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ORIGINAL ARTICLE

Effects of *Nigella sativa* and *Camellia sinensis* Water Extracts on Alloxan Induced Diabetic Nephropathy in Rats

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ABSTRACT

Nigella sativa L (Ranunculacea) (NS) leaves and camellia sinensis (Theaceae) (CS) seeds have been shown to contain distinct bioactive components, this study aimed at the effects of a binary mixture of NS and CS water extracts on some diabetic nephropathy (DN) complications. Alloxan-induced diabetic rats were received NS and CS water extracts as monotherapy or in a 1:1 combination at two doses of 50 and 100 mg/kg day by oral gavage. Serum levels of DN related parameters and the renal superoxide dismutase, catalase and glutathione peroxidase enzymes of treated groups were compared to that of both normal and diabetic control groups. Kidney also, was examined histologically in the studied groups. After six weeks of treatment, all applied treatments led to a recovery in the studied parameters but the mixture of NS and CS extracts dosed at 100 mg/kg was the best treatment on most of the studied parameters as recovered creatinine, albumin and superoxide dismutase levels of the diabetic rats to that of the normal group and 2.69 fold decreased serum level of urea as well. Also, the 100 mg/kg dosage of combined extracts increased the levels of catalase and glutathione peroxidase enzymes above the healthy control level (1.54 and 1.42 fold increase respectively). The mentioned treatment also improved the histopathologiacal state of the kidney. This study revealed that NS and CS extracts' mixture show more potent beneficial effects on biochemical and histological features of DN than their monotherapy.

Keywords: *Diabetic nephropathy, Creatinine, Albumin, Glomerular hypertrophy, interstitial inflammation, Antioxidant enzymes.*

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INTRODUCTION

Diabetic nephropathy (DN) is certainly one of the most severe complications of diabetes which develops in 15-25% of type 1 diabetic patients and in progressed cases can even lead to renal function failure [1-2]. DN in early stages is accompanied by glomerular hypertrophy, interstitial fibrosis, increased protein excretion and increase in serum levels of creatinine and urea [3-4]. Incidence of renal function failure is associated with numerous destructive factors existing in diabetes such as hyperglycemia and oxidative stress as hyperglycemia causes down regulation of proteoglycans in glomeruli and activates some pathways that eventually lead to increase in the levels of reactive oxygen species (ROS) as well as [5-7]. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) also were shown to be decreased in diabetic kidney tissue [8].

Nigella sativa L (Ranunculacea) (NS) or black cumin is an ancient folk medicine that is well-known all around the world especially in eastern countries [9, 11]. NS is reported to contain bioactive components

like thymoquinone, dithymoquinone, thymohydroquinone and thymol [12] which had showed various biological activities such as antidiabetic effects, bronchodilator, antibacterial and hypotensive properties in several previous studies [13-17]. NS also is known to exert some healing effects on DN conditions such as decreased kidney antioxidant enzymes, deregulated glomerular filtration rate (GFR) and disturbed histological architecture of glomeruli [18-20].

Camellia sinensis (Theaceae) (CS) or green tea is a rich source of flavanols, flavadiols, flavonoids and phenolic acids as they consist up to 30% of CS dry weight. Catechins as a group of flavonoids have been shown to exist in CS and are the most bioactive flavonoids among CS ingredients [21]. CS contains other bioactive components such as gallic acid, quercetin, kaempferol, myricetin and caffeic acid as well as [22]. CS components are reported to have several biological activities including antifungal, antitumor, antioxidant, antidiabetic effects and plasma cholesterol lowering action through several studies [23-25]. Some studies had aimed on the impact of CS extract on DN in several models and had showed that CS has beneficial effects on various DN related parameters such as serum creatinine, urea, albumin, renal oxidative stress and the levels of some antioxidant enzymes [25-28].

Regarding the effects of these two herbal medicines on DN complications and as for different bioactive constituents existing in these two plants, the aim of this study was to investigate the effects of a 1:1 (w/w) combination of CS and NS water extracts on some DN related parameters along with their monotherapy.

MATERIALS AND METHODS

Plant material

CS leaves and NS seeds were obtained from the Research Center of Agriculture and Natural Resources, Tabriz, Iran on Nov, 2013 and were verified by the Tabriz Reference Herbarium, University of Tabriz according to the voucher specimens. Extraction procedure was done using the Clevenger method [29]. The dried extracts were suspended in distilled water at a final concentration of 43 % (w/v) and were used at 50 and 100 mg/kg doses.

Experimental animals

Male Wistar rats weighting 180 ± 7 g were obtained from the Animal House of Institute of Pastor, Tehran, Iran. Animals were maintained on a standard laboratory diet *ad libitum* with free access to water and standard condition (23 ± 1 °C and a 12h light/12h dark cycle) in the facilities of Higher Educational Center of Jahad-e-Keshavarzi, Tabriz, Iran. Ethical approves for the animal experiments were taken from the Institutional Ethics Committee, Higher Educational Center of Jahad-e-Keshavarzi, Tabriz, Iran. All protocols for the care and handling of the animals were designed and performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (Sigma-Aldrich) suspended in 0.1 M citrate buffer (pH=4.5) at a dose of 110 mg/kg [30]. After 4 days, animals with a fasting blood glucose of 230 mg/dl or above were considered diabetic and included in the study. Glucose levels were assessed using Accu-chek glucometer (Roche Diagnostics, Mannheim, Germany).

Biochemical study

Serum biochemical parameters including urea, creatinine, total protein and albumin were measured by commercial available kits (Bionik, Tehran, Iran). The levels SOD, CAT and GPx also were assessed in kidney tissues through standard methods [31-33] at the Danesh Pathobiology Laboratory, Tabriz, Iran.

Histopathology of kidney

Paraffinized tissue samples were cut with a thickness of 5 μm and then were fixed on slides overnight. The sections were deparaffinized using xylene and by rehydration in graded ethanol series (99%, 96% and 70%), they were stained with hematoxylin and eosin. Glomeruli space and interstitial inflammation were examined on tissue samples. All histopathological examinations were carried out at the Danesh Pathobiology Laboratory, Tabriz, Iran.

Experimental design

The following eight groups each containing five rats (n = 5) were constituted for a six weeks lasting treatment by gavage: Group 1, normal untreated rats given 0.5 ml of physiologic saline per day; group 2, alloxan-induced diabetic control rats given 0.5 ml of physiologic serum per day; groups 3 and 4, diabetic rats treated with NS extract dosed at 50 and 100 mg/kg per day respectively; groups 5 and 6, diabetic rats treated with CS extract dosed at 50 and 100 mg/kg per day respectively; group 7 and 8, diabetic rats treated with the mixture of NS and CS extracts dosed at 50 and 100 mg/kg per day respectively.

Statistical analysis

Statistically differences were assessed by one-way ANOVA, followed by a least significant difference (LSD) test. Data are presented as means ± SD. All analyses were performed using the SPSS software (SPSS version 16). $P<0.05$ was considered statistically significant.

RESULTS

Serum parameters

As shown in table 1, normal and diabetic groups had significant difference in all measured serum parameters ($p<0.001$). Serum levels of urea and creatinine were shown to be decreased by all treatments ($p<0.01$) and the mixture of NS and CS extracts dosed at 100 mg/kg led to better improvement in the urea level. The creatinine level of serum also was shown to be recovered to the normal level after treatments with both CS extract (100 mg/kg) and the mixture of NS and CS extracts (100 mg/kg) ($p<0.01$). Total proteins and the albumin levels of serum also were decreased by all applied extracts. However the mixture of NS and CS extracts (100 mg/kg) caused to absolute remission of the albumin level ($p<0.01$).

Glomerular hypertrophy and interstitial inflammation

As figure 1b illustrates, diabetic kidney showed severe glomerular hypertrophy and interstitial inflammation. Although all administered treatments caused recovery in the pathological state of the kidney, the mixture of NS and CS extracts dosed at 100 mg/kg showed the highest efficiency as recovered the glomerular hypertrophy from the severe state to the mild and eliminated the observed interstitial inflammation as well as (Figure 1c).

Levels of antioxidant enzymes

Compared to the normal group, diabetic rats had lower levels of antioxidant enzymes ($p<0.001$). As shown in table 2, SOD levels of kidney were increased in NS, CS and the mixture of NS and CS (all 100 mg/kg) treated groups so the latter treat was the most efficient ($p<0.01$). Table 2 also shows that all applied treatments had beneficial effects on both CAT and GPx levels of kidney ($p<0.01$) but treatment with the mixture of NS and CS dosed at 100 mg/kg increased the CAT and GPx levels even more than the normal level.

Table 1. Multiple comparison of serum parameters among studied groups

Groups	Means ± SD				
	mg/dL			g/dL	
	Glucose	Urea	Creatinine	Total protein	Albumin
Normal control	83.32 ± 2.13[b]	9.77 ± 0.29[b]	0.576 ± 0.03[b]	9.86 ± 0.23[b]	5.20 ± 0.17[b]
Diabetic control	252.80 ± 2.94[a]	29.47 ± 0.49[a]	1.546 ± 0.04[a]	6.36 ± 0.31[a]	2.98 ± 0.07[a]
Diabetic + NS (50 mg/kg)	201.58 ± 3.38[a,b]	17.90 ± 0.76[a,b]	1.003 ± 0.02[a,b]	7.33 ± 0.25[a,b]	3.32 ± 0.15[a,b]
Diabetic + NS (100 mg/kg)	139.99 ± 3.49[a,b]	13.46 ± 0.45[a,b]	0.778 ± 0.04[a,b]	7.98 ± 0.89[a,b]	4.58 ± 0.05[a,b]
Diabetic + CS (50 mg/kg)	166.30 ± 2.28[a,b]	21.00 ± 0.80[a,b]	1.182 ± 0.04[a,b]	7.70 ± 0.16[a,b]	3.63 ± 0.60[a,b]
Diabetic + CS (100 mg/kg)	136.98 ± 3.23[a,b]	15.55 ± 0.70[a,b]	0.580 ± 0.02[b]	8.63 ± 0.11[a,b]	4.83 ± 0.11[a,b]
Diabetic + Mix* (50 mg/kg)	151.38 ± 2.03[a,b]	15.32 ± 0.66[a,b]	0.988 ± 0.03[a,b]	7.88 ± 0.12[a,b]	4.40 ± 0.19[a,b]
Diabetic + Mix (100 mg/kg)	122.16 ± 2.23[a,b]	10.93 ± 0.46[a,b]	0.592 ± 0.03[b]	9.01 ± 0.07[a,b]	5.11 ± 0.76[b]

* NS and CS extracts' mixture; [a] $p < 0.01$ compared to the values of normal group; [b] $p < 0.01$ compared to the values of diabetic group.

Table 2. Levels of antioxidant enzymes of kidney in studied groups

Groups	Means ± SD		
	U/mg protein		
	SOD	CAT	GPx
Normal control	12.19 ± 0.22[d]	30.08 ± 0.20[d]	40.19 ± 0.23[d]
Diabetic control	8.88 ± 0.25[b]	19.80 ± 0.44[b]	30.35 ± 0.22[b]
Diabetic + NS (50 mg/kg)	9.67 ± 0.11[a]	24.85 ± 0.24[b,d]	33.77 ± 0.31[b,d]
Diabetic + NS (100 mg/kg)	10.87 ± 0.14[c]	27.94 ± 0.17[b,d]	37.11 ± 0.07[b,d]
Diabetic + CS (50 mg/kg)	8.34 ± 4.07[b]	24.38 ± 0.34[b,d]	35.14 ± 0.16[b,d]
Diabetic + CS (100 mg/kg)	11.25 ± 0.17[c]	28.67 ± 0.11[b,d]	36.76 ± 0.14[b,d]
Diabetic + Mix* (50 mg/kg)	10.52 ± 0.07	25.24 ± 0.23[b,d]	34.57 ± 0.54[b,d]
Diabetic + Mix (100 mg/kg)	12.38 ± 0.31[d]	30.67 ± 0.10[b,d]	43.26 ± 0.21[b,d]

* NS and CS extracts' mixture; [a] $p < 0.05$ and [b] $p < 0.01$ compared to the values of normal group; [c] $p < 0.05$ and [d] $p < 0.01$ compared to the values of diabetic group.

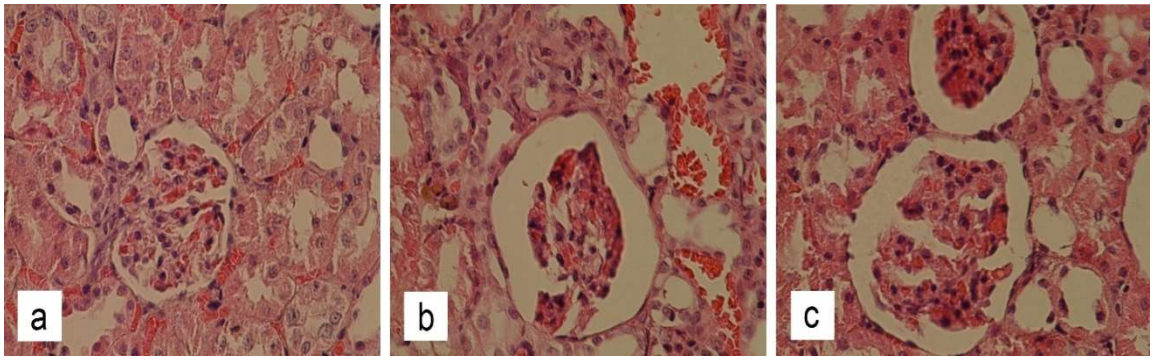

Figure 1. Microscopic structure of kidney of a (a) normal rat, (b) diabetic rat, (c) diabetic rat treated with 100 mg/kg of NS and CS extracts' mixture. All slides stained with hematoxylin and eosin (×400).

DISCUSSION

The water extracts of NS and CS especially their combination showed curative effects on some nephropathy related parameters of serum and tissue so the mixture of NS and CS recovered the creatinine, albumin and SOD levels of the diabetic rats exactly to their normal states.

Our observation of decreasing creatinine level in NS treated diabetic group is along with a previously reported study but in contrast, urea was not seen to be decreased in the same group of the mentioned study [34]. Also, the present observation of the recovery in the glomeruli space and antioxidant enzymes of the NS treated group are along with the earlier reports in these matters [18, 20].

Consistent with the current study, the glucose, urea, creatinine and the total protein of serum were seen to be decreased under the influence of CS administration [25, 35]. The observed refinement in the histological state of CS treated diabetic group is along with a previous study as well as [36].

To our knowledge, this is the first report indicating the effects of a combination of NS and CS extracts on some DN related parameters and complications. Along with the research hypothesis, we found the binary mixture more efficient in several cases. However, magnifying on the specific active components and incorporating urine indexes in similar studies are need to be performed in the future.

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