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Original Article

Hypolipidemic effects of alcoholic extract of eucalyptus (*Eucalyptus globulus* Labill.) leaves on diabetic and non-diabetic rats

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Abstract

Background: In traditional medicine, leaves of eucalyptus (*Eucalyptus globulus Labill.*) possess interesting biological properties such as antioxidant, antibacterial and antiviral effects. The aim of this study was to evaluate the hypolipidemic effects of eucalyptus ethanolic extract in non-diabetic and streptozotocin-induced diabetic rats.

Methods: In the present study, oral administration of 0.05, 0.1, 0.2 and 0.4 g/kg of eucalyptus leaves alcoholic extract for 21 days on the level of triglyceride and cholesterol in non-diabetic and streptozotocin-induced diabetic rats were evaluated. Six rats were arranged in each experimental group. A comparison was made between the effects of the alcoholic extract and a known antidiabetic agent, glibenclamide (600 µg/kg). Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test.

Results: The results showed that oral administration of the eucalyptus alcoholic extract caused a significant reduction of serum triglyceride and cholesterol in diabetic rats ($P<0.05$); whereas did not significantly change the levels of serum triglyceride and cholesterol in non-diabetic rats ($P<0.05$). The hypolipidemic effects of the extract were similar to that observed for glibenclamide.

Conclusion: It can be suggested to using leaves of eucalyptus as an adjuvant in the treatment of diabetes, however, further biochemical and pharmacological investigations are warranted to precisely elucidate the possible mechanism of action of this plant.

Keywords: Eucalyptus, Lipids, diabetes

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Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and/or by decreased responsiveness of the organs to secreted insulin [1]. DM is currently accounted as one of the most costly and burdensome chronic diseases and is notoriously increasing in epidemic proportions throughout the world [2]. Diabetes affects about 5% of the global population [3] and the management of diabetes without emerging any side effects is still a challenge to the medical system [4, 5]. DM is a disease characterized by hyperglycemia, and its complications have been attributed to the long duration of facing this condition due to the abnormality of glucose metabolism [6, 7].

At the same time, hyper-lipidemia has been frequently observed in type 1 [8,9] and type 2 diabetic patients [10]; as it was observed in experimental insulin-dependent [11,12] and non-insulin-dependent diabetic animal models [13]. Therefore, hyperlipidemia in addition to hyperglycemia, has been also thought to be a major risk factor for the development and progression of diabetic complications such as vasospastic angina [14], nephropathy [15,16], cataract [17] and peripheral neuropathy [18].

Renewed enthusiasm, in recent decades, has been directed to the alternative medicines and natural therapies and rapidly growing researches have been targeted traditional practices. The plant kingdom has become a target for the search for new drugs and biologically active "lead" compounds [19]. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes [20, 21], but only a few have scientifically scrutinized.

Eucalyptus globulus Labill. (Family: Myrtaceae) is a lofty tree of about 90 meter in height and is grown in various parts of Iran. Eucalyptus is traditionally used to treat diabetes in Iran [22]. Leaves from eucalyptus are reported to contain a high content of eucalyptol (cineol) together with rutin, terpineol, sesquiterpene, alcohols, aliphatic aldehydes, isoamyl alcohol, ethanol, terpenes and tannins [23]. In traditional medicine, leaves of eucalyptus possess interesting biological properties, such as antioxidant, antibacterial and antiviral effects [24-26].

In the present study, we investigated the hypolipidemic effects of eucalyptus leaves on normal and streptozotocin-induced diabetic rats.

Methods

Animals

Adult male Wistar rats weighing 200-230 g were used in this experimental study. The animals were maintained in an air-conditioned colony room at a temperature of 22ºC ±2ºC, relative humidity of 57±2% and photo-cycle of 12:12 h light and dark cycles. Animals supplied with standard pellet diet and tap water ad libitum. The study was conducted in accordance with ethical procedures and policies.

Preparation of eucalyptus extract

Fresh eucalyptus leaves were separated in summer then cleaned, and shed dried at 25°C, and the dried leaves of the plant were grounded with a blender. Dried and grounded leaves (about 60 g) were submitted to extraction with 300 ml ethanol (80%) in a Suxhlet apparatus (Hashemi, Iran) for 72 h. After extraction, the solvents were filtered and then evaporated by Rotavapor (Heidolph, Germany). The extract yield was 19%. The obtained eucalyptus alcoholic extractwas stored at −20°C until being used.

Experimental induction of diabetes in rats

Diabetes was induced by a single intraperitoneal injection of streptozotocin (70 mg/kg, i.p.) [27]. Five days after injection, diabetes was confirmed by determining plasma glucose levels as higher than 180 mg/dl. Six rats were used in each experimental group. Each animal was only used once in all of experiments. The food was removed from cages 12 h before testing.

Drug administration
Eucalyptus leaves extract was suspended in distilled water and administered orally through orogastric tubes with various doses including: 0.05, 0.1, 0.2 and 0.4 g/kg.

Experimental design
A total number of 66 rats (36 diabetes-induced rats, 30 normal rats) were arranged in 11 groups each contains 6 rats. Diabetes was induced in rats 5 days before starting the treatment. Group 1: normal control rats were administered 1 ml of distilled water, Groups 2, 3, 4 and 5: normal rats were daily administered eucalyptus leaves alcoholic extract (0.05, 0.1, 0.2 and 0.4 g/kg) via intragastric tube for 21 days, Group 6: diabetes-induced control rats were administered 1 ml of distilled water, Groups 7, 8, 9 and 10: diabetes-induced rats were daily administered eucalyptus leaves alcoholic extract (0.05, 0.1, 0.2 and 0.4 g/kg) via intragastric tube for 21 days, Group 11: diabetes-induced rats were daily administered glibenclamide orally (600 μg/kg) in aqueous solution via intragastric tube for 21 days.

Biochemical assays
After 21 days of treatment, blood samples were drawn from the heart. Serum total cholesterol and triglycerides levels were determined via Rifai method, 1999 [28].

Statistical analysis
All the data were given as mean ± SEM. Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test. Statistical P-value less than 0.05 was considered as significant.

Results

The effects of the eucalyptus leaves extract on serum total cholesterol in normal and diabetic rats is shown in Figure 1. One-way ANOVA showed that serum total cholesterol of diabetic control rats increased when compared with normal control rats ($P<0.001$). The administration of the eucalyptus leaves extract at doses of 0.2 ($P<0.05$) and 0.4 ($P<0.01$) g/kg and glibenclamide ($P<0.001$) significantly decreased total serum cholesterol toward normal values, while normal rats did not exhibit any significant alterations in the total cholesterol levels during the experiment. The effect of eucalyptus leaves extract was similar to that observed for glibenclamide. On the other hand, there were no significant changes in serum triglyceride levels of treated diabetic rats with eucalyptus leaves extract at dose 0.4 g/kg and glibenclamide ($P<0.05$).
The effect of the eucalyptus leaves extract on serum triglyceride in normal and diabetic rats presented in Figure 2. One-way ANOVA showed that serum triglyceride of diabetic control rats increased when compared with normal control rats ($P<0.001$). The administration of the eucalyptus leaves extract at doses of 0.2 ($P<0.05$), 0.4 ($P<0.01$) g/kg, and glibenclamide ($P<0.001$) significantly lowered serum triglyceride levels toward normal values; whereas, normal rats did not exhibit any significant alterations in the triglyceride level during the experiment. The effect of eucalyptus leaves extract was similar to that observed for glibenclamide. Nonetheless, there were no significant changes in serum triglycerides levels of diabetic rats treated with eucalyptus leaves extract at dose 0.4 g/kg, and glibenclamide ($P<0.05$).

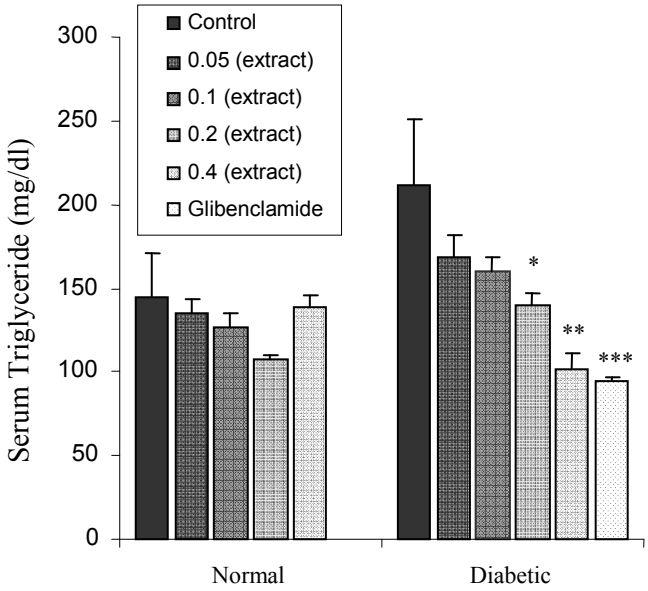

Figure 1. Effect of oral administration of eucalyptus leaves extract at doses of 0.05, 0.1, 0.2 and 0.4 g/kg on serum triglyceride levels (mg/dl) in normal and diabetic rats.

Glibenclamide (600 μg/kg) was administered only in diabetic rats. Each column represents mean ± SEM. for 6 rats. Control group was administered distilled water as a vehicle. One-way analysis of variance was used to test statistical significance. Differences were considered significant at $P < 0.05$. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ different from control diabetic group.

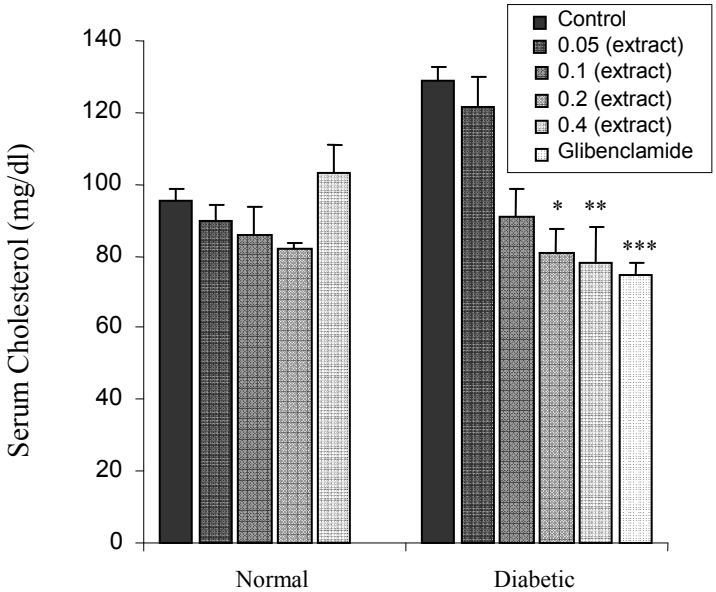

Figure 2. Effect of oral administration of eucalyptus leaves extract at doses of 0.05, 0.1, 0.2 and 0.4 g/kg body weight on serum total cholesterol level (mg/dl) in normal and diabetic rats.

Glibenclamide (600 μg/kg) was administered only in diabetic rats. Each column represents mean ± SEM. for 6 rats. Control group was administered distilled water as a vehicle. One-way analysis of variance was used to test statistical significance. Differences were considered significant at $P < 0.05$. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ different from control diabetic group.

Discussion

The present results indicated that the eucalyptus alcoholic extract significantly decreased serum triglyceride and cholesterol levels in treated diabetic rats as compared with control diabetic rats, but not in normal rats ($P<0.05$). The hypolipidemic effect of the extract was similar to that observed for glibenclamide.

The levels of plasma lipids are usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease [29, 30]. Lowering plasma lipid levels through dietary measures or drug therapy seems to be accompanied with a decrease in the risk of cardiovascular diseases [31]. Increase levels of plasma cholesterol, phospholipids, free fatty acids and triglyceride have been reported in alloxan-induced diabetic rats. The abnormally high concentration of plasma lipids in diabetes is frequently caused by increase in the mobilization of fatty acids from the peripheral depots, wherein insulin inhibits the hormone sensitive enzyme, lipase. On the other hand, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidemia that usually accompany by diabetic state, may therefore be regarded as a consequence of the unopposed actions of lipolytic hormone on the fat depot [32]. It has long been known that in the STZ-induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in plasma cholesterol, triglycerides and urea [33, 34].

It is reported that aqueous extract of eucalyptus increased peripheral glucose utilization in the mouse abdominal muscle and stepwise enhanced insulin secretion from the clonal pancreatic beta cell lines by 70-160%. The insulin-releasing effect of the aqueous extract of *Eucalyptus globulus* is probably responsible for the antihyperglycemic effects [35]. Administration of *Eucalyptus globulus* leaves diet for 21 days to normal rats did not resulted in hypoglycemia. In addition, STZ administration to these pre-treated rats did not produce hyperglycemia as severely as it was seen in controls. In addition, pre-treated rats also showed less polydypsia and body weight loss [36].

Medicinal plants are frequently considered to be less toxic and freer from side effects than synthetic agents [37]. The synthetic oral hypoglycemic agents can produce some side effects including hematological, gastrointestinal reactions, hypoglycemic coma and disturbances of liver and kidney. In addition, they are not suitable for using during pregnancy [38].

Taken together, *Eucalyptus globulus* leaves revealed significant dose-dependent hypolipidemic effects, which is comparable to the standard antidiabetic drug, glibenclamide.

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References

1. Matsui T, Tanaka T, Tamura S, et al. Alpha-glucosidase inhibitory profile of catechins and theaflavins. J Agric Food Chem 2007; 55: 99–105.
2. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025; prevalence, numerical estimates, and projections. Diabetes Care 1998; 21: 1414–31.
3. WHO. Traditional medicine strategy 2002-2005. WHO Publications 2002:1–6.
4. Chakraborty R, Rajagopalan R. Diabetes and insulin resistance

associated disorders: Disease and therapy. Curr Sci 2002; 83: 1533-8.
5. Kameswararao B, Kesavulu MM, Apparao C. Evaluation of antidiabetic effect of Momordica cymbalariafruit in alloxan-diabetic rats. Fitoterapia 2003; 74: 7-13.
6. Brownlee M. Glycation products and the pathogenesis of diabetic complications. Diabetes Care 1992; 15: 1835-43.
7. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 1988; 318: 1315-21.
8. Nikkila EA, Hormila P. Serum lipid and lipoproteins in insulin treated diabetes. Demonstration of increased high density lipoprotein concentrations. Diabetes 1978; 27: 1078-85.
9. Nikkila EA, Kekki M. Plasma triglyceride transport kinetics in diabetes mellitus. Metabolism 1973; 22: 1-22.
10. Reaven GM, Greenfield MS. Diabetic hypertriglyceridemia. Evidence for three clinical syndromes. Diabetes 1981; 30(2):66-75.
11. Hirano T, Mamo JCL, Takeuchi H, et al. Correlation of insulin deficiency and hypertriglyceridemia in diabetic rats. Diabetes Res Clin Pract 1991; 12: 173-80.
12. Ito M, Kondo Y, Nakatani A, et al. Characterization of low dose streptozotocin -induced progressive diabetes in mice. Environ Toxicol Pharmacol 2001; 9: 71-8.
13. Man ZW, Zhu M, Noma Y, et al. Impaired h-cell function and deposition of fat droplets in the pancreas as a consequence of hypertriglyceridemia in OLETF rat, a model of spontaneous NIDDM. Diabetes 1997; 46: 1718-24.
14. Garcia MJ, McNamara PM, Gordon T, et al. Morbidity and mortality in diabetics in the Framingham population. Diabetes 1974; 23: 105-11.
15. Ravid M, Brosh D, Ravid-Safran D, et al. Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. Arch Intern Med 1998; 158: 998-1004.
16. Dominguez JH, Tang N, Xu W, et al. Studies of renal injury III: lipid-induced nephropathy in type II diabetes. Kidney Int 2000; 57: 92-104.
17. Tsutsumi K, Inoue Y, Yoshida C. Acceleration of Development of diabetic cataract by hyperlipidemia and low high-density lipoprotein in rats. Biol Pharm Bull 1999; 22: 37-41.
18. Gottsater A, Ahmed M, Fernlund P, et al. Autonomic neuropathy in type 2 diabetic patients is associated with hyperinsulinemia and hypertriglyceridemia. Diabet Med 1999; 16: 49-54.
19. Evans WC. Trease and Evans's Pharmacognosy, 14th edition. Saunders, London; 1996. p. 124-43.
20. Pushparaj P, Tan CH, Tan BKH. Effects of Averrhoa bilimbi leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J Ethnopharmacol 2000; 72: 69-76.
21. Alarcon-Aguilara FJ, Roman-Ramos R, Perez-Gutierrez S, et al. Study of the anti-hyperglycemic effect of plants used as antidiabetic. J Ethnopharmacol 1998; 61: 101-10.
22. Zargari A. Medicinal Plant, vol. 2. Tehran University Press, Iran, 1997. p. 307-315.
23. Duke JA. CRC Handbook of Medicinal Herbs. CRC Press, Florida, US, 1985.
24. Amakura Y, Umino Y, Tsuji S, et al. Constituents and their antioxidative effects in eucalyptus leaf extract used as a natural food additive. Food Chemistry 2002; 77: 47-56.
25. Hou AJ, Liu YZ, Yang H, Lin ZW, et al. Hydrolysable tannins and related polyphenols from Eucalyptus globulus. J Asian Nat Prod Res 2000; 2: 205-12.

26. Takasaki M, Konoshina T, Fujitani K, et al. Inhibitors of skin-tumor promotion. VIII. Inhibitory effects of euglobals and their related compounds on Epstein-Barr virus activation. Chem Pharm Bull 1990; 38: 2723-39.
27. Eidi A, Eidi M, Sokhteha M. Effect of fenugreek (Trigonella foenum-graecum L) seeds on serum parameters in normal and streptozotocin-induced diabetic rats. Nutr Res 2007; 27: 728-33.
28. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. 3rd edition, In: Burtis CA, Ashwood ER, editors. Tietz textbook of clinical Chemistry. Philadelphia: W.B. Saunders Company; 1999. p. 809-61.
29. Chatterjea MN, Shinde R. Metabolism of carbohydrates. Part II. Text book of medical biochemistry. 1st edition. Jay Pee Brothers Medical Publishers Pvt. Ltd; 1994. p. 421.
30. Scott M, Grundy L. Diabetes and cardiovascular disease. Circulation 1999; 100: 1134-46.
31. Kannel WB, McGee DL. Diabetes and cardiovascular risk factors. The Framingham study. Circulation 1979; 59: 8-13.
32. Murray RK, Granner DK, Mayes PA, Rodwell VW, eds. Harper's biochemistry. 25 CT: Appelton and Lange; 2000. p. 610-7.
33. Cam CM, Cros HG, Serrano JJ, et al. In vivo antidiabetic actions of naglivan, an organic vanadyl compound in streptozotocin-induced diabetes. Diabetes Res Clin Pract 1993; 20: 111-21.
34. Pari L, Saravanan G. Antidiabetic effect of Cogent db, a herbal drug in alloxan-induced diabetes mellitus. Comp Biochem Physiol Part C 2002; 131:19-25.
35. Gray AM, Flatt PR. Antihyperglycemic actions of Eucalyptus globules (Eucalyptus) are associated with pancreatic and extrapancreatic effects in mice. J Nutr 1998; 128: 2319-23.
36. Swanston-Flatt SK, Day C, Flatt PR, et al. Glycemic effects of traditional European plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetes Res 1989; 10(2): 69-73.
37. Pari L, Umamaheswari J. Antihyperglycaemic activity of Musa sapientum flowers: effect on lipid peroxidation in alloxan diabetic rats. Phytother Res 2000; 14: 1-3.
38. Larner I. Insulin and oral hypoglycaemic drugs Glucagon. In: Gilman AG, Goodman LS, Rall TW, Murad F. (Eds.), The Pharmacological