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Toxicity and anti-oxidant activity of the essential oil of *Nigella sativa*

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ABSTRACT

The antioxidant properties of essential oils are very little studied. Oxidative stress occurs when the imbalance between the production of free radicals and antioxidant enzymes is related to the onset of serious diseases. The antioxidant activity of the essential oil of Nigella sativa against the DPPH radical has been evaluated by spectrophotometry. The results show an antioxidant power with an IC_{50} of 0.056 mg/ml. This activity is mainly due to the combined action of various endogenous antioxidants contained in the essential oil. The study of the acute toxicity of the essential oil of Nigella sativa showed no mortality in groups of used animals. The work of [1] *confirms our results. However, the author of* [2] *found a toxicity of this essential oil, due, according to him, to the presence of thymoquinone.*

INTRODUCTION

The nature hides a multitude of wonders which too often no attention is reach. The vast plant world for millennia provides the elements necessary for the survival of the human species. Indeed, it not only provides food and lodging, but also, what is treatment.

Thanks to their beneficial effects on health, polyphenols studies are becoming increasingly important. Indeed, they are involved in the prevention and treatment of diseases related to oxidative stress such as cancer, cataracts, atherosclerosis, diabetes, hypertension, neurodegenerative diseases and arthritis [3].

Anti-oxidant activity of *Nigella sativa*

Material and methods

In order to characterize the essential oil extracted from *Nigella sativa* seeds, one of total polyphenols and flavonoids assay was performed. The main reason for the choice of these substances resides in the fact that most of the antioxidant properties of plants are allocated to them. The method of assay of total polyphenols is the Folin-Ciocalteu reagent. Gallic acid was used as standard. The flavonoids assay was performed according to the method using aluminum trichloride as standard quercetin.

According to Boizot et al. [4], if the Folin-Ciocalteu assay is simple to implement and very sensitive, it is however not specific to polyphenols because it reacts with the amino acids tyrosine and tryptophan protein. Such interference can be ignored because these aromatic amino acids are too low in proportions relative to nonproteinaceous phenolic compounds in the extracts.

MATERIALS AND METHODS

The amount of phenolic compounds in plants depends mainly on the origin, variety, culture season of the harvest season, climatic and environmental conditions, the geographical location and different diseases that can affect the

plant. Quantitative determination of total flavonoids in terms of quercetin, the aluminum trichloride method reveals that the essentiaial oil of Nigel is rich in flavonoids with gradesof 6.005 µg.

If we compare our results with those of [5] who used the method in Prussian blue, there is a certain similarity of results in terms of content in phenolic compounds and flavonoids. Similarly, the flavonoids rate in both studies is relatively comparable. Furthermore, the content of phenolic compounds is more or less different. However, it is important to note that the use of different assay methods reduces the reliability of the comparison between the two studies. In fact, phenolic compounds, more particularly flavonoids are recognized as potentially antioxidant substances having the ability to trap the radical species and reactive oxygen species.

The assay results in terms of polyphenols equivalent gallic acid show that *N. sativa* essential oil contains 10.674 µg of extract. Phenolic compounds are composed of three main categories: phenolic acids, flavonoids and tannins. The Folin-Ciocalteu method was chosen for dosing the phenolic compounds.

Essential oils possess antioxidant and radical-scavenging properties that improve the lifetime of the food. Thus the incorporation of essential oils directly in food in the form of vapor or in the form of active packaging helps preserve the food from oxidation. It is in this context that we evaluated the antioxidant activity of the essential oil.

The antioxidant activity of the essential oil of *Nigella sativa* activity against the DPPH radical was evaluated by spectrophotometry following the reduction in this group which is accompanied by its shift from purple to yellow color measured at 517 nm.

The IC_{50} has been determined to better characterize the antiradical power in comparison with standard antioxidants such as quercetin (structural analog of thymoquinone) that showed a very potent anti-radical activity with IC_{50} of about 39.29 µg/ml [6]. The essential oil of Nigel has a radical scavenging activity with an IC_{50} of about 56.88µg/ml, much more interesting than those of the control value used, BHT (788 µg/ml) and ascorbic acid (4424 µg/ml) (see Table I).

Table I: IC_{50} for the essential oil of *Nigella sativa*, BHT and ascorbic acid.

Witness IC_{50} (µg/ml)		**Sample IC_{50} (µg/ml)**
BHT	Ascorbic acid	Essential oil of*Nigella sativa*
788	4424	56.88

The anti-radical activity of oil from the seeds of nigel was studied by [6]. The study was conducted on the fixed oil and its fractions, using the DPPH. The anti-radical activity shown by the crude oil was interpreted by a combined action of the various endogenous antioxidants in oil and is correlated with the content of polyunsaturated fatty acids, phospholipids and unsaponifiable compounds, as well as the initial value of peroxide crude oil.

The essential oil of Algerian nigel as well as the commercial oil have a fairly substantial with anti-radical activity with an IC_{50} of about 88,65±0,64 and 88,01±1,26 µg/ml respectively. In comparison with the standard antioxydant activity, the BHT (IC_{50} of 29,14 ±0,52 µg/ml), these oils are three times less active.

Work on the evaluation of the essential oil of nigel ability to trap the DPPH radical yielded different results varying from one to another ecotype. The study of [7] found that the essential oil of the Australian Nigella is capable of trapping the radical DPPH with an IC_{50} of about 460µg/ml, whereas the turkish variety presents an IC_{50} of 515 µg/ml.

Furthermore, in [8] it was demonstrated that the essential oil of Tunisian Nigella exerts a powerful anti-radical effect with an IC_{50} of 14 µg/ml. This difference in the IC_{50} can be attributed to the difference in the concentration of DPPH used in the test on the one hand [9], and secondly to the influence of other factors that may affect the chemical composition and therefore the antioxidant capacity of the sample, as the variety of seed, the growth conditions of the plant, the conditions of storage of the seed and oil and the extraction methods used [10].

The thymol, the thymoquinone and dithymoquinone, constituents of *Nigella sativa* have a role in neutralizing reactive oxygen species (ROS). Their antiradical activity against superoxide anion, hydroxyl radical or singlet oxygen was determined by spectrophotometry [11].

According to [12], the thymoquinone (majority compound in the essential oil of *Nigella sativa*) inhibits non-enzymatic lipid lipoperoxidation in liposomes. The thymoquinone, carvacrol, t-anethole and 4-terpineol (composed of the same oil) have significant scavenging effect of free radicals.

Fixed oil and its fractions (neutral lipids, glycolipids and phospholipids, the thymoquinone) decrease the rate of alanine aminotransferase and alkaline phosphatase on isolated hepatocytes oxidized by the tert-butyl hydroperoxide, thereby determining its hepatoprotective share [13].

Another phytochemical and biochemical study shows the anti-radical activity of polymorphonuclear leukocytes on thymoquinone [14].

A comparative study of the action of seven medicinal plants, including *N. sativa* on the deformation of the erythrocytes, their osmotic fragility at 37°C for 60 minutes in a hydrogen peroxide solution at 10 mM, and their protein degradation lipid peroxidation, shows a protective effect of Nigella degradation erythrocyte due to oxidative stress. *Nigella sativa* antioxidant and anti-free radical activity, may thus be beneficial in the rheological pathologies [15].

A study, implementing the toxicity of carbon tetrachloride (CCl4) in mice, showed that *Nigella sativa* oil has restored the serum lipid profile and played a protective role against hepatotoxicity [14,16]. The abnormally high levels of potassium and calcium, and complete blood count lowered by CCl4 were restored by black cumin oil [9]. It decreased the elevated liver enzymes and increased the diminished antioxidant enzymes. *N. sativa* fought against liver fibrosis by CCl4 [17].

Another study shows that black cumin oil increases the glutathione concentration and the antioxidant defense system in the renal cortex, in a dose-dependent manner biochemical and histological point of view, which implies protection against nephrotoxicity [18].

Other research teams have investigated the particular thymoquinone. Pretreatment with the thymoquinone showed rats hepatoprotective action after a CCl4 injection, in contrast to p-cymene and α-pinene which had no protective antioxidant effect [7, 19].

Nephrotoxicity, cardiotoxicity and oxidative stress induced by doxorubicin in rats, were treated with the thymoquinone; it slowed nephrotic hyperlipidemia and proteinuria and induced biomarkers acting against oxidative stress [20]. The administration of the oil of *Nigella sativa* and thymoquinone rats protects against hyperhomocysteinaemia induced by methionine by blocking the accumulation of homocysteine, one of the causes of the condition of oxidative stress, leading the protection against lipid peroxidation and oxidative status changes [18].

Moreover, treatment of rats fed a diet contaminated with aflatoxin, with the oil of *Nigella sativa*, results in significant protection against the hepatonephrotoxicity and oxidative alterations (reduction of the rate of SOD, increased peroxidation lipid and DNA damage) induced during aflatoxicosis. This protective effect of *Nigella sativa* oil can be attributed to its free radical scavenging effect according to [21].

In 2003, a study on rats put in ischemia-reperfusion status, comparing the effect of *Nigella* oil to thymoquinone, showed the superiority of thymoquinone; the parameters being measured LDL levels, SOD and GSH [22].

Schistosoma mansoni infection causes an increase in chromosome failures and therefore genotoxicity. Rats infected with *S. mansoni* were treated with oil and black cumin oil extracted thymoquinone. A decrease in abnormal chromosomes, deletion and tetraploid, and therefore an anti-genotoxic effects were observed [18, 23].

The work carried out show that three essential oils studied have a significant antioxidant activity; that of black seed is the most interesting (IC_{50}=56 µg/ml) by comparing it with the other two plants.

Toxicity of the essential oil of *Nigella sativa*

Material and methods

To verify the safety and efficacy of the essential oil of *Nigella sativa* plant, our in vivo study involved white Wistar rats (20 rats) of both sexes whose weight ranges between 180-300 grams and 95 albino mice (NMRI) of both sexes whose weight ranges from 17-23 grams (USP 35).

RESULTS AND DISCUSSION

In Table II, we do not observe any mortalityfor all the used doses. Hence, the DL50 cannot be determined.

Table II: Distribution of the lots and their submission to test the acute toxicity of the essential oil of *Nigella sativa*

Dose mg/Kg	1	0.8	0.6	0.4	0.2
Nbre of animals/ lot (n)	05	05	05	05	05
Nbre of dead / lot	00	00	00	00	00
% of dead / lot	00	00	00	00	00
A		4.5	3	2	1
B	0.2	0.2	0.2	0.2	0.2
AB		0.9	0.6	0.4	0.2
Σ AB	2.1				

A=average of the sumof deads for two successive doses; B=difference betweentwo successive doses; N=numberof animalsused by lot.

The work of Vahdati-Mashhdian [1] and Orsi-Llinares [24] confirm our results. By cons, other researchers were able to determine some toxicity to nigella. Indeed, [2] shows that the toxic properties of thymoquinone (TQ) and thymohydroquinone (THQ), which are major components of the black cumin were studied in rats by intraperitoneal injection to determine their LD50 (Median Lethal Dose). TQ with an LD50 of 10 mg / kg of body weight, would be more toxic than the THQ to 25 mg / kg [25].

In 2008, Al-Ali et al. [26] conducted a revaluation of the LD50 thymoquinone conducted on mice and rats. In mice, LD50 TQ after administration intraperitoneally of 104 mg / kg and 870 mg / kg body weight orally. Values are 57 mg / kg and 794 mg / kg in rats after intraperitoneal injection and oral administration respectively. The values of LD50 obtained after intraperitoneal injection and oral administration are respectively 10 to 15 times and 100 to 150 times higher than the thymoquinone doses required to achieve an anti-inflammatory, antioxidant or anticancer agents.

Other studies have demonstrated the safety of the aqueous extract of seeds of *N. sativa* composed of proteins, polyphenols and saponins. Administered orally for 14 days to rats, the aqueous extract caused no toxicity up to 5g / kg. Thereafter, the administration of cumin seeds to chickens 7 days for 7 weeks in a dose of between 20 and 100 g / kg did not affect growth [27, 28].

Although many experiments have reported a non-toxic effect of nigella seeds, one study showed that consumption of *N. sativa* seeds over a long period would be harmful to health. The LD50 obtained for single doses of fixed oil nigella seeds oral and intraperitoneal mice were 28.8 and 2.06 mg / kg respectively [2].

The study of chronic toxicity of the fixed oil of *Nigella* was carried out on rats for 12 weeks with daily doses of 2mg / kg body weight. No histopathological changes of heart tissue, liver, kidney and pancreas were observed. Liver enzymes were not changed either after 12 weeks of treatment [2].

However a significant decrease in serum parameters such as cholesterol, triglycerides, glucose, leukocytes and platelets, was noticed compared to the control group, and conversely, hemoglobin and hematocrit increased. These results suggest suggest that *N. sativa* fixed oil has a high therapeutic index, but the changes in the hemoglobin metabolism as well as the fall in the number of leukocytes and platelets are to be considered [29].

According to [2], works have consisted in the evaluation of the acute and subacute effects of possible toxicity of the seeds of *Nigella sativa* through the use of aqueous extracts, methanolic and chloroform. The extracts were administered orally to rats at 4 different doses: 6, 9, 14 and 21 g / kg. The death rate and weight changes were measured, respectively, for 3 and 7 days. No mortality was recorded. The methanolic extracts all doses and chloroform to 21 g / kg significantly reduced the weight mass of animals.

With the aqueous extract, degenerative phenomena in liver cells were observed. The results allow to conclude that these extracts are relatively nontoxic under acute toxicity test, but we must take into consideration the liver damage produced by aqueous extracts [1].

The work of Zaghlol et al. [30] confirms the toxic effect of *N. sativa* plant oil. Daily doses of 15 and 25 mg / kg body weight were administered to rats orally for a month. Changes in histological structure of the renal cortex and to a lesser extent in liver cells have been reported.

CONCLUSION

Nigel has an IC_{50} of 56.88µg/ml, which is related primarily to the presence of thymoquinone and hydrothymoquinone. Nigel does not contain toxic compounds.

Essential oils can be considered as drugs that can be substituted at least partially to antibiotics but must be subject to medical prescription in order to benefit from their therapeutic properties and avoid their toxic effects.

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