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Original Article

Effects of fenugreek, *Nigella*, and termis seeds in nonalcoholic fatty liver in obese diabetic albino rats

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ABSTRACT

Background and study aim: Nonalcoholic fatty liver disease (NAFLD) occurs in approximately 80% of cases of type 2 diabetes mellitus (T2DM). This study investigated the effects of some plants used in Saudi Arabia as antidiabetic agents on T2DM and associated fatty liver.
Materials and methods: A total of 150 adult male albino rats were divided into six experimental groups, each consisting of 25 rats. Twenty-five rats were considered as the control group. Experimental diabetes was induced in the remaining rats by administering a subcutaneous injection of 120 mg/kg of freshly prepared alloxan solution; these rats were classified into five groups: one group did not receive any treatment; the second group was treated with an aqueous extract of a mixture containing fenugreek, *Nigella*, and termis seeds; the third group was treated with an aqueous extract of *Nigella sativa* seeds; the fourth group was treated with an aqueous extract of fenugreek seeds; and finally the fifth group was treated with an aqueous extract of termis seeds at a dose of 100 mg/kg body weight. After 4 weeks of treatment, biochemical parameters were calculated, including blood sugar and serum insulin levels. Pancreatic and liver samples were obtained and processed for microscopic evaluation.
Results: The usage of each plant alone or a mixture of the plants corrected the glucose and insulin levels. Microscopically, a definite improvement in the number and diameter of β-cells in the diabetic group was observed. Furthermore, considerable improvement in fatty changes occurring in the liver of experimental animals was observed. The activity of the mixture was the most effective.
Conclusions: The aqueous extract of the seed mixture of the used plants appeared to be a useful agent in improving fatty changes in the liver texture associated with T2DM by reducing hyperglycaemia through an increase in insulin levels, regeneration of β-cells of the pancreas, and an amelioration of associated dyslipidemia.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognised condition that may progress to end-stage liver disease, affecting approximately 30% of Western populations. Due to increasingly sedentary lifestyles and the rising prevalence of obesity, NAFLD has become a common cause of chronic liver disease. NAFLD encompasses a spectrum of histological findings that range from macrovesicular steatosis alone (simple steatosis) to macrovesicular steatosis with hepatocyte ballooning and/or lobular inflammation (steatohepatitis) [1,2]. NAFLD occurs due to accumulation of lipids in the liver, mainly of triglycerides (TGs) [2]. Diabetes mellitus (DM) per se may generate NAFLD, in association with obesity and dyslipidemia. NAFLD is now considered as the hepatic manifestation of the metabolic syndrome, and it is present in approximately 80% of cases of type 2 diabetes mellitus (T2DM) [3].

In NAFLD, DM constitutes a risk factor for nonalcoholic steatohepatitis (NASH). NASH is the severe and progressive form of NAFLD, and it may develop into cirrhosis, hepatocellular carcinoma, and ultimately death [4,5]. Mechanisms leading to lipid accumulation are not completely understood, but it could potentially result from insulin resistance [6] and decreased disposal of fatty acids from impaired mitochondrial β-oxidation or deficient

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production of very low-density lipoprotein (VLDL) [7]. The proinflammatory and profibrogenic properties of lipid peroxidation end products such as serum malondialdehyde (MDA) potentially account for all of the typical histological features observed in this disorder [8]. DM is possibly the world's fastest-growing metabolic disease; hence, there is a need for more appropriate therapies [9]. Many plants have been investigated for their beneficial use in DM and reports occur in numerous scientific journals.

The active principles present in medicinal plants have been reported to possess the properties of regenerating pancreatic β-cell, increasing insulin secretion, enhancing glucose uptake by adipose or muscle tissues, inhibiting glucose absorption from the intestine and glucose production from the liver, and antagonising the problem of insulin resistance [10]. The literature survey revealed that *Nigella sativa* oil lowered blood glucose concentration in diabetic rats, and the hypoglycaemic effect of *N. sativa* may be mediated by extra-pancreatic actions rather than by a stimulated insulin release [3,4]. Oral administration of an ethanolic extract of *N. sativa* seeds to streptozotocin-induced diabetic rats for 30 days reduced the elevated levels of blood glucose and improved the altered levels of lipid peroxidation products; in addition, because of its antioxidant effects, its administration may be useful in controlling diabetic complications [5,6]. Fenugreek (*Trigonella foenum-gracaeum*) may increase the plasma insulin level in vivo [7–9]. Similarly, various hypotheses about the mechanism of the hypoglycaemic activity of fenugreek have been postulated, including delayed gastric emptying and an agonist effect on insulin receptors [10]. The major free amino acid constituent of fenugreek, 4-hydroxyisoleucine, stimulates insulin secretion from perfuse pancreas in vitro [11]. Termis seeds (lupine, a medicinal plant) have potential value in the management of diabetes with antihyperglycaemic activity, which is present in extracts of the whole seed. In white mice, extracts of seeds of the white lupine (*Lupinus albus* (*L. termis*)) were associated with increased tolerance to oral glucose [12].

Despite considerable progress in the treatment of diabetes by oral hypoglycaemic agents, the search for newer drugs continues because the existing synthetic drugs have several limitations. Alternatives are clearly needed because of the inability of current therapies to control, as well as high cost and poor availability of current therapies for many rural populations, particularly in developing countries [13]. This study was designed to examine the effects of an aqueous mixture extract of *N. sativa*, fenugreek, and termis and an extract of each of these plants alone on diabetic rats as well as the possible effects of these plants on the pancreatic cell types and numbers and on the liver.

Materials and methods

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

Plant material

The dried seeds of *N. sativa*, fenugreek, and termis were purchased from a local market in Al-Taif, KSA, in January 2012. A voucher specimen of each plant was kept in the Department of Pharmacognosy, College of Pharmacy, Taif University, KSA.

Preparation of plant extract

An exact weight (50 g) of the dried powdered seeds of *N. sativa*, fenugreek, and termis and a mixture of the powdered seeds in equal ratio (16.66 g *N. sativa* + 16.66 g fenugreek + 16.66 g termis) were separately extracted with 100 ml distilled water each, using the decoction method at a temperature of 100 °C for 5 min (boiling time). Each extract was separately filtered through cotton followed by refiltration through a filter paper. Using a rotary evaporator, the obtained aqueous extract of each sample was concentrated under vacuum to about 10 ml each followed by complete drying by the lyophilisation method using a freeze-dryer to afford dried extracts as follows: *N. sativa*, 5.56 g; fenugreek, 6.48 g; termis, 5.23 g; and the mixture, 5.04 g. All dried extracts were kept at −40 °C before use. Each of the obtained dried aqueous extracts of *N. sativa*, fenugreek, and termis seeds and the mixture was suspended in 0.5% carboxymethyl cellulose (CMC) sodium solution immediately before use and was orally administered to male albino diabetic rats at a dose of 100 mg/kg body weight.

Animal material

In the current work, 150 adult male albino rats 10–12 weeks of age were used – 25 rats with body weight ranging between 180 and 200 g (control) apart from 125 rats with body weight >250 g [14]. The rats were obtained from the Laboratory Animal Unit, King Fahd Medical Research Centre, King Abdulaziz University, Jeddah. All experiments were conducted in the research laboratories of the College of Medicine, Taif University. The animals were housed individually in clean rodent cages, in a room with relative humidity not less than 30% and not exceeding 70%, at a room temperature of 22–30 °C, with artificial lighting in a sequence of 12 h light and 12 h dark. Control animals were fed a standard rat chow (Ralston Purina, St. Louis, MO, USA). Diabetic animals were fed a semisynthetic diet enriched with sucrose (63 g sucrose/100 g) containing 20% (wt/wt) vitamin-free casein, 60% sucrose, and 5% lard [15] administered after induction of DM with an unlimited supply of drinking water. The animals were randomly selected and 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 carried out in 125 rats by administering a subcutaneous injection of 120 mg/kg of freshly prepared alloxan solution (powder from BDH Chemical Ltd., London, UK), dissolved in acetate buffer (pH 5.5) prepared immediately before use. After an overnight fast, and then 48 h later, the blood glucose level was determined using a glucometer for all animals. The glucometer measurements were validated using a glucose-oxidase method. Rats with a blood glucose level ranging from 180 to 250 were considered diabetic. Aqueous extracts of *N. sativa*, fenugreek, termis seeds, and a mixture of the powdered seeds in equal ratio, each in 0.5% CMC sodium, were separately administered by oral gavage to diabetic rats at a dose of 100 mg/kg body weight daily.

The experiment was carried out on six groups; each group contained 25 rats as follows:

- *The first group (control group, group I)* consisted of 20 normal rats that were administered a subcutaneous (sc) saline solution (0.01 ml/100 g body weight).

The diabetic rats were classified as follows:

- *Group II* received no treatment, and only CMC was administered.
- *Group III* was treated with an aqueous extract of the mixture of the seeds under investigation in equal ratio.
- *Group IV* was treated with an aqueous extract of *N. sativa* seeds.
- *Group V* was treated with an aqueous extract of fenugreek seeds.
- *Group VI* was treated with an aqueous extract of termis seeds.