

Evaluation of the protective effect of *Nigella sativa* oil's unsaponifiable fraction in carbon tetrachloride-kidney damage model

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Abstract

Background/Aim: Kidney diseases is a global public health problem. This study was conducted to evaluate the renoprotective effect of the total oil's unsaponifiable fraction (UF) of a medicinal plant called *Nigella sativa*.

Methods: The extraction of unsaponifiables from the total seed oil of the medicinal plant *Nigella sativa* was carried out according to the standardized methods of the European Pharmacopoeia (Edition 5.0). Carbon tetrachloride (CCl₄) is known to induce kidney toxicity. Acute poisoning of Male albino rats with 1:1 (v/v) mixture of CCl₄ and olive oil (3 ml/Kg b.w. of rat by subcutaneous injection) induces considerable kidney pain.

Results: *Nigella sativa* UF is found to be rich in tocopherols, its administration as a curative or preventive treatment to poisoned animals results in a very significant reduction in renal markers (CREA, URE). Histological study shows that kidneys are in better condition than those of intoxicated rats.

Conclusion: Measurable biological evidence has been given to the use of such extract as effective substance against kidney diseases.

Keywords: CCl₄, Renoprotective activity, *Nigella sativa*, total oil, Unsaponifiable fraction

INTRODUCTION

Exposure to CCl₄ can lead to nephrosis, nephritis, and kidney failure. Renal failure is the leading cause of death in many patients with acute CCl₄ exposure ¹, it is important that work be undertaken to provide a basis scientific use of traditional medicines by the population for the care of renal disorders.

Carbon tetrachloride (CCl₄) is used to induce experimental kidney toxicity which includes kidney damage: tubular and glomerular alterations, it induces encountered pathology in clinic: acute tubulopathy, often of the anuric type ². It is generally accepted that the toxicity of CCl₄ is the result of cytochrome P-450- dependent reductive dehalogenation to form a highly reactive trichloromethyl free radical, CCl₃• ³. CCl₄-induced damage is characterized by cell membrane damage, caused by lipid peroxidation ⁴. Natural products plant derived have received considerable attention in

recent years due to their diverse pharmacological properties including antioxidant and renoprotective activities ⁵. It is therefore vital to seek efficient plants, with low toxicity and low cost.

Nigella sativa, an herbaceous annual plant (family Ranunculaceae), is one of the important medicinal plants which has long been used as a natural remedy for a number of human illnesses and disorders ⁶. Therefore, this panacea has been widely studied with regard to its composition and its biological activities. *Nigella sativa* seeds have been reported to possess anti-inflammatory ⁷, anti-diabetic ⁸, immunopotentiating ⁹, anti-tumoral ¹⁰, antihepatotoxic ¹¹, analgesic ⁷ and antinephrotoxic properties ¹².

The unsaponifiables of vegetable oils are considered natural antioxidants. They are capable of minimizing the oxidation of oils during storage. The unsaponifiable fraction (UF) contains carbohydrates, phospholipids,

liposoluble vitamins, pigments, tocopherols, phytosterols and polyphenols. These natural antioxidants may function (a) as reducing agents, (b) as free radical scavengers, (c) as complexes of pro-oxidants metals, and (d) as inhibitors of the formation of singlet oxygen¹³. Through relatively simple laboratory tests, this is to provide biological evidence of renoprotective activity attributed to plant extracts which are the subject of the study.

2. MATERIALS AND METHODS

2.1. Vegetal material

The *Nigella sativa* seeds used come from the same batch of seeds procured from an herbalist. They are local, grown in the highlands of Northeastern Algeria (Batna and Biskra).

The taxonomy of the used plant has been confirmed and validated by Prof. H. Laouar, botanist, laboratory of plant biology (University of Setif 1, Algeria).

2.2. Total oil extraction

Seed powder was extracted in a Soxhlet-extractor with methanol solvent to get the methanol extract, to which we added n-hexane for obtaining the total oil. During the extraction, the extracted lipids required the addition of 0, 75 % aqueous sodium chloride solution. The solvent was removed on a rotary evaporator at 40°C. Total oil was collected in a flask and stored at 4°C¹⁴.

2.3. Unsaponifiable fraction extraction

UF was carried out according to the standardized methods of the European Pharmacopoeia (Edition 5.0). In a 250 ml flask fitted with a condenser, we introduced 5 g of the total oil of *Nigella sativa* seeds, which we added 50 ml of alcoholic potassium hydroxide solution 2 M. The mixture is heated in a water bath for 1 hour and circular stirring. After cooling to a temperature below 25 ° C the content was transferred into a separatory funnel and 100 ml of water was added. The mixture was washed (3 x 100 ml) with petroleum ether 40-60 ° C. Ethereal liquids are then combined in another separatory funnel containing 40 ml of water. After separating the two phases, the ethereal phase is recovered and washed initially with 40 ml of a potassium hydroxide solution at 30 g / l then with 40 ml of water. This washing is repeated several times until the aqueous phase is not alkaline anymore. The ethereal phase is transferred to another flask. After removal of the ether on a rotary evaporator at 30°C, the residue is dried at 100-105 ° C to constant mass¹⁵. The yield in all samples was 2,5%. Unsaponifiables are particularly rich in tocopherols; their characterization was performed by TLC and HPLC.

2.4. Animals

Albino male rats weighing between 180 and 220 g were used. They were placed in groups of six rats in cage and nourished by the granules produced by the national office of cattle feed. Their drink is water ad Libitum. The cages are placed in the enclosure of the animal where the temperature varies between 20-24 °C; the humidity is 50%

and a light cycle of 10h / 24 h. These animals are kept in adaptation phase for two weeks before the start of the experiment.

2.5. Induction of kidney toxicity

The experimental kidney pathology is obtained by subcutaneous injection of a massive dose of CCl₄. The chosen dose is equal to 3 ml CCl₄ / Kg b.w. of rat. For experimental convenience, the CCl₄ was dissolved in olive oil¹⁶.

2.6. Administration of plant extracts

The treatments are carried out every day at the same time, with the extracts obtained than 24 hours previously. Administrations are done by gavage. The extracts are therefore thawed in a water bath set at 37 °C. The UF was dissolved in sunflower oil¹⁷ and administered at 20 mg / Kg b.w. of rat. Animals are treated for preventive or curative manner over a period of 10 days. Administrations are carried out 30 min before (prevention mode) or after poisoning (curative mode).

2.7. Distribution of animals

Six groups of 6 rats each were formed and each batch corresponds to a particular treatment:

Group T: batch control treated with sunflower oil and dilution CCl₄ vehicle (olive oil).

Each rat received 1 ml of sunflower oil by gavage for 10 days and a subcutaneous injection of olive oil (3 ml / kg b.w. of rat.) is done the 10th day. Euthanasia on the 11th day. **Group C:** intoxicated lot with CCl₄ (3 ml / kg b.w. of rat.), euthanasia after 24 hours.

Preventive administrations excerpt:

Group UFC: group treated with the unsaponifiable fraction, each rat received 20 mg / kg b.w. of rat dissolved in 1 ml of sunflower oil for 10 days, and a subcutaneous injection of CCl₄ (3 ml / kg b.w. of rat) is done the 10th day, euthanasia on the 11th day.

Curative administration excerpt: Group CUF: each rat undergoes a CCl₄ subcutaneous injection (3 ml / kg b.w. of rat) the first day and receives by gavage 20 mg / kg b.w. of the unsaponifiable fraction dissolved in 1 ml of sunflower oil for 10 days, euthanasia on the 11th day.

2.8. Dosage of biological markers

The renal markers measured are creatinine (CREA) and urea (URE), these assays were performed at the central laboratory of the University hospital center of Sétif.

2.9. Histological study

The organ harvesting was carried out according to the protocol of the Biochemistry Applied Laboratory of the University of Setif.

The organs were immediately removed and washed with 1.15% KCl solution, weighed then cut into pieces and

stored at -80 ° C. Prior to use, liver homogenates were prepared by grinding the liver pieces in KCl buffer (0.15 M) at 4 ° C. They are centrifuged at 3000 rpm for 10 min and the supernatants are recovered for biochemical assays. The histopathological study was established at the service of the pathological anatomy of the University hospital center of Sétif according to the experimental protocol of the laboratory.

2.10. Statistical analysis

The results were expressed as mean±SD. The data were subjected to one-way analysis of variance (ANOVA), where the differences between groups and standards were determined by Student’s test, using SPSS 9,0 program, p value

≤ 0,05 was regarded as significant.

3. RESULTS

In the present study, the renoprotective activity of *Nigella sativa* total oil’s unsaponifiable fraction was investigated and to our knowledge this is the first study that dealt with it.

3.1. Biological parameters

They are creatinine (CREA) and urea (URE). The results are summarized in Table 1.

These results show the increase in blood levels of creatinine (0.47±0.09 mg/dl compared to 0.39±0.03 mg/dl in the control group) and urea (0.60±0. 08 g/l compared to 0.50±0.01 g/l in the control group) in rats poisoned with CCl₄ reflecting renal damage which is an acute tubulopathy of the anuric type, a renal anomaly described in the literature concerning the toxic effects of CCl₄ (National Academy of Medicine of Paris, ATSDR, US EPA, Stephen O. Adewole et al., 2007; B. Mégarbane, N. Deye, F. Baud, 2007); During our experience, we actually noted a very significant decrease in urinary secretion in this batch of rats (anuria).

On the other hand, in rats having undergone curative or preventive treatment with the fraction of unsaponifiables, all the parameters were within the norm compared to the control group, apart from the urea level which remained high in the IC group not reflecting necessarily renal dysfunction given that the creatinine level in the rats in this batch is normal (0.39±0.00 mg/dl).

The restoration of blood levels of creatinine and urea to normal, after their increase following CCl₄ poisoning, was better with curative treatment.

Table 1: Evolution of renal markers in rats treated with the unsaponifiable fraction of *Nigella sativa*

	T	C	UFC	CUF
CREA (mg/dl)	0,39±0,03	0,47±0,09	0,39±0,00	0,35±0,01
URE (g/l)	0,50±0,01	0,60±0,08	0,58±0,06	0,44±0,04

3.2. Histological studies

Figures 1 to 4 show the kidney histological sections of various experimental animal groups. Through the histological section of the kidney of rats from group T (control) (figure 1), we observe the cortical zone which is entirely delimited by a connective capsule, arrowed at 1; we see in 2 small spherical masses, these are the renal glomeruli, or Malpighian corpuscles where glomerular filtration occurs, they are scattered throughout the cortex. The majority of acidophilic renal tubes like those arrowed in 3, are located in the cortex (These are the first order convoluted tubes), which explains the more reddish coloring of this cortical area. The medulla in 4 is a less colored central zone located deep and made up of the pyramids of Malpighi and those of Ferrein.

In animals poisoned with CCl₄ (lot C) (Figure 2), histological examination shows damage to the tubular epithelium, focal necrosis of the renal tubes, a mononuclear inflammatory infiltrate and congestion.

After administration of a curative or preventive treatment of the unsaponifiable fraction of *Nigella sativa* seeds, all signs of acute tubulopathy disappeared and a completely normal kidney was observed as was already described in the rats from the batch control (figures 3 and 4).

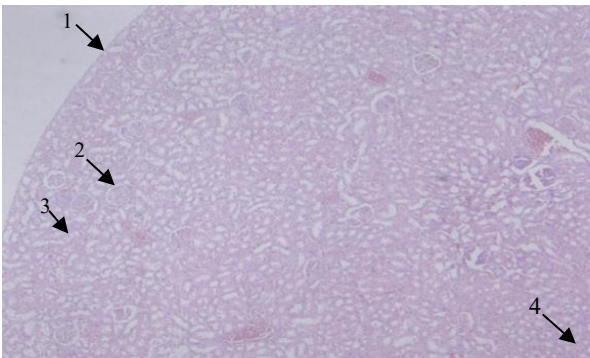


Figure 1: Kidney tissue from control lot treated with sunflower oil and olive oil (GX10)

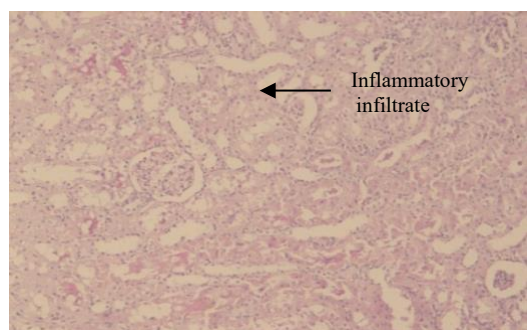
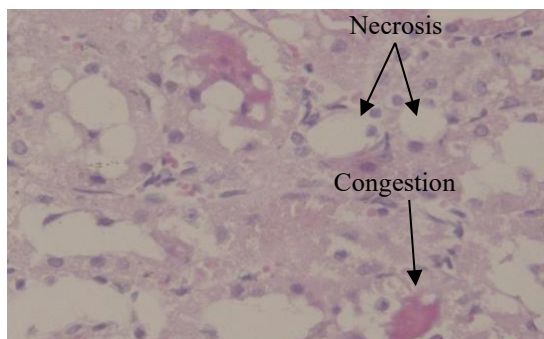
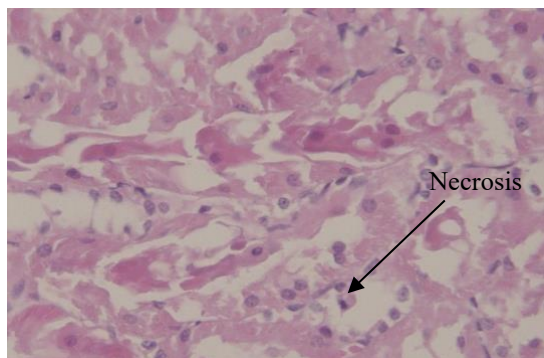


Figure 2: Renal tissue from the batch poisoned by CCl₄.

A: Congestion (GX100), B: Cellular necrosis of the renal tubes (GX400), C: Congestion + tubular necrosis (GX400), D: Tubular necrosis + inflammatory infiltrate (GX100).

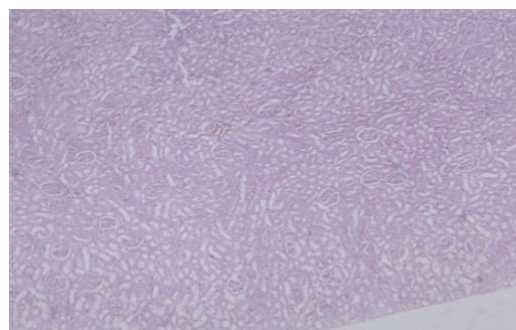


Figure 3: Renal tissue from the batch treated with UF then poisoned with CCl₄ (GX100)

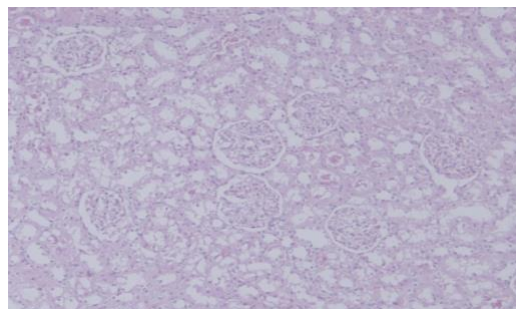


Figure 4: Renal tissue poisoned by CCl₄ then treated with UF (GX100)

4. DISCUSSION

The content of our oil in unsaponifiables has been estimated at 2.5%, a rate much lower than that of *Nigella sativa* oil from Denizli in Turkey (5.38%)¹⁸ of 1 (4%), Kutahya (Turkey) (3.47%) and Konya (Turkey) (4.0%) but high compared to that of the seeds of *Nigella sativa* from Egypt (0.66%)¹⁸. This fluctuation in the content of unsaponifiables is related to the extraction method and the origin of the seed¹³.

The extraction of unsaponifiables require a major importance given their richness in active biological compounds, it is phytosterols, liposoluble vitamins and other lipophilic secondary metabolites different from conventional lipids. Five major groups of substances are present in most unsaponifiables of vegetable fats: saturated or unsaturated hydrocarbons, aliphatic or terpenic alcohols, sterols, tocopherols and tocotrienols, and carotenoid pigments, especially xanthophylls, tocopherols figure as crucial constituents in the unsaponifiable fraction thanks to their vitamin and antioxidant activities. so despite its low content in the oil, the unsaponifiable fraction is of paramount importance thanks to the noble compounds it contains¹⁹.

The significant reduction in renal markers after treatment with Algerian *Nigella sativa* UF suggests that the extract is renoprotective. It has been suggested that the protective effect of plant extracts against CCl₄-induced kidney damage may be attributed to the presence of constituents including flavonoids, tannins, triterpenoids and alkaloids. Flavonoids are known to be antioxidants, free radical scavengers and

antilipoperoxidants which cause renoprotection²⁰. *N. sativa* has been reported to have antioxidant activities in vitro²¹ and in vivo²², principally related to lipidic compounds of the seeds. GC and HPLC analysis of the total oil allowed to reveal its composition of fatty acids, triglycerides and sterols and higher alcohols. These results indicate its rich bioactive lipid compounds, which would explain the assigned pharmacological properties²³.

Olive oil used to prepare the solution of intoxication is without disadvantage in rats; it may even have a protective effect in causing an increase in the activity of antioxidant enzymes and a decrease of the liver damage signs²⁴.

The observed renoprotection by UF suggests that the extract tends to prevent kidney damage and suppress the leakage of enzymes into the blood stream by preserving cells membranes. Studies have already mentioned that *Nigella sativa* showed an important in vitro antihemolytic activity²².

CONCLUSION

The present study has validated the renoprotective properties of *Nigella sativa* total oil's unsaponifiable fraction. These properties seem to be due to the presence of sterols, polyphenols and tocopherols that are endowed with known antioxidant and pharmacological properties; the evidence of such activity is reducing the rate of renal markers, kidney histology of animals showing kidneys in better morphological and architectural state than those of poisoned animals.

List of Abbreviations

CCl₄, Carbon tetrachloride; TO, Total oil; UF, Unsaponifiable fraction; FAMES, fatty acid methyl esters; ELSD, evaporating light scattering detector; HPLC, High Performance Liquid Chromatography; GC, Gas

Chromatography; FID, flame ionization detector.

Conflicts of Interest: none

Significance Statement:

The present study sheds light on the renoprotective effect provided by the unsaponifiable fraction extracted from the seeds of *Nigella sativa* in male albino rats poisoned by CCl₄, and this is a first to our knowledge, which support the use of such substances in therapy

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