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Original Article

Hypoglycemic and Hypolipidemic Effects of *Myrtus communis*, *Trachyspermum copticum* and *Ferula gummosa* Essential Oils on Streptozotocin Induced Diabetic Rats

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ABSTRACT

Background and Objectives: Diabetes is one of the major health challenges in world. Herbal medicines are widely used for the treatment of diabetes. The current study assessed the effects of oral administration of essential oils from Myrtus communis, Trachyspermum copticum and Ferula gummosa on blood glucose and lipid profiles in streptozotocin-induced diabetic rats and inhibitory effects of these oils on α-glucosidase activity *in vitro*.

Materials and Methods: Forty-eight male Wistar rats were divided into six groups of healthy control, diabetic control, healthy control received corn oil and three experimental diabetic groups treated by the essential oils. Four weeks after intraperitoneal injections of 45-mg/kg streptozotocin doses, experimental groups were gavaged with 200 mg/kg/day of the oils for thirty days, then serum glucose and lipid profiles of the rats were assessed. Data were analyzed using one-way ANOVA and Tukey test. Study was carried out in Animal Laboratory of the Translational Ophthalmology Research Center, Tehran, Iran, 2016.

Results: Compared to healthy control group, serum glucose, triglyceride (TG) total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) increased in diabetic control group significantly ($P < 0.001$). No significant differences were observed in high-density lipoprotein cholesterol (HDL-C) between the healthy and diabetic control groups. The *M. Communis* oil showed the most significant inhibitory effects on α-glucosidase than those two other oils did. Furthermore, M. communis significantly decreased glucose (478±24 vs. 355±48; $p<0.001$) , TG (167±13 vs. 118±13; $p<0.001$), TC (107±11 vs. 83±13; $p<0.01$), and LDL-C (70±8 vs. 47±4; $p<0.001$) while increased HDL-C (37±5 vs. 53±9; $p<0.01$). F. gummosa and T. copticum had no effect on glucose levels in diabetic rats. T. copticum lowered TC (107±11 vs. 89±12; $p<0.05$) and (LDL-C (70±8 vs. 43±10; $p<0.001$) while increased HDL-C (37±5 vs. 49±8; $p<0.05$). F. gummosa just decreased TG (167±13 vs. 105±12; $p<0.001$) and LDL-C (70±8 vs. 30±4; $p<0.001$) levels in diabetic rats.

Conclusions: In general, lipid profile improvement was demonstrated using the three essential oils in diabetic rats; of these essential oils, only *M. Communis* oil included hypoglycemic effects possibly due to its α-glucosidase inhibitory activity.

Keywords: Diabetes, Glucose, Hypolipidemic agents, Medicinal plants, α-glucosidase

Introduction

Diabetes is a chronic metabolic disorder that affects many people worldwide. The disease is characterized by chronic hyperglycemia and impaired metabolism of carbohydrates, lipids and proteins (1). Diabetes is one of the leading causes of mortality in world resulting in long-term complications such as retinopathy, nephropathy, neuropathy and cardiovascular dysfunctions (2). Despite the

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beneficial effects of anti-hyperglycemic drugs, the majority of diabetic patients do not achieve an optimal blood glucose control; accordingly, there is a renewed interest in use of herbal medicines as alternatives in treatment of diabetes. Medicinal plants have been popular since ancient times because of their fewer toxic and side effects, widespread availability and relatively low costs (3). A number of medicinal plants have been used extensively in treatment of diabetes mellitus (4, 5). Anti-hyperglycemic activities of *Myrtus communis* oil in type-2 diabetic patients (6), hypolipidemic effects of *Trachyspermum copticum* seeds in patients with primary hyperlipidemia (7) and effects of *Ferula gummosa* species in treatment of diabetes (8) have already been shown in a few studies. However, specific ingredients of most herbs with therapeutic effects are not well defined (9). Findings of several medicinal plant studies have shown that phenolics, alkaloids, terpenoids, flavonoids, carotenoids, tannins and saponins are the most substantial phytoconstituents with antidiabetic effects which have been used empirically in antidiabetic and antihyperlipidemic remedies (5, 10, 11).

Medicinal plants may reduce blood glucose due to their ability to restore the function of pancreatic tissues and increase insulin output, inhibit intestinal glucose uptake or modulate inflammatory and oxidative stress-dependent pathways which show critical roles in pathogenesis of diabetes (11). Various constituents of antidiabetic medicinal plants improve glucose metabolism and lipid profiles in diabetic animals through various mechanisms including antioxidant, immunomodulatory and hepatopancreatic protective mechanism (5).Demonstrating antidiabetic and antihyperlipidemic effects of medicinal plants in stablished animal models of diabetes and further pharmaceutical mechanism studies are necessary. Antioxidant, anti-atherosclerosis and hepato-protective effects of *M. communis* extracts have been demonstrated in STZ-induced diabetes rats (12, 13). According to the authors' best knowledge, no studies have assessed effects of *F. gummosa and T. copticum* essential oils on glucose and lipid profile in diabetic rats. Therefore, the aim of the current study was to assess and compare therapeutic effects of *M. communis*, *T. copticum* and *F. gummosa* essential oils on serum glucose and lipid profiles in diabetic rats as well as the herbal effects on *in vitro* α-glucosidase activity.

Materials and Methods

Animals: A total of 48 2-month-old male Wistar rats with 250–300 g weight were purchased from Royan Institute, Tehran, Iran and used in this study. Each two animals were housed in a cage on a 12-hour light/dark cycle with chow and water *ad libitum*. Standard rodent chow was purchased from Behparvar (Iran). Food composition is shown in details in Table 1. All experimental procedures were approved by the Ethics Committee of the Translational Ophthalmology Research Center, Tehran University of Medical Sciences, Tehran, Iran (Approval No. IR.TUMS.VCR.REC.1395.688).

Table 1. Rat diet compositions

Nutrients	Total
Crude protein %	22-23
Crude oil %	3.5-4.5
Crude fiber %	4-4.5
Ash %	up to 10
Calcium %	0.95-1
Phosphorus %	0.65-0.7
Sodium chloride %	0.5-0.55
Moisture %	up to 10
Lysine %	1.15
Methionine %	0.33
Methionine and cysteine %	0.63
Threonine %	0.72
Tryptophan %	0.25
Energy (Mj/Kg)	16.16-17

Experimental groups: A total of 48 rats were randomly divided into six groups of eight animals as follows: Group 1, healthy control received 0.5 ml of distilled water (D.W.) per day for 30 days using gastric tube; Group 2, diabetic control intraperitoneally injected with 45 mg/kg of STZ and received 0.5 ml of D.W. per day for 30 days using gastric tube; Group 3, control group and corn oil received 0.3 ml of corn oil per day for 30 days using gastric tube. Corn oil was used in this study as a solvent for the essential oils; Group 4, diabetic and *M. communis* received 200 mg/kg of *M. communis* essential oil dissolved in corn oil per day for 30 days using gastric tube; Group 5, diabetic and *T. copticum* received 200 mg/kg of *T. copticum* essential oil dissolved in corn oil per day for 30 days using gastric tube; and Group 6, diabetic and *F. gummosa* received 200 mg/kg of *F. gummosa* essential oil dissolved in corn oil per day for 30 days using gastric tube (Fig 1).

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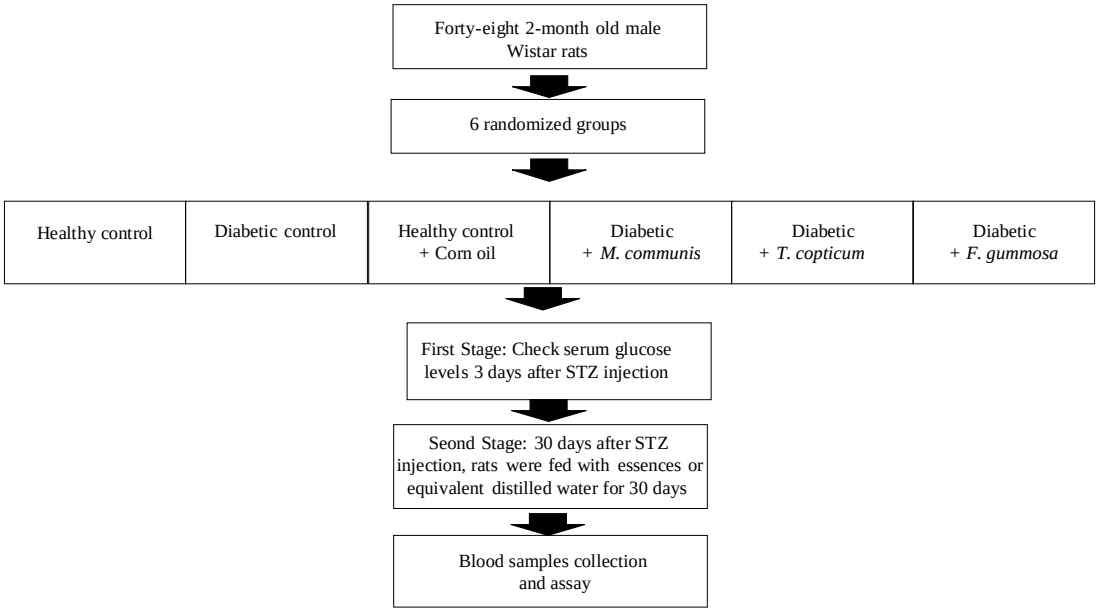


Fig 1. The experimental design

Induction of diabetes mellitus: Type I diabetes mellitus (DM) was induced using a single intraperitoneal (IP) injection of 45 mg/kg of STZ (Sigma-Aldrich, USA); dissolved immediately before use in 0.1 M of cold citrate buffer, pH 4.5 and used in 12-h fasting rats. Three days after DM inducement, fasting blood glucose (FBS) was assessed using blood samples from tails with glucometer (Arkray, Japan). Rats with FBS ≥ 250 mg/dl were considered diabetic and included in the study after four weeks.

Preparation of extract: All three essential oils were purchased from Barij Essence, Iran. Leaves of *M. communis*, gums of *F. gummosa* and seeds of *T. copticum* were used for oil extraction in the company. Compounds of *M. communis* included cineole, α-pinene, β-pinene, liminene and sabinene using gas chromatography (GC). Compounds of *F. gummosa* included α-pinene, β-pinene and myrtenol using GC. The major compound of *T. copticum* included thymol using GC.

Serum samples and biochemical tests: Thirty days after starting treatments, overnight fasting rats were anesthetized using ketamine and xylazine (100 and 15 mg/kg, respectively) and blood samples were collected directly from hearts. Sera were separated using centrifuge of blood samples at 1300 g for 20 min. Serum glucose, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were assessed using commercially available kits. All biochemical kits were purchased from Pars Azmun, Iran.

***In vitro* α-glucosidase inhibition assay:** Enzymatic assay of α-glucosidase was carried out using p-nitrophenyl α-D-glucoside (pNPG; Sigma-Aldrich, USA) as substrate. The α-glucosidase hydrolyzes pNPG to α-D-glucose and p-nitrophenol. Enzymatic activity was assessed using measurement of the p-nitrophenol absorbance. Ten microliters of 0.25-U/ml α-glucosidase (Sigma-Aldrich, USA) 130 µl of 100-mM phosphate buffer (pH 6.8) and 10 µl of each sample at various concentrations were mixed in microplate wells and incubated at 37 °C for 15 min. Then, 20 µl of 5-mM pNPG were added to the mixture and incubated at 37 °C for 15 min. Reaction was terminated using 80 µl of 0.2-M sodium carbonate solution. Absorbance of the wells was measured at 405 nm using plate reader (Autobio Diagnostics, China). Reactions without plant extracts and α-glucosidase were used as control and blank, respectively. Each experiment was repeated three times. The following formula was used to calculate the enzyme inhibitory rate of the samples (14):

$$\text{Inhibition (\%)} = \frac{\text{Abs 405(cotrol)} - \text{Abs 405 (extract)}}{\text{Abs 405 (control)}} \times 100$$

The α-glucosidase inhibitory activity of each essential oil was demonstrated by IC50 value (ppm). The IC50 is the concentration of an inhibitor required to decrease enzymatic reaction rates by 50%.

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Statistical analysis: The Shapiro-Wilk test was used for the normality test. Data with *P* values greater than 0.05 indicated the normal distribution of data. Data were analyzed using SPSS software v.21 (IBM Analytics, USA). One-way analysis of variance and Tukey test were used to show significant differences between the groups. *P* values < 0.05 were considered statistically significant.

Results

All data are expressed as mean ±SD. Table 2 and 3 show significant differences in body weight, food and water intakes, glucose, TG, TC, HDL-C and LDL-C in animals of normal control, diabetic control and treated diabetic groups after 30 days. No significant differences were seen between the healthy control and control groups that received corn oil. A significant difference was observed between the parameters (except HDL-C) in diabetic control group, compared to that in healthy control group. The mean differences of glucose, TG, TC and LDL-C between diabetic and healthy control groups were 341, 52, 24 and 37 mg/dl; these were higher in diabetic control group than healthy control group. *In vitro* α-glucosidase inhibitory activity of the three essential oils is shown in Fig. 2. The *M. Communis* essential oil showed more significant inhibitory effect on α-glucosidase than that the other oils did.

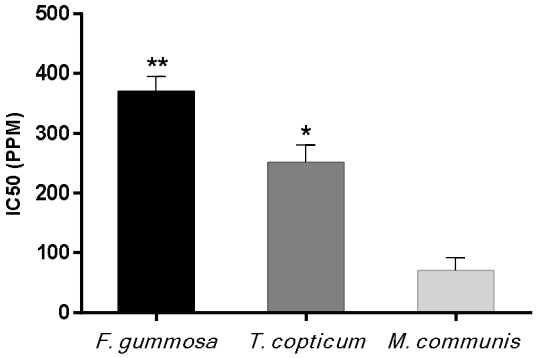


Fig 2. Differences in inhibitory effects of the essential oils on α-glucosidase activity. *Myrtus Communis* essential oil showed more significant inhibitory effects on α-glucosidase than that the other oils did. Values are given as mean ±SD. **P* < 0.01, ***P* < 0.001 vs *Myrtus Communis* essential oil

Table 2. Effect of essential oils on body weight, food and water intake in STZ-induced diabetic rats after 30 days of treatment

Groups	Weight (g)	Water (ml/day)	Food (g/day)
Control	337.42 ±14.39	45 ±13.4	17.25 ±4.56
Diabetes	218.57 ±27.81*	145 ±42.6*	27.92 ±6.22*
Control and corn oil	327.28 ±24.13†	52.6 ±18.6†	16 ±3.78†
Diabetes and *M. communis*	237 ±37.78*	146 ±44.5*	27.71 ±5.93*
Diabetes and *T. copticum*	238.28 ±26.22*	132 ±37.8*	24.07 ±6.42*
Diabetes and *F. gummosa*	230.14 ±38.84*	150 ±45.3*	31 ±5.74*

Values are given as mean ±SD, (n = 8 per group), *control *vs* other groups* : *P* < 0.001, †diabetes vs other groups†: *P* < 0.001

Table 3. Effect of essential oils on glucose level and lipid profile in STZ- induced diabetic rats after 30 days of treatment

Groups	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Control	137.10 ±21.30	115.27 ±16.24	82.98 ±10.20	33.27 ±4.15	46.46 ±8.12
Diabetes	478.43 ±24.43**	167.03 ±13.22**	106.94 ±11.31*	69.96 ±7.80**	37.05 ±5.02
Control and corn oil	187.18 ±22.92††	120.09 ±9.75†††	87.41 ±7.72†	32.65 ±2.13†††	39.43 ±5.82
Diabetes and *M. communis*	355.18 ± 48.40**†††	118.36 ±12.86†††	83.06 ±12.52††	47.51 ±3.98†††	53.24 ±8.87††
Diabetes and *T. copticum*	436.81 ±39.43**	147.82 ±9.91**	89.22 ±11.75†	42.70 ±9.73†††	49.31 ±7.86†
Diabetes and *F. gummosa*	429.91 ±46.14**	105.18 ±12.13†††	91.02 ±11.95	29.59 ±3.76†††	42.07 ±9.68

Values are given as mean ±SD, (*n* = 8 per group), *control vs other groups, $^{*}P$ < 0.01, $^{**}P$ < 0.001, †diabetes vs other groups $^{\dagger}P$ < 0.05, $^{\dagger\dagger}P$<0.01, $^{\dagger\dagger\dagger}P$<.001

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Discussion

Results of the current study showed the significant effects of *M. communis* in improvement of TC, TG, LDL-C, HDL-C and glucose in diabetic rats. *T. copticum* essential oil decreased TC, LDL-C and increased HDL-C in diabetic rats significantly. However effects on TG and glucose were not significant. The *F. gummosa* essential oil significantly decreased TG and LDL-C in diabetic rats. *M. Communis* showed the maximum α-glucosidase inhibitory activity *in vitro*. Intraperitoneal administration of STZ resulted in symptoms such as weight loss, polyphagia, polydipsia and polyuria and laboratory abnormalities such as hyperglycemia and dyslipidemia which are prominent alterations of diabetes (2). Streptozotocin alkylates DNA after taken up by pancreatic B cells via glucose transporters. DNA damage induces activation of poly ADP-ribosylation that leads to decreased ATP contents and subsequent inhibition of synthesis and secretion of insulin. Furthermore, STZ generates reactive oxygen species (ROS) which contributes to DNA damages. The ADP-ribosylation is likely more important for diabetogenicity of STZ than the generation of ROS (15). Moreover, diabetic rats showed lipid abnormalities such as hypertriglyceridemia, hypercholesterolemia, increased LDL and decreased HDL in response to increases blood glucose. Lipid disorders are a relatively common problem associated with diabetes mellitus. Studies have shown that diabetes and insulin deficiency result in increased activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, high triglyceride production, activated hormone-sensitive lipase and reduced lipoprotein lipase activity, which are possible reasons for lipid disorders in diabetes (16, 17).

In this study, *M. communis* essential oil inhibited activity of α-glucosidase *in vitro*. Furthermore, the essential oil showed a significant decrease in plasma glucose, compared to that in diabetic group. No significant differences were observed in glucose levels between the diabetic group and those received *T. copticum* and *F. gummosa* essential oils. A therapeutic approach to decrease postprandial hyperglycemia is the slow absorption of glucose in gastrointestinal tract (GI) by inhibition of carbohydrate hydrolyzing enzymes such as α-glucosidase (18). In 2004, Sepici et al. reported that the myrtle oil significantly increased glucokinase enzyme activity and glycogen in liver of alloxan-diabetic rabbits (18). They showed that the plasma insulin did not change after the *M. communis* oil administration; therefore, they suggested that α-glucosidase inhibitory effect of *M. communis* extract in brush-borders of the small intestinal epithelia could decrease the intestinal absorption of glucose (19). Oxidative stress plays an essential role in pathogenesis of both types of diabetes mellitus (20). Therefore, chemicals with antioxidative properties can decrease STZ toxicity due to preserve pancreatic β-cell integrity and may also decrease DNA fragmentation (15, 21). Snoussi et al. showed that the essential oils and ethanol extracts of myrtle included various natural antioxidants such as polyphenolic acids and flavonoids (22). Evidence show that plant/food polyphenols and their active metabolites include anti-hyperglycemic effects in diabetes. Hypoglycemic effects of these compounds can be attributed to decreased intestinal absorption of carbohydrate, carbohydrate metabolism regulation, improvement of pancreatic tissue regeneration and B-cell function and their antioxidative and anti-inflammatory properties (23). Moreover, polyphenol compounds have been shown to include effects on glucose tolerance and insulin sensitivity; as shown by Anderson (24).

In the current study, administration of the three essential oils provided positive effects on lipid profiles in STZ-diabetic rats. Antihyperlipidemic effects of aqueous extracts of *M. communis* (25) and seed powders of *T. ammi* have been shown in hyperlipidemic albino rabbits (26). A possible mechanism of lipid alteration might include inhibition of HMG-COA reductase in liver. The HMG-CoA reductase is a rate-limiting enzyme for cholesterol synthesis (27). The *M. communis* essential oil has been shown as a potent inhibitor of HMG-CoA reductase (28). The HMG-CoA reductase inhibition induces the upregulation of LDL receptor expression in liver while increases the catabolism of plasma LDL and decreases the plasma concentration of cholesterol (27). Phytosterols, found naturally in plants, are other important compounds that inhibit intestinal absorption

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of cholesterol and reduce LDL-C. Competing for the cholesterol incorporation into micelles in the intestinal lumen is a possible mechanism of the cholesterol-lowering effects of plant sterols (29). Although both chemical compounds include close chemical similarities, plant sterols include a greater affinity to form mixed micelles due to a more bulky hydrophobic group (29). Javed et al. detected significant quantities of flavonoids, tannins and sterols in *T. ammi* (30). Antioxidants seem to improve dyslipidemia in diabetes (31). The major components of *F. gummosa* include terpenoids. Previous studies have suggested that *F. gummosa* root extracts can scavenge H_2O_2 and this ability is associated to phenolic and terpenoids components of this plant (32). The major constituent of the essential oils from *T. copticum* includes thymol (33). Thymol and carvacrol, as natural monoterpene phenols, are well-known natural antioxidants, which are responsible for decreasing serum cholesterol possibly due to increased microsomal geranyl pyrophosphate pyrophosphatase activity (34, 35). Jalbani et al. concluded that Ajwain seeds were effective in primary hyperlipidemia by decreasing LDL-C by 8.9 and increasing HDL-C by 13.1% within two months (7). All essential oil treated groups showed increased HDL-C. A possible mechanism may include the effect of polyphenolic compounds that increases the activity of lecithin cholesterol acyl transferase (LCAT). The LCAT is responsible for the synthesis of cholesteryl esters and plays a critical role in maturation of HDL particles (36).

Conclusion

To the best of the authors' knowledge, the current study is the first study investigating effects of *T. copticum* and *F. gummosa* oils on glucose and lipid profiles in diabetic rats. Findings have demonstrated that the hypoglycemic effect of *M. communis* essential oil may link to its inhibitory effect on α-glucosidase activity. Furthermore, positive effects of the three essential oils on lipid profiles may attribute to antioxidant and polyphenol components of the oils. In the present study, the plant essential oils, and not their effective ingredients, have been used. Identification of the effective ingredients and studies of their mechanisms will result in further valuable knowledges. Moreover, human studies must be designed to generalize the current results.

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The authors declare no conflict of interests.

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