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Protective Effect of *Nigella sativa* Seed Extract on Alloxan-Induced Mice Kidney Histology

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Abstract

Nigella sativa (*N. sativa*) has been used in traditional medicine, and several studies have been performed in the last decades to reveal its effects of it on different medical disorders such as diabetes, dyslipidemia, hypertension, and obesity. This study aimed to evaluate the effects of *Nigella sativa* extract in an alloxan-induced mice model. This research was a true experimental post-test-only control group design. Eight-weeks old male Mus musculus were treated with alloxan (150 mg/ kg) by a single intraperitoneal injection to induce diabetes mellitus. At three days of injection, *N. sativa* extract (150 and 300 mg/kg) was administered via gavage for two weeks. The result of this study showed that *N. sativa* seed extract had a significant ($P<0.05$) nephroprotective effect on the kidneys based on the degree of kidney damage in alloxan-induced mice. This effect may be due to *Nigella sativa'*s antioxidant properties.

Keywords: *Nigella sativa*, histology kidney, oxidative stress

INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycaemia due to impaired insulin secretion, impaired insulin action, or both [(1)]. Chronic hyperglycaemia can cause complications and multi-organ disorders such as the eyes, nerves, heart, blood vessels and kidneys [(2)]. As an effort in the treatment of diabetes mellitus, medicinal plants have started to be widely used. One example is the *Nigella sativa* plant. Nigella sativa is a flowering plant that belongs to the Ranunculaceae family [(3)].

(*Nigella sativa*) [(4)]

This plant grows to a height of 20-90 cm, with finely divided leaves, and segments linear to a threadlike line. The flowers are very delicate and usually white, yellow, pink, pale blue or pale purple, with 5-10 petals [(5)].

METHOD

Type and Design of Research

This research was true experimental, with the research design used was Post Test Only Control Group Design, which taking measurements after the treatment is given.

Extraction and Fraction

One thousand grams of N.sativa seeds in a dry state, ground using a grinder. Then, the refined sample seeds were soaked in 5000mL of 96% ethanol solvent with a ratio of 1000gr: 5000mL of 96% ethanol solvent.

Samples soaked in 96% ethanol were shaken using a mixer for 2-3 hours, and then allowed to stand for 24 hours. After that, the sample was filtered using a fil-

ter. The results of the filtering filtrate were then put in the rotary evaporator. While in the evaporator rotator, 96% ethanol solvent was vacuumed and later distilled to become liquid. The 96% ethanol solvent from the distillation was collected. If the 96% ethanol solvent has all evaporated, a thick extract of N. sativa seeds will be obtained.

Experimental Animals

The experimental animals used were Mus musculus mice purchased from PUSVEPMA. Twenty male mice were separated according to the study groups, namely the control group (K), the DM group, the DM group and N.sativa extract dose of 150 mg (DM+ENS1), and the DM group and N.sativa extract 300 mg (DM+ENS2). Before testing, the mice were adapted (acclimatized) for one week in the cage at UNUSA Faculty of Medicine Research Laboratory. Prior to treatment, the test animals were weighed and tagged. This test animals followed the criteria for the use of test animals and have passed ethical clearance and then grouped according to treatment.

Alloxan Induction

Alloxan induction used a dose of 120 mg/kg BW by injection via the intraperitoneal route. The induction was carried out with the help of a syringe. The aim of alloxan induction was to make the mice in hyperglycemic state.

Histopathological Examination

After 14 days of treatment, samples of kidney organ were taken. The samples then embedded in paraffin wax, sectioned at five µm and stained with haematoxylin stain. Representative areas were selected for qualitative light microscopic analysis of the inflammatory cellular response at 10x, 40x and 100x magnifications.

Data Analysis

Research data are presented as mean ± standard error by analyzing with ANOVA test. If it did not meet the ANOVA test, then a non-parametric test would be used, namely the Kruskal Wallis Test and proceed with the Mann-Whitney test.

RESULT

Based on the results of observations from the kidney H&E staining, scoring was carried out by looking at kidney damage characterized by reduced normal tubules on histopathological observations. The normal proximal tubules were summed, and the average value was calculated for each mouse. Then the average value for each mouse was summed, and the average group value was made. It was observed that N.sativa seed extract at doses of 150 mg/kg ($P<0.05$) and 300 mg/kg ($P<0.01$) significantly reduced renal tubular damage compared to the DM group with n=10/ group and repeated twice.

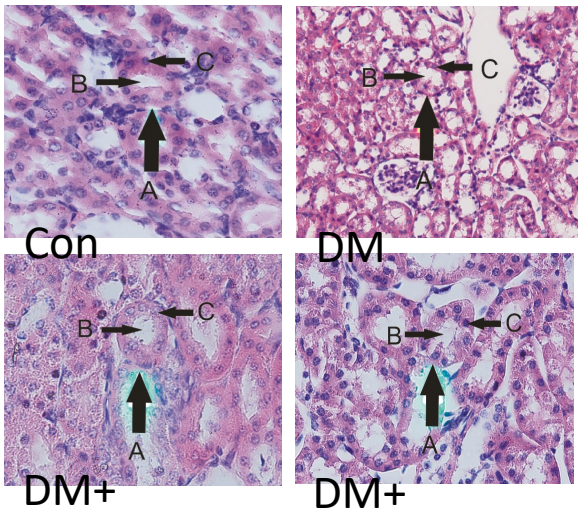

Figure. 1 Histopathological picture of the kidney after administration of Nigella Sativa. HE Extracts, objective 40X. (A) is tubule, (B) tubule lumen, and (C) hydropic degeneration.

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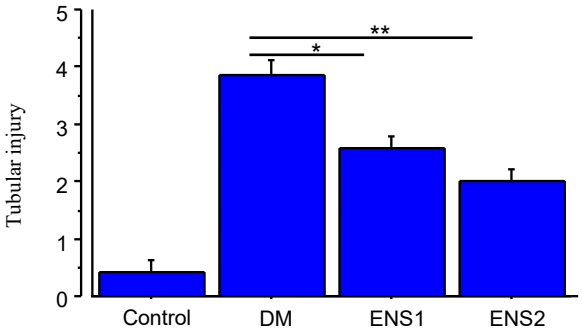

Figure 2 Calculation results for the number of normal proximal tubules (±SD). N=10, per group. DMs; diabetes mellitus, ENS1; N. Sativa Extract 150mg/kgBB, ENS2; N.Sativa Extract 300mg/kgBB.*;P<0.05, **;P<0.01

DISCUSSION

This study showed renal histological changes in the alloxan-induced diabetes mellitus model. However, alloxan-induced kidney damage underwent improvement in renal histology after the administration of N. sativa seed extract. These results indicate that the ethanol extract of Nigella sativa seeds has a protective effect against kidney damage in alloxan-induced diabetes mellitus.

Alloxan has been widely used to induce diabetes mellitus in experimental animals through hyperfiltration in the glomerulus via the Glut-2 receptor and oxidative stress processes [(12).]. Oxidative stress activity will trigger kidney nephron damage and eventually cause kidney inflammation [(13).]

In this study, it has been found that alloxan induction on the histological appearance of the kidney indicates damage to the kidney due to inflammation from the process of free radical oxidative stress. This is in line with previous research by Lan Yao[(14)], which found that alloxan injection could damage kidney function; however, the administration of N. sativa extract in this study showed that kidney histological changes improved.

Administration of Nigella sativa seed extract for two weeks showed a significantly better histological appearance of the renal tubules at doses of 150 mg/kg and 300 mg/kg compared to the alloxan-induced diabetes group, although not the same as the control group. The results of this study indicate that the administration of Nigella sativa seed extract to mice with a diabetic model can prevent kidney damage due to complications of diabetes mellitus. This can be caused by the protective effect of Nigella sativa, which has a strong antioxidant and can suppress oxidative stress due to hyperglycemia [(15)]. These results also support previous studies' findings that Nigella sativa extract has a hypoglycemic effect in vivo studies using Mus musculus mice. [(16)]

CONCLUSION

Administration of Nigela sativa seed extract provides a synergistic protective effect against nephrotoxicity in alloxan-induced mice. To find out the exact mechanism, it is necessary to carry out an IHC examination to see the specifications of the damage.

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