

Fatty acid composition, antioxidant activity, brine shrimp toxicity, and *in vivo* irritation potential of egyptian flaxseed oil in rabbits

© KATIBA BEROUAL^{1,2}, © MOHAMED CHERIF ABDEL DJELIL³, © SALIHA TORCHE⁴,
© SABER BEGHOUL³, © SIHEM HALMI¹, © SEDDIK KEBBAL², © MOHAMED ZAOUANI⁵,
© NORA MIMOUNE^{2,6}

¹Toxicology and Pharmacology Research Laboratory, Institute of Veterinary Sciences,
University Frères-Mentouri, Constantine 1, Algeria

²Animal Biotechnology Research Laboratory (LBA), Institute of Veterinary Medicine, University of Blida 1, Algeria

³PADESCA Research Laboratory, Institute of Veterinary Sciences, University Frères-Mentouri, Constantine 1, Algeria

⁴Management of Animal Health and Productions Laboratory, Institute of Veterinary Sciences,
University of Frères Mentouri Constantine 1 - El-Khroub, Constantine, 25000, Algeria

⁵Laboratory of Research Management of Local Animal Resources (GRAL), Higher National Veterinary School,
Abbes Street, Oued-Smar, 16000, Algiers, Algeria

⁶Higher National Veterinary School, Algiers, Algeria

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Beroual K., Abdeldjelil M. C., Torche S., Beghoul S., Halmi S., Kebbal S., Zaouani M., Mimoune N.
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Summary

Flaxseed oil, also known as linseed oil, is a widely used traditional remedy obtained by grinding and pressing the seeds of flax or linseed plant (*Linum usitatissimum*). This study investigated the fatty acid profile and antioxidant activity of this oil. Its safety was also investigated by studying its cytotoxicity on *Artemia salina* larvae and its irritation potential on a rabbit model. The analysis of the GC-MS chromatogram showed three major unsaturated fatty acids representing 84.38% of the oil's fatty acid composition: linolenic acid (45.11%), oleic acid (22.04%), and linoleic acid (17.23%). The other less represented constituents were saturated fatty acids: stearic acid (8.60%) and palmitic acid (7.03%). In the cupric reducing antioxidant capacity test (CUPRAC method), flaxseed oil showed antioxidant activity. However, it was less than that of the other antioxidant standards. As part of the brine shrimp lethality test, LC_{50} was not reached within the concentration range tested and was therefore considered to be greater than 80 $\mu\text{g/mL}$. *In vivo* tests did not show any ocular irritation effect of the oil, while a slight irritation was observed on the abraded skin of rabbits. Thus, our data indicate preliminary cutaneous tolerance after a single application, which should be confirmed in repeated-application and skin-sensitization studies before recommending any dermatological or cosmetic use. In addition, the absence of visible irritation after a single ocular application suggests that flaxseed oil may be considered a good candidate for further investigation as an ophthalmic vehicle.

Keywords: flaxseed oil, fatty acid profile, antioxidant activity, brine shrimp lethality test, *in vivo* irritation tests

Flax (*Linum usitatissimum*) is an important crop cultivated either for its fiber, or its seeds and the oil produced from them (18). Flaxseed oil, or linseed oil, is obtained by pressing the plant's seeds to release their natural oil. The oil contains around 96% triacylglycerides (TAG) and 1.4% polar lipids, glycolipids, and phospholipids (53). Flaxseed oil produced worldwide is used for industrial purposes, as well as for human and animal food production. This oil is also used as a tra-

ditional remedy to treat various ailments (9, 50). The positive health effects of this oil are probably linked to its unique fatty acid profile, rich in polyunsaturated fatty acids, particularly alpha-linolenic acid (ALA), an omega-3 fatty acid (26). Potential benefits are also related to the high content of lignans, high-quality proteins, and dietary fiber; lignans are phenolic compounds that act as both antioxidants and phytoestrogens (28). The wide use of this oil in traditional remedies

raises the question of its safety. The results of a previous study conducted to evaluate the potential toxicity risk linked to the use of flaxseed as a feed supplement in rabbits (5) showed that such supplementation was not only safe, but also beneficial in enhancing body weight and carcass yield. The supplementation did not affect liver or kidney function parameters, and had no deleterious effects on their histology.

This study investigated the fatty acid profile of Egyptian flaxseed oil and its antioxidant activity. It also assessed the toxic effect of the oil by evaluating its cytotoxicity on *Artemia salina* larvae and its irritation potential following ocular and cutaneous applications in a rabbit model. Unlike previous studies that focused primarily on nutritional or dietary supplementation, the present work provides an integrated evaluation of fatty acid composition, antioxidant capacity, and multiroute safety assessment of flaxseed oil using standardized *in vitro* and *in vivo* models. Specifically, the current study combines chemical characterization (fatty-acid profile and cupric-reducing antioxidant capacity) with a preliminary safety screen – brine shrimp cytotoxicity together with acute ocular and dermal tolerance after a single application in rabbits – intended to detect any overt acute hazard and to define safe dose ranges for future pharmacological studies.

Material and methods

Ethical approval. All procedures were approved by the Scientific Council of the Institute of Veterinary Sciences, University of Constantine 1, Algeria, and were carried out in accordance with the guidelines of the Institutional Animal Care Committee of the Algerian Ministry of Higher Education and Scientific Research (agreement no. 45/DGLPAG/DVA.SDA.14) and with the guide for the care and use of laboratory animals (29). The study was designed as a preliminary safety assessment rather than as a fully OECD-(35,36) compliant investigation. The experimental procedures were adapted from established methods, and all relevant deviations were reported explicitly.

Plant material. Brown linseeds cultivated in Egypt were purchased from a local market in Cairo, Egypt. The seeds were manually cleaned to remove any foreign matter and cold-pressed. The oil obtained was subjected to 24 hours (h) of decantation, then filtered and kept in a dark brown glass bottle at refrigeration temperature. Specimens of the products (seeds and oil) were deposited in the Laboratory of Pharmacology-Toxicology, Institute of Veterinary Sciences, University of Constantine 1, Algeria.

Gas Chromatography coupled with Mass Spectrometry Analysis. Fatty acids were derivatized to form fatty acid methyl esters (FAMES), which were then analyzed on a Hewlett Packard Agilent 6890 plus Gas Chromatograph coupled with a Hewlett Packard Agilent 5973 Mass Detector and equipped with the Agilent Mass Hunter Workstation Qualitative Analysis software. An HP-WAX Column with a length of 60 meters, an inner diameter of 0.25 mm, and film thickness of 0.25 μm was used for separation.

Parameters for acquisition were as follows: 1 μL of the sample was injected using a split/splitless injector with an inlet temperature of 250°C. The carrier gas was helium at 1.5 mL/min.

Temperatures of the GC oven throughout the run were as follows: initial temperature of 140°C, held for 5 min, a ramp in temperature of 2°C/min to 240°C maintained for 9 min, for a total run time of 70 min.

A solvent delay of 8 min was set before MS acquisition began. The transfer line from the GC column to MS was set to 260°C and the source 230°C. Source fragmentation was done by electron ionization (EI) at 70 eV, with a scan range of 35 to 550 amu.

Fatty acids were identified by searching the NIST 17 Database with the NIST Mass Spectral Search Program version 2.3.

Antioxidant activity. The antioxidant activity was determined using the cupric reducing antioxidant capacity (CUPRAC method) (2). The reducing capacity of our oil was compared with those of α -tocopherol, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) used as antioxidant standards for comparison. The results were given as $A_{0.50}$ ($\mu\text{g/mL}$), which corresponds to the concentration producing 0.500 absorbance.

Brine shrimp lethality assay. Brine shrimp (*Artemia salina* L.) eggs were provided by the National Center for Biotechnology Research (CRBT, Constantine, Algeria). Oil samples used in this assay were dissolved in 10% dimethylsulfoxide (DMSO) (v/v). From this first mix, four different concentrations (10 $\mu\text{g/mL}$, 20 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$, and 80 $\mu\text{g/mL}$) were prepared using artificial seawater. The control vial was left with sea salt water and 10% DMSO (v/v) only, serving as the drug-free control (25).

As part of this assay, described by Meyer et al. (24), brine shrimp eggs were hatched after 24 h incubation in artificial seawater. Ten shrimp larvae (nauplii) were transferred to each of the vials of different concentrations with a Pasteur pipette, and the total volume in the vial was adjusted to 5 mL with seawater. Three replications were used for each concentration. All tubes were incubated for a period of 36–48 h with suitable conditions of salinity, temperature, pH, and oxygenation. After incubation, the percentage of mortality was determined, and the lethal concentration (LC_{50}) was calculated by probit analysis. Results in this study were interpreted as previously described (25) (Tab. 1).

Tab. 1. Median lethal concentration ranges of the product for brine shrimp larvae (25)

LC_{50}	Toxicity
$\geq 100 \mu\text{g/mL}$	– (non-toxic)
$100 \mu\text{g/mL} > LC_{50} \geq 50 \mu\text{g/mL}$	+ (moderate)
$50 \mu\text{g/mL} > LC_{50} \geq 10 \mu\text{g/mL}$	++ (toxic)
$< 10 \mu\text{g/mL}$	+++ (highly toxic)

In vivo rabbit model testing. Six adult male Californian rabbits (aged 3.5 months and weighing 2.0–2.5 kg), obtained from the same breeding farm, were housed individually under standardized conditions with *ad libitum* access to food and water. To minimize animal use in accordance with the reduction principle of the 3Rs, we performed ocular and

dermal irritation procedures sequentially on the same six rabbits rather than using separate animals for each test. The ocular assessment was performed first. The dermal assessment followed after an interval of 7 days. This sequential design reduced the number of animals required and allowed recovery between procedures.

Outcome assessment was not blinded, which may have introduced observer bias. We also acknowledge the ethical limitations of conducting the abraded-skin procedure without analgesia. Future studies should prioritize validated *in vitro* and *ex vivo* irritation models. Where *in vivo* assessment remains necessary, refined protocols should avoid skin abrasion whenever possible and incorporate appropriate pain management in accordance with the replacement and refinement principles of the 3Rs.

In vivo assessment of ocular irritation. The test was carried out based on OECD guidelines (36). The lower lid of the left eye of each animal was gently pulled away from the eyeball, and 0.1 mL of flaxseed oil was instilled in the conjunctival sac. Eyelids were gently held together for about one second to prevent the loss of material. The other eye, which remained untreated, served as a control. One hour after oil application and for the next seven days, the degree of eye irritation/corrosion was evaluated by scoring lesions of conjunctiva, cornea, and iris according to the Draize scale (14). A score of less than 15 was considered to show that the oil was not irritant (45).

In vivo skin irritation/corrosion test. The test was carried out adapted from OECD guidelines (35). The back and sides of each animal were closely clipped to remove fur 24 hours before the test. Three parallel abrasions were performed on one flank, while the other flank was left undamaged. The scarifications of the epidermis were performed (the dermis was not reached), without anesthesia or analgesia. This procedure reflects the historical Draize design and is acknowledged as an ethical and methodological limitation. The abrasions were superficial and confined to the epidermis. Analgesic, anesthetic, and anti-inflammatory agents were not applied to the test sites, because they could alter erythema and edema, the predefined endpoints of the irritation assessment (This methodological consideration does not remove the ethical limitation).

For four hours, a gauze patch covered with flaxseed oil was held in place with non-irritating tape to cover abraded and intact skin areas. Dermal reactions were recorded 1 h after bandage removal, then 24, 48, and 72 h after application (13).

Dermal reactions (erythema and edema) were scored according to the Draize scoring system (14). The combined scores given for the areas of intact and abraded skin were used to calculate the Primary Irritation Index:

For each animal, erythema grades (0-4) and edema grades (0-4) for intact and abraded skin recorded at 24, 48, and 72 h were summed, and the PII was obtained by dividing this sum by the total number of readings. With six rabbits, two skin sites (intact

and abraded), and three reading times, the divisor was $6 \times 2 \times 3 = 36$.

According to Draize (14), irritation is categorized as negligible for a PII of 0-0.4, slight for 0.5-1.9, moderate for 2.0-4.9, and severe for 5.0-8.0.

Statistical analysis. Statistical analyses were carried out using XLSTAT (version 7.5.2; Addinsoft, Paris, France). Data are reported as the mean $\pm$ standard deviation (SD) of three technical replicates (triplicate wells) obtained within a single assay run ($n = 3$). Because the three replicates were technical (wells from a single plate) rather than independent experimental repeats, and because the *in vivo* irritation tests used a small number of animals in a descriptive, non-comparative design, all results are presented descriptively – as mean $\pm$ SD or as individual-animal Draize scores – and no inferential hypothesis testing was performed. This choice reflects the preliminary, screening nature of the study and avoids the pseudo-replication that would arise from treating technical replicates as independent observations; it is acknowledged as a limitation.

For the CUPRAC assay, absorbance at 450 nm at each of the seven concentrations (3.125-200 $\mu\text{g/mL}$) and the $A_{0.50}$ value are reported as mean $\pm$ SD. The $A_{0.50}$ value is the concentration producing an absorbance of 0.500, estimated for each replicate curve by linear regression over the linear portion of the dose-response relationship. Differences between the four samples (BHA, BHT, α -tocopherol, and flaxseed oil) are described in terms of the magnitude of the values measured rather than tested inferentially. Flaxseed oil was treated as a right-censored value ($A_{0.50} > 200 \mu\text{g/mL}$) because its absorbance did not reach 0.500 within the concentration range tested.

Results and discussion

Fatty acid profile. Figure 1 shows the GC chromatogram and mass spectra of fatty acids composing flaxseed oil, and Table 2 presents the peaks identified.

GC-MS analysis showed that unsaturated fatty acids represented 84.37% of the total, against 15.63% for the saturated ones. The principal unsaturated fatty acid was linolenic acid (18:3), followed by oleic acid (18:1) and linoleic acid (18:2). The main saturated fatty acids were palmitic acid (16:0) and stearic acid (C18:0).

The fatty acid composition of flaxseed oil reported in the literature varies considerably from one study to another. This variation is due to differences in the varieties studied, analysis methods, experimental setups, and processing treatments applied to the product (32). The fatty acid profile of linseeds cultivated in

Tab. 2. Fatty acid composition of flaxseed oil (The values are relative area percentages)

Peak	Retention time (min)	Name	Formula	Area	Content (%)
1	33.53	Palmitic acid, methyl ester	$\text{C}_{17}\text{H}_{34}\text{O}_2$	270174267	7.03
2	42.89	Stearic acid, methyl ester	$\text{C}_{19}\text{H}_{38}\text{O}_2$	330321121	8.60
3	43.35	Oleic acid, methyl ester	$\text{C}_{19}\text{H}_{36}\text{O}_2$	846840590	22.04
4	44.96	Linoleic acid, methyl ester	$\text{C}_{19}\text{H}_{34}\text{O}_2$	662292646	17.23
5	47.76	Linolenic acid, methyl ester	$\text{C}_{19}\text{H}_{32}\text{O}_2$	1733453183	45.11

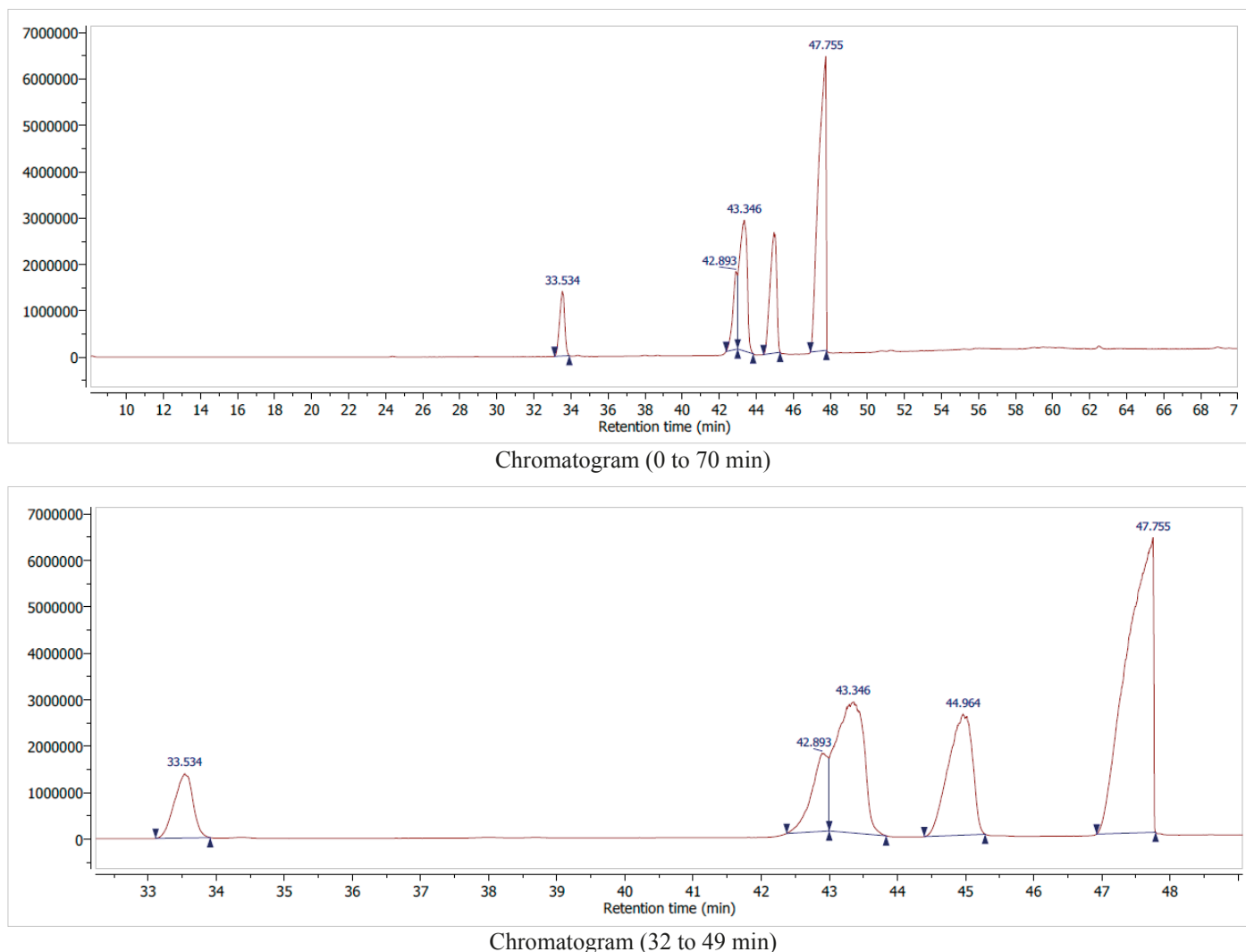


Fig. 1. GC chromatogram of fatty acid methyl esters from the GC-MS analysis of flaxseed oil

Romania showed a similar distribution of the predominant fatty acids, with 88.97% for the unsaturated acids and 11.01% for the saturated ones (40). In the same study, flaxseed oil was found to contain high levels of linolenic acid (53.21%), followed by oleic acid (18.51%) and linoleic acid (17.25%), while the dominant saturated acids were palmitic acid (6.58%) and stearic acid (4.43%). The high content of linolenic acid makes flaxseed oil a valuable health-promoting oil. However, unsaturated fatty acids make it unsuitable for cooking and susceptible to rancidity (10). This fact has led to many attempts to create varieties with low contents of linolenic acid (32). Low-linolenic-acid flax lines known as Linola™ or Solin produce edible flaxseed oil with a substantially reduced linolenic acid

(less than 2% of total seed fatty acids). This variety resists rapid oxidation and is well-suited for culinary use (18). In the same context, Lukaszewicz et al. (23) report that in order to avoid rancidity, flaxseed oil is often supplemented with vitamin E, stored in dark containers, and may not be used for frying.

Antioxidant activity – Cupric reducing antioxidant capacity (CUPRAC). The results of CUPRAC assays, compared with those of BHT, BHA, and α -tocopherol (Tab. 3), showed that activity (absorbance) increased linearly with the increasing amount of flaxseed oil. However, the oil did not exhibit higher activity than that found in the antioxidant standards.

The antioxidant activity of the flaxseed oil was tested by the CUPRAC method (cupric reducing antioxidant

Tab. 3. CUPRAC absorbance (450 nm) at each concentration and $A_{0.50}$ values for the three reference antioxidants and flaxseed oil

Sample	3.125	6.25	12.5	25	50	100	200	$A_{0.50}$ ($\mu\text{g/mL}$)
BHA	0.46 ± 0.00	0.78 ± 0.01	1.34 ± 0.08	2.36 ± 0.17	3.45 ± 0.02	3.76 ± 0.03	3.93 ± 0.01	3.64 ± 0.19
BHT	0.19 ± 0.01	0.33 ± 0.04	0.66 ± 0.07	1.03 ± 0.07	1.48 ± 0.09	2.04 ± 0.14	2.32 ± 0.28	9.62 ± 0.87
α -tocopherol	0.26 ± 0.01	0.33 ± 0.03	0.42 ± 0.04	0.58 ± 0.04	0.95 ± 0.06	1.71 ± 0.23	2.43 ± 0.32	18.75 ± 1.91
Flaxseed oil	0.17 ± 0.01	0.20 ± 0.01	0.21 ± 0.02	0.23 ± 0.01	0.27 ± 0.01	0.30 ± 0.02	0.35 ± 0.02	> 200

capacity). This simple and versatile antioxidant capacity assay is used for various antioxidant compounds (37).

Other studies have investigated the antioxidant activity of flaxseed oil by different techniques. Obranović et al. (33) measured the antioxidant activity of four varieties of flaxseed oils by determining free radical scavenging activity (DPPH). All oil samples had values between 59 ± 0 and $66 \pm 0\%$ of reduced DPPH.

Previous studies report various antioxidant bioactive components in different parts and products of *Linum usitatissimum*. These compounds include lignans, phenolic acids, anthocyanin pigments, flavonols, flavones, and phytic acid (56). Deng et al. (12) found that the lignans and free phenolic acids cooperatively constituted the primary material basis of the endogenous antioxidant system in 32 flaxseed varieties. Flaxseed shells studied by Han et al. (16) demonstrated effective antioxidant activity compared with α -tocopherol and BHT used as positive controls in different antioxidant assays. After oil extraction, several antioxidant compounds remain present in the oil, especially in cold-pressed oil obtained without the use of heat or chemical treatments. In such oil, Siger et al. (49) reported the presence of flavonoids (12.7-25.6 mg/100 g), phenolic acids (76.8-307.3 mg/100 g), α -tocopherols (0.55-9.11 mg/100 g), γ -tocopherols (10.56-15.00 mg/100 g), and plastochroman-8 (3.37-5.53 mg/100). Basically, tocopherols are the primary antioxidants in flaxseeds. These lipid-soluble compounds consist of four tocopherols (α -, β -, γ - and δ -) and four tocotrienols (α -, β -, γ - and δ -). The main representative tocopherol is γ -tocopherol (33).

Brine Shrimp Assay. No mortality was observed at 10, 20, or 40 $\mu\text{g/mL}$. At 80 $\mu\text{g/mL}$, one larva died in one of the three replicate vials, corresponding to a mean mortality of $3.33 \pm 5.77\%$ (Tab. 4; Fig. 2). The LC_{50} was not reached within the concentration range tested and was therefore considered to be greater than 80 $\mu\text{g/mL}$ (a formal toxicity category cannot be assigned without evaluating concentrations above 80 $\mu\text{g/mL}$). Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$), used as the positive control, produced concentration-dependent mortality with an LC_{50} within the 10-40 $\mu\text{g/mL}$ range, indicating that the assay responded as expected.

Artemia nauplii is a model for several preliminary studies of pharmacological and ecotoxicological activities of various compounds (54). Flaxseed oil did not show any toxicity for *Artemia salina* larvae within the concentration range tested ($\text{LC}_{50} > 80 \mu\text{g/mL}$). Parikh et al. (38) found no toxicity for the plant's seeds used

as a dietary supplement. Another study on flaxseed oil reported a murine oral median lethal dose (LC_{50}) of 5.656 g/kg (1). This value, well above 5 g/kg, indicated a very low acute oral toxicity. In the same context, it is known that flaxseeds contain potentially toxic bioactive ingredients, including cyanogenic glycosides, linatine, phytic acid, trypsin, lignans, isoflavones, and cadmium (7, 11, 27, 44).

Cyanogenic glycosides are nitrogenous secondary metabolites. The intestinal metabolism of these compounds produces hydrogen cyanide (11), which is toxic for the respiratory, nervous, and endocrine systems (38, 42, 47). Phytoestrogens, such as lignans and isoflavones, are abundant in flaxseed and have chemical structures resembling endogenous estrogens. In postmenopausal women, these compounds may alter estrogen metabolism more significantly than supplementation with the same amount of soy (8). Finally, the metal cadmium present in flaxseeds is potentially toxic for humans. Its accumulation in the kidney can cause renal dysfunction and pulmonary emphysema and may even compromise mineral reabsorption, making the organism susceptible to osteo-malacia (3). Taken together, the findings indicate that the safety of flaxseed-derived products is dose- and context-dependent. Although several constituents of flaxseed oil warrant caution at high exposure levels, evidence from previous studies conducted at conventional doses also supports the potential health benefits of flaxseed oil.

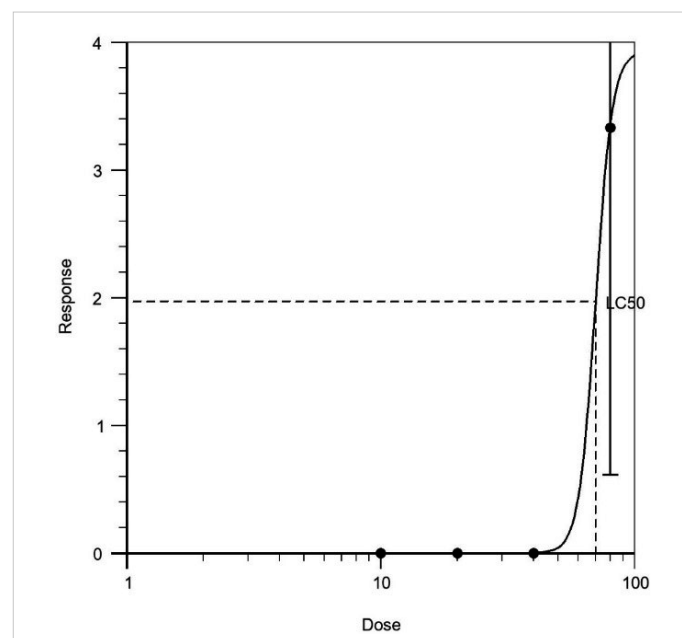


Fig. 2. Cytotoxicity of crude flaxseed oil

Tab. 4. Mortality of *Artemia salina* larvae exposed to flaxseed oil and potassium dichromate (positive control)

Extracts	Mortality rate (%)					Toxicity
	10 μg	20 μg	40 μg	80 μg	$\text{LC}_{50} \mu\text{g/mL}$	
Flaxseed oil	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 5.77	$\text{LC}_{50} > 80 \mu\text{g/ml}$	Not reached
$\text{K}_2\text{Cr}_2\text{O}_7$	0.00 ± 0.00	50.00 ± 10.00	80.00 ± 0.00	100.00 ± 0.00	$40 \mu\text{g} > \text{LC}_{50} \geq 10 \mu\text{g}$	++

Flaxseed is commonly acknowledged as a superfood that provides potential health benefits due to its abundant content of essential fatty acid (α -linolenic acid, ALA), high-quality protein, soluble dietary fiber, and bioactive compounds (19, 21). Ocaña-Sánchez et al. (34) report that the oral administration of flaxseed oil at fixed doses prevents cognitive and motor impairments as well as neuronal alterations in rats with hyperammonemia, which supports the potential use of this oil to counter changes that occur in hepatic encephalopathy. According to Tang et al. (53), flaxseed, which is rich in-linolenic acid (ALA), lignans, proteins and dietary fibers, is considered as an important food ingredient. Moreover, flaxseed oil could provide many potential health benefit: it can serve as a cardiovascular and liver protector, an anti-tumoral and anti-inflammatory agent, and can also be used to prevent diabetes (17, 31, 41, 50, 51). However, at very high doses and over long periods, an excess of alpha-linolenic acid (the main lipid component of flaxseed), can cause gastrointestinal disorders and bleeding (22). Overall, toxicity, whether acute or chronic, remains dose-dependent.

***In vivo* tolerance assessment**

The findings of the ocular and dermal irritation assessments are presented below.

***In vivo* ocular safety of use.** Following a single ocular instillation, only low and transient Draize scores were recorded (Tab. 5). The mean score was highest at 1 h (2.67) and declined progressively to zero by day 7, remaining well below the irritation threshold of 15. Under the conditions of this study, flaxseed oil did not produce an ocular irritation response.

According to our results, flaxseed oil is safe for topical eye instillation. Other oils, such as olive oil, maize oil, and castor oil, scored respectively 0, 1, and 2 in the same *in vivo* ocular irritation test (45). The commonly used lipophilic vector in the formulation of eyedrop

drugs is castor oil. However, this oil is known for its low stability and presumed ocular cytotoxic effects. According to Said et al. (45), replacing castor oil with another lipophilic vector could result in better tolerance of eyedrops. Flaxseed oil seems to be a good candidate to fulfill this role because of the safety of use and its unique composition of fatty acids.

Some authors have reported on treatment of experimentally induced kerato-conjunctivitis sicca (KCS) in rabbits with eyedrops consisting of flaxseed oil alone or as a vehicle for drugs used to treat that condition. Neves et al. (30) evaluated the effectiveness of flaxseed oil alone in treating experimentally induced KCS in rabbits. The authors compared various routes of administration (oral, topical, and combination of oral and topical). Topical instillation of flaxseed oil increased tear production and corneal ulcer resolution without causing any ocular side effects, such as irritation, hyperemia, or discomfort. Flaxseed oil administered orally was also effective in treating KCS in rabbits, although the concomitant use of flaxseed orally and topically did not show additional benefits beyond those obtained with a single route of administration (30). To treat the same condition (KCS), Parrilha et al. (39) evaluated the effectiveness of topical 1% cyclosporine eyedrops diluted in olive oil versus the same drug diluted in flaxseed oil. The results showed that flaxseed oil, used alone or as a diluent for cyclosporine, was more effective at treating KCS than olive oil. A similar study conducted by Sgrignoli et al. (46) used another immunosuppressive agent called tacrolimus with two types of oily vehicles: almond oil, a usual vehicle for this drug, and flaxseed oil as a vehicle tested for the first time for tacrolimus. Data showed that tacrolimus dissolved in almond oil and flaxseed oil decreased edema as well as both corneal and conjunctival degeneration. It also increased the number of caliciform cells. When used alone, both oils accelerated the resolution of corneal ulcers and effectively increased aqueous tear production, with the flaxseed oil treatment showing the highest increase. These studies revealed that the anti-inflammatory activity of flaxseed oil used as

a vehicle enhanced the effect of immunosuppressive drugs (cyclosporine or tacrolimus) (39, 48). This potential of flaxseed oil is due to its unique fatty acid composition of ω -3 and ω -6. A study conducted by Rashid et al. (43) showed that the topical use of ω -3 and ω -6 successfully controlled inflammatory signs in mice with experimentally induced KCS.

Cutaneous safety of use. Only a very slight, transient erythema appeared on non-abraded (intact) skin. On abraded skin, there also occurred a very slight, transient erythema (grade 1) without edema, which re-

Tab. 5. *In vivo* ocular irritation score

1 h	24 h	48 h	72 h	7 d
2.67	1.67	1.33	0.67	0

Tab. 6. Results of the primary skin irritation index

Rabbit	Abraded skin						Non-abraded skin					
	24 h		48 h		72 h		24 h		48 h		72 h	
	Ery	Ede	Ery	Ede	Ery	Ede	Ery	Ede	Ery	Ede	Ery	Ede
1	1	0	1	0	0	0	1	0	0	0	0	0
2	1	0	1	0	0	0	1	0	0	0	0	0
3	1	0	1	0	0	0	1	0	0	0	0	0
4	1	0	1	0	0	0	1	0	0	0	0	0
5	1	0	1	0	0	0	1	0	0	0	0	0
6	1	0	1	0	0	0	1	0	0	0	0	0
Total	12						6					
IP	18/36 = 0.5											

Explanations: Ery – erythema; Ede – edema.

solved within 48 h. The Primary Irritation Index of the oil, calculated from the individual animal scores (at 24, 48, and 72 h) was 0.5 (Tab. 6).

This score indicates that flaxseed oil was slightly irritating to the abraded rabbit skin. It is worth noting that abraded skin treated with flaxseed oil healed faster than untreated control skin.

A single application caused only a very slight and transient erythema on intact and abraded skin, with no edema observed. Even abraded skin tolerates the topical application of this oil. Thanks to its unique fatty acid composition and tocopherol content, flaxseed oil is safe for the skin and helps protect it (A slight irritation that is reversible within two days is considered insignificant and of no concern.).

The fatty acids and triglycerides in flaxseed oil can increase skin hydration by reducing trans-epidermal moisture loss (15). Tocopherols present in flaxseed oil are among the most important lipid-soluble antioxidants in food as well as in human and animal tissues. For more than 50 years, vitamin E has been used in dermatology. It is a major ingredient in many cosmetic products. It protects the skin from various deleterious effects due to solar radiation by acting as a free-radical scavenger. Experimental studies suggest that vitamin E has antitumorogenic and photoprotective properties (20).

Flaxseed oil is also safe for burnt skin. This result is in accordance with that of a previous study conducted to assess the effect of crude flaxseed oil applied topically on burn wounds in rabbits (4). Burns treated with flaxseed oil healed significantly faster compared to those in control and standard drug groups. Healed burns showed less inflammatory cells, complete re-epithelialization, and discreet fibrosis associated with increased neovascularization, collagen fibers, fibroblasts, and myofibroblasts (4).

Furthermore, flaxseed oil is also safe for the hair. Hair growth and health are strongly linked with the skin health. In a study by Beroual et al. (6), who investigated the effect of flaxseed oil on hair growth, flaxseed oil application on rabbit's shaved skin did not cause any erythema or edema during four weeks of use. At the end of the experimental period, topical application of flaxseed oil had a significant positive effect on hair width, suggesting a hair growth-promoting potential. The pharmacological properties indicate that flaxseed mucilage is a promising natural and cost-effective therapeutic candidate for the management of atopic dermatitis and other inflammatory skin disorders (17).

In conclusion, Egyptian flaxseed oil was characterized by a high proportion of unsaturated fatty acids, mainly linolenic, oleic and linoleic acids, and showed measurable, but relatively low, antioxidant activity in the CUPRAC assay compared with standard antioxidants. In the brine shrimp assay, the oil did not show any acute toxicity within the range tested ($LC_{50} > 80 \mu\text{g/mL}$). Following a single ocular instillation,

only low and transient Draize scores were observed, indicating a good short-term ocular tolerance in this rabbit model. The absence of visible irritation after a single ocular application suggests that flaxseed oil may be considered a possible candidate for further investigation as an ophthalmic vehicle. Before such use can be recommended, it is necessary to conduct studies on repeated-dose ocular tolerance, sterility, microbiological quality, oxidative stability, physico-chemical characterization, preservative compatibility, and pharmaceutical formulation. Under the conditions tested, flaxseed oil caused only very slight, transient erythema on intact and abraded skin, with no edema. These findings indicate preliminary cutaneous tolerance after a single application, but should be confirmed in repeated-application and skin-sensitization studies before any dermatological or cosmetic use can be recommended.

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Corresponding author: nora.mimoune@gmail.com