

The Comparison of Eucalyptus Aqueous Extract and Insulin on Blood Sugar and Liver Enzymes in Diabetic Male Rats

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Article information	Abstract
<p>Article history: Received: 13 Nov 2011 Accepted: 18 Jan 2012 Available online: 29 Oct 2012 ZJRMS 2013; 15(6): 25-28</p> <p>Keywords: Eucalyptus Lipoprotein Male rat Blood glucose Liver enzymes</p> <p>*Corresponding author at: Department of physiology, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran E-mail: m_shahrakim@zaums.ac.ir</p>	<p>Background: Since eucalyptus is a traditional plant which has been consumed as antidiabetic in herbal medicines, the aim of this survey was to compare the effect of eucalyptus aqueous extract and insulin on serum blood sugar and liver enzymes in streptozotocin (STZ) diabetic male rats.</p> <p>Material & Methods: The experiment was performed on four groups of rats: sham control (A), diabetic control (B), diabetic (C) which received insulin and diabetic group (D) which consumed <i>Eucalyptus aqueous</i> extract in drinking water for 4 weeks (n=8). Sham control and B group did not receive any agents. At the end, animals were deep anesthetized by diethyl ether, sacrificed and blood samples were collected. Blood sugar, serum lipids and liver enzymes were measured by ordinary methods. Obtained data were analyzed using ANOVA and Tukey tests. Results were expressed as mean±SD. Statistical difference of $p<0.05$ was recognized significant.</p> <p>Results: Results showed that blood sugar in group C significantly decreased compared with that of group B but ALT, AST and ALP activity value significantly increased compared with those of other groups.</p> <p>Conclusion: These results indicated that <i>Eucalyptus aqueous</i> extract caused decreased blood sugar but increased liver enzymes activity in STZ diabetic male rats</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Diabetes mellitus is a metabolic disorder which is accompanied by carbohydrates, fats and proteins abnormality and there are two types of real insulin-dependent and non-insulin-dependent [1]. In insulin-dependent diabetes (type I), β cells in pancreas are impaired and insulin secretion decreases but in non-insulin-dependent diabetes (type II), insulin secretion is high but insulin receptor, sensitivity in skeletal muscle and adipose tissue reduce [2, 3]. Chronic diabetes mellitus is accompanied by complications in the eyes, nerves, kidneys and blood vessels [4].

The prevalence and incidence of diabetes is increasing in most populations, being more prominent in developing countries [5]. According to WHO forecast, more than 100 million people suffer from diabetes mellitus in the world [6]. Many herbal products have been described for the treatment of diabetes mellitus [7]. Plant products are frequently considered to be less toxic and freer from side effects than synthetic ones [7, 8]. Eucalyptus is a herbal medicine whose leaves, seeds and other parts have hypoglycemic effects on animals and human [8].

This plant is native in Australia but it can grow all around the world, such as Mediterranean and Iran [9]. While more than 700 species of eucalyptus exist in the world, components of 500 species of eucalyptus have herbal medicine effects. Eucalyptus leaves product has

analgesic effects and an anti-inflammatory effect in respiratory tract [10, 11]. Experiment results show that the compounds of eucalyptus extract have antimicrobial effects [12] and are also useful for erythrocytes activity [13]. Surveys show that eucalyptus extract components cause decreased blood sugar in diabetic mice induced by streptozotocin (in vivo) and in intestine absorption cell culture (in vitro) [14-16].

In addition, materials obtained from the eucalyptus leaves have antioxidant effects [17]. Binduja et al. in 2000, investigated that many materials obtained from the leaves of eucalyptus have hepatocytes protective effects and stabilize serum levels of liver enzyme activity (AST and ALT) in rats [18, 19]. Since Eucalyptus is a traditional plant which has been consumed as an antidiabetic in herbal medicine, the aim of this survey was to compare the effect of *Eucalyptus aqueous* extract and insulin on blood sugar and liver enzymes activity in streptozotocin (STZ) diabetic male rats.

Materials and Methods

This experiment was performed on 32 adult Wistar-Albino male rats, which were purchased from Razi Institute in Karaj and were kept in animal house of Zahedan University of Medical Sciences. Rats weighed

200±20gr, aged 5-7 and were separately housed in cages (one rat in each cage) and had free access to water and food. Animals were maintained in a room at 23±2°C with a fixed 12-h artificial light period (6 Am to 6 Pm), humidity of 45-70% and the air was adequately recycled. All animals were fed with a standard rodents' diet and experiment duration was 30 days (4week) (20). After a 5-day habituation, animals were randomly divided into three groups (n=8): Sham control group (A), just receiving rodent's diet and tap water, diabetic test group (B) which were made diabetic by STZ receiving rodent's diet and tap water but were not given any agents during experiment treatment. Diabetic test group (C) which received 3 IU/kg/ip insulin daily. And finally, diabetic test group (D) which received drinking water containing 2.5 mg/ml of aqueous eucalyptus extract in treatment duration.

Eucalyptus leaves were collected and then identified in the department of biology in Faculty of Science, Sistan and Balouchestan University, Zahedan, Iran.

The leaves were dried at room temperature and ground into powder. Extraction was performed by mixing 2.5 gr of powder in 1000 ml of distilled water and put on a hot plate Magnet for 24 hours. The prepared extract was filtered through a gauze followed by filtration through a regular filter paper Watman no.1 [24].

The product was a dark brown aqueous extract which later was dried in incubator for 1 day at 40°C. Rats in Diabetic test group (D) consumed tap water supplemented with 2.5 mg/ml of aqueous eucalyptus extract per day in treatment period [16, 22]. In a fasting situation (14-16 h), tests groups animals were given intra peritoneal (ip) injection of 65 mg/kg STZ (the solution was prepared in cold citrate buffer, pH=4.5). Streptozotocin purchased from sigma was dissolved in citrate buffer immediately before use. All of the test animals were diabetic and showed diabetic symptoms by polydipsia, polyuria and polyphagia. After 7 days, the diabetic condition was found to be established when Fasting Blood Sugar (FBS) was higher than 126 mg/dl [3] and put in three groups A, B and C.

At the end of treatment, the body weight of all the rats was measured (final weight), then all animal were anesthetized under high dose of ether and sacrificed by cervical decapitation and blood samples were collected from cervical vein. Blood sugar and liver enzyme activity (AST, ALT, ALP) were measured using standard methods (Technicon,

USA). Water intake and food consumption were carefully measured during the treatment duration.

Obtained data were analyzed by SPSS (v.11). Analysis of variance was used for the comparison of the groups. Tukey test as a post hoc multiple comparison test was applied to compare healthy control and diabetic groups. Data were expressed as mean±SD and P value less than 0.05 was considered significant.

Results

The results obtained from this survey showed that AST, ALT and ALP activity significantly increased in group D compared with those of other groups A, B and C (Table 1). In addition, the results obtained in the present survey revealed that blood sugar significantly increased in group D compared with those of other three groups of A, B and C ($p=0.001$, $p=0.002$, $p=0.01$, table 1).

The amount of water consumption in group D (133.54±18.6) was significantly higher in group D compared with group A (41.22±12.29) but this value did not show a difference with those of group B (144.5±11.56) and group C (79.17±13.22). Food intake in diabetic groups of D, C, and B (25.76±5.16, 21.04±2.79 and 27.28±5.16 gr, respectively) significantly increased compared to that of group A (16.11±2.3 gr). The comparison of this value did not show any difference among diabetic groups. Initial body weight was similar in all the three groups but final weight in group A, B, C and D were (255.11±11.23), (175.18±15.16), (192.5±2.79) and (178.76±22.5) gr, (Table 1).

Based on ANOVA and TUKEY tests ALT value in group D significantly increased compared with those of other groups ($p=0.003$, $p=0.001$). Based on ANOVA and TUKEY tests AST value in group D significantly increased compared with those of other groups ($p=0.001$).

Based on ANOVA and TUKEY tests Alp value in group D significantly increased compared with those of other groups ($p=0.001$, $p=0.002$). Based on ANOVA and TUKEY tests blood sugar value in group D significantly decreased compared with those of other groups ($p=0.01$, $p=0.002$, $p=0.001$).

Discussion

In the present study all test animals were made diabetic by single dose of intra peritoneal injection of streptozotocin (STZ, 65 mg/kg, ip). The diabetic symptom were detected and observed with polyurea, polydipsia polyphasia and hyperglycemia.

Table 1. The Comparison of insulin and eucalyptus aqueous extract on blood sugar and liver enzymes in diabetic male rats

Groups treatment	Blood sugar	ALT iu/l	AST iu/l	Alk Pho/iu/l
Sham control (A)	93.85±8.27	83.79±18.09	195.37±18.53	77.87±12.6
Diabetic control (B)	347.5±26.17	101.12±22.69	218.25±11.16	96.12±11.06
Diabetic & insulin (C)	148.62 ±18.56	87±11.2	204.37±39.29	91.5±6.25
Diabetic & Extract (D)	245.75±36.07*	227.5 ±30.34 *	456.6±41.03*	140.75±20.18*

*= $p<0.05$

Diabetic condition was established in the tests groups with high FBS measurement (more than 126 mg/dl). The results of this study showed that oral administration of *Eucalyptus aqueous* extract caused increased liver enzyme (ALT, AST and ALP) activity but it significantly decreased blood sugar in diabetic male induced by streptozotocin. The results of the present study is in agreement to those by Gallagher et al. reported in 1998 that added eucalyptus leaves to food and administration of eucalyptus extract in drinking water caused decrease blood sugar in diabetic male rats [16].

In addition, Gallagher et al. investigated that administration of eucalyptus extract on intestinal absorption cultured in rats cells caused decreased glucose in the environment culture and increased glucose uptake in these cells [16]. Gallagher and et al. did not provide any explanation in their report to suggest why blood sugar decreased in the intestinal cell culture. The present study results revealed that blood glucose decreased in group D which received *Eucalyptus aqueous* extract. This decrease may be due to water-soluble compounds which probably existed in *Eucalyptus aqueous* extract.

These components probably have effected on glucose metabolism in fat or skeletal muscle cells and decreased blood sugar by increasing the glucose influx in these cells. Moreover, this component may affect glycolysis and increase glucose consumption in fat and skeletal muscle cells in group D and cause decreased blood sugar. In addition, this component may decrease glucose absorption in duodenum absorbs cell in diabetic male rats (group D) and reduce blood sugar in this group. Our findings showed that eucalyptus leaves aqueous extract administration caused increased liver enzyme (ALP, ALT, AST) activity.

These findings are in disagreement with those of Binduja and Saraswat, 1996, who reported that the uracilic acid obtained from eucalyptus leaves extract, had a protective effect on rat hepatocytes [19]. These conflicting reports may be due to the following reasons: in Binduja and Saraswat's study, animals were normal

and β cells were used in vitro obtained from healthy animals pancreas but in the present study, diabetic male rats were used for administered eucalyptus aqueous extract. Since diabetes alters cells mechanisms, serum pH [3] and cell sensitivity alter the effects of the compounds which exist in *Eucalyptus aqueous* extract on the group D. (We could not study liver tissue and it was our work deficiency).

Our result in the present study are in disagreement with those of Binduja et al. reported in 2000 suggesting that eucalyptus leaves extract has an uric acid which has protective effects on the liver hepatocytes in diabetic rats. This report unlike our finding indicated that administration of uracilic acid obtained from eucalyptus leaf extract cause a significant decrease in liver enzymes (ALT, AST, and ALP) activity compared to those of control group [18].

Such difference between these two studies may be due to different components used in these surveys. Binduja et al. studied the uracilic acid obtained from eucalyptus leave extract in diabetic rats but in the present study we used complete aqueous extract of eucalyptus leaves on diabetic male rats. These results indicated that *Eucalyptus aqueous* extract caused decreased blood sugar but increased liver enzyme activity in STZ diabetic male rats.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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