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The Effect of Dietary Raw and Roasted *Nigella sativa* L. on Streptozotocin-Induced Changes of Serum Glucose and Body Weight in Rats.

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ABSTRACT

Nigella sativa L. (NS) has been known for many health benefits, but its glucose-lowering and body weight-controlling activities in diabetes still remain unresolved. This study was performed to investigate effects of raw and roasted NS seeds on serum glucose, body weight, and food and water intakes in normal and diabetic rats. Twenty-seven streptozotocin-induced diabetic and 18 normal male Sprague-Dawley rats, each of which were randomly divided into 3 groups; each group was assigned to one of 3 diets containing 0%, 2% raw or 2% roasted NS and given *ad libitum* for 6 weeks. Serum glucose was quantified and other biological parameters were assessed. Compared to normal rats, diabetic rats had significant (P<0.05) hyperglycemia and weight loss, higher food and water intakes, and lower food efficiency ratio. In normal rats, compared to 0% NS, these variables were not affected (P≥0.05) by raw or roasted NS feeding. In diabetic rats, compared to 0% NS, raw or roasted NS feeding produced similar food and water intakes, whereas roasted NS caused significant (P<0.05) rise in weight gain (28.5 ± 12.8 g) and food efficiency ratio (3.8 ± 1.7) compared to 0% (-6.6 ± 12.8 g; -0.5 ± 1.7), or raw (-13.7±12.8 g; -1.81.7) NS feeding respectively. In both normal and diabetic rats, compared to 0% NS, serum glucose (184.3 ± 21.7; 475.9 ± 17.8 mg/dl) evidently tended (P<0.08) to fall as a result of raw (158.2 ± 21.7; 450.9 ± 17.8 mg/dl) or roasted (145.3 ± 21.7; 447.4 ± 17.8 mg/dl) NS feeding respectively. Differences in serum glucose and food and water intakes of rats fed NS diets were not significant. Results suggest that raw or roasted NS attenuates serum glucose in normal and diabetic rats, and roasted NS is seemingly capable to improve body weight in a role that is likely to have dietetic applications in diabetes care.

Keywords: *Nigella sativa*, roasting, serum glucose, body weight, food intake, diabetes, rats.

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INTRODUCTION

Diabetes mellitus is a serious health problem worldwide. The epidemic of this disease is a global issue, and continues to be one of the most prevalent causes of death and disability [1]. Diabetes causes marked aberrations in assimilation and metabolism of carbohydrates, lipids and proteins leading to several clinical symptoms such as hyperglycaemia, hyperlipidaemia, ketoacidosis, and body weight disturbances [2]. Given the public health burdens of this disease [3], its management is becoming a major challenge, urging to identify potential means of therapy [4].

Current nutritional and medical care of diabetes involves various dietary regimes, lifestyle modifications and the use of drugs [5]. However, therapeutic complexity, side effects and cost are among many reasons that may limit the use of drugs in diabetes [6]. Nowadays, there is a growing interest in the consumption of plant foods, especially functional seeds for managing serum glucose and body weight and reducing the risk of diabetes [7].

Nigella sativa L is one of the ancient cultivated spice plants. It is originated in the Middle Eastern region and is central to the local diet [8].The seeds of the plant are commonly known as black cumin seeds, and have been used for many nutritional and medicinal purposes. They are traditionally consumed as a food with or without honey, and are usually incorporated into many food preparations as condiments or flavorings. Raw seeds or more preferably roasted seeds are consumed especially in foods such as bakery products to enhance desirable aroma and flavor [9]. Consumption of *Nigella sativa* seeds is related to higher-quality diets, including higher intakes of protein, essential fatty acids, fiber, antioxidants and a number of vitamins and mineral elements [10]. *Nigella sativa* seeds are also associated with a broad range of beneficial health effects, particularly anti-diabetic, anti-inflammatory, cardiovascular-protective, anti-microbial, gastro-protective, anti-oxidant, anti-asthmatic, nehpro-protective, anti-cancer and neuro-protective activities [11].

Effects of *Nigella sativa* seeds on serum glucose in humans and animals have been extensively studied, as shown by several comprehensive reviews [12-15]. However, evidence for possible antihyperglycemic activity of the seeds has been mixed. Several studies have shown that *Nigella sativa* seeds do not or variably affect serum glucose [16-18], whereas other studies failed to support this [19-21]. Here, it is important to emphasize that most of the nutritional and clinical studies linking *Nigella sativa* seeds with glycaemia in normal or diabetic situations have been mainly devoted to the effects of the seeds' fixed and essential oil and its bioactive components such as thymoquinone, and have often paid little or no attention to the method of preparation of the seeds as a possible mechanism for explaining such link. It is well known that *Nigella sativa* seeds are usually consumed as a roasted food. In fact, roasting improves flavor, color, texture and palatability of the food product, and may also affect its bioactive components [22]. From nutritional point of view, functionality of a food is primarily based on its being a whole, conventional, natural or a processed food and not pills, capsules, extracts or supplements [23], a matter that was not the focus of the previous studies. Nevertheless, controlled human or animal studies that link consumption of dietary raw or roasted *Nigella sativa* seeds with serum glucose, body weight and other biological parameters are generally lacking. Therefore, we investigated whether the consumption of diets containing raw or roasted *Nigella sativa* seeds had any effect on serum glucose concentrations, body weight homeostasis and food intake in normal and streptozotocin-induced diabetic rats fed such a dietary regimen for a period of 6 weeks.

MATERIALS AND METHODS

Preparation of Raw and Roasted *Nigella sativa* Seeds Powder

One batch (10 kg) of *Nigella sativa* L. seeds of Turkish origin was purchased from the local market in Amman, Jordan. The seeds were freed from foreign materials, cleaned and then divided into two equal portions, one was left as raw and the other was conventionally dry roasted [9]. In this method, seeds were spread on aluminum trays with a depth of 2 cm and roasted in a hot oven (Memmert, Karl lob, Germany) at 180 ºC for 10 minutes with occasional stirring. After roasting, the seeds were allowed to cool to room temperature. Physical and sensory tests were performed to confirm roasting efficiency. Raw and roasted seeds were ground separately to pass a sieve with circular openings of 1 mm in diameter (Retsch GmBH, Haan, Germany), and each powder was homogenized in a stainless steel blender (Kenwood®, Hampshire, England) for 15 minutes. The resultant powders were placed desiccated in sealed polythene bags and kept refrigerated at 4

ºC until further use. The macronutrient content of the raw and roasted *Nigella sativa* powders was determined by the Weende method [24]. These nutrients were: moisture, protein, fat, carbohydrate, ash and fiber.

Experimental Diets

Three isocaloric and isonitrogenous diets were prepared; they differed in their content of *Nigella sativa* powders (0%, 2% raw or 2% roasted, w/w). This dose (2%) was chosen as it is close to the usual intake of the seeds in Jordan (Jordan Department of Statistics: Personal communication, 2016). The protein and carbohydrate contents of *Nigella sativa* powders were taken into consideration in the calculation of nutrient composition of the diets. Ingredient composition of the diets is described in Table 1. All diets contained the same amount of calories, carbohydrate, protein, fat, vitamins and mineral elements. Dietary supplies of nutrients were in accordance with the dietary recommended allowances for rats from the American Institute of Nutrition [25]. The macronutrient and energy contents of the diets are described in Table 1. Diets were freshly prepared once a week and placed desiccated in sealed polythene bags and then kept refrigerated at 4 °C.

Table 1. Composition of the experimental diets ($g.kg^{-1}$)

Ingredient/Nutrient	Control diet	Raw NS diet	Roasted NS diet
Ingredient composition ($g.kg^{-1}$)			
Nigella sativa (NS) powder	0	20	20
Cornstarch	650.7	645.0	644.4
Egg albumin	180.0	175.1	175.2
Corn oil	90	83.6	84.0
Vitamin mix (AIN-93)*	30	30	30
Mineral mix (AIN-93)*	40	40	40
DL-Methionine	3	3	3
Tert-Butylhydroquinone	0·008	0·008	0·008
Nutrient and energy content			
Carbohydrate (%)	65.7	65.7	65.7
Protein (%)	18.0	18.0	18.0
Fat (%)	9.0	9.0	9.0
Energy ($kcal.100g^{-1}$)	415.8	415.8	415.8

*AIN: American Institute of Nutrition [25]

Animal Experimentation

Fifty-four male Sprague- Dawley rats were obtained from the Experimental Animal Unit of the Faculty of Medicine, The University of Jordan, Amman, Jordan. The animals were acclimatized for 7 days prior to the start of the experiment, during which they were fed on chow diet with free access to tap water. They were individually housed in plastic cages with stainless steel wire-mesh bottom (North Kent Plastic Cages, Ltd, Dartford, England) under controlled temperature (22±2 ºC) and hygienic conditions with 12-hour light, 12-hour dark cycle. Diets were offered in glass jars and water was given in glass bottles fitted with melamine stoppers. All the experiments involving animals were approved by the Institutional Animal Ethics Committee of the Deanship of Academic Research, University of Jordan, Amman, Jordan) and carried out according to the recommended guidelines for animal care and use [26].

Diabetes was partially induced in 36 rats by a single intraperitoneal injection of a freshly prepared streptozotocin (Sigma Chemical Co., Saint Louis, MO, USA) solution (32.25 mg/ml in 0.05 M citrate buffer, pH

4.5) at a dose of 32.25 mg/kg [27]. The remaining 18 normal rats received only citrate buffer in the same volume and through the same route. Four days after streptozotocin injection, animals were checked for diabetes by testing the presence of glucose in the urine, using reagent test strips (Glkotest, Roche Germany). Twenty-seven rats developed glucosuria and justified diabetes, and 9 had glucose-free urine; hence they were excluded from the study.

At the beginning of the experiment, animals weighed 200.4 ± 2.1 g and they were consisting of 18 normal and 27 streptozotocin-induced diabetic rats, each of which were randomly divided into 3 groups, and each group was then assigned to one of the 3 dietary regimens described above. During the experimental period, which lasted for 6 weeks, experimental diets and tap water were given *ad libitum.* Body weight and food intake were monitored weekly. Water intake was measured daily for the diabetic and weekly for the normal rats. Food efficiency ratio as body weight gain (g) per 100 (g) food intakes was also calculated. On the termination day and after an overnight fast, animals were anesthetized using chloroform. Blood was collected by performing cardiac puncture and the serum was isolated and stored frozen at −20 °C until chemical analysis.

Biochemical Analysis

Concentrations of serum glucose were determined by using standard kits and in accordance to the manufacturer's instructions (Boehringer Mannheim GmbH, Germany). Analysis was performed at the Medical Laboratories of the Islamic hospital, Amman, Jordan, using a pre- calibrated automated clinical chemistry analyzer (Roche/Hitachi 912 chemistry analyzer).

Statistical Analysis

Data analysis was performed using the statistical analysis software (SAS version 22, USA). Results were expressed as means ± standard errors of the mean (SEM). Significance was set at $P < 0.05$ and tendency was accepted at $P < 0.08$. Statistical significance was assessed by two-way ANOVA followed by the Duncan's multiple range tests. Partial correlations were used to test the associations between the various studied parameters for the whole normal or diabetic animals.

RESULTS

The macronutrient composition of raw and roasted *Nigella sativa* (NS) powders used in this study is presented in Table 2. On dry matter basis, raw NS powder was found to contain high content of fat (32.1 g/100g), protein (24.6 g/100g), carbohydrate (28.5 g/100g) and energy (501.3 kcal/100g). Moisture content was 4.8 % and 1.2 % for raw and roasted powders respectively. Roasting had a slight effect on the nutrient and energy content of the seeds.

Table 3 shows serum glucose and certain biological parameters of normal and diabetic rats fed raw and roasted NS powders for six weeks. Initial body weights were essentially similar in all normal and diabetic rats. Compared to normal states, streptozotocin injection significantly ($P < 0.05$) induced hyperglycemia (476 ± 18 versus184 ± 22 mg/dl), weight loss (-6.6 ± 12.8 versus 91.4 ± 15.7 g), higher food (912 ± 31 versus 595 ± 38 g) and water (6.86 ± 0.29 versus 0.98 ± 0.35 l) intakes, and lower food efficiency ratio (-0.5 ± 1.7 versus 15.4 ± 2.1). In normal rats, compared to 0% NS, raw or roasted NS feeding did not significantly ($P \geq 0.05$) influence these variables. In diabetic rats, compared to 0% NS, raw or roasted NS feeding produced similar food and water intakes, whereas roasted NS resulted in significant ($P < 0.05$) rise in weight gain (28.5 ± 12.8 g) and food efficiency ratio (3.8 ± 1.7) compared to 0% NS (-13.7 ± 12.8 g; -1.8 ± 1.7), or raw NS (-6.6 ± 12.8 g; -0.5 ± 1.7) feeding, respectively. In fact, rats fed roasted NS were the only among diabetic rats which continued to gain weight, though in a lesser magnitude than normal rats (Table 3). In all animals, final body weight, body weight change and food efficiency ratio behaved similarly in response to NS feeding. Among the experimental animals, these variables were the lowest in diabetic rats fed 0% NS diet. In both normal and diabetic rats, compared to 0% NS (184 ± 22; 476 ± 18 mg/dl), serum glucose evidently tended ($P < 0.08$) to fall as a result of raw (158 ± 22; 451 ± 18 mg/dl) or roasted (145 ± 22; 447 ± 18 mg/dl) NS feeding, respectively. Differences in serum glucose and food and water intakes of rats fed NS diets were not significant.

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Table 2. Macronutrient and energy content of *Nigella sativa* seeds powder

Component*	Raw *Nigella sativa* ($g.100g^{-1}$)	Roasted *Nigella sativa* ($g.100g^{-1}$)
Carbohydrate	28.5	31.7
Protein	24.6	23.8
Fat	32.1	30.1
Ash	4.5	4.3
Fiber	10.3	10.1
Energy ($kcal.100g^{-1}$)	501.3	492.9

*Mean of triplicates with less than 5% coefficient of variation, on dry matter basis.

Table 3. Serum glucose and certain biological parameters of rats fed raw and roasted *Nigella sativa* seeds powder.

Variable	Normal groups$^{\$\#\alpha}$			Diabetic groups$^{\$\#\alpha}$		
	Control	Raw NS	Roasted NS	Control	Raw NS	Roasted NS
Initial weight (g)	207.1±4.6^{a}	206.8±4.6^{a}	206.7±4.6^{a}	194.9±3.8^{a}	194.9±3.8^{a}	200.3±3.8^{a}
Final weight (g)	298.5±14.6^{a}	301.4±14.6^{a}	300.5±14.6^{a}	188.8±12.0^{c}	181.2±12.0^{c}	228.7±12.0^{b}
Weight change (g)	91.4±15.7^{a}	94.7±15.7^{a}	93.8±15.7^{a}	-6.6±12.8^{c}	-13.7±12.8^{c}	28.5±12.8^{b}
Food intake (g)	595±38^{b}	603±38^{b}	606±38^{b}	912±31^{a}	880±31^{a}	886±31^{a}
FER$^{\#}$	15.4±2.1^{a}	15.4±2.1^{a}	15.3±2.1^{a}	-0.5±1.7^{c}	-1.8±1.7^{c}	3.8±1.7^{b}
Water intake (l)	0.98±0.35^{b}	0.88±0.35^{b}	0.94±0.35^{b}	6.86±0.29^{a}	6.58±0.29^{a}	6.34±0.29^{a}
Glucose (mg/dl)	184.3±21.7^{b*}	158.2±21.7^{b}	145.3±21.7^{b}	475.9±17.8^{a*}	450.9±17.8^{a}	447.4±17.8^{a}

$^{\$}$ Values are means ± SEM

$^{\#}$ NS: *Nigella sativa*; FER: food efficiency ratio: body weight change/100g food intake

$^{\alpha}$ Values in rows with different superscripts are significantly different ($P < 0.05$)

* Values in rows with asterisk (*) tend to differ ($P < 0.08$) from raw and roasted NS

Overall partial correlation tests in normal rats revealed no associations between the various studied parameters, except that between weight change and food intake (r = 0.66, $P<0.01$; n=18). In diabetic rats, serum glucose correlated positively (r = 0.38, $P<0.05$; n = 27) with water intake and negatively with each of weight change (r= -0.44, $P<0.02$; n = 27) and food efficiency ratio (r= -0.46, $P = .01$; n = 27); and tended to associate positively with food intake (r= 0.24, $P<0.08$; n = 27). In these rats, a positive correlation (r= 0.82, $P<0.01$; n = 27) was found between water and food intakes.

DISCUSSION

In this study, streptozotocin-diabetic rats were used as a model of insulin insufficiency to examine the influence of raw or roasted NS powder when incorporated into the diet on the levels of serum glucose, body weight and food and water intakes. Streptozotocin is a nitrosourea derivative of glucosamine capable to selectively induce multiple DNA strand beaks leading to pancreatic β–cells destruction, and thus diabetes [28]. It has been documented that streptozotocin produces mild to severe types of diabetes depending on the dosages used (< 50 or > 50 mg/kg, respectively) that mimic type-1 or type-2 diabetes [29]. We used a single intraperitoneal injection (32.25 mg/kg) of streptozotocin that led to marked hyperglycaemia, weight loss, hyperphagia, polydipsia, and a fall in food efficiency ratio. These results were substantiated by remarkable inter-correlations recorded between various manifestations. Such signs are consistent with those reported in this model elsewhere [27-30]. Hyperglycaemia, weight loss and reduced food utilization are primarily attributed to defective carbohydrate, protein and lipid assimilation and metabolism associated with increased substrate catabolism and osmotic diuresis as a consequence of insulin deficiency [2].

The present study shows that in rats, the incorporation of 2% raw or roasted NS powder into the diets of normal or streptozotocin-diabetic rats lessened serum glucose, but did not influence food and water intakes in contrast to rats fed NS-free diet. Unlike in normal rats, roasted NS prevented weight loss and ameliorated

food efficiency ratio of diabetic rats compared to those fed NS-free or raw NS-diets. The different NS diets had only apparently random effects on serum glucose and food and water intakes.

The energy and macronutrient composition of the NS powder used in this study was comparable to the literature range values [10,12]. However, relative variability in the nutritional properties of NS has been reported. This variability may be attributed to a number of factors, such as differences in genotype or variety, geographic or climatic circumstances under which the plants were grown, maturity stage, postharvest handling and storage conditions, product quality and analytical procedures [10,31]. Dry roasting is usually applied to foods, particularly seeds for the main purpose of enhancing desirable aroma and flavor [9]. Differences in the obtained energy and macronutrient values for raw and roasted NS powder were slight; however, such effect has not previously been documented.

It is noteworthy that in the current study, unlike raw NS, roasted NS was able to reverse weight loss and to mend disturbed food efficiency ratio induced by streptozotocin without noticeable effects on food and water intakes. Several animal studies have investigated the effect of NS oils or extracts, but not its powder on body weight; however, no human studies are available. In agreement with our results, injecting 0.2 ml/kg/day aqueous NS extract intraperitoneally for 32 days has been shown to markedly improve body weight gain in streptozotocin-induced diabetic rats [32]. Similar results have been obtained in diabetic rats receiving a diet containing 5% NS oil for 3 weeks [33]. Furthermore, attenuation in body weight loss induced by streptozotocin has been documented in rats treated intraperitoneally with 5 mg/kg hydroalcholic NS extract for 32 days [19]. It has been shown that NS may have an ameliorating action on β-cells leading to increased insulin secretion and prevented protein wasting, thus providing a plausible explanation for such results [19]. In this regard, effects of roasted NS or its components on body weight homeostasis in normal or diabetic humans or animals are yet to be elucidated.

There is a large literature linking NS with diabetes in humans and animals [16-21], which has expanded rapidly since 1980s, but findings are debatable. This might be due to the apparent discrepancy between the various experimental approaches used. In fact, the type and complexity of the NS source, variety, preparation methods or chemical structure, the form and amount consumed, feeding mode and duration, basal diet composition and other lifestyle patterns are among many potential confounders that may contribute to this inconsistency. Moreover, there appears to remain a general lack of studies investigating the effect of roasted NS in diabetes. Roasting is the method of choice for preparation and consumption of NS, especially in the communities of the Middle East [9]. The NS seeds are also exposed to different sorts of heat treatment when incorporated into many food preparations as condiments or flavourings [9]. It may be also noticed that the aim of most of the previous studies was to evaluate effects of NS extracts, oils or NS bioactive phytochemicals, particularly thymoquinone in various normal or pathological conditions. However, studies involving roasted NS inclusion to standard diets or those examining such diets in normal or diabetic humans or animals are not available. This certainly limits the discussion of the present results with those of the other studies.

In diabetic animal models, oral feeding of 400-500 mg/kg/day NS oil for 1-4 weeks has been reported to cause a significant fall in blood glucose with [34] or without rise in insulin [35]. Similar results have been shown in diabetic animals injected intraperitoneally with 10-100 mg/kg/day NS oil for few hours-30 days [36,37]. Marked reduction in blood glucose has been also shown in diabetic animals receiving intraperitoneal injection of 0.2 ml/kg/day aqueous NS extract [2], or 5 mg/kg hydroalcholic NS extract [19], or an oral dose of 300 mg ethanol NS extract for 21-32 days [20]. Improvements in the antioxidant status and preservation of islet structure as a result of NS administration have been repeatedly documented in diabetic animals [19,20,32,36,37]. Oral or intraperitoneal administration of various doses of NS powder, extract or oil to normal animals for different periods has been shown to variably affect blood glucose [12,17,18]. In humans, oral administration of capsules containing 0.5-3.0 g powdered NS daily to patients with diabetes type 2 for periods of 2-3 months has been shown to reduce blood glucose and improve glycemic control [21,38], or to produce no effects on these variables [16]. Apparently, some of these results are consistent with our findings; however, a remarkable variation in the experimental protocols still exists.

It is important to understand that the study substance for the aforementioned human and animal studies was NS powder, extract or oil. In this respect, varied NS administration modes, doses and duration found in different experimental approaches will certainly influence studies' results [12-15]. Interaction of NS or its components with other medication strategies, diet composition and lifestyle patterns in diabetic patients

may also complicate interpretation of results. Unlike these studies, we used solely NS powder and incorporated it into an isocaloric-isonitrogenous standardized rat diet. In view of these facts, in contrast to NS components, whole NS itself has been recommended as a therapeutic and protective strategy in many chronic diseases including diabetes [13-15]. We also experimented the NS after being dry roasted in accordance to the NS preparation and eating custom in the Middle East [9]. This goes in line with the recent view of functionality of a food that is primarily based on its being a whole, conventional, natural or a processed food and not pills, capsules, extracts or supplements [3,39], limiting the possibility of excess doses and toxicity.

CONCLUSIONS

Taken together, when incorporated into a standardized diet, raw and roasted NS tend to exert a favourable effect on serum glucose, and the particular roasted NS improves body weight homeostasis in streptozotocin-induced diabetic rats. The roasted NS is seemingly capable to counteract the diabetogenic effect of streptozotocin in rats. It is obvious that a sort of interaction between roasted NS and the mechanisms underlying glucose metabolism took place, though it was not addressed. Thus, it would be of great importance to explore the nature of such interaction that may modify glucose assimilation and metabolism under streptozotocin conditions. This is likely to have great dietetic applications in human diabetes therapy and prevention.

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