PROTECTIVE EFFECTS OF NIGELLA SATIVA OIL AGAINST METHOTREXATE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Background

Use of Methotrexate has been related with toxic effects on a variety of systems and organs such as the gastrointestinal tract, liver, kidneys, lung, and bone marrow. *Nigella sativa* extracts have shown many beneficial effects in recently conducted clinical and experimental trials where it found to act as the immunomodulator, anti-inflammatory, anti-tumor, and antibacterial agents.

Objectives

The aim of the research is to assess the effect of *Nigella sativa* oil (NSO) in the protection of Methotrexate (MTX)-induced liver toxicity in rats.

Materials and Methods

Twenty four Sprague-Dawley rats were assigned into 4 groups of 6 animals each as follow: GroupI presented as control negative; Group II presented as liver toxicity without treatment, Group III presented as NSO treated group, and Group IV presented as control positive group that received N-acetyl cysteine (NAC). The state of serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST),Alkaline Phosphatase (ALP), and Total Antioxidant Capacity (T-AOC) were determined. The homogenates from liver tissue was used for figuring of malondialdehyde (MDA) and glutathione (GSH), and for histopathological examinations.

Results

The results distinctly showed that NSO provides significant protection against MTX-induced toxicity in the liver of rats through reduction in ALT, AST, and ALT activities, increase in T-AOC, improvement in the state of oxidative stress induced by MTX, and improvements in the histopathological picture of the liver.

Conclusion

Orally administered NSO protects the liver against MTX-induced hepatotoxicity in rats.

Keywords: *Nigella sativa* oil, Methotrexate toxicant, Hepatotoxicity, Animal study.

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INTRODUCTION

Methotrexate (MTX) is an immuno-suppressant agent used as prophylaxis and in the management of many diseases, including psoriasis, rheumatoid arthritis, systemic lupus erythematosus, graft-versus-host disease, or together with other drugs, for the treatment of neoplastic diseases [1]. Although, MTX is used in low doses in these diseases and shows low levels of toxicity [2, 3], but hepatotoxicity is still considered as a major problem even at these minor doses [4]. On the other hand, long-term use of MTX has been related with sincere toxic effects on a variety of systems and organs such as the gastrointestinal tract (GIT), liver, kidneys, lungs, and bone marrow [5]. Other researchers found the pivotal role of oxidative stress in MTX hepatotoxicity [6-7]. Hence, high production of reactive oxygen and nitrogen species (ROS/RNS) along with ablated antioxidant defense mechanism encourage the development and progression of hepatotoxicity [8].

The seeds of *Nigella sativa* known as the black seed or black cumin in English,Habatul Barakah or Habatul Sawda in Arabic, and Rashka in Kurdish. It has been used for centuries in a traditional medicine for the treatment of a variety of ailments worldwide such as headache, cough, prolixity, choleretic, antispasmodic, and uricosuric [9-11]. *Nigella Sativa* seeds contain 36 - 38% fixed oils, proteins, alkaloids, and saponin and 0.4 - 2.5% essential oil [10].The main component of fixed oil is unsaturated fatty acids (UFA) such asarachidic and eicosadienoic acids [12]. Other elements that also were identified are thymoquinone (27.8-57.0%), ρ-cymene (7.1-15.5%), carvacrol (5.8-11.6%), t-anethole (0.25-2.3%), 4-terpineol (2.0-6.6%), and longline (1.0-8.0%) [13] .

Nigella sativa (NS) extracts have shown many beneficial effects in recently conducted clinical and experimental trials where it found to act as the immunomodulator, anti-inflammatory, anti-tumor, and antibacterial agents [14-15]. Additionally, oral and topical use of *Nigella sativa* oil (NSO) was effective in ameliorating psoriatic lesions [16-17]. Moreover, NS was found in many studies to exert hepatoprotective effects against many drugs such as isoniazid (INH) [18], or chemicals such as CCL_4 [19]. Accordingly, our research work is planned to evaluate the hepatoprotective activity of *Nigella sativa* oil against MTX-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals

Twenty four male Sprague-Dawley rats weighing 180-220g and aging 7-9 weeks were kept for 1 month during the period of the study in the animal house of the College of Pharmacy, University of Sulaimani. Animals were housed over sawdust beds and retained on a 12-hr light/dark cycle in a humidity- and temperature-controlled facility; and allowed to feed on commercial rat pellets and tap water ad libitum.

Animal grouping and experimental design

The 24 rats were randomly assigned into 4 groups of 6 animals each. All animal groups except group I injected intraperitonealy with a single dose of MTX (20 mg/Kg). Next day, all animal groups received the oral treatment using gavages' tube once a day for 7 days as follow: group I: 1.0 mL normal saline (control negative); group II: 1.0 mL normal saline (Vehicle); group III: 10 mL/kg NSO; group IV: 50 mg/Kg/day N-acetyl cysteine (NAC). The experimental protocol was approved by the Ethical Committee– College of Medicine/ University of Sulaimani, Republic of Iraq.

Animals were sacrificed at day 8 with diethyl ether after overnight fasting. Blood samples were obtained from the heart by cardiac puncture to set up serum for calculation the activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), and Total Antioxidant Capacity (T-AOC) using specific assay kit for each test according to the instructions provided by Manufacturer Company and the results were read by a colorimetric method using Visible Spectrophotometer at 520 nm. Liver tissues were excised immediately after opening of the carcass and liver homogenate was prepared directly for estimation of malondialdehyde (MDA) [20], glutathione (GSH) [21], and for histopathological examinations using hematoxylin and eosin (H & E) staining protocol [22]. All the aforementioned tests have been performed at College of Pharmacy, University of Sulaimani.

Statistical analysis was performed using analysis of variance (ANOVA) followed by Student's t-test. The data were expressed as mean ± SEM and P value ≤ 0.05 considered as significant.

RESULTS

Free radical and lipid peroxidation assessment

As shown in Table 1, administration of MTX significantly (P<0.05) elevates liver tissue homogenate MDA levels (372.3±19.2) compared to control group (126.9± 15.4), while GSH level in the liver tissue was significantly decreased (P<0.05) (10.2 ± 2.2) compared to control group (28.2 ± 1.3). Meanwhile, administration of NSO after inducing liver damage by MTX results in significant depletion (P<0.05) of MDA level (133.8 ± 18.7) and significant (P<0.05) elevation of GSH level (23.5 ±1.7) compared to the group treated with MTX. The effect of NSO was better than that produced by NAC treated group, yet non significantly (P>0.05) different.

Serum biochemistry analysis

Table 2 showes that administration of MTX significantly (P<0.05) elevates ALT, AST and ALP activities (78.2 ± 7.8, 17.3±0.71, and 129.6±12.3 respectively) compared to control group (18.2 ± 0.46, 5.4±0.44, and 44.4 ±2.6 respectively). Treatment with MTX significantly decreased (P<0.05) serum level of Total Antioxidant Capacity (T-AOC) (0.31±0.06) compared to control group (0.98±0.09). Treatment with NSO resulted in significant (P<0.05) decrease in serum liver enzymes (23.5 ±1.7, 5.9±0.56, and 36.4 ±1.9 respectively), and significant elevation (P<0.05) in serum T-AOC (1.12± 0.04) compared to treated MTX group.

Histopathological assessment

Regarding histopathological results, administration of MTX resulted in hydropic degeneration and coagulation necrosis in the hepatocytes and the centrilobular zone, while administration of NSO with MTX results in the decrease in the histopathological changes induced by MTX. The effect of NSO was comparable to that produced by NAC (Figure1).

Table1. Effects of treatment with *Nigella sativa* oil (NSO) on the levels of liver glutathione (GSH) and malondialdehyde (MDA) in rats intoxicated with methotrexate (MTX).

Group (n=6)	GSH (µg/L)	MDA (µg/L)
Normal saline	28.2 ± 1.3 [a]	126.9± 15.4 [a]
MTX + Normal saline	10.2 ± 2.2 [b]	372.3±19.2 [b]
MTX + NSO	23.5 ±1.7 [a]	133.8 ± 18.7 [a]
MTX + NAC	25.1± 1.9 [a]	149.3 ± 14.3 [a]

Values presented as Mean ± SE; n= number of animals; values with non-identical superscripts (a, b) are considered significantly different (P<0.05).NAC:N-acetyl cysteine.

Table 2. Effects of treatment with Nigella sativaoil (NSO) on the activities of liver enzymes in rats intoxicated with methotrexate (MTX).

Group (n=6)	ALT (Unit/L)	AST (Unit/L)	ALP (Unit/L)	T-AOC
Normal saline	18.2 ± 0.46 [a]	5.4±0.44 [a]	44.4 ±2.6 [a]	0.98±0.09 [a]
MTX + Normal saline	78.2 ± 7.8 [b]	17.3±0.71 [b]	129.6±12.3 [b]	0.31±0.06 [b]
MTX + NSO	23.5 ±1.7 [a]	5.9±0.56 [a]	36.4 ±1.9 [a]	1.12± 0.04 [a]
MTX + NAC	26.1 ± 1.9 [a]	6.02 ± 0.3 [a]	39.8 ± 2.3 [a]	1.01± 0.04 [a]

Values presented as Mean ± SE; n= number of animals; values with non-identical superscripts (a, b) are considered significantly different (P<0.05). ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, and T-AOC: Total Antioxidant Capacity, NAC: N-acetyl cysteine.

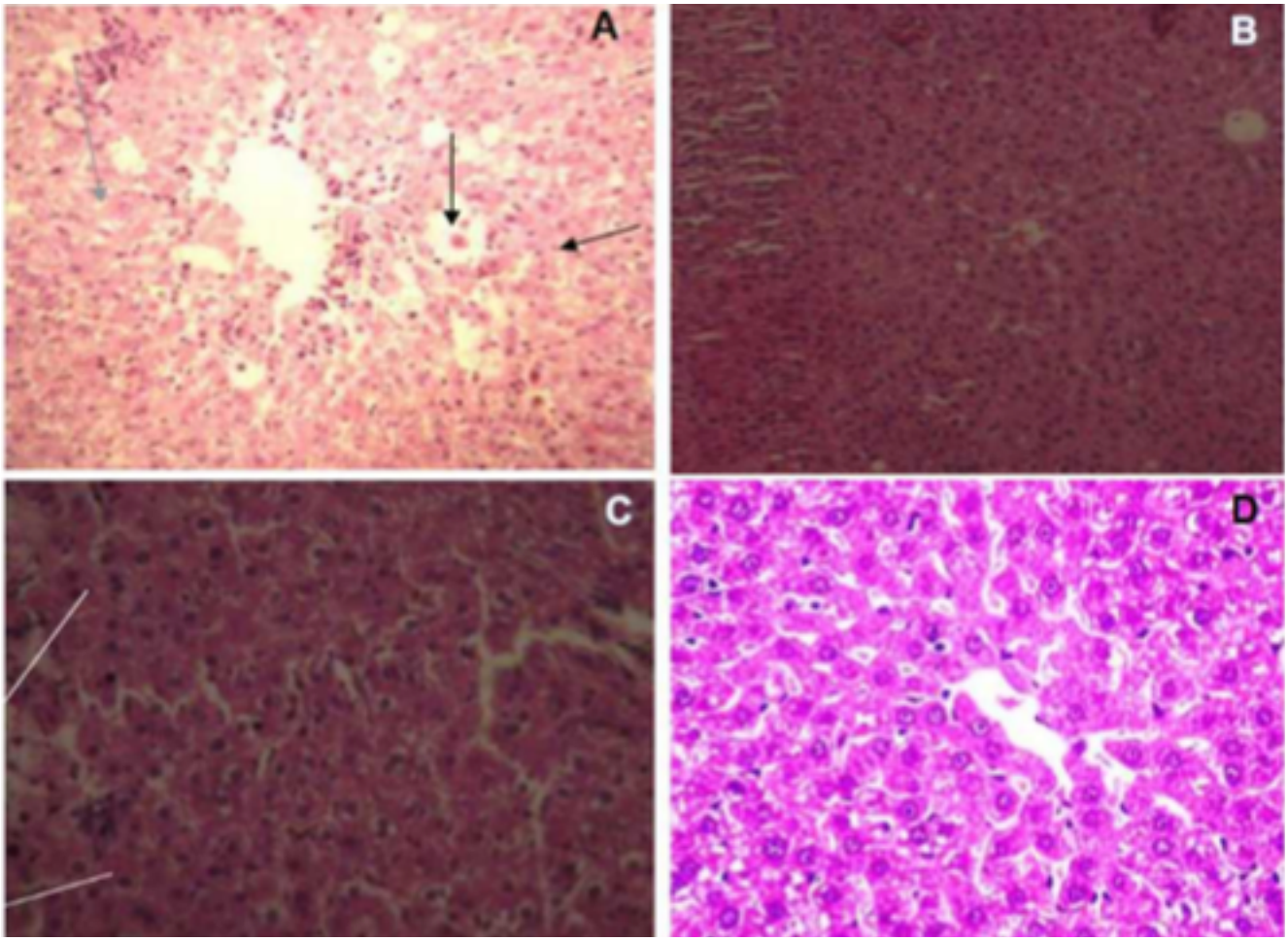

Figure 1. (A) Liver section of rats treated with normal saline. Normal appearance of liver parenchyma and sinusoidal area (X80), (B) Liver section of rat treated with methotrexate (MTX) and normal saline. Hydropic degeneration (black arrow) and coagulative necrosis (gray arrow) in the hepatocytes and the centrilobular zone (X80), (C) Liver section of rat treated with MTX and NS oil. Normal appearance of liver parenchyma with sparse coagulative necrosis (white arrow) (X200), and (D) Sections of liver tissue from a rat treated with MTX and NAC. There is a marked reduction of eosinophils in lobular parenchyma compared with liver from rats that have received MTX alone. However, there is still some degree of Kupffer cell hyperplasia (X100), H&E stain.

DISCUSSION

Methotrexate-induced toxicity mainly occurs as an outcome of the interplay of various factors such as the dose and the duration of treatment; patients' risk factors, type of disease, and presence of genetic and molecular apoptotic factors [23].

In the current study, MTX produced oxidative damage through increasing lipid peroxidation manifested by increasing MDA level and reducing GSH level. However, these levels have been almost normalized by the use of NSO and the results were comparable to that produced by NAC. Lipid peroxidation, generated by oxygen free radicals considered as an important cause of destruction and deterioration to cell membranes and may lead to the development of MTX mediated tissue damage [24].

The fixed oil of NS is known for its antioxidant activity [25]. The results of the present study revealed a protective effect of NSO against MTX induced hepatotoxicity through restoring antioxidant capacity which was the inconsistency with other studies [26]. The activity of hepatic enzymes was also normalized by the use of NSO and the effect was even more pronounced than that produced by NAC; this finding

was in tune with other studies [27]. Additionally, NSO decreased lipid peroxidation and liver enzymes, and increased antioxidant capacity in the CCl4-induced hepatotoxicity in rats.

Histopathological examination showed slight hepatocellular degenerative and necrotic changes with no obvious fibrosis and/or cirrhosis in an NSO-treated group. NSO prevents liver fibrosis and cirrhosis possibly through immunomodulation and antioxidant activities [28] .

The main proposed mechanism for these actions could be attributed to the activity of thymoquinone via inhibition of Ecosanoids generation in white blood cells (WBC) [12]. Thymoquinone, the main ingredient of NSO, has been reported to exhibit hepatoprotective activity possibly by antioxidant and immunostimulant effects [29].

In conclusion, the results of this study showed anti-inflammatory, anti-oxidant, and cytoprotective effects of NSO against MTX-induced hepatotoxicity in rats.

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Disclosure Statements

The author has no conflict of interest to declare.

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