Modulatory Effects of Ginger and Clove Oils on Physiological Responses in Streptozotocin-Induced Diabetic Rats

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Abstract: The present study was conducted to compare the efficiency of ginger, clove and ginger plus clove oils supplementation in streptozotocin (STZ)-diabetic and non-diabetic male Wistar rats. In comparison with control, highly significant increases in the values of blood glucose (273.72%), triglycerides (34.97%), cholesterol (65.79%), low density lipoprotein LDL-cholesterol (201.07%), total protein (21.09%), creatinine (74.31%), urea (82.08%), uric acid (81.23%), alanine aminotransferase (74.36%) and aspartate aminotransferase (34.99%) were observed in STZ-diabetic rats, while the value of high density lipoprotein HDL-cholesterol was markedly declined (21.68%). Administration of ginger oil to diabetic rats resulted in mild increases of the levels of blood glucose, triglycerides, cholesterol, LDL-cholesterol, total protein, urea, uric acid and aspartate aminotransferase, while the value of HDL-cholesterol was significantly decreased. Moreover, the treatment with ginger oil noticeably restored the values of blood creatinine and alanine aminotransferase activity to the control levels. Supplementation of tested oils significantly decreased the haematobiochemical changes in STZ-diabetic rats. In comparison with control, administration of ginger oil or ginger plus clove oils significantly reduced the levels of blood glucose in non-diabetic rats. Reducing effect of ginger oil on the level of blood triglycerides was notably observed in non-diabetic rats. From the present new findings, it was suggested that ginger, clove and ginger plus clove oils supplementation may act as antioxidant agents and these oils could be an excellent adjuvant support in the therapy of diabetic mellitus and its complications.

Key words: Streptozotocin, diabetes, ginger oil, clove oil, haematobiochemical parameters, rats

INTRODUCTION

Streptozotocin (STZ), a glucoseamine-nitrosourea compound, has a chemical name of 2-deoxy-2-(3-methyl-5-nitrosoureido)-D-glucopyranose (C7H10N3O2). Streptozotocin has been used as a diabetogenic agent in experimental animals. The mechanisms of STZ-induced hyperglycaemia are considered as follows: (1) STZ causes DNA strand breaks in pancreatic islets and stimulates nuclear poly (ADP-ribose) synthetase and thus depletes the intracellular NAD⁺ and NADP⁺ levels, which inhibit proinsulin synthesis and induces diabetes (Wilson et al., 1988). (2) Activated oxygen species, such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen, have been implicated to play important roles in diabetes, especially diabetic angiopathy (Sato et al., 1979).

Diabetes mellitus is probably the fastest growing metabolic disorder in the world and it is a major source of morbidity in developed countries. Moreover, Diabetes can strike any one, from any lifestyle and it does-in numbers that are dramatically increasing. For example, in the last decade, the cases of people with diabetes jumped more than 40% to 21 million Americans. Worldwide, it afflicts 150 million people. World Health Organization estimates that by 2025, that number will be more than double. Today, diabetes takes more lives than Acquired Immune Deficiency Syndrome (AIDS) and breast cancer combined—claiming the life of one American every three minutes. Annually, diabetes costs the American public more than $132 billions. Generally, diabetes mellitus is an endocrine and a chronic metabolic disorder characterized by hyperglycaemia resulting from defects in insulin secretion or action or both (Georg and Ludvik, 2000; Nyh blohm et al., 2000). It is associated with serious complications like polyurea, polyphagia, polydyspia, ketosis, nephropathy, neuropathy and cardiovascular.

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disorders (Gandjbakhch et al., 2005) and at present, it is
known as syndrome (Zimmes, 1997). In modern
medicine, no satisfactory effective therapy is still
available to cure diabetes mellitus. Though, insulin
therapy is used for management of diabetes mellitus
but there are several drawbacks, which include insulin
allergy, insulin antibodies, lipodystrophy, autoimmunity
and other delayed complications like morphological
changes in kidney and severe vascular complications
Additionally, pharmaceutical drugs like sulfonylureas and
biguanides are used for the treatment of diabetes but
these are either too expensive or have undesirable side
effects or contraindications (Rang et al., 1991). Moreover,
the number of patients with diabetes mellitus, who exhibit
insulin resistance, is increasing recently all over the
world (Bonora et al., 2004). The major causes have been
suggested to be functional disorders in insulin secretion
capacity and in carbohydrate metabolism deterioration
with aging (Lipson, 1986; Jaber et al., 2004).

The uses of natural products properties is as
ancient as human civilization and for a long time, mineral,
plant and animal products were the main of drugs
(Hernandez-Ceruejos et al., 2002). The exploitation of
plants by man for the treatment of diseases has been
in practice for a very long time. Over the years, a variety
of medicinal plants has been very popular for the
cure of a number of both human and animal diseases
(Lamia, 1981; Sofowora, 1984; Gill, 1992). The plant
Kingdom is an important potential source of effective oral
hypoglycaemics. More than 400 species have been
reported to display hypoglycaemic effects, but only a few
have been investigated in any detail (Miura and Kato,
1995; Miura et al., 1996, 1997). Ginger (Zingiber officinale,
family: Zingiberaceae) is a natural dietary component,
sweet, pungent and aromatic herb that has expectorant
properties. The herb increases perspiration, improves
digestion and liver function, controls nausea, vomiting
and coughing. It stimulates circulation, relaxes spasms
and relieves pain. Additionally, ginger or gingerol, the
major pungent constituent of ginger, has antioxidant,
antiinflammatory, antifungal, antimycobacterial and
anticarcinogenic properties (Shadmani et al., 2004;
Manju and Nanini, 2005; Bidiontto et al., 2006). Cloves are
the dried, unopened inflorescence of the clove tree,
Syzygium aromaticum, which is a member of the
Myrtaceae family. Cloves are strongly pungent due to
their high content of eugenol, which can be extracted by
distillation to yield the essential oil. Clove buds have been
regarded as safe when taken orally for medicinal use
(Duke, 1985). Cloves have been used by humans for
medicinal applications for over two thousand years, being
chewed to alleviate the pain of toothache and are also
widely use to disinfect root canals in temporary fillings
(Duke, 1985) and as an oral anesthetic. Clove, especially
eugenol, is a natural antibiotic with broad antimicrobial
activities against bacteria and fungi (Suresh Babu and
Madhavi, 2001; Yano et al., 2006). It is worth to mention
that the influence of ginger, clove or ginger plus clove oils
supplementation on diabetic and non-diabetic rats has not
been established. Therefore, the aim of this study was to
find if the administration of ginger oil, clove oil and ginger
oil plus clove oil could have beneficial effects on
physiological parameters in STZ-induced diabetic rat.
The physiological parameters including blood glucose,
triglycerides, cholesterol, high density lipoprotein
HDLC-cholesterol, low density lipoprotein LDL-
cholesterol, total protein, creatinine, urea, uric acid,
alanine aminotransferase (ALT) and aspartate
aminotransferase (AST). Finally, these parameters were
chosen on the basis that they are closely related to the
diabetic syndrome.

MATERIALS AND METHODS

Experimental protocol: Healthy eighty male Wistar rats,
weighing 225-252 g were provided by the Animal
Experimental Unit of King Fahd Medical Research Center,
King Abdul Aziz University, Jeddah, Saudi Arabia. The
experimental animals were assigned to one of eight groups
each of 10 rats. Animals were allocated 5 per cage in a
temperature (24±1°C) and light: dark cycle was 12 h: 12 h.
Rats of group 1 were intraperitoneally injected with
0.5 mL of sodium citrate buffer solution (pH 4.5), served
as controls, fed ad libitum on normal commercial chow
and had free access to water. The animals of groups 2,
3, 4 and 5 were intraperitoneally injected with STZ
(Sigma Chemical Company, St. Louis, Mo, USA) at a dose
of 30 mg kg⁻¹ in 0.5 mL sodium citrate buffer solution
(pH 4.5). Four days after STZ injection, the blood samples
were collected from orbital venous plexus in the fasted rat
(two samples from each groups 2, 3, 4, 5), water was not
restricted and the level of serum glucose was determined.
The serum glucose level of over 277 mg \(\text{dL}^{-1}\) was defined
as diabetic model rats. Rats of group 2 were fed with the
same diet given in group 1. Groups 3, 4 and 5 were fed
with the diets containing 5% ginger oil, 5% clove oil and
2.5% ginger oil plus 2.5% clove oil, respectively. Groups
6, 7 and 8 received sodium citrate buffer solution (pH 4.5)
at the same dose given in group 1 and fed with diets.
containing 5% ginger oil, 5% clove oil and 2.5% ginger oil plus 2.5% clove oil, respectively. After 2 weeks, the experimental animals were fasted for 8 h, water was not restricted and then anaesthetized with ether. Blood samples were collected from orbital venous plexus in non-heparinized tubes, centrifuged at 2000 rpm for 20 min and blood sera were then collected and stored at 4°C prior immediate determination of glucose, triglycerides, cholesterol, high density lipoprotein HDL-cholesterol (HDL-C), low density lipoprotein LDL-cholesterol (LDL-C), total protein, creatinine, urea, uric acid, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). All of these parameters were measured using an automatic analyzer (Architect e8000 Clinical Chemistry System, USA).

**Statistical analysis:** All experimental data of haematobiochemical parameters were expressed as mean±SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 12.0 and differences between means were detected by Student’s t-test. p-values of less than 0.05 were considered significant.

**RESULTS**

Supplementation effects of ginger, clove and ginger plus clove oils on the haematobiochemical parameters of diabetic and non-diabetic rats are represented in Table 1. In comparison with control, highly significant increases in the values of blood glucose (273.72%), triglycerides (34.97%), cholesterol (65.79%), LDL-cholesterol (20.07%), total protein (21.09%), creatinine (74.31%), urea (82.08%), uric acid (81.23%), ALT (74.36%) and AST (34.99%) were noted in STZ-diabetic rats, while the value of HDL-cholesterol was markedly decreased (21.68%). Administration of ginger oil to diabetic rats resulted in mild increases of the levels of blood glucose (35.05%), triglycerides (9.82%), cholesterol (26.08%), LDL-cholesterol (79.22%), total protein (9.77%), urea (25.83%), uric acid (15.51%) and AST (10.17%), while the value of HDL-cholesterol (7.39%) was significantly decreased. Moreover, the treatment with ginger oil noticeably restored the values of blood creatinine and ALT activity to the control levels. Treatment with clove or ginger plus clove oils induced mild increases in the values of blood glucose, triglycerides, cholesterol, LDL-cholesterol, total protein, creatinine, urea, uric acid, ALT and AST in diabetic rats, while the value of HDL-cholesterol was markedly increased. Supplementation of the tested oils significantly decreased the haematobiochemical changes in STZ-diabetic rats. In comparison with control, supplementation with only ginger oil significantly reduced the level of blood glucose (9.38%) and triglycerides (12.27%) in non-diabetic rats. Lowering effect of ginger plus clove oils on blood glucose (6.47%) was notably observed in non-diabetic rats. From Table 1, it was pronounced that the administration of ginger oil exhibited a notable improvement role, as evidenced by its modulating effects on the studied parameters of diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>STZ</th>
<th>STZ+ ginger oil</th>
<th>STZ+ clove oil</th>
<th>STZ+ ginger plus clove oil</th>
<th>Ginger oil</th>
<th>Clove oil</th>
<th>Ginger plus clove oils</th>
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<tbody>
<tr>
<td>Glucose (mg/dL⁻¹)</td>
<td>8.60±2.41</td>
<td>10.60±3.17</td>
<td>12.00±4.82</td>
<td>14.70±6.82</td>
<td>18.40±8.82</td>
<td>17.40±9.82</td>
<td>15.40±8.82</td>
<td>13.40±9.82</td>
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<tr>
<td>Triglycerides (mg/dL⁻¹)</td>
<td>65.20±3.03</td>
<td>88.00±2.52</td>
<td>81.40±3.02</td>
<td>74.60±3.91</td>
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<tr>
<td>Cholesterol (mg/dL⁻¹)</td>
<td>83.60±5.13</td>
<td>138.60±5.98</td>
<td>105.40±5.46</td>
<td>120.80±5.36</td>
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<tr>
<td>HDL-C (mg/dL⁻¹)</td>
<td>40.60±2.41</td>
<td>31.80±1.79</td>
<td>37.60±1.81</td>
<td>34.80±0.84</td>
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<tr>
<td>LDL-C (mg/dL⁻¹)</td>
<td>29.40±4.38</td>
<td>89.40±4.82</td>
<td>53.40±5.79</td>
<td>76.70±7.28</td>
<td>69.40±6.70</td>
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<tr>
<td>Total protein (g/dL⁻¹)</td>
<td>5.12±0.38</td>
<td>6.20±0.28</td>
<td>5.40±0.20</td>
<td>5.50±0.25</td>
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<tr>
<td>Creatinine (mg/dL⁻¹)</td>
<td>1.00±0.10</td>
<td>1.00±0.12</td>
<td>1.66±0.11</td>
<td>1.72±0.08</td>
<td>1.54±0.11</td>
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<td>Urea (mg/dL⁻¹)</td>
<td>20.56±1.49</td>
<td>38.20±1.64</td>
<td>26.40±1.68</td>
<td>31.66±1.64</td>
<td>28.54±1.28</td>
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<td>Uric acid (mg/dL⁻¹)</td>
<td>4.90±0.25</td>
<td>8.88±0.37</td>
<td>5.60±0.56</td>
<td>7.48±0.58</td>
<td>6.40±0.27</td>
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<td>ALT (U/L⁻¹)</td>
<td>39.50±1.87</td>
<td>60.80±7.90</td>
<td>46.20±6.46</td>
<td>59.40±2.90</td>
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<tr>
<td>AST (U/L⁻¹)</td>
<td>80.60±2.70</td>
<td>108.60±3.49</td>
<td>88.80±1.79</td>
<td>93.40±3.68</td>
<td>90.20±2.86</td>
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</table>

a: Indicates a significant difference between control and treated groups. b: Indicates a significant difference between STZ group and groups treated with STZ+ginger, STZ+clove or STZ+ginger plus clove oils. c: Indicates a significant difference between group treated with STZ+ginger and groups treated with STZ+clove or STZ+ginger plus clove oils. d: Indicates a significant difference between group treated with STZ+clove oil and group treated with STZ+ginger plus clove oils. e: Indicates a significant difference between group treated with STZ+ginger and group treated with ginger oil only. f: Indicates a significant difference between group treated with STZ+ginger plus clove oils and group treated with ginger plus clove oils only. g: Indicates a significant difference between group treated with STZ+ginger plus clove oils and groups treated with ginger plus clove oils only. h: Indicates a significant difference between group treated with ginger oil only and groups treated with clove oil only or ginger plus clove oils only and I: Indicates a significant difference between group treated with clove oil only and groups treated with ginger plus clove oils only.
DISCUSSION

The present investigation showed that the supplementation of ginger, clove or ginger plus clove oils significantly inhibited the haematobiochemical changes in STZ-diabetic rats. The most changes were pronounced in diabetic rat treated with ginger oil. Generally, it is obviously from the present data that the STZ-induced several disturbances on carbohydrate, lipid and protein metabolism in experimental rats. Bolkent et al. (2004), Prakash et al. (2004), Ravi et al. (2005), Singh et al. (2005), Yanardag et al. (2005) and Rajasekaran et al. (2006) reported that in the STZ-diabetic rats, the levels of blood glucose, total lipid, cholesterol, triglycerides, LDL-cholesterol, creatinine, urea, uric acid, ALT and AST activities were significantly increased, while the levels of HDL-cholesterol were markedly decreased. In the study of Shinde and Goyal (2003), histopathological investigations of kidney and liver showed several changes included the increases in the intensity and incidence of vacuolations, cellular infiltration and hypertrophy in STZ-diabetic rats. Additionally, they showed that STZ-induced an elevation of serum creatinine and urea levels as well as an elevation of serum level of hepatic enzymes in diabetic rats. Moreover, Murali et al. (2003), Sato et al. (2005) and Yoshida et al. (2005) reported that kidney damage in STZ-induced diabetic rats includes glomerular expansion, renal hypertrophy, glycosuric degeneration of distal tubules, fatty degeneration of glomerular endothelium. It is worth to mention that the above previous studies demonstrated that the administrations of several herbal extracts could restore the alterations in the levels of blood and tissue parameters, morphological and histological structure. In the present investigation, it can not be excluded that the possibility that diabetes-induced liver and kidney damage. However, the increases of serum ALT, AST, creatinine, urea and uric acid levels are considered as obvious indicators for liver and kidney damage and dysfunctions. ALT and AST are directly associated with the conversion of amino acids to keto acids and the increased protein catabolism accompanying gluconeogenesis and urea formation that are seen in diabetic state might be responsible for the elevation of these aminotransferases. The diabetic hyperglycaemia induces elevations of the blood levels of creatinine, urea, uric acid which are considered as significant markers of renal dysfunction (Almdal and Vilstrup, 1988). It has been documented that several medicinal plants show their hypoglycemic effects associated with a significant alteration in the activity of liver hexokinase (Bopanna et al., 1997; Santhakumari et al., 2006), glucokinase (Kumari et al., 1995; Lee et al., 1997). It has been reported that treatment with the herbs caused an improvement in the activities of liver glucose-6-phosphatase, glycogen synthetase, glycogen phosphorylase, glucose-6-phosphate dehydrogenase and phospho-fructokinase. Diabetes mellitus is also grossly reflected by profound changes in protein metabolism and by a negative nitrogen balance and loss of nitrogen from most organs (Almdal and Vilstrup, 1987). Increased urea nitrogen production in diabetes may be accounted by enhanced catabolism of both liver and blood proteins (Jorda et al., 1981, 1982). The effect of diabetes mellitus on lipid metabolism is well established. The association of hyperglycaemia with an alteration of lipid parameters presents a major risk for cardiovascular complications in diabetes. Many secondary plant metabolites have been reported to possess lipid-lowering properties (Rajasekaran et al., 2006). The serum cholesterol and triglycerides were significantly decreased in diabetic rats supplemented with ginger, clove and ginger plus clove oils. These oils supplementation also result the significant attenuation in the levels of HDL-cholesterol and LDL-cholesterol in serum toward the control level which again strengthen the hypolipidaemic influence of these oils. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, is responsible for the observed accumulation of lipids (Rajalingam et al., 1993; Sharma et al., 2003). The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. Further, it has been reported that diabetic rats treated with insulin show normalized lipid levels (Pathak et al., 1981). We suggest that the present effects of these oils-treated diabetic rats may be due to its role in normalization of insulin secretion, lowering activity of lipid biosynthesis enzymes, especially cholesterol and or lowering level of lipolysis.

Concerning the previous studies on the role of ginger and clove extracts, but not their oils, in diabetic status, Al-Amin et al. (2006) and Ojojewole (2006) stated that the extracts of ginger possess hypoglycaemic, hypocholesterolaenic and hypolipidaemic potential in STZ-induced diabetic rats and mice. Additionally, Al-Amin et al. (2006) demonstrated that ginger is effective in reversing the diabetic proteinuria observed in the diabetic rats. Thus, ginger may be of great value in managing the effects of diabetic complications in human subjects. Bhandari et al. (2005) reported that the extract of Zingiber officinalis produced significant antihyperglycaemic effect in STZ-induced diabetic rats. Further, the extract treatment also lowered serum total cholesterol, triglycerides and increased the HDL-cholesterol levels when compared with pathogenic
REFERENCES


