



Research Article

The Effect of *Nigella Sativa* Extract on Glucose Regulation and Renal Parameters in Streptozotocin-Induced Diabetic Rats

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Abstract:

Ethnopharmacological relevance: *Nigella sativa* is a widely used medicinal plant and its' antihypertensive, antidiabetic, renal protective and antioxidant properties were demonstrated in several studies. In this study we wanted to determine its' possible favourable effects on glucose regulation and renal parameters in streptozotocin induced diabetic rats.

Materials and Methods: Rats were divided into four groups: [1] streptozotocin induced diabetic rats [2] diabetic with *Nigella sativa* [3] nondiabetic with *Nigella sativa*, and [4] control with standard diet. *Nigella sativa* was prepared as extract and administered as 200 mg/kg/day for second and third groups. Serum glucose, urea and creatinine were measured and initial and final values were compared eachother.

Results: A positive effect in terms of glucose regulation was not detected in diabetic with *Nigella sativa* group. The final plasma creatinine was lower in nondiabetic with *Nigella sativa* and control with standart diet groups.

Conclusion: For glucose regulation *Nigella sativa* was not effective as in used dose and therapy duration. To determine the potential positive effect on renal functions, only urea and creatinine measuements absolutely are not enough and further evaluation methods are required to test the potential therapeutic and preventive effects of *Nigella sativa*.

Keywords: Diabetic Kidney Disease; Diabetes Mellitus; *Nigella Sativa*; Oxidative Stress

Introduction: Diabetic Kidney Disease (DKD) is the leading cause of end stage renal disease [1]. Chronic hyperglycaemia induces oxidative stress, the formation of advanced glycation end products and the activity of renin angiotensin system (RAS). With the proinflammatory cytokines, endothelial cell injury with podocyte damage, extracellular matrix deposition and thickening of the basal membrane follows each other and eventually tubulointerstitial fibrosis with glomerulosclerosis developed [2,3].

For early detection of DKD we have a few laboratory parameters and one of them is microalbuminuria. In kidney disease process, permeability to plasma proteins increase with glomerular damage and result in their excretion in the urine. And also abnormalities of extracellular matrix synthesis can lead to increased urinary excretion of matrix proteins that reflecting

glomerular injury. Microalbuminuria is defined as albumin excretion rate is 30-300 mg/day and is independently associated with cardiovascular risk and also underlying renal disease [4]. The studies have indicated that a reduction of microalbuminuria in type 2 diabetic patients provide renal and cardiovascular risk reduction [5]. For this purpose reduction in dietary protein, improvement in glycaemic control, lipid lowering therapy and blockade of the RAS are proven benefit treatment modalities [6].

Along with these modalities, the preventive and therapeutic approaches are still investigated. Especially in last years studies regarding vitamin D receptor activation and Transforming Growth Factor (TGF) - β and endothelin 1a receptor inhibition are really promising [7-9]. Medicinal plants are other preventive modalities. *Artemisia campestris* (Ac), *Ligustrazine*, a bioactive component contained in *Chuanxiong* and *Euryale ferox* seeds showed a renoprotective role in animal models [10-12].

Nigella sativa (*N.sativa*) from the Ranunculaceae family is a commonly known traditional medicine. The biological activity of it is related to the composition of its essential oil, which is containing of 30 to 48% Thymoquinone (TQ), 7 to 15% P-cymene, 6 to 12% Carvacrol, 2 to 7% 4-terpineol, 1 to 4% Tanethole and 1 to 8% Sesquiterpene [13]. Pharmacologically, TQ and its derivatives are the most important components [14]. Due to the presence of fatty acids and compounds such as tocopherols, carotenoids, metal ions and phosphorus components in smaller amounts, fresh and intact plant oil is resistant against to oxidation [15]. *N.sativa* has been detected useful as antimicrobial (against *Staphylococcus aureus*, *S. epidermidis*, *Helicobacter pylori*, *Candida albicans*, *Trichophyton* spp., and *Schistosoma mansoni*), antiinflammatory, gastroprotective, antiasthmatic, antihypertensive, antidiabetic and renal protective [16-18]. Particularly with antioxidant activity, it is believed that the seeds of this plant are capable to protect the kidneys against oxidative stress.

The effect of TQ on the nephropathy and oxidative stress induced by Doxorubicin in rats were evaluated and triglycerides, total cholesterol and lipid peroxides in the kidneys of TQ treated rats decreased compared with Doxorubicin alone [19]. Additionally with TQ, a complete reversal of gentamicin-induced increase in blood urea, creatinine and lipid peroxides was observed. TQ supplementation prevented gentamicin-induced injury in kidney [20].

In this year another study with exciting results was presented that based on the potential molecular mechanisms of TQ in the diabetic kidney. Albuminuria, podocyte injury and extracellular matrix proteins were investigated. In that study by the preservation of the podocyte function, decreased albuminuria was observed. Also in diabetic kidney, prevention with *N.sativa* for over expressions of Type IV collagen, TGF- β 1 and vascular endothelial growth factor-A was presented [21].

In this study firstly we wanted to investigate the possible favorable effect of *N.sativa* in terms of plasma glucose regulation. At the same time we wanted evaluate the possible useful effect on renal functions of this extract in diabetic and nondiabetic rat models.

Materials and Methods:

Experimental procedure: *N. sativa* seeds were purchased from a local herb store in Istanbul, Turkey. The specimen was washed, dried and waited for one day in room temperature. Whole kernels were extracted. They crushed using a

grinder. Subsequently, powdered seeds were subjected with methanol for 24 hours at 40°C. The methanol was evaporated using Heidalf Hei-VAP version - 1,0 ; 072019, *Germany*.

The extract was obtained after lyophilisation at - 85°C using Labconco Free Zone 2,5 plus, *USA*. Finally with this method, 0.79 g of extract was obtained from 10 g *N. sativa* seeds because of technical convenience.

Because of inadequate equipment, analytical chemistry could not be performed.

Streptozotocin-induced diabetes: Streptozotocin (STZ) and other chemicals were purchased from Makrosel Group Chemical Co. DM was induced in rats by a single intraperitoneal injection with 55 mg/kg STZ (freshly diluted with isotonic NaCl) according to standardized protocol [22]. The one day and 7 days after STZ injection, blood samples were obtained from anesthetised rats with intracardiac injection because of technical convenience. Diabetic status was confirmed by monitoring blood glucose 7 days after the injection. According to procedure of animal studies, rats with sustained blood glucose levels up to 200 mg/dl were considered for install the diabetic groups.

Animals: In this study, thirty number 15-week-old male Sprague-Dawley rats, each weighing 300-400 g. were used. The animals were housed in individual polycarbonate cages under standard conditions and acclimitized properly. Rats were kept on a 12 h light/dark cycle at 21 \pm 1°C and 50 \pm 10% humidity. The animals were maintained under standard conditions and fed with standard rat diet and water. All animals were cared for according to the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health [23]. The Institutional Local Ethics Committee approved the study protocol. (*No: 2013/57*)

Experimental Design: Thirty rats were randomly divided into four groups as follows; STZ diabetic with no *N. sativa* [6], STZ diabetic with *N. sativa* for 5 weeks [8], nondiabetic with *N. sativa* with same dose for 5 weeks [8], control with standard diet [8].

In literature different administration routes were used in rat models but generally intraperitoneal and per oral route were observed. In this study per oral route was used.

Because of limitation in terms of dose quantification, *N.sativa* dosage ranges varies almost in every study [24]. In our study *N. sativa* was diluted with one ml distilled water and administered at a dose of 200 mg/kg/day per oral

using gavage injector for five weeks in STZ diabetic and nondiabetic with *N. sativa* groups. Because of in diabetic groups some of rats were died with weight loss, for ethical convenience the duration of study was determined as five weeks. At the end of the study, the rats were euthanized by a lethal dose of sodium pentobarbital 100 mg/kg intraperitoneal.

Serum glucose, urea and creatinine levels were tested with commercial kits using by automatic biochemistry analyzer (Cobas 8000 Roche Hitachi, USA).

Statistical analysis: Statistical analysis of the data was tested with IBM SPSS 22 statistical pocket program. For to determine the normal dispersion or not, Shapiro-Wilk test was used. For descriptive statistics of datas, variables with no normal distribution are presented as median (minimum-maximum). For the comparison of more than two independent groups with continous data with no normal dispersion Kruskal Wallis test and as Post-Hoc test, test of Dunn was used.

The comparison of time-dependent measurements between groups was tested percentage change compared to baseline values for variables obtained with the measurement -percentage change = ((the final data- first data)/first data)x100 - Statistical significance was determined as $p < 0.05$.

Results: For initial median glucose, statistical difference was detected between control with extract and two diabetic groups ($p=0.003$, $p=0.003$). Between control with standart diet with other groups there is no statistical difference. Glucose percent change was not different between all groups ($p > 0.05$). Initial median urea was not different between four groups ($p > 0.05$). But in terms of urea percent change the difference was detected between diabetes with no extract and control with extract and control with standart diet ($p=0.035$, 0.041). For initial median kreatinin and creatinin percent change any difference was not observed for all groups ($p > 0.05$). All related datas are presented in Table 1.

Variable	Diabetes with no N.Sativa	Diabetes with N.Sativa	Control with N.sativa	Control with standard diet	p^*	p^{**}
Initial Median glucose (mg/dl)	481 (406-571)	438 (295-590)	274 (228-308)	307 (272-350)	<0.001	1-2: >0.05 1-3:0.003 1-4:>0.05 2-3: 0.003 2-4:>0.05 3-4: >0.05
Glucose percent change	0.34 (0.31-0.78)	0.76 (-0.83-0.96)	-0.10 (-0.33-0.27)	-0.69 (-0.38-0.12)	0.027	>0.05
Initial Median urea (mg/dl)	34 (31-41)	38 (26-47)	32 (20-35)	34 (31-41)	>0.05	1-2: >0.05 1-3:>0.05 1-4: >0.05 2-3:>0.05 2-4:>0.05 3-4: >0.05
Urea percent change	0.90 (0.62-1.09)	0.61 (-0.51-1.17)	-0.036 (-0.22-0.42)	-0.02 (-0.19-0.15)	0.014	1-2: >0.05 1-3:0.035 1-4: 0.041 2-3: >0.05 2-4: >0.05 3-4: >0.05
Initial Median creatinine (mg/dl)	0.45 (0.43-0.54)	0.45 (0.36-0.50)	0.38 (0.36-0.45)	0.40 (0.37-0.44)	0.012	1-2: >0.05 1-3:>0.05 1-4: >0.05 2-3:>0.05 2-4:>0.05 3-4: >0.05
Creatinine percent change	-0.20 (-0.26/-0.03)	-0.05 (-0.24-0.24)	-0.20 (-0.50/-0.06)	-0.11 (-0.24/-0.01)	>0.05	1-2: >0.05 1-3:>0.05 1-4: >0.05 2-3:>0.05 2-4:>0.05 3-4: >0.05

*Statistical difference between four groups for initial glucose, urea and creatinin parameters

**Statistical difference between each groups for initial glucose, urea and creatinin parameters

Table 1: The initial glucose, urea and creatinine measures and percent change of each parameters between four groups.

For all groups, the comparison eachother in terms of initial and final glucose change, statistical difference was not observed ($p>0.05$). Only diabetes with extract group the change of urea measurement was statistically different ($p=0.036$). For two groups, control with extract and control

with standart diet, the change in terms of creatinin was statistically different ($p=0.012$, $p=0.012$). The initial and final values and change of glucose, urea and creatinine measurement for each groups are presented in Table 2.

Variable	Diabetes with no N.Sativa	Diabetes with N.Sativa	Control with N.sativa	Control with standard diet
Initial Median glucose (mg/dl)	481 (406-571)	438 (295-590)	274 (228-308)	307 (272-350)
Final Median glucose (mg/dl)	721 (634-769)	779 (48-865)	245 (189-296)	288 (200-346)
p^*	0.068	0.069	0.123	0.263
Initial Median urea (mg/dl)	34 (31-41)	38 (26-47)	32 (20-35)	34 (31-41)
Final Median urea (mg/dl)	67 (58-80)	64 (12-101)	30 (26-34)	33 (30-37)
p^{**}	0.068	0.036	0.575	0.575
Initial Median creatinine (mg/dl)	0.45 (0.43-0.54)	0.45 (0.36-0.50)	0.38 (0.36-0.45)	0.40 (0.37-0.44)
Final Median creatinine (mg/dl)	0.42 (0.34-0.46)	0.43 (0.35-0.49)	0.30 (0.23-0.36)	0.36 (0.30-0.39)
p^{***}	0.068	0.327	0.012	0.012

*Statistical difference between initial and final glucose for each group

**Statistical difference between initial and final urea for each group

***Statistical difference between initial and final creatinine for each group

Table 2: The initial and final values and change of glucose, urea and creatinine measurement for each groups

Discussion: As expected, the initial glucose was higher in diabetic groups according to control groups. But difference was significant between diabetic groups and control with N sativa. At the final of the study any positive effect in terms of glucose regulation was not detected in diabetic *N.sativa* group. Although the initial creatinin measurement was minimal level in control with N sativa, inter group analysis was not detected any significance.

Only diabetes with *N.Sativa*, the statistical difference was detected between initial and final urea variation. But this parameter was rising direction and did not provide any beneficial information. In terms of creatinine, this variation was observed in two control groups. In that groups, the final creatinine was in statistically lower levels according to inital. But this change was at the same level and did not determine a possible beneficial effect of *N.sativa* between nondiabetic groups.

DKD is a progressive and irreversible renal disease, which is characterized by oxidative stress associated with hyperglycaemia. Hyperglycaemia causes tissue damage through five mechanisms: increased flux of glucose and other sugars using the polyol pathway, increased advanced glycation end products (AGE), increased expression of the AGE receptor and its' activating ligands, activation of protein kinase C isoforms and overactivity of the hexosamine pathway. All of these mechanisms are activated by mitochondrial overproduction of reactive oxygen radicals [25]. The polyol pathway is based on aldo - ketoreductase enzymes that use carbonyl compounds as substrates. Aldose reductase is found in tissues such as retina, nerve and glomerulus. Glucose uptake in the vascular cells of these tissues is mediated by insulin-dependent glucose transporters, and intracellular glucose concentration levels increase with hyperglycaemia [26].

AGEs are formed by the nonenzymatic reaction of glucose and other compounds derived from glucose and from increased fatty acid oxidation in arterial endothelial cells. In diabetes, increased AGEs are found in the extracellular matrix, and these precursors can damage cells by several mechanisms. Peripheral arterial diseases, heart failure and increased mortality after ischaemic events are associated with this pathogenetic process [27]. Activation of protein kinase C isoforms is another pathogenetic mechanism that impacts the accumulation of microvascular matrix protein by inducing expression of TGF - 1, fibronectin and type IV collagen [28]. Hyperglycemia and increased fatty acid oxidation appear to contribute to diabetes mellitus- related complications by increasing the flux of fructose 6 - phosphate into the hexosamine pathway [29].

Along with this process, for these patients, lipotoxicity plays another important role in islet dysfunction. The studies suggest that patients with diabetes have increased IL-1 β expression and macrophage recruitment in their islets [30]. β -cell failure in type 2 diabetes has an inflammatory component. For islet inflammation and β -cell dysfunction, the role of cholesterol accumulation was observed in rats that are deficient in cholesterol transporters. In that model, excessive cholesterol in islets lead to macrophage recruitment, increased IL-1 β expression and defective glucose stimulated insulin secretion [31].

For prevention of patient from these pathogenetic process and finally DKD, tight glycaemic control, lipid lowering therapy and blockade of the RAS are proven benefit and used treatment modalities. A various pharmacological and herbal material are currently evaluated for this purpose also.

One of them, *N. sativa* is studied with different experimental methods and dosage intervals for possible positive effects on glucose regulation and lipid profile. Its' activity for reduction of oxidative stress through modulation of hepatic enzyme expression was observed and decreased serum sodium dismutase and catalase enzymes and increased hepatic antioxidant enzymes such as glutathione peroxidase, reductase and transferase activities was determined [32].

In a diabetic rat model with combination of α - lipoic acid, L-carnitine and *N. Sativa*, an improvement of the carbohydrate metabolism and to less extent lipid metabolism was observed [33]. With different dosages, the effects of *N. sativa* extract on glucose concentrations was investigated also. In a rat model with extract, 5 mg/kg, 10mg/kg and 20 mg/kg dosages for 32 days were studied and hypoglycemic and ameliorative effect on

regeneration of pancreatic islets at low doses with 5 mg/kg has been observed [34].

In one of the clinical studies with 94 patients, capsules containing *N. sativa* with different dosages were administered orally for three months for plasma glucose regulation and a dose of 2 gr/day was presented as a beneficial adjuvant to oral hypoglycemic agents in patients with diabetes [35]. In last year with 114 type 2 diabetes patients, *N.sativa* glucose lowering effect was compared with placebo. In that study along with fasting blood glucose, hemoglobinA_{1c} and C- peptide, changes in total antioxidants capacity, superoxide dismutase and catalase activity were studied. Improvement on glucose homeostasis and antioxidant defense system was observed with long term supplementation of *N sativa* [36].

N. sativa treatment also showed a reduction in total cholesterol, triglyceride and low density lipoprotein and an increase of high density to low density lipoprotein ratio in patients with diabetes [37]. In addition, *N. sativa* was associated with a reduction in waist and hip circumferences and weight loss [38].

Consequently *N. Sativa* is currently studied for antiglycemic effect and it can improve glycemic status and lipid profile in diabetes models. But more clinical trials are necessary to clarify its' effective type and dosage for diabetes management and its complications [39].

In this study we firstly wanted to evaluate this plants' possible favorable effect on glisemic regulation in a rat model. But between diabetic versus *N.sativa* diabetic group, no favourable result was observed.

In terms of possible nephroprotective features, *N.sativa* was studied in a few models. Possible protective effect was associated with a dose-dependent improvement in biochemical and histological indices in rats with gentamycine related nephrotoxicity [40]. The effect of *N. sativa* on bromobenzen - induced hepatorenal toxicity showed remarkable improvement in liver and kidney architecture also [41].

Despite these datas, DKD associated experimental studies are insufficient. In a recent study, *N. sativa* - related renal morphological and immunohistochemical improvement was examined in a DKD model by assessing the mesenchymal markers, Fsp1, desmin and MMP - 17 as well as the epithelial marker, ZO - 1 [42]. In another experimental model of the diabetic nephropathy TQ treatment was presented with albuminuria reduction and suppression of enhanced extracellular matrix gene expression [21].

In our study only, diabetes with *N.sativa* groups' final urea statistical higher than initial and in control with *N.sativa* and control with standart diet groups, final creatinine was statistically lower according to initial.

These results reflect the several limitations of this study. First of all we think that, the used total dosage and treatment duration of extract was not enough or not appropriate for providing glisemic regulation. Also the initial glucose measurements of control groups did not meet the inclusion criterias of the study. Its' possible reason was personel inexperience. In diabetes with *N.sativa* group, rising of urea was evident and it was possibly associated with insufficient hydration and in two control groups the decrease of creatinine at the same level did not provide any additional information for this parameter.

Conclusion: In this study, any positive data was not observed in terms of plasma glucose regulation. We unabled to determine the protective effect of *N. sativa* on DKD because of only urea and creatinine measurements were not enough for evaluation of renal function. Further studies for activities of *N. sativa* and its bioactive constituents should be conducted. Also to determine the compounds responsible for its biological effects, the preparation and administration methods and the potential negative drug interactions must be investigated.

Study Limitation: In this study we observed some defects after the end of trial. The first of all at the initial of study, plasma glucose measurements were up to 200 mg/dl in nondiabetic groups. It was associated with because of the presence of overweight rats. Exactly these animals should be excluded from the study at the initial.

Secondly because of there is no consensus for effective dose and therapy duration for *N. sativa*, applied protocol probably was not inappropriate. In this study with relevant literature we identified duration but especially in diabetic groups severe weight loss was observed and some of rats died. We have to supplement new rats in that duration with same care rules and same maintenance time with ethic committee approval.

Also for *N. sativa* therapeutic dosages, there is no consensus in literature. We think that maybe used dosages are not sufficient for preffered useful effect.

The other defect of this study that, althought we evaluated the possible protective effect of *N.sativa* on DKD process, we tested only possible inducative markers. Actually if it was possible, we

could have more detailed information with comparison renal tissue light and electron microscopic apparence and immunohistochemical analysis in terms of kidney involvement.

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