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FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN
2217-5369
(print version ceased in 2023)
2217-5660 (online)

UDK 582.795.13:547.915:616.831
DOI: 10.5937/ffr0-56751

Original research paper

PREVENTIVE EFFECT OF *NIGELLA SATIVA* L. AGAINST CEREBRAL OXIDATIVE ALTERATIONS INDUCED BY A HIGH-FAT, IRON-ENRICHED DIET IN MICE

Halima Benabdallah[*1,2], Rachida Benaraba[1,2], Mostefa Belarbi[3], Hemida Houari[4]

[1]University of Tiaret, Laboratory of Research on Local Animal Products, 14000 Tiaret, PO Box 78 Zaaroura, Algeria
[2]University of Tiaret, Faculty of Natural and Life Science, 14000 Tiaret, PO Box 78 Zaaroura, Algeria
[3]University of Tiaret, Laboratory of Mathematics and Computer Science, 14000 Tiaret, PO Box 78 Zaaroura, Algeria
[4]University of Tiaret, Institute of Veterinary Sciences, 14000 Tiaret, PO Box 78 Zaaroura, Algeria

Abstract: Recent research underscores the pivotal role of oxidative stress in cerebral alterations, prompting investigations into strategies such as the utilization of natural substances derived from medicinal plants, including *Nigella sativa* L. (NS) for their therapeutic potential. NS is known for its bioactive compounds that play a significant role in disease prevention and treatment. This study employed Fourier Transform Infrared Spectroscopic (FT-IR) analysis to identify functional groups and compounds in NS seed and to examine its preventive effects on cerebral oxidative changes induced by a high-fat diet (HFD) supplemented with iron in NMRI mice. The focus was on lipid oxidation, Ferric Reducing Antioxidant Power (FRAP), thiol groups, catalase activity, and Reduced Glutathione (GSH) levels. Mice were randomly assigned to four experimental groups (six mice per group): control (ST), control + NS seed powder (ST+NSP), high-fat diet with $FeCl_3$ (HFD/Fe^{3+}), and HFD/Fe^{3+} + NSP. Following an 11-week experimental period, lipid oxidation, FRAP, thiol groups, and CAT activity were measured in plasma and brain, while GSH levels were assessed exclusively in the brain. NS significantly reduced lipid peroxidation in HFD/Fe^{3+} mice and restored FRAP, thiol groups, CAT activity, and GSH levels, which were markedly reduced in HFD/Fe^{3+} mice compared to the ST group. The HFD/Fe^{3+} regimen increased lipid peroxidation products relative to the ST group. These findings suggest that NSP supplementation mitigates cerebral oxidative stress and enhances antioxidant enzyme activity.

Key words: *black cumin, lipid oxidation, oxidative stress, hyperlipidic diet, $FeCl_3$, brain alterations*

INTRODUCTION

The emergence of oxidative stress as a metabolic disorder is attributed to changes in lifestyle, social and economic conditions, and increased exposure to environmental pollutants and toxins. This chronic, multifactorial, and complex phenomenon involves both genetic and environmental influences. Oxidative stress is associated with numerous health conditions, including diabetes, hypertension, cardiovascular diseases, kidney disease, cancer, and neurodegenerative disorders (Mamun et al., 2019). It is characterized by an overproduction

Corresponding author: Phone: +213782030641
E-mail address: halima.benabdellah@univ-tiaret.dz

of reactive oxygen species (ROS), which are toxic due to their potential to damage vital cellular components such as lipids, proteins, and DNA, ultimately leading to cell death (Bonnefont-Rousselot, 2014). Neurons are particularly susceptible to oxidative stress because of their high oxidative metabolism, rich lipid content, and dependence on astrocytes for antioxidant support (Ré, Nafia, Nieoullon, Le Goff & Had-Aissouni, 2005; Chen, Guo & Kong, 2012). Oxidative stress can lead to neuronal death in degenerative diseases -both acute (ischemia, trauma) and chronic (including amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and Huntington's disease) (Ré et al., 2005).

The brain's high oxidative metabolism results in substantial ROS production (Juurlink & Paterson, 1998). Additionally, the brain contains large amounts of iron, which can catalyse ROS production (Ré et al., 2005). The brain is also abundant in polyunsaturated fatty acids, making it susceptible to lipid peroxidation. Furthermore, antioxidant enzyme activity in the brain is significantly lower than in the liver or kidneys (Ré et al., 2005). In the brain, GSH is the primary defence against ROS. However, GSH synthesis in neurons is heavily reliant on metabolic exchanges with astrocytes, and any disruption in the supply of GSH precursors from glial cells could compromise the neurons' antioxidant defences. Moreover, as neurons are post-mitotic cells, they are generally irreplaceable if they suffer irreversible damage (Ré et al., 2005).

A commonly accepted definition of this cerebral alteration involves the progressive degeneration of neurons, typically affecting a specific subset of nerve cells. This phenomenon can arise from various factors, including genetic, environmental, biological, autoimmunity, and ageing (Gaeta & Hider, 2005).

Recent evidence indicates a significant association between a high-fat diet and oxidative stress in brain alteration. In experimental models, an HFD is frequently employed to induce dietary-related oxidative stress (OS). HFD not only impact the immune system by altering inflammatory responses but also promotes adiposity (Gil-Cardoso et al., 2017). Furthermore, HFD has been demonstrated to induce changes in gut microbiota composition, increase intestinal permeability and impair mucosal barrier function (Custers & Kiliaan, 2022). Prolonged consumption of a diet high in saturated fats is recommended as a model for inducing OS, as it significantly reduces hepatic antioxidant enzyme activity and elevates lipid peroxidation (LPO) levels (Chung, Gurtu, Chakravarthi, Moorthy & Palaniasamy, 2018). Moreover, HFD can directly affect the brain by increasing the permeability of the blood-brain barrier (BBB), allowing dietary lipids and inflamematory molecules to penetrate the central nervous system (Custers & Kiliaan, 2022). This promotes the infiltration of free fatty acids and lipopolysaccharides (LPS) into the brain, which subsequently triggers neuroinflammation and oxidative stress (Chianese et al., 2018; Melo, Santos & Ferreira, 2019). Once inside, dietary lipids, particularly saturated and trans fats, integrate into neuronal membranes, altering their composition and fluidity (Custers & Kiliaan, 2022). These alterations can disrupt membrane-bound receptor function, impair synaptic signalling, and modify lipid raft organization, all of which are crucial for neurotransmission and cognitive processes. Consequently, HFD-induced modifications to the BBB and neuronal membrane composition contribute to neurodegenerative mechanisms and cognitive decline (Custers & Kiliaan, 2022; Melo et al., 2019).

In addition to high-fat consumption, excessive dietary iron intake, often linked to overconsumption of meat, may also enhance prooxidant effects (Hininger-Favier, Osman, Roussel, Intes & Montanari, 2016). Transition metal ions, including iron, are recognized as crucial catalysts in the initial production of free radicals (Hininger-Favier et al., 2016). Concerning this potential prooxidant effect, high iron levels have been associated with an increased risk of type 2 diabetes, cardiovascular disease, and brain alterations (Berg &Youdim, 2006; Chen, Kung & Gnana-Prakasam, 2022).

Iron is indispensable for life and essential for brain function, serving as a key cofactor in vital biological reactions. However, the same electron transfer properties that render iron indispensable can also lead to toxicity when moderated by OS (Berg &Youdim, 2006). This dual capability of iron to accept and donate electrons facilitates the formation of ROS through Fenton and Haber-Weiss reactions. Specifically, Fe^{3+} and Fe^{2+} ions react with superoxide and hydrogen peroxide (H_2O_2), gene-

rating hydroxyl free radicals. These highly reactive radicals play a significant role in the pathogenesis of neurodegenerative diseases (Berg &Youdim, 2006). In this context, the human diet is crucial in mitigating the onset of OS. Recent trends highlight the integration of natural products into daily nutrition. Notably, nutraceuticals derived from botanical sources have attracted considerable interest as dietary supplements, offering potential benefits for the prevention of chronic diseases (Alu'datt et al., 2024) with minimal side effects. Consequently, numerous studies have focused on introducing natural products with antioxidant and free radical-scavenging properties. Among these, *Nigella sativa* L. is acknowledged in traditional medicine as an adaptogenic herb with disease-preventing benefits (Gholamnezhad, Keyhanmznesh & Boskabady, 2015). Furthermore, researchers have identified *NS* and its compounds as promising candidates for addressing both physiological and neurological disorders (Cascella et al., 2018). NS, commonly known as black cumin, belongs to the *Ranunculaceae* family and has a long-standing history in both medicine and religion. Its seeds often referred to as black seeds, have been investigated for their therapeutic properties (Balbaa, El-Zeftawy, Ghareeb, Taha & Mandour, 2016). Clinical and animal studies have demonstrated that NS provides a wide range of benefits, including bronchodilation, immunomodulation, antibacterial effects, hypotension reduction, antidiabetic activity, hepatoprotection, gastroprotection, antihistaminic effects, as well as antioxidative and neuroprotective properties (Desai, Saheb, Das & Haseena, 2015; Tavakkoli & Hosseinzadeh, 2020; Jarmakiewicz-Czaja, Zielińska, Helma, Sokal & Filip, 2023).

In this study, an HFD enriched with iron was used as an original animal model to induce OS, stimulating certain aspects of deleterious dietary habits and serving as an *in vivo* inducer of oxidative stress. This approach is grounded in the well-established relationship between HFD, iron overload, and OS. To the best of the authors' knowledge, no prior study has explored the potent neuroprotective effect of *N. sativa* seed powder (NSP) in oxidative stress induced by an HFD enriched with iron. In this regard, the objective of the present study was to elucidate the antioxidant and neuroprotective effects of NSP in OS by incorporating it into an HFD enriched with $FeCl_3$.

MATERIALS AND METHODS

Chemicals

To conduct this study, tripyridyl-s-triazine (TPTZ), N-acetylcysteine (NAC), 2,6-Di-tert-butyl-4-methylphenol (BHT), 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), 1,1, 3, 3-tetraethoxypropane (TEP) and trichloroacetic acid (TCA), were procured from SigmaAldrich Company, St. Louis, MO, USA. All other chemicals used in this study were of high quality.

Plant materials

N. sativa seeds were purchased from a local herb store in Tiaret, Algeria. The seeds were subsequently ground into a fine powder (NSP) using an electric grinder (Fritsch, Germany). The NSP was used as a supplement in the experimental diet and for Fourier Transform Infrared Spectroscopic (FT-IR) analysis**.**

N. sativa seed powder (NSP) concentration

NSP was incorporated into the HFD at a concentration of 4 g per 100 g of feed, based on a preliminary meta-analysis which included the study by Kehili, Saka and Aouacheri (2018) that investigated the effects of an experimental diet with 5% of *N. sativa* powder on oxidative stress induced by cadmium chloride.

$FeCl_3$ concentration

The integration of $FeCl_3$ into the HFD aims is to expedite the onset of OS. This compound was incorporated at a concentration of 32 mg per 100 g of the diet, as documented by Hininger-Favier et al. (2016).

Animals, housing conditions and diets

This study involved twenty-four healthy adult male NMRI mice, aged 6 to 8 weeks and weighing between 25 and 30 g, sourced from the Pasteur Institute of Algiers, Algeria. Upon arrival, the mice were randomly allocated into four groups, each comprising six mice, and housed individually in cages within the animal room of the Clinical Autopsy Laboratory at the Institute of Veterinary Sciences, University of Tiaret, Tiaret, Algeria. The environmental conditions were meticulously controlled, maintaining a temperature of 24 ± 2 °C, a 12-hour light/dark cycle, and a relative humidity of 60 ± 10%. During a 2-week acclimatization period, the mice had *ad libitum* access to food

and water. Following this period, to investigate the effects of NSP supplementation in the high-fat $FeCl_3$ diet, the mice were divided into four groups (six mice per group) as follows:

G1 (ST): Animals in this group received a standard diet (Tab. 1).

G2 (ST+NSP): Animals in this group received a standard diet supplemented with 4% of *Nigella sativa* powder (Table 1).

G3 (HFD/Fe^{3+}): Animals in this group were fed an HFD consisting of 48% animal fat derived from ovine sources, supplemented with 0.032% $FeCl_3$ (Table 1).

G4 (HFD/Fe^{3+}+NSP): Animals in this group were fed an HFD consisting of 48% animal fat of ovine origin, supplemented with 0.032 % $FeCl_3$ and 4% of NSP (Tab. 1).

The mice were housed in polyethylene cages with wood shavings as bedding, which were replaced weekly throughout the study. After 11 weeks, the mice underwent an overnight fasting period before being sacrificed. On the day of sacrifice, all four groups were humanely euthanized using an anaesthetic overdose. Blood samples and specific organs, including the brain, were collected and stored at -20 °C until analysis.

Throughout the study, all animals received specialized veterinary care in accordance with the internationally accepted guidelines of the European Union on Animal Care (CEE Council 86/609 (CEC, 1986) and Directive 63/2010 on the protection of animals used for scientific purposes) (European Union, 2010). The experimental procedures were carried out in the animal house of the Veterinary Institute in Tiaret, Tiaret, Algeria, under the supervision of veterinarians. The authors are affiliated with the Algerian Association of Sciences in Animal Experimentation (AASEA) (Agreement Number: 45/DGLPAG/DVA.SDA.14).

Fourier Transform Infrared Spectroscopic (FT-IR) analysis

An ALPHA-p BRUKER FT-IR spectrometer was employed for the analysis. To prepare the samples, 100 mg of dispersive KBr (potassium bromide) was mixed with 0.5 to 2 mg of the material to create the finished pellets, which were then placed in the sample holder. During the measurement, FT-IR spectra were obtained in the frequency range of 400–4000 cm.

Assessment of oxidative stress markers in plasma and brain

Brain tissue samples (0.2 g) were homogenized in 2 mL of 1X PBS buffer (pH 7.4) using an UltraTurrax T25 (Janke & Kunkel GmbH & Co. KG, IKA Labortechnik, Staufen, Germany). The homogenate was then centrifuged at 3.000 rpm for 10 minutes at 4 °C. The supernatant was collected, incubated on ice for 1 hour, and subsequently stored at −20 °C until further analysis. These samples were used to assess oxidative stress markers as outlined below.

Determination of total antioxidant power using the FRAP technique

The assessment of ferric reducing antioxidant power in plasma and brain homogenates was conducted using the method outlined by Benzie and Strain (1996). The FRAP assay is a colorimetric method that measures the change in absorbance at 593 nm, which occurs due to the reduction of Fe^{3+} to Fe^{2+}, forming a blue Fe^{2+}-tripyridyltriazine complex. This reduction is facilitated by antioxidants that donate electrons. The procedure involves the use of the TPTZ reagent (Sigma Aldrich, Germany).

Table 1.
Composition (g/100g diet) of the mice diets used in this study

	Type of diets			
Diet composition (g/100g diet)	**Standard diet (ST)**	**Standard diet +NSP**	**High-fat diet (HFD)**	**High-fat diet + NSP**
Fat	8.05	8.05	48.00	48.00
Carbohydrates	45.36	45.36	6.10	6.10
Proteins	19.07	19.07	20.00	20.00
$FeCl_3$	/	/	0.032	0.032
NSP	/	4.00	/	4.00
Energy value (kcal/100g diet)	330.2	330.2	591.9	591.9

NSP-*N. sativa* powder

A standard curve was obtained from a stock solution of $FeSO_4$, $7H_2O$ at 1 mM. Subsequently, 100 µL of the sample solution was mixed with 900 µL of FRAP solution. Absorbance readings were taken using a UV/VIS Spectrophotometer (Shimadzu, UV -1202, Japan) at 593 nm, following 30-minute incubation at 37°C.

Determination of thiol groups

The quantification of thiol groups, which serves as an indicator of protein oxidation in plasma and brain homogenates, was performed using the method described by Ellman (1959). In the presence of thiol groups, Ellman's reagent (5,5'-dithiobis (2-nitrobenzoic acid)) is reduced, resulting in the formation of colored aromatic thiol derivatives (5-thio-2-nitrobenzoic acid) with absorbance between 412 and 415 nm. A standard curve was prepared using a solution of NAC (1 mM). A sample of 250 µL was combined with 750 µL of phosphate buffer (pH=8) and 250 µL of DTNB (Sigma Aldrich, Germany). The mixture was incubated in the dark for 15 minutes and the absorbance was measured at 412 nm using a Shimadzu spectrometer.

Determination of reduced glutathione (GSH) concentration

The concentration of reduced glutathione (GSH) in brain tissue homogenates was determined using the colorimetric method described by Ellman (1959). GSH reacts with DTNB (5,5'-dithiobis (2-nitrobenzoic acid)) to form TNB^-, a yellow chromophore. The rate of TNB^- formation is measured spectrophotometrically at a wavelength of 412 nm. A standard curve was generated using a solution of reduced GSH (1 mM). Subsequently, a sample of 250 µL was added to 750 µL of phosphate buffer (pH=8) and 250 µL of DTNB (Sigma Aldrich, Germany). The mixture was incubated in the dark for 15 minutes, and the absorbance was recorded at 412 nm using a Shimadzu spectrometer.

Determination of MDA levels

The quantification of malondialdehyde (MDA) levels was conducted in both plasma and brain homogenates. This method employs an indirect strategy to assess the effects of free radicals by measuring substances formed from lipid peroxidation, which react with TBA. The measurement of MDA serves as an indicator of lipid peroxidation and oxidative stress. This assay followed the protocol established by Yagi (1976). Specifically, 800 µL of a mixture containing TBA at 0.375% (w/v), TCA at 20%, BHT at 0.01% and hydrogen chloride (HCl) 1N were added to 200 µL of the sample solution.

After stirring for 2 minutes, the mixture was incubated in a water bath at 100 °C for 15 minutes. To terminate the reaction, the tubes were placed in ice, and the resulting complex was extracted with butanol for 2 minutes. Following centrifugation at 4000 rpm for 10 minutes at 4 °C (Sigma, 3K10, Laborzentrifugen, Germany), the supernatant was collected, and the absorbance of the pink chromogen was measured at 532 nm using a UV/visible light spectrophotometer (Shimadzu 1202, Japan). Additionally, the plasma and brain MDA concentrations were calculated using a standard curve of TEP.

Estimation of catalase activity (CAT)

Catalase activity was assessed using the method described by Aebi (1984), with minor modifications. For this assay, 20 µl of haemolysate or homogenate was added to 1255µl of phosphate buffer, and the reaction was initiated by the addition of 725 µl of H_2O_2 at 54 mM. The blank contained 20µl of haemolysate and 1980 µl of phosphate buffer (50 mM, pH 7.0).

One unit CAT activity is defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per minute. The rate of H_2O_2 decomposition was measured spectrophotometrically at 240 nm and 25 °C. Catalase activity was expressed as µmoles of H_2O_2 consumed per minute per millilitre of erythrocyte and as µmoles per minute per gram of tissue in brain homogenate**.**

Statistical analysis

The results are presented as the mean ± standard error of the mean (SEM). Statistical analyses were performed using STATISTICA software version 6.1 (StatSoft, Tulsa, OK). Comparisons between the different groups were conducted using a one-way analysis of variance (ANOVA). The least significant difference (LSD) test was used for post-hoc analysis. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Bioactive compounds and antioxidants in *N. sativa* L. (NSP)

FT-IR spectroscopy is recognized as a rapid and effective technique for identifying various functional groups within a sample (Nivetha & Prasanna, 2016; Bruce and al., 2021). This method is particularly beneficial due to its capacity to detect bands associated with organic molecules, such as acids and polyphenols (Nivetha & Prasanna, 2016; Bruce and al., 2021). In this study, FT-IR analysis was performed on NSP. The results, including FT-IR peak values and their corresponding functional groups, are depicted in Fig.1 and Table 2. A peak at 3282 cm^{-1} indicates the presence of a phenolic functional group. Peaks at 2917 cm^{-1} and 2849 cm^{-1} are indicative of C-H stretching vibrations, suggesting the presence of alkane groups. The absorption peak at 1644 cm^{-1} is associated with C=C aromatic stretching. The peak at 1030 cm^{-1} suggests C-H bending, indicative of alcohol or acid groups. Furthermore the C-H bending observed at this frequency further supports the presence of an aromatic group.

Effect of NSP supplementation on plasma reducing power (PRP) and cerebral reducing power (CRP)

The antioxidant properties of *N. sativa* have been extensively studied, underscoring its significant role in mitigating oxidative stress.

In this research, NSP supplementation inhibited free radical formation in an HFD enriched with $FeCl_3$ and enhanced free radical scavenging in a normal diet.

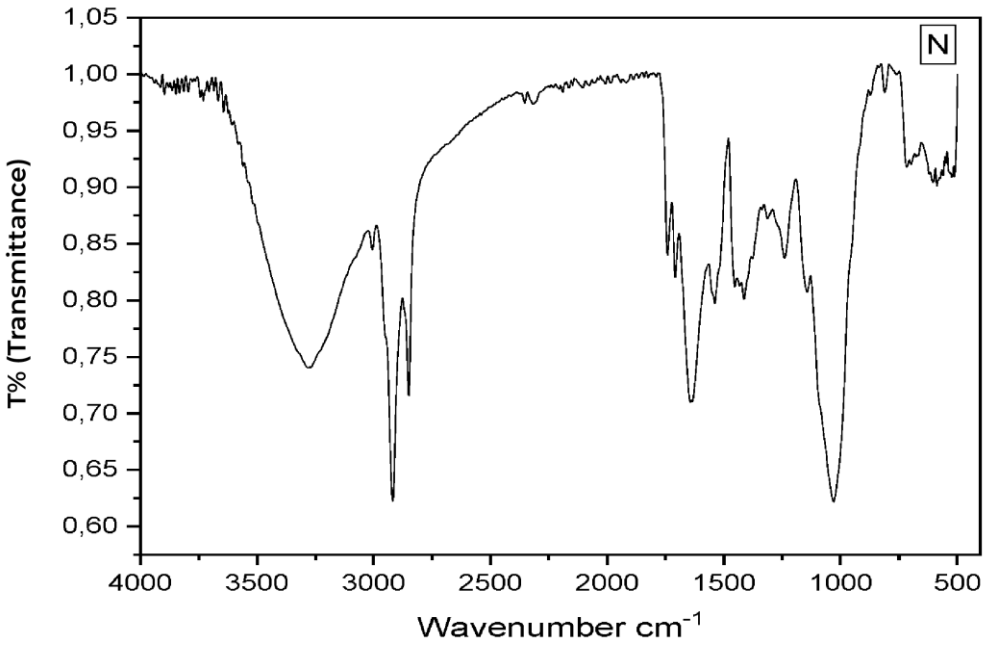

Figure 1. FT-IR spectra of NSP

Table 2.
The main peaks in the FT-IR spectrum of NSP

#	Characteristic absorption	Identified functional groups	Compound class
1	3282	O-H stretching/H-bonded	phenol/alcohol
2	2917	C-H stretching	alkan
3	2849	C-H stretching	alkan
4	1644	C=C aromatic stretching	aromatic
5	1030	C-O stretching	alcohol/acid
6	697	C-H bending	aromatic

These effects are attributed to the polyphenols and flavonoids in *N. sativa*, which act as potent natural antioxidants by scavenging free radicals through their multiple hydroxyl groups (Alu'datt et al., 2024).

The results of the plasma-reducing power (PRP) and cerebral reducing power (CRP) (Table 3) reveal that the HFD/Fe^{3+} significantly impacts reducing power (PR) as measured by the FRAP technique. Mice receiving the HFD/Fe^{3+} exhibited a significantly lower reducing power compared to the ST ($p < 0.05$).

Incorporating NSP into the standard diet significantly enhanced antioxidant activity in both plasma and the brain. The ST+NSP group demonstrated a highly significant increase in antioxidant activity compared to the HFD/Fe^{3+} group ($p < 0.001$). Similarly, the addition of NSP to the HFD/Fe^{3+} significantly improved plasma-reducing power (PRP) ($p < 0.05$) and cerebral-reducing power (CRP) ($p < 0.01$), with notable enhancements, observed relative to the HFD/Fe^{3+} group.

These findings align with prior research, which attributes the observed protective effects to the presence of phenolic antioxidants and a high concentration of phytochemicals in *NS*. These components are crucial in mitigating various degenerative diseases. Dietary polyphenols are recognized for their remarkable bioactivity, including their capacity to traverse the blood-brain barrier and reach neuronal cells (Grabska-Kobyłecka et al., 2023).

These com-pounds positively impact brain health by enhancing plasticity, improving cognitive functions, and modulating mood. Numerous studies have investigated the mechanism through which plant-derived polyphenols neutralize reactive oxygen species, support signal transduction, and promote neuronal plasticity. Furthermore, polyphenols enhance neuronal health by elevating levels of neurotransmitters such as dopamine, serotonin, and noradrenaline in brain tissue (Grabska-Kobyłecka et al., 2023).

Additionally, polyphenols act as effective metal chelators, providing neuroprotection against oxidative stress and neurotoxicity induced by iron and copper. This neuroprotective effect is facilitated by their metal-chelating properties, modulation of signal transduction, and reduction of oxidative stress (Bhullar & Rupasinghe, 2013).

Flavonoids, a subclass of polyphenols, exhibit additional pharmacological properties, including iron chelation and inhibition of lipid peroxidation, further supporting their role in maintaining neuronal health and protecting against oxidative damage (Juurlink & Paterson, 1998). Dong et al. (2020) demonstrated in their study on NS seeds that the plant enhanced the total antioxidant capacity in mice subjected to a high-fat diet and developing type 2 diabetes. Meziti, Meziti, Boudiaf, Mustapha and Bouriches (2012) reached a similar conclusion, suggesting that daily oral administration of the crude extract (500 and 800 mg/kg/day) and the essential oil (2 and 4 ml/kg/day) of *NS* for 21 days in Swiss albino mice significantly improved plasma antioxidant capacity.

Recent studies also emphasized the antioxidant properties of thymoquinone (TQ), a compound in NS that exhibits notable free radical scavenging activity, enhanced by its redox features, ability to bypass biological barriers, and capacity to penetrate subcellular compartments (Cascella et al., 2018). Ismail, Al-Naqeep and Chan (2010) demonstrated that both the TQ-rich fraction and TQ significantly improved plasma antioxidant status by inhibiting hydroxyl radical formation.

Furthermore, compounds isolated from N. sativa, including thymoquinone, carvacrol, anethole, and 4-terpineol have been shown to possess notable free radical scavenging properties (Hosseinian et al., 2018).

Effect of NSP supplementation on protein oxidation (thiol group) and GSH concentration

The evaluation of protein oxidation in plasma and brain tissues (Fig. 2 and 3) indicated that the HFD/Fe^{3+} group exhibited significant alterations in protein oxidation. This is evidenced by a highly significant reduction in thiol group content in the HFD/Fe^{3+} group compared to both the ST group and the ST+NSP group in the plasma ($p < 0.001$). Similarly, the brain results corroborate these findings, showing significantly lower thiol group levels in the HFD/Fe^{3+} group compared to the ST group ($p < 0.05$) and the ST+NSP group ($p < 0.001$).

Table 3.
Effect of NSP supplementation on plasma reducing power (µmol/l) and cerebral reducing power (µmol/g of tissue) in different groups of mice after 11 weeks

FRAP	Experimental groups			
	G1 (ST)	G2 (ST+N)	G3 (HFD/Fe^{3+})	G4 (HFD/Fe^{3+}+N)
Plasma FRAP	1271.20± 86.94$^{\#}$	1633.74± 58.92	893.39± 101.66$^{*\ \#\#\#\ \P}$	1266.90± 147.08$^{\#}$
Brain FRAP	727.05±49.01	924.70±139.82	498.82±35.52$^{*\ \#\#\#\ \P\P}$	849.41 ±44.64

Values are means ± SEM (n = 6) * *p < 0.05 versus ST group;* $^{\#\#\#}$ *p < 0.001 versus G2 (ST+NSP) group;* $^{\#}$ *p < 0.05 versus G2 (ST+NSP group);* ¶ *p < 0.05 versus G3 (HFD/*Fe^{3+}*+NSP);* ¶¶ *p <0.01 versus G3 (HFD/*Fe^{3+}*+NSP)*

The integration of NSP into the HFD exhibits a protective effect against protein oxidation, as demonstrated by a very significant increase of thiol group content in the HFD/Fe^{3+}+NSP group compared to the HFD/Fe^{3+} group at both plasma ($p < 0.01$) and the brain levels ($p < 0.001$). However, this protective effect is consistently less pronounced than that observed in the ST and ST+NSP groups. Notably, both the ST and ST+NSP groups show a very significant increase in thiol content compared to the HFD/Fe^{3+}+NSP group at the plasma level ($p < 0.01$). A similar trend is observed at the brain level, particularly in the ST+NSP group, although this increase does not reach statistical significance.

GSH, the most abundant antioxidant in cellular compartments, is implicated in a feedback loop involving iron accumulation, reduced GSH levels, and oxidative stress (Yarjanli, Ghaedi, Esmaeili, Rahgozar & Zarrabi, 2017). In this study (Fig. 4), GSH levels were found to be depleted in the brain tissue of HFD/Fe^{3+}-fed animals compared to the ST group ($P < 0.01$) and the ST+NSP group ($p < 0.001$). Supplementation with NSP significantly restored the reduced GSH levels in HFD/Fe^{3+}-fed animals ($p < 0.001$).

Sulfhydryl (SH) groups, highly reactive components of both protein and non-protein molecules, play a crucial role in scavenging oxygen free radicals (Hosseinian et al., 2018). In this study, HFD/Fe^{3+} promotes ROS generation by reducing the antioxidant enzyme activity and depleting intracellular GSH concentrations in the brain. NSP supplementation acts as a free radical scavenger, enhancing non-enzymatic GSH levels and total thiol groups in both plasma and brain. Indeed, small thiols like GSH may also play a direct role in scavenging strong oxidants and can inactivate the hydroxyl radical.

Several studies underscore the role of *NS* in increasing the total thiol content of serum and tissues. For instance, Hosseinian et al. (2018) and Salama, Abd-El-Hameed, Abd-El-Ghaffar, Mohammed and Ghandour (2011) demonstrated that administering NS extract at doses of 100 and 200 mg/kg and 50 mg/kg (I.P.), respectively, significantly increased the total thiol content in the serum and tissues of Cisplatin-treated Wistar rats. Furthermore, daily oral administration of *NS* extract for two months in experimentally induced diabetic rabbits increased GSH levels (Meral, Yener, Kahraman & Mert, 2001). Additional research suggests that *N. sativa* powder, when administered orally, can normalize glutathione (GSH) and nitric oxide (NO) levels in rats, indicating significant efficacy in mitigating oxidative stress (Javidi, Razavi & Hosseinzadeh, 2016).

Effect of NSP supplementation on lipid peroxidation (MDA level)

Lipid peroxidation represents a significant consequence of hydroxyl radicals and other potent oxidants, leading to disruptions in brain function by altering the fluidity and integrity of neuronal cell membranes (Juurlink &Paterson, 1998). In this study, the HFD/Fe^{3+} group was used to induce lipid oxidation, as evidenced by MDA levels (Table 4). A highly significant increase in MDA concentration was observed in the HFD/Fe^{3+} group compared to the ST group ($p < 0.01$).

Furthermore, MDA levels in the HFD/Fe^{3+} group were significantly higher than those in the ST+NSP group ($p < 0.001$) at the plasma level. At the brain level, a similar highly significant rise in MDA levels was noted in the HFD/Fe3+ group compared to both the ST group ($p < 0.001$) and the ST +NSP group ($p < 0.01$).

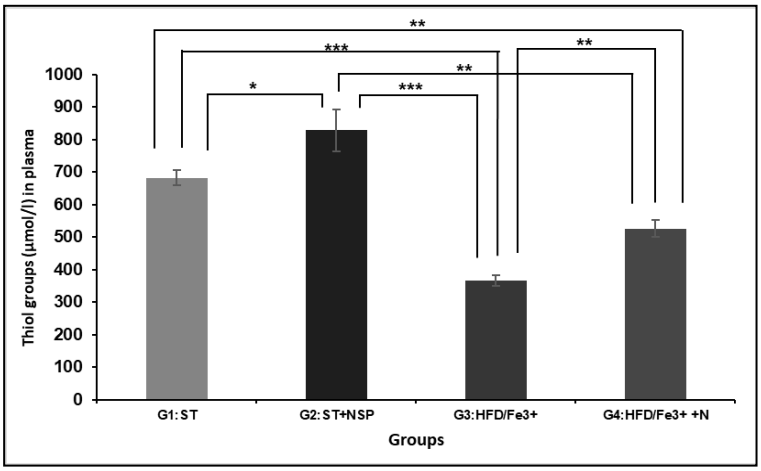

Figure 2. Effect of NSP supplementation on protein oxidation in plasma (thiol group) (µmol/L) in the different groups of mice after 11 weeks
(n = 6 mice in each group). Data are presented as mean ± standard error. * P < 0.05*; **p < 0.01; ***p < 0.001.*

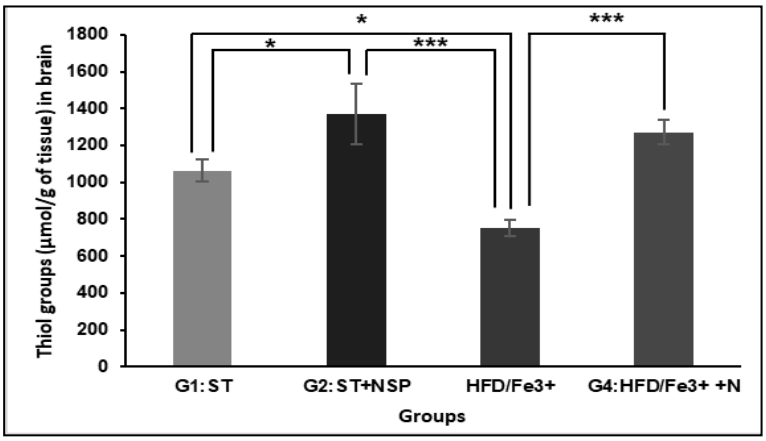

Figure 3. Effect of NSP supplementation on protein oxidation in brain (µmol/g of tissue) in different groups of mice after 11 weeks (n = 6 mice in each group). Data are presented as mean ± standard error
* P < 0.05*; **p < 0.01; ***p < 0.001*

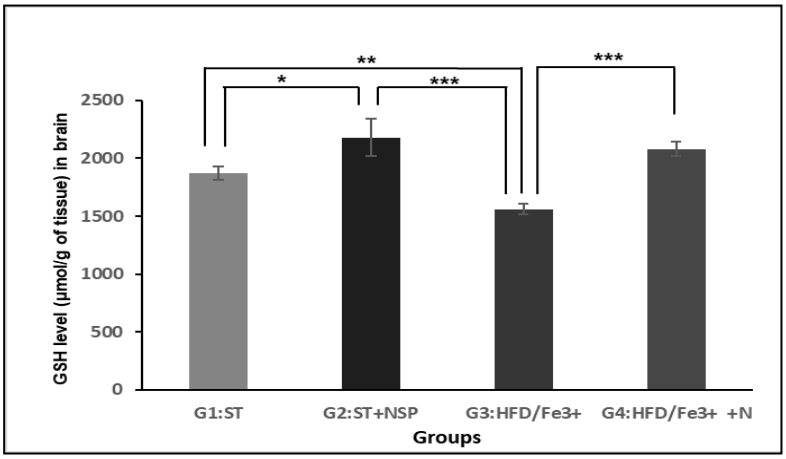

Figure 4. Effect of NSP supplementation on cerebral GSH concentration (µmol/g of tissue) in different groups of mice after 11 weeks (n = 6 mice in each group). Data are presented as mean ± standard error
* P < 0.05; **p < 0.01; ***p < 0.001

Table 4.
Effect of NSP supplementation on plasma lipid peroxidation (µmol/l) and brain lipid peroxidation (µmol/g of tissue) in different groups of mice after 11 weeks

MDA level	Experimental groups			
	G1 (ST)	G2 (ST+N)	G3 (HFD/Fe^{3+})	G4 (HFD/Fe^{3+}+N)
Plasma MDA	4.92±0.43**	3.32±0.56***	7.05±0.51	2.33±0.64***
Brain MDA	16.15±1.00***	20.13±2.21**	29.98±2.12	18.24±2.82**

Data are presented as means ± SEM (n = 6 mice); all $^{***}p < 0.001$; $^{**}p < 0.01$, comparison between HFD/Fe^{3+} and the other groups

The addition of NSP to the HFD/Fe^{3+} regimen significantly reduced the MDA levels induced by this diet. This reduction was evident in the HFD/Fe^{3+}+NSP group compared to the HFD/Fe^{3+} group at the plasma level ($p < 0.001$). A similar decrease in MDA levels was observed in the HFD/Fe^{3+}+NSP group compared to the HFD/Fe^{3+} group ($p < 0.01$) at the brain level.

These results suggest that NSP confers a protective effect, particularly in the context of elevated MDA levels induced by the high-fat diet with iron, providing superior protection compared to the control groups at both the plasma and brain levels.

This finding is corroborated by Balbaa, Abdulmalek and Khalil (2017), who demonstrated that *N. sativa* reduces oxidative stress markers by decreasing TBARS in the brain tissue of HFD/STZ-induced rats.

Additionally, *N. sativa* has been shown to decrease high MDA levels and enhance antioxidant enzyme activity (Ismail et al., 2010). Kanter, Coskun, Kalayc, Buyukbas and Cagavi (2006) also investigated the effect of black seed on lipid peroxidation and the antioxidant defence system, finding that treatment with the volatile oil of *N. sativa*, reduced spinal cord tissue MDA levels following experimental spinal cord injury in rats.

A recent study also confirmed that *NS* seed powder (300 mg/kg BW) significantly decreased MDA levels in diabetic albino rats induced by streptozotocin (Desai et al., 2015). Moreover, Mehri, Shahi, Razavi, Hassani and Hosseinzadeh (2014) demonstrated that TQ exhibited a protective effect against acrylamide (ACR)-induced neurotoxicity in Wistar rats, primarily by reducing lipid peroxidation in the cerebral cortex.

Effect of NSP supplementation on Catalase content in erythrocyte and brain

This study explored the effects of HFD/Fe^{3+} and NSP supplementation on enzymatic antioxidants, specifically CAT activity in erythrocytes and brain tissues (Table 5). A highly significant decrease in CAT activity was observed in the HFD/Fe^{3+} group compared to both the ST group ($p < 0.001$) and the ST+NSP group ($p < 0.01$) at the erythrocyte level. Although the supplementation of NS to the high-fat feeding group increased CAT activity, this increase was not statistically significant compared to the HFD/Fe^{3+} group.

A highly significant reduction in CAT activity was observed in the HFD/Fe^{3+} group at the brain level in comparison to both the ST group ($p < 0.05$) and the ST+NSP group ($p < 0.001$). Furthermore, catalase activity was consistently higher in the control group treated with NS, as opposed to both the ST group ($p < 0.05$) and the HFD/Fe^{3+}+N group ($p < 0.05$). At the erythrocyte level, a significant decrease in catalase activity was detected in the HFD/Fe^{3+} group compared to the HFD/Fe^{3+} group treated with NSP whereas no significant difference was evident at the brain level. Catalase is a crucial enzyme that catalyses the decomposition of hydrogen peroxide into water and molecular oxygen, thereby serving as a vital defence against oxidative stress (Juurilink & Paterson, 1998). In this study, the administration of NSP in a high-fat diet enriched with $FeCl_3$ was found to enhance catalase activity in both blood and brain tissues. This finding aligns with other studies on NS, particularly when combined with vitamin C, which demonstrated the ability to mitigate the inhibition of catalase activity following experimental spinal cord injury in rats. Moreover, increased catalase activity has been reported as an indicator of the

Table 5.
Effect of NSP supplementation on catalase content in erythrocyte (µmol/min/ml) and brain (µmol/min/g of tissue) in different groups of mice after 11 weeks

Catalase content	Experimental groups			
	G1: ST	G2: ST+NSP	G3: HFD/Fe^{3+}	G4: HFD/Fe^{3+} +N
Eryth CAT	1244.38±44.52	895.75±145.79	416.78 ±104.66 $^{***\,\#\#}$	677.53±120.19**
Brain CAT	50383.02±203.14$^{\#}$	52887.61±1374.83	48423.54±383.80$^{*\#\#\#}$	49304.39±219.69$^{\#\#\#}$

Values are means ± SEM (n = 6); *** p < 0.001 versus group G1(ST); ** p < 0.01 versus group G (ST); * p < 0.05 versus group G1(ST); $^{\#\#\#}$ p < 0.001 versus group G2 (ST+NSP); $^{\#\#}$ p < 0.01 versus group G2 (ST+NSP); $^{\#}$ p < 0.05 versus group G2 (ST+NSP

antioxidant properties of *NS* (Javidi et al., 2016).

The administration of TQ has also been shown to restore CAT activity in the rat brain to normal levels (Leong, Rais Mostafa & Jaarin, 2013). The study by Rasoli et al. (2018), which involved adult female Wistar rats subjected to stress and treated with *N. sativa* extract, indicates that *N. sativa* extract possesses the chemical properties necessary to enhance antioxidant capacity, increase CAT activity, and reduce lipid peroxidation.

CONCLUSIONS

After 11 weeks of experimentation, the findings suggest that the inclusion of NSP in an HFD enriched with $FeCl_3$ enhances plasma and brain-reducing powers, thiol levels, catalase activity, and GSH content. Concurrently, NSP supplementation significantly reduces the MDA levels induced by the HFD/Fe3+. These results imply that *N. sativa* contains essential biomolecules capable of traversing the BBB and exerting a neuroprotective effect against oxidative imbalance and lipid peroxidation caused by an iron-enriched hyperlipidemic diet. However, further studies and more comprehensive investigations are necessary to validate these findings.

Future research should focus on measuring iron concentration in the brain, characterizing the bioactive molecules likely to cross the BBB, and exploring the potential of NSP as an alternative treatment for mitigating brain damage caused by oxidative stress.

AUTHOR CONTRIBUTIONS

Conceptualization, B.R.; Methodology, B.M. and H.H.; Investigation, formal analysis, validation, writing-original draft preparation, B.H.; Writing-review and editing, B.H.; Supervision, B.R.

DATA AVAILABILITY STATEMENT

Data contained within the article.

ACKNOWLEDGEMENTS

This research was highly supported by DGRST of the Algerian Ministry of Higher Education and Scientific Research.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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PREVENTIVNI EFEKAT *NIGELLA SATIVA* L. PROTIV CEREBRALNIH OKSIDATIVNIH PROMENA IZAZVANIH ISHRANOM BOGATOM MASTIMA I OBOGAĆENOM GVOŽĐEM KOD MIŠEVA

Halima Benabdallah[*1,2], Rachida Benaraba[1,2], Mostefa Belarbi[3], Hemida Houari[4]

[1]Univerzitet u Tijaretu, Laboratorija za istraživanje lokalnih animalnih proizvoda, 14000 Tijaret, Alžir
[2]Univerzitet u Tijaretu, Fakultet prirodnih nauka, 14000 Tijaret, Alžir
[3]Univerzitet u Tijaretu, Laboratorija za matematiku i računarske nauke, 14000 Tijaret, Alžir
[4]Univerzitet u Tijaretu, Institut za veterinarske nauke, 14000 Tijaret, Alžir

Sažetak: Nedavna istraživanja naglašavaju ključnu ulogu oksidativnog stresa u cerebralnim promenama, podstičući istraživanja strategija poput korišćenja prirodnih supstanci dobijenih iz lekovitih biljaka, uključujući *Nigella sativa* L. (NS), zbog njihovog terapijskog potencijala. NS je poznata po svojim bioaktivnim jedinjenjima koja igraju značajnu ulogu u prevenciji i lečenju bolesti. Ova studija koristila je Fourier Transform Infrared Spectroscopic (FT-IR) analizu za identifikaciju funkcionalnih grupa i jedinjenja u semenu NS, kako bi ispitala njene preventivne efekte na cerebralne oksidativne promene izazvane ishranom bogatom mastima (HFD) uz dodatak gvožđa kod NMRI miševa. Fokus je bio na oksidaciji lipida, Ferric Reducing Antioxidant Power (FRAP), tiolnim grupama, aktivnosti katalaze i nivoima redukovanog glutationa (GSH). Miševi su nasumično podeljeni u četiri eksperimentalne grupe (po šest miševa u grupi): kontrola (ST), kontrola + prah semena NS (ST+NSP), ishrana bogata mastima uz $FeCl_3$ (HFD/Fe^{3+}), i HFD/Fe^{3+}+NSP. Nakon eksperimentalnog perioda od 11 nedelja, oksidacija lipida, FRAP, tiolne grupe i aktivnost katalaze merene su u plazmi i mozgu, dok su nivoi GSH procenjivani isključivo u mozgu. NS je značajno smanjila lipidnu peroksidaciju kod HFD/Fe^{3+} miševa i obnovila FRAP, tiolne grupe, aktivnost katalaze i nivoe GSH, koji su bili znatno smanjeni kod HFD/Fe^{3+} miševa u poređenju sa ST grupom. Režim HFD/Fe^{3+} povećao je proizvode lipidne peroksidacije u odnosu na ST grupu. Ovi nalazi sugerišu da suplementacija ishrane sa NS u prahu ublažava cerebralni oksidativni stres i poboljšava aktivnost antioksidativnih enzima.

Ključne reči: *crni kumin, lipidna oksidacija, oksidativni stres, hiperlipidna ishrana, $FeCl_3$, promene na mozgu*

Received: *18 February 2025/* ***Received in revised form:*** *15 March 2025/* ***Accepted:*** *17 March 2025*

Available online: *April 2025*