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Original Article

Therapeutic effect of *Nigella sativa* extract on folic acid-induced acute hepatorenal injury: influences and underlying mechanisms

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ABSTRACT

Nigella sativa is utilized to treat many ailments and to maintain and improve global health as it has numerous pharmacological activities such as anti-inflammatory, antidiabetic, antimetastatic, anxiolytic, hepatoprotective and immunomodulatory. We tried to assess the disorders in the liver, the main organ for folate metabolism, and the kidney in response to high-dose folic acid (HDFA) supplementation, and we tested if the herbal *Nigella sativa* extract (NSE) could alter these disturbances and the likely underlying mechanisms. Eighteen adult male rats were separated into three groups: Control, HDFA and HDFA+NSE. Both kidney and liver dysfunctions induced by HDFA administration significantly improved by NSE which was supported by histological examination. Thus, NSE being an ample source of antioxidants has a hepatorenal protecting effect.

KEYWORDS: *Nigella sativa*, folic acid, kidney, liver, antioxidants.

1. INTRODUCTION

Folic acid (FA) is a vital element of water-soluble vitamin B9. It is well-thought-out to be a non-toxic vitamin and it has an indispensable role in nucleic acid synthesis [1]. Fortification of food with FA commenced in the United States because the folate has an essential role in preventing neural tube defects, and reducing the risk of vascular diseases, and cancers [2-4] but recent data showed a negative effect of high-dose folic acid (HDFA) supplementation on several organs of the body especially the kidney [5], and the liver [6] particularly for those not initially targeted for fortification.

Ezzat *et al*. reported that HDFA induced acute kidney injury (FA-AKI), by prompting inflammation, oxidative stress, and apoptosis [7]. Currently, there are no satisfactory therapies for AKI or acute liver injury and all the available medications are chemicals with multiple adverse effects [8], which heightened the need for substitute treatments and the therapeutic usage of natural products, especially those of plant origin with scarcer side effects related to chemical medications [9-11].

Nigella sativa (NS), also known as black seed, is a flavoring herb usually cultivated in the Middle East, Eastern Europe, Mediterranean regions and Western Asia [12]. It has been utilized to treat many ailments and to maintain and improve global health as it has numerous pharmacological effects such as anti-inflammatory, antidiabetic, antimetastatic, anxiolytic, hepatoprotective, immunomodulatory,

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and muscle relaxing, besides having a reno-protective effect in diabetics [13, 14]. Also, it can decline the nephrotoxic complications of some drugs such as cisplatin [15]. So, we assumed that NS could have a defensive impact on acute renal and hepatic impairment induced by HDFA. Therefore, the major objective of this research was to inspect the efficacy of *Nigella sativa* extract (NSE) against renal and hepatic injury in adult male rats induced by HDFA and the probable mechanisms.

2. MATERIALS AND METHODS

2.1. Chemicals

Folic acid, methanol, and pentobarbital were purchased from Sigma-Aldrich, USA. The chemical kits were purchased from Biodiagnostic Company (El Moror St, Dokki, EGY).

2.2. Preparation of NSE

250 g of NS seeds was bought from a herbal shop in Giza, Egypt, then cleaned, dried and crushed to obtain Nigella sativa powder (NSP). 100 g of this powder was extricated thrice with 300 mL of 96% ethanol and filtered. After evaporation of the solvent *via* a rotary, the blackish-brown liquid concentrate of NSE was obtained. NSE was mixed with water and given to rats using a gastric tube [16].

2.3. Phytochemical qualitative analysis

The presence of phytochemical activity in plant extracts was determined according to the procedures of Lotfy *et al*. [17].

2.4. Ethical consideration

All experiments were carried out according to the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the Local Ethics Committee.

2.5. Animals

Eighteen healthy adult male Wistar rats weighing 160 ± 20 g from the National Research Center, Egypt were enrolled in this experiment. They were housed in wood shaving-bedded polyacrylic cages, in the well-ventilated animal house of the Department of Zoology, Faculty of Science, Cairo University, Giza, Egypt. The animals were kept for 7 days (12 h light/12 h dark, temperature: 25 ± 1 ^{o}C, and relative humidity: 50 + 15%) before the commencement of the research. Tap water and ordinary diet were supplied *ad libitum*.

2.6. Experimental design

Rats were haphazardly distributed into three groups (6/group): Control group: rats received 300 mM NaHCO3 (0.2 mL) single intraperitoneal injection. HDFA group: rats were injected with FA (250 mg/kg, single dose, i.p) dissolved in a vehicle (0.2 mL of 300 mM $NaHCO_3$) [18]. HDFA+NSE: rats were injected with FA (250 mg/kg, single dose, i.p) dissolved in 0.2 mL of 300 mM $NaHCO_3$, followed by administration of NSE (250 mg/kg/day) for 7 consecutive days [19].

2.7. Animal handling

Animals were euthanized with sodium pentobarbital (150 mg/kg, i.p) [20]. Blood was collected and serum was separated and centrifuged at 3000 rpm for 20 minutes, then kept at -80 °C till further analysis [21]. The liver and kidneys were excised, washed and blotted using filter paper to remove traces of blood, then frozen at -80 °C till analysis.

2.8. Liver and kidney tissue homogenate preparation

Liver and kidney tissues were weighed and homogenized (10% w/v) in ice-cold 0.1 M Tris-HCl buffers (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 min. and the resultant supernatant was kept in aliquots and stored at -20 °C for the biochemical analysis [22].

2.9. Serum biochemical parameters

The appropriate kits were bought from Biodiagnostic (Dokki, Giza, Egypt) for quantifying the following biochemical parameters in serum: aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cr), urea, uric acid, and albumin.

2.10. Oxidative stress parameters

The supernatant of the hepatic and renal tissue homogenates was employed for biochemical analysis according to the manufacturer's instructions using Biodiagnostic kits (Giza, Egypt). Malondialdehyde (MDA), reduced glutathione (GSH) and catalase (CAT) levels were measured in the liver and kidney tissues.

2.11. Histopathological analysis

Kidney and liver tissues were fixed in 10% formal saline, embedded in paraffin and sectioned. Then, the sections were stained with hematoxylin and eosin for histological examination using a light microscope. The qualitative score was applied to the detected histopathological alterations in the kidney as follows: (0) no lesion, (1) mild, (2) moderate and (3) severe lesion [21].

2.12. Statistics

Using SPSS (version 15.0), all values were expressed as means ± standard error. The comparisons were done by one-way analysis of variance (ANOVA) with the Duncan post hoc test, and $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Phytochemical qualitative analysis

Table 1 data indicates the components of NSE. Flavonoids, tannins, steroids, phenols, and alkaloid are all present in the extract, while it is devoid of anthraquinones, triterpenes, saponins, and cardiac glycosides.

Table 1. Quantitative determination of compounds in *Nigella sativa* extract.

Test	*Nigella sativa* extract
Flavonoids	++
Anthraquinones	-
Tannins	++
Phenols	+
Triterpenes	-
Steroids	+
Alkaloid	+
Saponins	-
Cardiac Glycosides	-

(-) negative reaction, (+) positive reaction, and (++) strong positive reaction.

3.2. Effect of NSE on the serum creatinine, urea and uric acid

There was a significant increase in serum Cr, urea, and uric acid concentrations in the HDFA group compared to the control group ($P<0.05$). Treatment with NSE significantly reduced these raised parameters as noted in Table 2.

3.3. Effect of NSE on the liver enzymes and serum albumin concentration

There was a significant rise in both AST and ALT levels but a significant reduction in albumin concentration in the HDFA group indicating acute liver injury compared to the control group ($P<0.05$). However, NSE administration standardized these values as revealed in Table 3.

3.4. Effect of NSE on oxidative stress markers

A significant ($p < 0.05$) elevation in MDA concentration and a significant reduction in both GSH and CAT concentrations was observed in HDFA group compared to control group but upon treatment with NSE, MDA declined significantly ($P < 0.05$) while GSH and CAT activities improved significantly ($P < 0.05$) as shown in Table 4.

3.5. Histopathological investigation

As demonstrated in Figure 1 and Table 5 there was severe congestion of the cortical blood vessels with perivascular edema, mononuclear inflammatory cells infiltration, severe interstitial nephritis with moderate nephrosis, marked proliferation of mesangial cells with an increase in the glomerular size, and glomerular Bowman's space area in HDFA group relative to control group but these

Table 2. Comparison between kidney function parameters among all studied groups.

Parameters	Control	HDFA	HDFA+NSE
Urea (mg/dl)	38.75 ± 0.66^{a}	60.8 ± 1.21^{b}	54.17 ± 0.83^{c}
Creatinine (mg/dl)	0.31 ± 0.03^{a}	1.27 ± 0.03^{b}	0.573 ± 0.01^{c}
Uric acid (mg/dl)	3.55 ± 0.05^{a}	4.92 ± 0.12^{b}	4.05 ± 0.16^{c}

HDFA: High-Dose Folic Acid, NSE: *Nigella Sativa* Extract. All values are represented as mean ± SE (n = 6). Values with different superscript letters are significantly different ($P < 0.05$).

Table 3. Comparison between liver function parameters among all studied groups.

Parameters	Control	HDFA	HDFA+NSE
ALT(U/ml)	138.1±2.01[a]	153.01±1.51[b]	142.82±2.51[c]
AST (U/ml)	6.80±1.07[a]	13.23±0.43[b]	10.1±0.50[c]
Albumin (g/dl)	7.82±0.13[a]	3.97±0.24[b]	5.99±0.19[c]

HDFA: High-Dose Folic Acid, NSE: *Nigella Sativa* Extract. All values are represented as mean ± SE (n= 6). Values with different superscript letters are significantly different ($P < 0.05$).

Table 4. Comparison between oxidant-antioxidant parameters among all studied groups.

Parameters	Organ	Control	HDFA	HDFA+NSE
MDA (nmol/g.tissue)	Kidney	4.55±0.81[a]	9.56±1.12[c]	6.12±0.40[b]
	Liver	3.15±0.32[a]	5.95±0.83[c]	4.01±0.37[b]
GSH (mg/g.tissue)	Kidney	15.7±0.44[c]	9.6±0.37[a]	14.23±0.52[b]
	Liver	9.11±0.52[c]	4.99±0.27[a]	7.00±0.11[b]
CAT (U/g.tissue)	Kidney	0.14±0.01[c]	0.04 ±0.01[a]	0.09±0.01[b]
	Liver	0.98±0.03[c]	0.25 ±0.02[a]	0.59±0.02[b]

HDFA: High-Dose Folic Acid, NSE: *Nigella Sativa* Extract. All values are represented as mean ± SE (n= 6). Values with different superscript letters are significantly different ($P < 0.05$).

Table 5. Comparison between qualitative scores of histopathological lesions in kidneys among all studied groups.

Lesions	Control	HDFA	HDFA+NSE
Congestion	0	3	2
Perivascular edema and inflammation	0	3	1
Nephrosis	0	2	1
Interstitial nephritis	0	3	1

HDFA: High-Dose Folic Acid, NSE: *Nigella Sativa* Extract.
(0) absent, (1) mild, (2) moderate and (3) severe.

pathological changes significantly improved by treatment with NSE.

Moreover, histological analysis of the liver tissues of rats in the HDFA group showed sporadic vacuolar degeneration of the hepatocytes with a focal aggregation of mononuclear cells; however there was a complete absence of these changes upon treatment with NSE except for mild congestion in portal blood vessels as demonstrated in Figure 2.

4. DISCUSSION

In the current study, HDFA induced nephrotoxicity in rats, which was confirmed by the significant increase in creatinine, urea, and uric acid. Similarly, Li *et al*. [23] reported that HDFA caused toxicity in the kidney, resulting in a substantial increase in serum Cr. and blood urea nitrogen (BUN), proteinuria, tubular epithelial cell loss and morphologic abnormalities. Poor solubility of FA at acidic pH and its precipitation intra-tubularly forming crystals cause local obstruction, hypoxia, tubular necrosis and even renal failure [24]. Besides, Malinow *et al.* recorded altered methionine trans-sulfuration cycle in response to HDFA supplementation secondary to impaired synthesis of several enzymes leading to accumulation of homocysteine that increases Col1a2 expression promoting fibrogenesis [25].

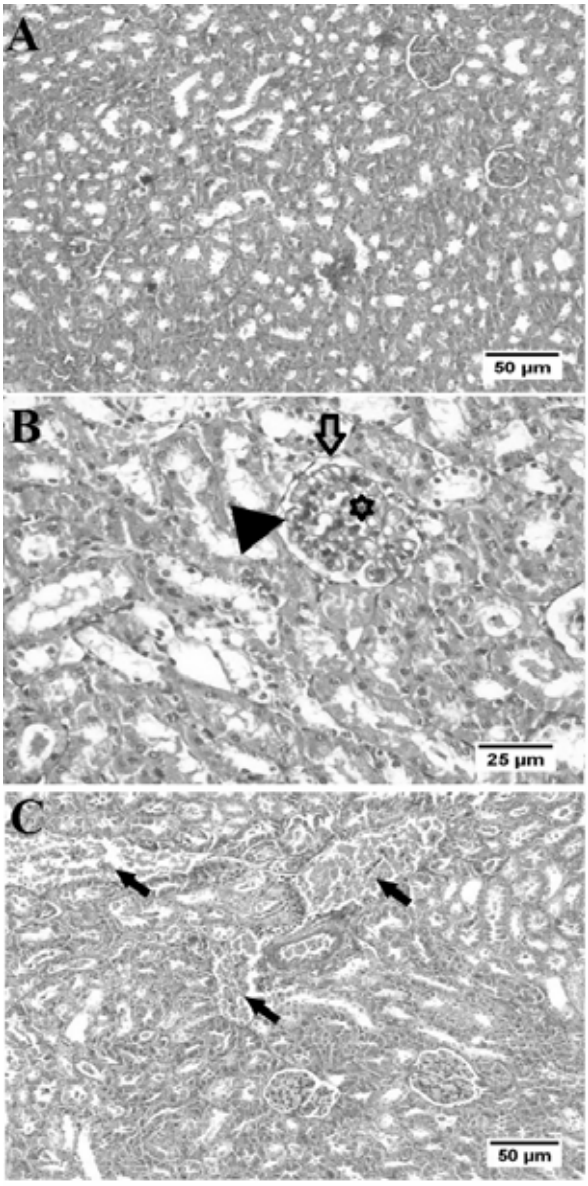

Figure 1. Light micrographs of the kidney**. A)** Control group: showing a normal renal histology, normal glomerular and tubular architectures (H&E). **B)** HDFA group: showing marked increase of the glomerular size (black arrow), mesangial cell proliferation (star) and increased glomerular Bowman's space area (arrowhead). **C**) HDFA+NSE: showing severely congested blood vessels (arrows) with apparently normal renal tubules (H&E).

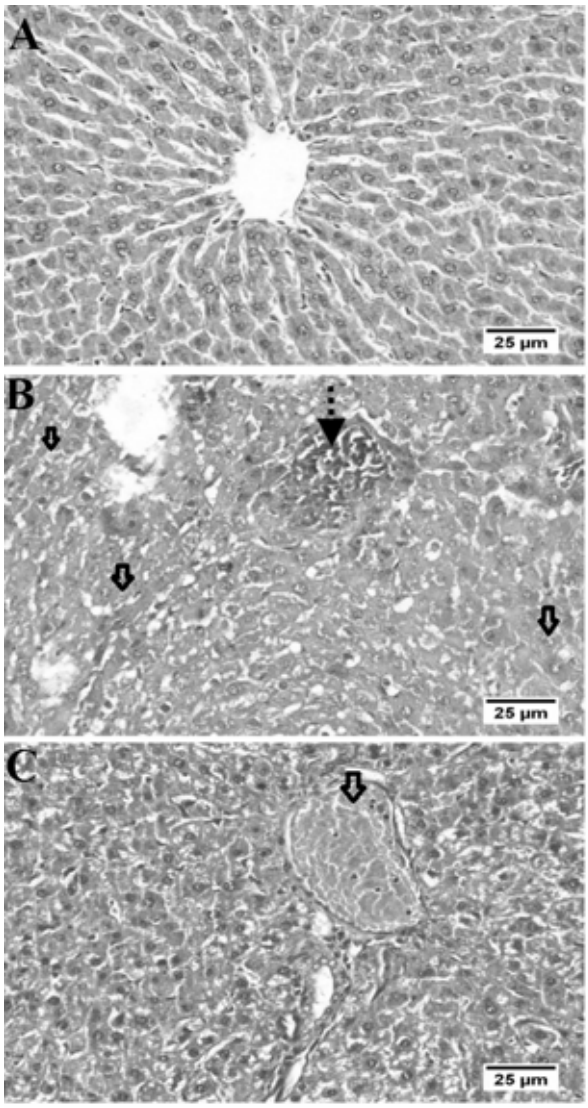

Figure 2. Light micrographs of the liver**. A)** Control group: showing normal hepatocytes surrounding the central vein (H&E). **B)** HDFA group: showing sporadic vacuolar degeneration of the hepatocytes (black arrow) with focal aggregation of mononuclear cells (dotted arrow) (H&E). **C**) HDFA+NSE: showing mild congestion in the portal blood vessel (arrow) (H&E).

The significant increase in ALT, AST and reduction in albumin content refer to liver injury following HDFA injection. The increase in hepatocyte membrane fluidity, which led to enzyme release into the circulation, may be linked to the rise of liver enzymes. Following HDFA administration, a high amount of unmetabolized folic acid resulted in the formation of unmetabolized homocysteine, which altered intracellular lipid metabolism and promoted hepatic fat storage [26].

HDFA induced oxidative stress in the present study, and caused autooxidation of GSH which resulted in increased lipid peroxidation and

production of MDA indicating apoptosis, altered membrane architecture and severe nephrotoxicity [27]. HDFA-induced mitochondrial dysfunction causes a reduction in ATP level and excessive production of ROS, which directly triggers cellular damage [28].

Our study proved that NSE had a potential effect on kidney and liver functions in addition to eliminating lipid peroxidation, thereby verifying its antioxidant role, which decreased the pathological changes in kidney and liver tissues. It was in line with Mousavi G, who stated that pre-treatment with NS in the hepatic ischemia and reperfusion injury rat model reversed all the functional and histopathological changes [29]. These beneficial effects may be owed to thymoquinone (TQ), which is the main component of NS seeds as it possesses the following properties: 1) Antioxidant [30] - decreases nuclear factor-kappa B (NF-κB) in the human proximal tubular epithelial cell [31]. 2) Anti-nitrosative - decreases the free radical Nitric oxide by downregulation of inducible nitric oxide synthase (i-NOS) expression in kidneys and liver [32]. 3) Anti-inflammatory [33] - it hinders cyclooxygenase and 5-lipo-oxygenase pathways thus decreasing interleukin-1β (1L-1β), interleukin-6 (1L-6), tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), NADPH oxidase 4, interferon-gamma (IFN-γ) and Prostaglandin E2 (PGE-2) [34] and 4) Anti-apoptotic - *via* decreasing caspase in the liver [35].

5. CONCLUSIONS

We concluded that the induction of a pro-oxidant state could be the key mechanism for the initiation of acute renal and hepatic damage following HDFA administration. Bearing in mind the plentiful positive effects, chiefly antioxidant activity, of NS on kidney and liver histopathology and function markers, it was believed that NS is highly protective, but further research is required to identify the different cellular and molecular mechanisms of its therapeutic effect, conceivable toxicity, optimum dosage and interaction with other drugs in human bodies.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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