of DM and direct action on associated dyslipidemia without added side effects. More studies on these plants are advised to be done with different doses and for different periods before recommending their use on a wide scale.

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