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PROTECTIVE EFFECT OF NIGELLA SATIVA OIL AND ITS NEUTRAL LIPID FRACTION ON ETHANOL-INDUCED HEPATOTOXICITY IN RAT MODEL

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ABSTRACT

Background: alcoholic fatty liver disease is the earliest stage of alcohol-related liver disease. *Nigella sativa* is one of the herbal medicines that show a significant effect on many human diseases. **Aim:** the aim of this study is to investigate the hepatoprotective effect of two fractions of *Nigella sativa*; total oil (TO) and neutral lipid fraction (NLF). **Material and methods:** in the present investigation, total oil (TO) and its neutral lipid fraction (NLF) extracted from the seed of the medicinal plant *Nigella sativa* were tested for their therapeutical effect on alcohol-induced liver injury in male albino rat model.

The malondialdehyde (MDA), superoxide dismutase (SOD), catalase and reduced glutathione (GSH) concentrations, in plasma and hepatic homogenate were determined.

Results: we noted a significant increase in SOD and CAT activities and GSH level with a decrease in MDA level in rats treated with either TO or NLF extracts.

As **conclusion:** both extracts of *Nigella sativa* seedspossess an important liver protective potential against ethanol induced hepatotoxicity in rats.

Keywords: *Nigella sativa*, total oil, neutral lipids fraction, alcohol-induced hepatotoxicity, antioxidant enzymes

INTRODUCTION

Heavy alcohol consumption is one of the most important causes of liver toxicity. Alcohol is primary metabolised in the liver and generates highly reactive oxygen species (ROS) (Cederbaum, 2003). By their ability to interact with many cellular structures, these species are the leading cause of cell and tissue damages (Molina *et al*., 2003).

Alcohol consumption enhances lipid peroxidation, mitochondrial dysfunction and generation of ROS by the cytochrome P450 2E1 enzyme (Balkan *et al*., 2001). However, it has been reported that iron overload (Bacon and Britton, 1990), cholestatic injury (Parola *et al*., 1992) and CCl_4 induced liver intoxication (Danladi *et al*., 2013), which is associated with liver fibrosis and cirrhosis, are among the main causes leading to the ROS production (Parola *et al*., 1992).

To prevent liver damages by ROS, our body has developed several endogenous antioxidant systems which are divided into two groups; enzymatic and non-enzymatic. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Lipid-soluble vitamin E (tocopherol), vitamin A (β-carotene) and carotenoïds, as well as vitamin C (L-ascorbic acid) and reduced glutathione (GSH) are among the non-enzymatic antioxidants (Halim *et al*., 1997). In certain pathological cases, such as severe inflammation and tissue damages, an exogenous supply of antioxidant molecules is required to protect our body. Plants appear to be the important source of these antioxidant molecules and have helped human in the preservation of his health for thousands of years.

In our days, traditional herbal medicines are receiving great importance in the health sector as source of antioxidant molecules. In this study, we focused on the seeds of the medicinal plant *Nigella Sativa.* The seeds are commonly known as “black cumin”, and the plant belongs to botanical family of Ranunculaceae (Saad *et al*., 2006). This plant, mainly by its oil, is one of the most effective with a very high therapeutic potential (Tariq, 2008). By its divers biological and pharmacological properties such as anti-inflammatory activity (Ali and Ammar, l997), diuretic and hypotensive activity (Zaoui *et al*., 2002) antiviral (Salem, 2005) activities, *Nigella sativa* is widely used in folk medicine for the treatment and/or the prevention of different human diseases. Much of its biological activities have been related to its fixed and essential oils (Houghton *et al*., 1995; Mansour *et al*., 2001).

One of the most important therapeutic potential of *Nigella sativa* oil is the hepatoprotective effect on damages induced by different agents; carbon tetrachloride CCl_4 (Danladi *et al.,* 2013), D-galactosamine (Ganiand John, 2013), paracetamol (Pal *et al*., 2011) and ethanol (Hamed *et al*., 2014). Such hepatoprotection is mainly the consequence of the antioxidant properties of *Nigella sativa* by scavenging ROS and reducing malondialdehyde and other oxidative stress biomarkers.

Thymoquinone, most active component of *Nigella sativa*, inhibits the expression of induced nitic oxide (iNOS), which participats in the oxidative stress, and hence can increase the expression of antioxidant enzymes such as GSHPx and SOD (Al-Okbi *et al*., 2013). Equally this molecule is able to reduce NADH which leads to the inhibition of lipogenesis in hepatocytes (Khalife *et al*., 2007).

For preventive and therapeutic reasons, in this study we investigated the hepatoprotective effect of two fractions; total oil (TO) and neutral lipid fraction (NLF), extracted from *Nigella sativa* seeds against ethanol-induced hepatotoxicity in rats fed with Lieber-DeCarli liquid diet. Many studies pointed out that this type of diet is suitable to investigate metabolic effects of ethanol in a rat model including those involving the fetal alcohol syndrome (Detering *et al*., 1979; Ghishan*et al*., 1982). N-acetyl cysteine (NAC), with its therapeutic effect on liver injury (Martin *et al*., 2011), was used as a positive control.

MATERIAL AND METHODS

1) VEGETABLE MATERIAL

Nigella sativa seeds were obtained, in march, from Bechar, a city situated in the Algerian desert and botanically identified by Professor H. Laouar, director of the Plant Biology Laboratory (PBL) at Sétif University, Algeria. Seeds were washed, dried and powdered with an electric micronizer.

2) EXTRACTION AND FRACTIONATION OF THE TOTAL LIPID

Seeds powder was extracted in a Soxhlet-extractor with methanol as solvent to get the methanol extract. To the latter, n-hexane was added and the total oil fraction was obtained. During the process, an addition of 0.75% aqueous sodium chloride solution to the lipids extracted was required. The solvent was removed on a rotary evaporator at 40°C. Total oil was collected in a flask and stored at 4°C for further analyses.

Obtained total oil was further fractionated on a silica gel 60 G (70-230 mesh) column (30 cm x 2 cm) where neutral lipids are eluted by chloroform (3 times, 100 ml) whereas polar lipids were eluted by acetone

to get glycolipids (GL) and methanol to get phospholipids (PL) following the protocol reported by Ramadan and Mörsel (2002) Solvents were evaporated on a rotary evaporator at 40°C.

3) ANIMALS AND TREATMENT

Male albino rats weighting between 120-180g were obtained from the animal house of Mentouri University, Constantine, Algeria. Animals were maintained, in collective cages (eight animals per cage), in controlled environment (12 hours light/dark cycles) at 22-25°C with 50 % humidity. Rats were fed with Lieber-DeCarli liquid diet during the period of treatment with ethanol (six weeks). The composition of both Lieber-DeCarli liquid and Control liquid diets contents are given in **table 1** bellow.

Table 1: Different components of Lieber-DeCarli liquid diet and Control liquid diet

Components	Amount/ 1litre (Lieber- DeCarli liquid diet)	Amount/ 1litre (control liquid diet)
Dextrin-maltose	27,91g	114 g
Casein	41,4g	41,4g
Oil mixture	43,8 ml	43,8 ml
Vitamin mixture	100 ml	100 ml
Salt solutions	100 ml	100 ml
l-cystine	0,5g	0,5g
methionine	0,6g	0,6g
ddH_2O	Approx 690 ml	Approx 760 ml
Ethanol	68,5 ml	/

4. EXPERIMENTAL INDUCTION OF HEPATOTOXICITY

Hepatotoxicity was induced in all rats by ethanol consumption with a specific diet (Lieber-DeCarli liquid diet) for six weeks. Rats were given a first dose of ethanol (l2 mg/kg/day) during the first week and this dose was increased to 17mg/kg/day during the five following weeks and the body weight was measured daily **(**Adeline *et al*., 2013). The control group was fed with the Control liquid diet. Six weeks after ethanol administration, rats received total oil (TO), neutral lipid fraction (NLF) and N-acetyl acetate (NAC) for four weeks. Ethanol and the different treatments (TO, NLF and NAC) were given daily, orally, by gavage.

a) EXPERIMENTAL DESIGN

Rats were randomly divided into five groups of eight animals each:

Ethanol group: treated by Ethanol with diet for 6 weeks,

TO group: treated by ethanol with diet (6weeks) + TO (400 mg/kg/day) for 4 weeks,

NLF group: treated by ethanol with diet (6 weeks) + NLF (300 mg/kg/day) for 4 weeks,

NAC group: treated by ethanol with diet (6 weeks) + NAC 1,2 g/kg/day (4 weeks),

Control group: treated with control liquid diet (6 weeks).

b) SAMPLE COLLECTION

Ethanol and control groups were sacrificed, under diethyl ether anesthesia, after six weeks of treatment and the remaining groups were sacrificed after ten weeks of treatment. The animals were fasted overnight, weighed and sacrificed by cervical dislocation. Blood samples were collected from the retro-orbital sinus of the eye by ocular puncture into heparinized tubes for biochemical analyses. The liver was removed from each rat, washed in ice-cold 1.15% KCl solution and weighted, then sliced into pieces and stored at -80°C until further use.

To prepare liver homogenate, samples were weighed and homogenized in 0.15 M KCl at 4°C. Homogenates were centrifuged at 3000 rpm for 10 minutes and the supernatants were aliquoted and used for biochemical estimations.

5. BIOCHEMICAL ANALYSIS

Stress oxidant parameters were estimated on blood plasma and liver homogenate of rats. These parameters included malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT). Hepatic and lipid parameters were assessed on plasma and liver homogenate.

a) DETERMINATION OF LIPID PEROXIDATION

Lipid peroxidation in plasma and liver homogenate was estimated spectro-photometrically at 535 nm, which is based on the estimation of MDA concentration, using 1,1,3,3-tetramethoxypropane as an external standard. Values were expressed as nanomoles of MDA formed per deciliter of plasma. MDA was determined by using molar extinction coefficient 1.56 x $10^5 M^{-1}$ cm^{-1} and values expressed as nM (Quantanilha *et al*., 1982).

b) ESTIMATION OF REDUCED GLUTATHIONE CONCENTRATION

The estimation of the reduced glutathione (GSH) in plasma and liver homogenate was carried out with a colorimetric method based on the GSH oxidation with 5.5-dithio-bis-6.2- nitrobenzoic acid (DTNB) using Ellman method (Ellman, 1959). The reaction product was measured at 412 nm and the level of GSH was determined on the standard curve obtained by different concentrations of GSH solution and the concentration was expressed as nmol/ml of plasma or mg of tissue.

c) ESTIMATION OF CATALSE ENZYMIC ACTIVITY

Catalase enzymic activity was estimated by the method of Aebi *et al*., (1984) which is based on the disappearance of H_2O_2 to give water and oxygen molecular in the presence of enzyme source (plasma or homogenate).

The reaction is followed by measuring the decrease in absorbance at 240 nm. Enzymatic activity was estimated by measuring the difference in absorbance per unit of time.

d) ASSESSMENT OF COPPER-ZINC SUPEROXIDE DISMUTASE (CU-ZN-SOD) ACTIVITY

Copper-zinc superoxide dismutase (Cu-Zn-SOD) activity was determined from rats'plasma and liver homogenate following the method of Sun *et al.* (1988) based on the reduction of nitroblue tetrasolium (NBT) to dark blue formazan. This obtained product is detected at 560 nm and one unit of the SOD is determined as the amount of protein that inhibited the rate of NBT. Results were expressed as unite/gram of liver tissue or ml of plasma.

E) PROTEIN ESTIMATION

Total protein in plasma and liver homogenate was determined by a Spin react BSIS30-1 KIT. In this assay, proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is included as an antioxidant. The intensity, by reading the absorbance at 540 nm, of the obtained color is proportional to the total protein concentration in the sample (Koller, 1984).

6. STATISTICAL ANALYSIS

All experiments were performed three times and analysis for each experiment was carried out in triplicates. Results were expressed as means ± standard error (SEM). Statistical analysis was performed using one way analysis of variance (ANOVA), followed by multiple comparison post hoc tests (Tukey). $p \leq 0.05$ was considered statistically significant.

RESULTS

A) ANTIOXIDANT ACTIVITY

To assess the oxidative stress in experimental rats, reduced glutathione (GSH), malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) were determined in plasma and liver homogenates.

In both plasma and liver homogenates, MDA levels decreased significantly (≤0.05) in rats from the groups treated either with TO, NLF or NAC (positive control) compared to the average level of the ethanol treated control group of rats. GSH levels increased significantly (≤0.05)in rats from the groups treated either with NLF or NAC (positive control), whereas no obvious change in GSH level in the group of animals treated with TO compared to the average level of the ethanol treated rats (**Tables 2 and 3).**

Treatment with NLF lead to a significant increase in SOD and CAT activities in plasma and liver homogenate of rats treated with NLF and NAC (positive control) compared to rats from the group treated

with ethanol. Although treatment with TO showed an increase in the activities of both enzymes but did not reach significance (**Tables 2 and 3).**

Table 2: Effect of the two extracts (TO and NLF) from *Nigella Sativa* seeds on few stress oxidant markers in the plasma of rats with ethanol-induced liver toxicity. Values are expressed as mean ± SEM, (N = 8); *significant difference (*: p≤0.05, **: p≤0.01, ***: p≤0.001) against ethanol treated group. Experiments were performed three times and analysis for each experiment was carried out in triplicates

Groups	Parameters			
	GSH (nmol/ml)	MDA (nmol/ml)	CAT (U/ml)	SOD (U/ml)
Ethanol	0,58 ± 0,13	59,01 ± 3,18	10,68 ± 1,91	6,67 ± 1,58
Control	1,17 ± 0,27	71,73 ± 5,38***	22,91 ± 4,00	10,59 ± 7,90
NAC	1,27 ± 0,40*	59,87 ± 4,05***	12,00 ±1,23	10,54 ± 7,90
HT	1,19 ± 0,20	60,33 ± 5,52**	19,54 ± 2,19	10,85 ± 4,16*
FN	1,41 ± 0,48***	54,34 3,46***	35,43 ± 8,45*	12,86 ± 4,49**

Table 3: Effect of the two extracts (TO and NLF) from *Nigella Sativa* seeds on few stress oxidant markers in the liver homogenate of rats with ethanol-induced liver toxicity. Values are expressed as mean ± SEM, (N = 8); *significant difference (*: p≤0.05, ** : p≤0.01, *** : p≤0.001) against ethanol treated group. Experiments were performed three times and analysis for each experiment was carried out in triplicates

Groups	Parameters			
	GSH (nmol/g)	MDA (nmol/g)	CATA (U/mg)	SOD (U/mg)
Ethanol	4,24 ± 0,135	55,28 ± 2,49	17,01 ± 2,43	72,12 ± 10,67
Control	4,43 ± 0,140	44,97 ± 4,23*	61,94 ± 16,87	78,23 ± 11,60
NAC	4,89 ± 0,111*	37,32 ± 2,66***	65,74 ± 19,88	83,00 ± 10,17*
HT	4,82 ± 0,091	33,77 ± 1,67***	66,67 ± 12,97	85,25 ± 3,17**
FN	5,63 ± 0,080***	36,74 ± 0,76***	101,64 ± 11,32**	90,07 ± 3,17**

DISCUSSION

Over the last few decades, human medical research geared for finding new molecules, mainly from medicinal plants, with important therapeutical potential and reduced harmful side effects. As it is well documented, *Nigella sativa* is a miracle medicinal plant with a large curative spectrum (Haq et *al.*, 1999; Hmza et *al.*, 2013; Ahmad et Beg, 2014). Despite the fact that many recent studies pointed out the different beneficial effects of various extracts of *Nigella sativa seeds* (Nagi et *al.*, 1999; Chakrabarty et Emerson, 2003; Sobhi et *al.*, 2011), up to date, little is known about the protective effect of neutral lipid fraction (NLF) extracted from the seeds of this plant, against induced liver toxicity.

In this study, we investigated the effect of both TO and NLF of *Nigella sativa* seeds on ethanol induced liver toxicity in rats fed with a specific diet (Lieber-DeCarli liquid diet). By allowing efficient consumption of ethanol, this diet is suitable to investigate metabolic effects of chronic-ethanol feeding, including fetal alcohol syndrome, in rats (Lieber and DeCarli, 1982; Lieber *et al*., 1989).Our results showed a significant decrease of SOD and CAT enzymatic activities. Levels of MDA increased and those of GSH decreased in ethanol liquid diet group of rats.

Treatment with TO and its NLF lead to an obvious improvement on the oxidative stress status markers in both plasma and liver homogenates of rats. A significant increase in both SOD and CAT activities, a

decrease in MDA titer and an increase in GSH level. These results are in agreement with those reported previously where an increased in GSH level, a decrease in MDA level and a clear amelioration in enzymatic activity of SOD in rat model were marked (Kim *et al*., 2008; Seval *et al*., 2014; Hamed *et al*., 2014).

Together these data confirm the protective effect of *Nigella sativa* oil, a result of an increase in the activity of the antioxidant defense systems GSH, MDA, SOD and CAT in both plasma and liver. NLF appeared to be more effective in lowering plasma transaminase activities, and in ameliorating the stress oxidative markers status in both plasma and liver homogenate, with a significant improvement in liver histology following ethanol administration.

TO and NLF are rich in molecules with a large spectrum of action making it difficult to relate the hepatoprotective effect to a single chemical compound. Therefore, deep investigations, at the molecular level, on these two fractions, are required. Effect of TO could be due to its other properties such as anti-inflammatory, immunomodulatory and anti-oxidant that reduce the oxidative stress. The latter is the main cause of different cell damages and is the most important mechanism in hepatotoxicity **(**Houghton *et al.,* 1995). This might be due to the antioxidative nature of the plant components which reported to have significant free radical scavenging properties (Burits, 2000).

CONCLUSION

To our knowledge, this study is the first of its type that investigated the hepatoprotective effect of NLF of *Nigella sativa* seeds against ethanol induced hepatotoxicity in rat model. Results showed that NLF has an important potential as a protective agents of liver against this kind of hepatotoxicity. Probably by decreasing lipid peroxidation and liver enzyme activities and increasing antioxidant defense system activity. These results supported our previously published data where we showed that TO of *Nigella sativa* seeds had a protective effect on rat liver using precision-cut rat liver slices (Sobhi *et al*., 2016).

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