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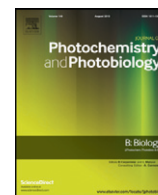

Modulation of gamma-irradiation and carbon tetrachloride induced oxidative stress in the brain of female rats by flaxseed oil

Amel F.M. Ismail [a,*], Asmaa A.M. Salem [b], Mamdouh M.T. Eassawy [b]

[a] Drug Radiation Research Department, National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Nasr City, Cairo, Egypt
[b] Regional Center for Food and Feed (RCFF), Agriculture Research Center, Giza, Egypt

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The activity of flaxseed oil (FSO) on gamma-irradiation (7 Gy) and/or carbon tetrachloride (CCl_4) induced acute neurotoxicity in rats' brain was investigated. The results revealed a significant decrease ($p < 0.05$) in superoxide dismutase (SOD), catalase (CAT), glutathione-peroxidase (GSH-Px) activities, reduced glutathione (GSH) and manganese (Mn) contents. Further, a significant elevation ($p < 0.05$) in malondialdehyde, nitric oxide (NO), Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1-beta (IL-1β), Interleukin-6 (IL-6), transforming growth factor-beta-1 (TGF-β1), iron (Fe), calcium (Ca), copper (Cu) and magnesium (Mg) levels were observed. Furthermore, the relative ratio of xanthine oxidase (XO) and inducible nitric-oxide synthase (iNOS) gene expression levels were elevated in the brain tissues of γ-irradiated and CCl_4 intoxicated animals. Those effects were augmented due to the effect of CCl_4-induced toxicity in γ-irradiated rats. The treatment of FSO displayed significant amendment of the studied parameters in the brain tissues of γ-irradiated and CCl_4 intoxicated animals. FSO has a neuroprotective effect against CCl_4-induced brain injury in gamma-irradiated rats. This effect is interrelated to the ability of FSO to scavenges the free radicals, enhances the antioxidant enzymes activity, increases GSH contents, down-regulates the inflammatory responses, ameliorates the iron, calcium, copper, magnesium, manganese levels and inhibiting the gene expression level of XO and iNOS in the brain tissues of intoxicated animals. In conclusion, this study demonstrated that the potent antioxidant and anti-inflammatory activities of FSO have the ability to improve the antioxidant status, suppress the inflammatory responses, and regulate the trace elements in the brain tissues of γ-irradiated, CCl_4, and their combined effect in intoxicated animals. Consequently, FSO exhibited neuroprotective activity on γ-irradiated, CCl_4, and their combined effect induced brain injury in rats.

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1. Introduction

Flaxseed oil (FSO), flax oil or linseed oil is derived from the seeds of the plant *Linium usitatissimum* (41 g oil/100 g flaxseed) [1]. Flaxseed has several nutritional properties and health benefits that gained worldwide awareness in research and in the food industry [2]. The flaxseed health benefits, including cardioprotective [1], neuroprotective [3], anti-diabetic [4], anti-inflammatory [5], immune-modulatory [6] and anticancer [7] activities are mainly attributed to its constituents of omega-3 fatty acids and the phenolic lignans [8]. FSO is rich in the polyunsaturated fats, which constitute about 75% of its weight [1]; approximately 40 to 60% are alpha-linolenic acid (ALA, 18:3, u-3) [9] with lower quantities of linoleic acid and oleic acid (each about 15%). Alpha-linolenic acid (18:3 n-3 omega-3 fatty acid), is a precursor to eicosapentanoic (C20:5n-3, EPA) and docosahexanoic acid (C22:6n-3, DHA), and it may have beneficial effects on health and in control of chronic diseases [3,10–12]. Phospholipid membranes of the brain are extremely enriched with EPA and DHA, which promote the brain function, behavior and central nervous system development [13,14]. In addition, FSO is the richest plant source of lignans, which are present in flaxseed in a higher concentration than in other edible sources [3,15] FSO contains approximately 1–4% of the phenolic compound lignan, secoisolariciresinol diglucoside (SDG) [16,17]. SDG is a lignan precursor, is converted by the bacterial flora of the human colon to two major mammalian lignans, enterodiol and enterolactone [18]. Very low quantities of matairesinol, isolariciresinol, lariciresinol, demethoxysecoisolariciresinol and pinoresinol are also present in FSO [19–23]. The significant antioxidant activity of these lignans is attributed to their high levels of free radical scavenger properties [24,25].

Ionizing radiation has evoked a lot of consideration due to its beneficial and achievable destructive effects of the human population [26]. The deleterious effects of ionizing radiation have been contributed to the generation of reactive oxygen species (ROS). Radiation exposure attenuates the endogenous antioxidant enzymes; the first line defense mechanism that maintains the redox balance and normal biochemical processes. ROS evoke imbalance of the pro-oxidant and antioxidant status in the cells, thus causing DNA, protein and lipid damage

* Corresponding author.
E-mail address: afmismail@gmail.com (A.F.M. Ismail).

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[27]. This damage generated by ROS is opposed by the antioxidant defenses, enzymatic and non-enzymatic. Antioxidant enzymes include superoxide dismutase (SOD) and catalase (CAT), while glutathione (GSH) is part of the non-enzymatic antioxidants. In normal situations, the human body produces antioxidants or procures them from the diet, and either way, they are capable of reducing the concentration of reactive species. The imbalance between the production of reactive species and amount of antioxidants characterizes oxidative stress. Oxidative stress in the central nervous system has been associated with the development of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease [28].

On the other hand, carbon tetrachloride (CCl_4) is a potent hepatotoxic agent used extensively to induce in vivo liver degeneration by oxidative stress. CCl_4 toxicity is attributed to its lipo-solubility. When CCl_4 bounds to proteins and lipids, thus induces tissue degeneration [29–31]. CCl_4 induced oxidative stress in the brain in the previous studies [28,32]. The molecular mechanism underlying the toxic effects of CCl_4 involves lipid peroxidation (LPO), mediated by the free radicals that are generated during its metabolism [33,34]. Free radicals - such as trichloromethyl (•CCl_3 and/or •CCl_3OO) and oxygen centered lipid radicals (LO• and/or LOO•) - are pivotal in CCl_4-induced toxicity and are generated during CCl_4 metabolism by the cellular cytochrome P450 [35].

This study has been oriented to investigate the possible neuroprotective mechanisms of flaxseed oil against γ-irradiation, CCl_4 and their collective effect that induced acute oxidative damage and inflammatory cascades in rats' brain.

2. Materials and Methods

2.1. Chemicals

The FSO was obtained from the herbal drugstore Imtenan Health Shop; Nasr City, Cairo, Egypt. Carbon tetrachloride (CCl_4) was obtained from Merck. All other chemicals and reagents used in this study were of analytical grade.

2.2. Radiation Facility

Whole-body gamma-irradiation was performed at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using Canadian Gamma Cell-40 biological irradiator (^{137}Cesium), manufactured by the Atomic Energy of Canada Limited, Ontario, Canada. The radiation dose rate was 0.456 Gy/min at the time of exposure. The total radiation dose was 7 Gy as a single dose of the whole body. Animals were not anesthetized before irradiation.

2.3. Animals

Female Wistar rats (weighing 100–120 g) were obtained from the Nile Pharmaceutical Co., Cairo, Egypt. They were housed at the animal facility at the National Center for Radiation Research and Technology. Upon arrival, the animals were allowed to acclimatize for one week before starting the experiment. The animals were kept under standard laboratory conditions of light/dark cycle (12/12 h), a temperature of 25 ± 2 °C and humidity of 60 ± 5%. The rats were housed in cages with free access to food and drinking water *ad libitum*. They were provided with a nutritionally adequate standard laboratory (pellet) diet. The study was conducted in accordance with international guidelines for animal experiments and approved by the Ethical Committee of the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt.

2.4. Experimental Design

The rats were divided into the following groups (6 animals/group): Control group (C): rats administered with water orally by gastric intubation, once daily for 7 days. Irradiated group (R): Rats were exposed to a single dose of whole-body γ-irradiation [36], then, they were administered water orally. CCl_4-treated group (CCl_4): rats were orally administered water, then they were intraperitoneally (IP) injected with an acute single dose; 2 ml/kg body weight of CCl_4 (prepared with olive oil 1:1 *v*/v) after 7 days from the beginning of the experiment [38]. Irradiated-CCl_4 treated group (R-CCl_4): Irradiated rats were orally administered water by gastric intubation for seven days, then they were administered intra-peritoneal (IP) with an acute single dose; 2 ml/kg body weight of CCl_4. FSO treated group (FSO): rats were orally administered FSO (500 mg/kg b. wt.) [37]. Irradiated-FSO-treated group (R-FSO): Rats were exposed to a single dose of whole body γ-irradiation (7 Gy), 30 min later was orally administered FSO, till the end of the experimental period. FSO-CCl_4 treated group (FSO-CCl_4): rats were orally administered with FSO, for seven days. Then they were administered IP with acute single dose, 2 ml/kg body weight of CCl_4. Irradiated-FSO-CCl_4 treated group (R-FSO-CCl_4): rats were exposed to a single dose of whole body γ-irradiation (7 Gy); 30 min later was orally administered with FSO for seven days, then they were administered IP with acute single dose; 2 ml/kg body weight of CCl_4.

The rats were kept overnight fasting period with free access to drinking water, mainly after 16 h of CCl_4 administration [38]; under light ether anesthesia, the blood was collected from the eyes in glass tubes, allowed to clot, centrifuged at 1200*g* using universal centrifuge (16R, Germany) and sera were separated. The brain of each animal was excised immediately and washed with physiological saline and stored at −80 °C.

2.5. Determination of Antioxidants and Oxidative Stress Parameters in the Brain Homogenate

The brain was weighed and homogenized (10%) in chilled 50 mmol phosphate buffered saline (pH 7.4), centrifuged at 1300*g*, at 4 °C for 15 min, using universal centrifuge (16R, Germany), then the supernatants were used for the determination of the following parameters:

Superoxide dismutase (SOD) activity was determined according to Nishikimi et al. [39]. The assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium (NBT) dye, which was followed photometrically at 560 nm. Briefly, 0.05 ml sample was mixed with 1.0 ml phosphate buffer (pH 8.5), 0.1 mL nitroblue tetrazolium (NBT), and 0.1 ml NADH. The reaction was initiated by adding 0.01 ml phenazine methosulphate (PMs). After then, the increase in absorbance was read at 560 nm for five minutes. SOD activity was expressed as U/g tissue.

Catalase (CAT) activity was assessed according to Aebi [40]. The method is based on the decomposition of H_2O_2 by catalase. The sample containing catalase is incubated in the presence of a known concentration of H_2O_2, then, incubated for exactly one minute at 25 °C. In the presence of 0.5 ml horse radish peroxidase (HRP), the remaining H_2O_2 was reacted with 0.2 ml 3,5-Dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample The catalase activity was expressed as U/g tissue.

Glutathione-peroxidase (GSH-Px) activity was measured according to Rotruck et al. [41] that based on indirect determination of GSH-Px, whereas GSH-Px react with known amount of GSH, then the residual GSH reacted with 5.5′-dithio-bis-2-nitrobenzoic acid (DTNB).

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