Nigella sativa seed fixed oil reduces cortisol secretion, lipid peroxidation and haemolysis induced by stress in rat

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Abstract

The present study investigates the anti-stress potential of Nigella sativa seed fixed oil (NFO) in rats. In untreated animals with the NFO, immobilization stress induced a significant decrease of lymphocytes percentage and plasma magnesium, and a significant increase of neutrophil to lymphocyte ratio, haemolysis, plasma cortisol (Cor) and erythrocytes malondialdehyde level. However, in pretreated rats with the NFO (0.2 ml/kg BW/d, i.p, for 8 weeks) the same stress induced a slight increase of haemolysis and plasma Cor without any significant modification of the other parameters. Our data suggest the presence of bioactive compounds in the NFO, capable of reducing the Cor secretion and erythrocytes lipid peroxidation resulting in protection of their membrane integrity against haemolysis, partially due to antioxidiant properties. Further elucidation of bioactive molecules and underlying mechanisms could lead to potential intervention in stress.

Citation:

1. Introduction

Nigella (Nigella sativa L.) is a spice plant belonging to the family Ranunculaceae, and it is cultivated in several countries in the Mediterranean regions and Asia (Ghedira, 2006). N sativa seeds contain 32 to 53% (w/w) of fixed oil (Houghton et al., 1994) and are known for their extensive use in the traditional medicine. According to several studies, the extracts of N. sativa seeds have showed bronchodilator, anti-bacterial, anti-ulcer, hypotensive, immunostimulant, anti-tumoral, Antidiabetic, analgesic, anti-inflammatory and insulin-sensitizing properties (Ali and Blunden, 2003; Ghedira, 2006). Other researchs have
reported that the seeds of *N. sativa* are also able to play an antioxidant role both in vitro and in vivo (Ali and Blunden, 2003; Aftab et al., 2013).

It is well known that stress induces an increase in circulating levels of adrenocorticotropic hormone and thus glucocorticoids, including cortisol which is the most reliable biomarker of stress situation (Hashimoto et al., 1988). Hypersecretion of glucocorticoids due to stress is associated with a change in the tissue redistribution of leukocytes as in the skin, and reduces the number of T cells, B cells and monocytes in the blood (Dhabhar, 2008). In addition, stress may decrease the blood levels of reduced glutathione and the activity of erythrocyte superoxide dismutase (Orzechowski et al., 2000) and increase the plasma levels of malondialdehyde (MDA) (Aleksandrovskii et al., 1988).

Oxidative stress (SO) refers to a disruption of cellular metabolic balance in which the generation of oxidants overwhelms the antioxidant defense system. Reactive oxygen species (ROS) have great potential to damage almost all types of cellular components of the body, which explains their involvement in the induction and/or amplification of several pathologies (Lipinski, 2012). The accumulation of ROS results in the appearance of often irreversible cell and tissue damage that the most vulnerable biological targets are proteins, lipids, and deoxyribonucleic acid (Halliwell and Whiteman, 2004; Valko et al., 2006).

On the other hand, due to their high polyunsaturated fatty acids, oxygen and hemoglobin, erythrocytes are highly susceptible to oxidative damage, and provide a reliable cellular model to study cellular changes due to SO (Burak Çimen, 2008). Indeed, exposure of erythrocytes to free radical attack during stress, leads to disruption of their plasma membranes with the release of their cell hemoglobin content whose measurement at 540 nm can test their osmotic fragility (Adenkola and Ayo, 2009; Minka and Ayo, 2010). The alteration of the erythrocyte membrane under the effect of SO, is also marked by lipid peroxidation (LP) of their cell membranes by ROS, responsible for the release of cytotoxic aldehydes such as MDA, which is considered a good indicator of the state of the SO (Mendanha et al., 2012.).

The present study was designed to evaluate the anti-stress potential of the *Nigella sativa* fixed oil (NFO) by analysing some parameters. To evaluate this potential, the neutrophil to lymphocyte ratio (N:L ratio) and plasma levels of magnesium (Mg) and cortisol (Cor) were analyzed. Furthermore, the erythrocytes osmotic fragility (EOF) and the erythrocytes production of malondialdehyde (MDA) were studied.

2. Materials and Methods

In the work reported here, we have used as indicators of stress situations N:L ratio (Browers et al., 2008; Dhabhar, 2008), haemolysis (Al-Naqeeb et al., 2009; Meziti et al., 2012), plasma Mg (Kuzniar et al., 2003; Srivastava et al., 2010) and Cor (Browers et al., 2008; Dhabhar, 2008; Kalaz et al., 2012) levels, and erythrocytes LP (Suboh et al., 2004; Al-Naqeeb et al., 2009). These parameters were analyzed in rats pretreated or untreated with the NFO, then exposed to an acute immobilization stress, and compared to those evaluated in control groups. After blood sampling, Ht, blood cells count and smears (LF and N:L ratio) were studied. Plasma was separated (minerals and Cor levels) and erythrocytes suspension (haemolysis and LP) were prepared after centrifugation. LP was analyzed by MDA production and plasma Mg and Cor were measured by using commercial kits.

2.1 Plant material

The seeds of *Nigella* (*Nigella sativa* L.) were purchased at a local market in the city of Casablanca in Morocco (North of Africa, latitude 33°34'42.44" N, longitude 7°36'23.89" O). After taxonomic identification of the plant, a reference specimen was deposited at the laboratory of physiopathology and molecular genetic, faculty of sciences Ben Msik, Hassan II-Mohammedia university, Casablanca. Seeds of *N. sativa* were mechanically crushed using a blender for 6 min. 80 g of the obtained powder was dissolved in petroleum ether and the fixed oil has been extracted using a Soxhlet extractor at a temperature of 60° C-90° C for 18h. The extract was then concentrated using a rotary evaporator for 30 min. To remove the rest of the ether, the extract was left in an open container for 24 h.

2.2 Animals

Twenty-four male white rats, Wistar strain weighing 186±14 g, in apparent good health and belonging to the pet of the faculty of sciences Ben M’sik, Casablanca (Morocco) were used in this experimentation. The temperature of the pet was maintained at
22±1° C, humidity around 50%±10 and circadian rhythm was 12 h of light and 12 h of darkness. The animals were fed a standard diet. 1 kg of this feed gives 160 g of crude protein, 20 g of fat, 70 g of mineral matter, 305 g of Pi, 9 g of Ca and vitamins A, D and E at the respective doses of 10 000, 1 500 and 10 IU. The food was distributed to the animals daily at 11 am, water was provided ad libitum.

2.3 Distribution and treatment of animals
The animals were randomized into four experimental groups of 6 rats each: two groups (Nigella NS and Nigella S) were intraperitoneally treated daily at 10 am by NFO (0.2 ml/kg BW) for 8 weeks and two groups (NaCl NS and NaCl S) were treated in the same way at the same time, during the same period and with the same volume of physiological saline solution at 0.9%. At the end of treatment, the groups Nigella S and NaCl S were exposed to an acute immobilization stress for 6 h, whereas the groups Nigella NS and NaCl NS were not subjected to any stress, and were considered as controls. Stress was induced for 6h, from 8h to 14h, in the absence of food and water and at an ambient temperature of 18° C, by setting the animals in individual plastic tubes (length, diameter).

2.4 Blood samples
At the end of the experiment, the blood of stressed and unstressed animals was collected by decapitation neck in two EDTA (ethylene diamine tetra acetic acid) tubes, one for the analysis of haematological parameters and the other to prepare a suspension of erythrocytes and isolate the plasma.

2.5 Measurement of haematocrit
The Ht was determined by centrifugation of a precise amount of blood in Ht calibrated tubes (Hettich Haematokrit D-7200), the cell mass/plasma ratio was expressed as % by direct reading on the tube: Ht (%) = [(level cap)/(overall height)]x100.

2.6 Erythrocytes and leucocytes count
By using a Malassez cell (1 mm²), erythrocytes count requires a blood dilution of 1/200 in Marcano solution, whereas for leukocytes count, a dilution of 1/20 in Hayen solution was used. Erythrocytes were counted in 4 rectangles composed of 20 small squares located at the 4 corners of the grid (= N) and the average of four values was m=N/4. As the volume of a rectangle is 10⁻² mm³ and the dilution was 1/200, the final result was: mx100x200 erythrocytes/mm³.

Leukocytes were counted in 5 horizontal bands (=N') and the volume of each was 10⁻¹ mm³. For 5 bands (1/2 mm)³, there was N'x2 leucocytes/mm³ of blood which was diluted at 1/20. So, the final result was: N'x2x20=N'x40 leucocytes/mm³.

2.7 Leukocytes formula
Blood smears were made in the min of collection. They were stained with May-Grünwald-Giemsa, ie 5 min of May-Grünwald and 5 min of Giemsa tenth diluted in water, to determine the FL. To do this we examined the smear by moving slots in the body of the smear (near the tail). Of 100 leukocytes, the percentage of neutrophils (neutrophils, eosinophils, basophils), lymphocytes and monocytes was determined. The neutrophil to lymphocyte ratio (N:L ratio) was calculated as an indicator of stress (Stull and Rodiek, 2002).

2.8 Preparation of the suspension of erythrocytes
The suspension of erythrocytes was prepared according to the procedure mentioned by Dacie and Lewis (1995). The blood was centrifuged at 1500xg for 5 min, the plasma was aliquoted and stored at -20° C until biochemical assays. The pellet was washed 3 times with a solution of phosphate buffered saline (NaCl 150 mM, NaH₂PO₄ 1.9 mM, Na₂HPO₄ 8.1 mM, pH 7.4). During each washing, the suspension of red cells obtained was homogenized with a simple twist of the tube, the supernatant and the interface layer was removed immediately after centrifugation. The pellet of the last centrifugation was added to the same phosphate buffer to obtain a 50% Ht. Suspensions of erythrocytes were prepared aliquoted and stored at -80° C until the osmotic fragility test and evaluation of LP.

2.9 Test of erythrocytes osmotic fragility
The test of osmotic fragility (Oyewale, 1993; Oladele et al, 2003) was performed after an incubation period (30 min at 37° C) of 0.2 ml of the suspension of erythrocytes in numbered Eppendorf tubes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 containing 1.8 ml of NaCl solution (pH 7.4) respectively at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 g/L. The tubes were then centrifuged at 1500xg for 10 min, and the absorbance (OD) of the supernatant at 540 nm was measured. The percentage of erythrocytes haemolysis (H%) was calculated using the following formula (Fraukner and King, 1970): H% = (DO of erythrocytes in NaCl/DO of erythrocytes in pure water)x100.
2.10 Evaluation of lipid peroxidation in erythrocytes

Lipid peroxidation was determined indirectly by measuring the production of MDA in erythrocytes by the method of Gilbert et al. (1984). In short, trichloroacetic acid 10% (w/v) was added to 1.5 ml of the suspension of erythrocytes and the whole was centrifuged for 10 min at 300xg. 1 ml of thiobarbituric acid (TBA 1% in NaOH 0.05 M) was added to the supernatant. The mixture was heated to 95°C for 30 min. After cooling to 4°C, 1 ml of n-butanol was added for extract the MDA-TBA complex. MDA concentration (µmol/L) was obtained by subtracting 20% of the absorbance at 453 nm from that measured at 532 nm using the molar extinction coefficient of 1.56x10^5 M^-1 cm^-1.

2.11 Minerals and cortisol analysis

Minerals (Ca, Pi and Mg) were analyzed using commercially available kits (CHRONOLAB AG Baarerstrasse 57, 6302 ZUG, Switzerland). Cor was analyzed in plasma by radioimmunoassay at the National Center of Energy, Sciences and Nuclear Techniques Maâmoura (Morocco), using commercial kits (DIAsource Immunoassays SA, Nivelles, Belgium).

2.12 Statistical analysis

The results were presented as mean more or less the standard deviation (X±SD). The significance of observed differences between animals was simultaneously determined by one-way analysis of variance followed by the Mann-Whitney test. The probability value p<0.05 was assumed significant.

3. Results and Discussion

3.1 Haematological parameters

In the untreated rats with the NFO (NaCl S), the subjection to an acute immobilization stress induced a significant (P<0.05) decrease of lymphocytes percentage and a significant (P<0.05) increase of N:L ratio by comparison with those measured in unstressed ones (NaCl NS) (respectively 49±4 vs 60±5 and 0.77±0.07 vs 0.51±0.06) (Table 3.1). On the contrary, in the pretreated groups with NFO (Nigella S), the stress induced no change of these parameters by comparison with control animals (Nigella NS) (Table 3.1). In treated and untreated rats, stress immobilization induced no significant change in Ht, number of erythrocytes and leukocytes, and percentage of neutrophils, eosinophils, basophils and monocytes by comparison with those observed in unstressed ones (Table 3.1).

Blood leukocyte populations are known to change under stress, so, several studies had used neutrophil and lymphocyte counts as an indicator of different stress situations (Browers et al., 2008; Dhabhar, 2008). In mice, Browers et al. (2008) had observed that stress induced by restraint, low temperature, forced swim, handling or isolation, reduced the lymphocyte count. This reduction is not an indication of cell death; rather, it reflects a redistribution of these cells to different organism areas. In fact, acute stress has been suspected to modulate the immune cell distribution as an adaptive response (Dhabhar, 2008). Furthermore, several authors had hypothesized that during stress, leukocytes traffic out of the blood and into the skin, lymphonodes, and bone marrow in order to prepare the organism for any injury or infection (Dhabhar, 2008).

The responses of the immune cell distribution could vary with the type, intensity and duration of the stress. An acute restraint stress didn’t change the leukocytes count (Suresh and Koner, 2012) and reduces T lymphocyte proliferation (Zafir et al., 2009) in rat, and markedly reduced spleen lymphocyte number and caused spleen atrophy in mice (Li et al., 2012). Whereas, a significant decrease of leukocytes count under a chronic restraint stress was observed in rat (Suresh and Koner, 2012; Moazzam et al., 2013).

In addition, in mice, restraint, low temperature, isolation, and handling resulted in more stress-induced changes in lymphocytes and monocytes than forced swim (Browers et al., 2008).

The dose of NFO used in the work reported here induced no alteration of haematological profile in the pretreated rats and was able to protect them against stress alterations, so, it may be considered as non-toxic. The Lethal Dose 50 of the same oil administered intraperitoneally in mice is 2.06 ml/kg BW (Zaoui et al., 2002). Furthermore, no toxic lesions were observed following administration of the NFO at an oral (10 ml/kg) (Khanna et al., 1993) or intraperitoneal (0.5 ml/kg) (El Khasmi et al., 2011) doses. According to Zaoui et al. (2002), in rats treated daily with an oral dose of 1 ml/kg BW of the NFO for 12 weeks, erythrocytes and leukocytes count, and Ht didn’t change during the first 8 weeks of treatment.

3.2 Haemolysis

Osmotic fragility curve expressing the correlation between the percentage of...
Stress immobilization used here may increase haemolysis in each cup and the corresponding salt concentration showed a sigmoid configuration (Fig. 3.2). In unstressed animals (controls), those pretreated with NFO (Nigella NS) showed a resistance of erythrocytes to haemolysis in hypotonic salt solutions (0.7, 0.6, 0.5 and 0.4%), significantly (p<0.05) higher when compared to that observed in control animals (NaCl NS) (Fig. 3.2). Our results showed that immobilization stress caused an haemolysis of erythrocytes in hypotonic salt solutions (0.8, 0.7, 0.6, 0.5 and 0.4%), significantly (p<0.05) higher in untreated animals (NaCl S) than that measured in treated ones (Nigella S) (Fig. 3.2).

Table 3.1: Erythrocytes (RBC) and leukocytes (WBC) count, haematocrit (Ht), leukocytes formula and neutrophil to lymphocyte ratio (N:L ratio) in rats subjected to immobilization stress after treatment with Nigella sativa seeds oil (0.2 ml/kg BW/d) for 8 weeks intraperitoneally. Mean ± SD, *P<0.05; comparison of stressed animals: treated (Nigella S) and untreated (NaCl S).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animal groups (n=6)</th>
<th>NaCl NS</th>
<th>NaCl S</th>
<th>Nigella NS</th>
<th>Nigella S</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (x10⁶/ml)</td>
<td>5.28± 1.12</td>
<td>7.11± 1.12</td>
<td>5.73± 1.16</td>
<td>6.54± 1.21</td>
<td></td>
</tr>
<tr>
<td>WBC (x10⁶/ml)</td>
<td>7.67± 1.65</td>
<td>6.42± 1.74</td>
<td>7.56± 1.34</td>
<td>6.12± 1.43</td>
<td></td>
</tr>
<tr>
<td>Ht (%)</td>
<td>39.82± 3.46</td>
<td>45.22± 3.44</td>
<td>40.65± 3.12</td>
<td>43.23± 3.45</td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>31±3</td>
<td>38±4</td>
<td>33±3</td>
<td>37±4</td>
<td></td>
</tr>
<tr>
<td>E (%)</td>
<td>3±1</td>
<td>5±1</td>
<td>4±1</td>
<td>4±1</td>
<td></td>
</tr>
<tr>
<td>B (%)</td>
<td>0.5±0.1</td>
<td>0.4±0.1</td>
<td>0.5±0.1</td>
<td>0.5±0.1</td>
<td></td>
</tr>
<tr>
<td>L (%)</td>
<td>60±5</td>
<td>49±4*</td>
<td>58±5</td>
<td>54±4</td>
<td></td>
</tr>
<tr>
<td>M (%)</td>
<td>5±1</td>
<td>7±1</td>
<td>4±1</td>
<td>4±1</td>
<td></td>
</tr>
<tr>
<td>N:L ratio</td>
<td>0.51± 0.06</td>
<td>0.77± 0.07*</td>
<td>0.56± 0.07</td>
<td>0.68± 0.07</td>
<td></td>
</tr>
</tbody>
</table>

NaCl NS: untreated and unstressed group.
Nigella NS: treated and unstressed group.

Figure 3.2: Haemolysis in hypotonic solutions of NaCl in rats subjected to immobilization stress after treatment with Nigella sativa seeds oil (0.2 ml/kg BW/d) for 8 weeks intraperitoneally. Mean±SD, *P<0.05; comparison of stressed animals (Nigella S) and untreated (NaCl S). #P<0.05; comparison of unstressed animals: treated (Nigella NS) and untreated (NaCl NS). Thus, causes oxidative damage of the membrane proteins and lipids then induces haemolysis (Hanzawa and Watanabe, 2000). Immobilization stress increases haemolysis, changes in blood pH and muscle consumption of oxygen (Szygula, 1990) resulting a free radical production (Filipovic et al., 2010), then, an erythrocytes destruction and LP (Tauler et al., 2003).

In the work reported here, the NFO showed a significant protection against haemolysis induced by immobilization stress. Exposure of rats used here to stress immobilization may impair antioxidant defenses which lead to oxidative damage of erythrocytes, but a pretreatment with the NFO was able to exhibit a protective effect against this damage traduced by haemolysis.

Several studies had reported of the Nigella seeds some protective effects on erythrocytes. In mice and rats, treatment with N. sativa seeds extract significantly protects from cisplatin-induced haemolysis (Nair et al., 1991; El-Daly, 1998). In the same context, in vitro studies had showed that N. sativa seeds extracts protect erythrocytes against increased haemolysis caused by the hydrogen peroxide (Suboh et al., 2004; Al-Naqeeb et al., 2009). In addition, treatment of mice for 21 days with...
NFO (2 ml and 4 ml/kg BW/d per.os) has caused a significant delay of haemolysis (Meziti et al., 2012). Similar results were obtained by Kökdil et al. (2006) which showed that oral administration of the fixed oil extracted from *Nigella unguicularis*, *Nigella orientalis* and *Nigella segetalis* seeds at a dose of 1 ml/kg in rats during 4 weeks, resulting in improved blood parameters.

### 3.3 Minerals, cortisol and malondialdehyde

In untreated rats, immobilization stress induced a significant (P<0.05) decrease of plasma Mg level (mM) by comparison with that observed in unstressed rats (0.4±0.1 vs 0.8±0.1) (Fig. 3.3A). However, in all animal groups (treated and untreated), the same stress didn’t induce any significant change in plasma levels of Ca and Pi, by comparison with those observed in unstressed groups (Fig. 3.3A).

**Figure 3.3A:** Plasma levels of calcium, phosphorus and magnesium in rats subjected to immobilization stress after treatment with *Nigella sativa* seeds oil (0.2 ml/kg BW/d) for 8 weeks intraperitoneally. Mean±SD, *P<0.05; comparison of stressed animals: untreated (NaCl S) and treated (Nigella S). NaCl NS: untreated and unstressed group. Nigella NS: treated and unstressed group.

Our results showed that in untreated animals, plasma Cort concentrations (ng/ml) and erythrocytes MDA levels (µmoles/L) were significantly (P<0.05) higher in stressed animals (NaCl S) than those measured in unstressed ones (NaCl NS) (respectively 560±40 vs 240±50 and 13.5±0.5 vs 10.1±0.4) (Table 3.3B). In addition, in stressed animals, those pretreated with NFO (Nigella S) showed a plasma levels of Cor and erythrocytes MDA concentrations (µmoles/L) significantly (p<0.05) lower than those observed in untreated rats (NaCl S) (respectively 340±60 vs 240±50 and 11.1±0.3 vs 13.5±0.5) (Table 3.3B).

**Table 3.3B:** Plasma cortisol (ng/ml) and malondialdehyde (MDA) (µmoles/L) level in untreated and treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NaCl NS</th>
<th>NaCl S</th>
<th>Nigelle NS</th>
<th>Nigelle S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol</td>
<td>240±50</td>
<td>560±40</td>
<td>210±50</td>
<td>340±60</td>
</tr>
<tr>
<td>MDA</td>
<td>10.1±0.4</td>
<td>13.5±0.5</td>
<td>9.6±0.4</td>
<td>11.1±0.3</td>
</tr>
</tbody>
</table>

The decrease of plasma Mg and the increase of plasma Cor under stress, may be implicated in the oxidant stress leading to an erythrocytes MDA production by LP, then an increase of haemolysis observed in our untreated, then stressed rats. In fact, Mg intake, body Mg storages and blood and intracellular Mg have been connected with the antioxidant protection of lipids from peroxidation together with vitamins C and E and selenium (Haenni et al., 1998). In mice, hypomagnesemia lowers the activity of superoxide dismutase and catalase, glutathione S-transferase and glutathione reductase activities in erythrocytes (Kuznir et al., 2003). In addition, Mg showed favorable effects on haematological and other biochemical parameters under oxidative stress induced by arsenic poisoning in humans (Srivastava et al., 2010).

The increase in plasma Cor level by an acute immobilization stress observed in untreated rats with the NFO corresponds with previous reports in mice (Browers et al., 2008; Dhabhar, 2008). Similarly, circulating corticosterone levels were observed to increase by subjection to immobilization stress in rat (Kalaz et al., 2012) and mice (Tsoi et al., 2011). These levels could vary with the type of the stress. Thus, in mice, forced swim elicited significantly higher corticosterone concentrations than isolation or handled stressors (Browers et al., 2008).

Immobilization stress used in our work, is able to activate the hypothalamic–pituitary–adrenal axis (HPA axis) and induce both psychological (escape reaction) and physical (muscle work) stress as a result of restricted mobility and aggression. The release of glucocorticoids is an indicator for the activation of the HPA axis (Herman et al., 2005) and is able to suppress immune system (Al-Naqeeb et al., 2009).
During stress, the hypothalamus receives direct stimulation of the limbic system and stimulation from the noradrenergic locus coeruleus and nucleus of the solitary tract. In response to these stimuli, the hypothalamus releases the corticotropin-releasing factor (CRF) in the hypothalamo-pituitary portal system for activate the adenohypophysis. This gland responds by secreting the corticotrope hormone (ACTH) which stimulates the synthesis and the release of glucocorticoids by adrenal glands (Herman et al., 2005). The reduction of Cor secretion under immobilization stress observed in our pretreated rats with the NFO, may be explained at least in part by an inhibitory action of this oil via the HPA axis, or the adrenal gland. To discriminate between these hypothesis, an analysis of CRF and ACTH should be needed.

In untreated rats with NFO, the high plasma levels of circulating Cor during immobilization stress may be involved in LP resulting in alteration of erythrocyte membrane then elevated haemolysis. In fact, in rat, three-fold Cor injection (25 mg/kg, once per day) potentiated stress-induced changes in the processes of free radical oxidation of lipids in the hypothalamus (Flerov and V'iushina, 2011). In addition, it has been reported in mammals that administration of glucocorticoid hormones caused LP by decreasing the nonenzymatic antioxidant capacity and suppressing the enzymatic antioxidant systems in the liver (Ohtsuka et al., 1998), erythrocytes (Orzechowski et al., 2000), skeletal muscle and lymphoid organs (Pereira et al., 1999).

In pretreated rats with the NFO, erythrocytes showed a significant protection against LP then haemolysis under stress immobilization.

In this investigation, we have evaluated LP of erythrocytes by MDA production which was reduced by pretreatment with the NFO in stressed rats. MDA is a major end product of polyunsaturated fatty acids peroxidation and is often used as an indicator of cell injury. Increase in the production of MDA may be due to the formation of reactive oxidants which leads to structural changes of the lipid molecules, and the changes are more severe as lipids are the main constituent of biological membranes.

In a series of in vitro tests on Nigella seeds antioxidant activity, Burits and Bucar (2000) had demonstrated that NFO acts as a donating agent in the diphenylpicrylhydrazyl assay and possessed hydroxyl radical scavenging properties in both the assay for non-enzymatic lipid peroxidation and the deoxyribose test.

In the same context, in vitro studies had showed that N. sativa seeds extracts protect erythrocytes against LP and protein degradation caused by the hydrogen peroxide (Suboh et al., 2004; Al-Naqeeb et al., 2009). In fact, the plasma contains a network of endogenous antioxidants such as (albumin, bilirubin, reduced glutathione and uric acid) as well as exogenous antioxidants derived from food. These antioxidants may act in a complementary and synergistic manner to provide a better protection against oxidant stress. The increase in plasma antioxidant capacity of our treated rats may be attributed to the elevated levels of exogenous antioxidants acquired following the chronic treatment of our rats with the NFO, such as phenolic compounds and flavonoids (Alzoreky and Nakahara, 2001; Mezit et al., 2012).

Phenolic compounds and flavonoids are recognized as potentially antioxidant substances with the ability to scavenge free radical species (Kumar et al., 2008) and to chelate free metal ions (iron, copper) (Morris et al., 1995; Brown et al., 1998). Mezit et al. (2012) reported that chloroform extract of N. sativa seeds contained the highest amount of total phenolic (Gallic acid equivalent GAE) and flavonoids (Quercelin equivalent QE) compounds (81.31 µg GAE/mg, 5.20 QE/mg) followed by ethyl acetate extract (72.43 µg GAE/mg, 4.19 µg QE/mg), methanol extract (33.64 µg GAE/mg, 3.80 µg QE/mg) and water extract (27.07µg GAE/mg, 2.45 µg QE/mg) while the hexane extract contains only (15.95 µg GAE /mg, 0.43 µg QE/mg). According to Alzoreky and Nakahara (2001), the antioxidant activity (Trolox Equivalent Antioxidant Capacity TEAC) and total phenolics of methanolic extracts of N. sativa seeds from Yemen are 1.1 mM/g dry weight and 0.1 mg/g respectively. In addition, Thymoquinone as one of the major compounds of N. sativa seeds oil, is able to inhibit the generation of O2• radical by xanthine/xanthine oxidase without having any effect on the activity of the enzyme (Badary et al., 2003). Furthermore, the NFO and its fractions (neutral lipids, glycolipids and phospholipids) are able to show a potent in vitro scavenging activity (Suboh et al., 2004). On the other hand, several extract of N. sativa seeds are able to
prevent radical formation and lipid oxidation of β-carotene (Meziti et al., 2012).

Conclusion

From the obtained data in the present work, we can suggest that NFO may have a considerable anti-stress activity by reducing Cor secretion, LP and haemolysis. Further research on the action of the NFO in the central nervous system, elucidation of bioactive molecules and underlying mechanisms against LP could lead to potential intervention in stress. In this context, further studies should be carried out on human to confirm the anti-stress and antioxidant efficacy of NFO.

Author’s Contribution

M.A., carried out the experiments, made calculations, participated in the discussion of results, and the writing. M.F., made the contribution for design, analysis and interpretation of data. N.E., carried out the RIA dosage of cortisol. A.B., calculations, presentation and statistical analysis of results. M.E., analysis, discussion and final writing.

Competing Interests

The authors have non-financial competing interests in an exclusive academic way.

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