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Original article

Flaxseed Prevents Interferon-alpha Induced Depressive Behavior in Mice: the α-Linolenic Acid is Essential

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Abstract

Background and objectives: Interferon-alpha (IFN-α) is a cytokine with various clinical applications, but it may induce depression by decreasing tryptophan level and producing neuroactive metabolites. Since *Linum usitatissimum* (flaxseed) is a valuable source for amino acids, α-linolenic acid (ALA), and lignans that could prevent inflammation and neurotoxicity, flaxseed effects on IFN-α induced depressant was evaluated. **Methods**: Flaxseed was applied either by whole ground flaxseeds in mice diet, or flaxseed oil by gavage feeding tube until effective antidepressant effects were observed. Seventy-eight male albino mice 25±3 g were used and divided in 13 groups, IFN-α 16×10^{5} IU/kg was injected for 6 days. After the locomotor test, the forced swimming test (FST) was used to measure the immobility time indicating despair behavior, and the sucrose preference test measured anhedonia. **Results**: There were only marginal differences in the locomotor activity; however, the immobility time increased by IFN-α (154.5±11.22 s, vs control 121.3±7.14 s; p=0.031), and sucrose preference was 65% indicating depression. The administration of flaxseed 30% or flaxseed oil 25% with IFN-α significantly reduced the immobility time (92.67±11.60 s and 94.17±10.12 s, respectively, vs IFN-α normal diet, p<0.01), sucrose preference also increased that supported the antidepressant effect. **Conclusion**: Flaxseed could prevent IFN-α induced depressive-like behavior in mice. Although interpretation from animal to human studies needs careful attention, this study supports the use of flaxseed in the diet as reasonable strategy to prevent depression in high-risk individuals, such as patients treated with IFN-α.

Keywords: alpha interferon; alpha linolenic acid; depression; flaxseed; *Linum usitatissimum*

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Introduction

Interferon-α (IFN-α) is a cytokine that plays an important role in innate immunity to virus infections. It has numerous therapeutic uses including hepatitis C and various types of cancer [1]. Recently, it has been shown that IFN-α could reduce respiratory COVID-19 virus load while decreasing the inflammatory biomarkers level in blood circulation [2]. Generally, IFN-α exposure induces 'flu-like' symptoms; in addition, it can induce neuropsychiatric side effects. Long-term treatments can exhibit depression symptoms within 2-3 months of usage [3]. The kynurenine/tryptophan ratio, which is an indicator of indoleamine 2,3-dioxygenase (IDO) activity, increases in patients under IFN-α treatment. IDO as an extrahepatic enzyme converts tryptophan into kynurenine, producing neuroactive metabolites such as kynurenic acid, 3-hydroxykynurenine and quinolinic acid [4]. Interestingly, it has been shown that quinolinic acid levels in cerebrospinal fluid directly contribute to the severity of depression [5]. Tryptophan as a precursor of serotonin or 5-hydroxytryptamine (5-HT) and a rate-limiting

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factor in the synthesis of 5-HT, has an important role in the neurobiology of affective disorders. Since less tryptophan concentration in the blood would cause reduced 5-HT availability inside central nervous system (CNS), it is associated with depression in susceptible patients [6].

Omega-3 polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are crucial nutrients with promising antidepressant effects. Populations that include a larger amount of omega-3 in their diet have a lower incidence of major depressive disorder [7].

Flax (*Linum usitatissimum* L.) from the Linaceae family is an annual plant with seeds known as flaxseed. It has become one of the substantially popular healthy foods in global market because it contains approximately 40% lipids, 30% dietary fiber, 20% protein, and 1% lignans. It also contains B and C vitamins, as well as multi minerals [8]. Flaxseed is the richest plant source of the omega-3 fatty acid, α-linolenic acid (ALA) that contains between 40-60% of its fatty acid content [7]. ALA is converted to EPA, and DHA with different percentage in the body [9]. Flaxseed amino acid profile is comparable to that of soybean; it contains essential amino acids such as tryptophan [8,10].

Studies on the antidepressant effect of soybean following IFN-α induced depression, revealed that the mice with soybean diet were 50% less inactive in FST test. Probably, the tryptophan existing in soybean could have induced 5-HT synthesis leading to an increased 5-HT level in the CNS, playing a part in helping to prevent depression [11]. Common antidepressant drugs should not be proposed for prevention of depression in patients using IFN-α. In addition to side effects, they can expose patients to multiple drug adverse effects [12]. Improving the patients' diet could be a rational approach, since nutritional neuroscience is developing and elucidating the point that nutritional factors are interrelated with human cognition, behavior, and emotions. In the present study the aim was to evaluate the effect of flaxseed on IFN-α induced depression in mice model of despair and anhedonia. For this purpose, first different percentage of ground flaxseed was added to the animal diet to obtain the best antidepressant result, then its effectiveness was evaluated following IFN-α induced depression. Since ALA is found mostly in plants oil, the flaxseed oil antidepressant efficacy was also examined.

Material and Methods

Ethical consideration

All animal procedures were performed in accordance with guidelines for the Care and Use of Laboratory Animals issued by The National Ethical Committee of Iran (Date: 2019-09-21, Ethical Number: IR.MUI.REC.1398.375). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments, at the end of experiments animals were euthanized in a CO_2 chamber.

Chemicals

Depression was induced by IFN-α (Pooyesh Darou, Iran; 3×10^6 IU), a selective serotonin reuptake inhibitors (SSRIs) fluoxetine (Sigma-aldrich, Germany) was chosen as the reference drug, and sunflower oil (Oila, Iran) was applied as a common oil used in the community for comparison with flaxseed oil.

Plant material

Flaxseed (Zardband industry, Iran) containing ALA 50.5% of its total polyunsaturated fatty acids (standardized by Hoortash Apadanna laboratory, Isfahan) was used in the study. The flaxseed regime was prepared by adding ground flaxseed to ground normal mice chow at amounts of 15, and 30% (W/W) and carefully converting them to pallets. Flaxseed oil (Barij industry, Iran) containing 35 % ALA, was administered by gavage feeding tube in concentrations of 12.5%, and 25% (W/W) [13].

Experimental design

Animals

Male albino mice weighing 25±3 g (6-8 weeks old) were housed six in each cage with free access to normal mice chow or flaxseed regime and tap-water at room temperature 21± 2 ºC, on a 12-12 h light-dark cycle (lights on at 6 AM). Totally, 78 animals were used that were divided in 13 groups. Animals were placed in the experimental room 24 h before the test for acclimatization. All experiments were performed between 8 AM and 1 PM in the pharmacology laboratory.

Treatment design

IFN-α (16×10^5 IU/kg body weight) was injected subcutaneously for 6 consecutive days, while control animals received normal saline; tests were performed on day 7 [11]. In a separate group after IFN-α administration a single dose of fluoxetine

20 mg/kg was injected intraperitoneally 30 min before testing on day 7 [14].
Animals were fed with the flaxseed regime for one or two weeks and the tests were performed on the 7th or 14th day until antidepressant effects were observed, control animals were fed with normal mice chow. Since the two-week flaxseed regime was effective, in a separate group it was administered for two weeks while IFN-α was injected from day 9 to 14. Flaxseed oil 12.5%, and 25% were prescribed by gavage feeding tube daily for a week. The effect of, sunflower oil 25% (W/W) was also evaluated, sunflower oil was applied as a common oil used in the community for comparison with flaxseed oil. The sham group received water by gavage feeding tube, this group was applied in order to exclude any possible stress induced by gavage feeding tube in normal animals. In a separate group in addition to flaxseed oil, depression was induced by IFN-α administered from day 2-7. The tests were performed after prescriptions ended on the following day (ie, on day 8 of flaxseed oil ingestion, or day 15 of flaxseed regime).

Locomotor test
This was the first test applied in the study. The locomotor activity was assessed in an open arena (Borj Sanat, Iran) that was divided into 15 zones by red beams. The mice were places at the corner of the arena and allowed to explore for 3 min; by passing through the beams the number of zone entries were counted automatically while rears on hind-legs were recorded manually. The total activity equal to the sum of zone entries (horizontal exploration) and rears (vertical exploration) was considered for each animal.

Forced swimming test (FST)
After the locomotor test, the FST was performed for each animal that evaluates animal despair behavior . Mice were placed in a 2-liter Pyrex beaker (diameter 12.5 cm, depth 12 cm) filled with water (25 ºC) and forced to swim for six min. The measurements were performed during the last four min of the trial after habituation was considered at the first 2 min. The immobility time was defined when no additional activity was observed other than that required to keep the animals' head above the water. Horizontal movement throughout the beaker which involved at least two limbs was measured as the swimming time; upward movements of the forepaws along the side of the beaker were recorded as climbing behavior [15]. The whole experiment was recorded by a camera and analyzed later. After six min, the mice were dried carefully to avoid hypothermia and returned to their home cage.

Sucrose preference test
Anhedonia was measured as another depression phenotype. The test was performed in three consecutive days; the first two days were for habituation. On the first day two bottles of sucrose solution (5 % w/v) were placed in the animals' home cage, and on the second day one bottle of sucrose solution was replaced with water. On the third day, mice had access to the two bottles containing a consistent amount of sucrose solution and tap water that were finally measured after 24 h (i.e., on day 8 of flaxseed oil ingestion, or day 15 of flaxseed regime). The percentage for sucrose preference was calculated according to the sucrose solution and water consumption. A decrease in sucrose preference measured to a level below 65% was taken as a criterion for anhedonia [16].

Data processing and statistical analysis
All statistical evaluation and data processing were carried out by using Excel 2010 and the GraphPad Prism 6 software (La Jolla, USA). The results are expressed as mean ± SEM. In order to compare total activity in the locomotor test and behaviors in the FST, one-way analysis of variance (ANOVA) was performed to compare flaxseed regime with the chow group and flaxseed regime plus IFN-α to IFN-α+chow; flaxseed oil with the sham or sunflower oil group and flaxseed oil plus IFN-α to IFN-α+chow group. The test was followed by Tukey's multiple comparison tests. Values of $p<0.05$ were defined as statistically significant.

Results and Discussion
The flaxseed regime 15% and 30% (as shown in Table 1) increased the animals' appetite during a week compared to the normal chow diet ($p=0.0123$); however, there was only marginal difference in food consumption between flaxseed regime 30% and the normal chow group after 14 days.
These results showed that the flaxseed regime was palatable and animals got familiar to the new taste after a week.

Table 1. Food intake and body weight changes

Group (n=6)	Chow (W1)	FxR 15% (W1)	FxR 30% (W1)	Chow (W2)	FxR 30%(W2)
Food intake (mg/g body weight)	118.00±9.21	160.00±7.85 *	163.00±11.60 *	174.00±12.30	142.00±7.84
% Body weight change	5.75±1.02	13.10±1.69 **	7.58±1.34	8.17±2.45	9.54±3.92

Ground flaxseed in regime (FxR 15 and 30 %) was evaluated after 1 or 2 weeks of ingestion. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison tests; FxR: flaxseeds regimen; W: week; * p<0.05, ** p<0.01 compared with chow (1W) group

Table 2. The effect of flaxseed regime on locomotor and activity during forced swimming test

Groups (n=6)	Locomotor total activity (count)	FST swimming time (s)	FST climbing time (s)
Chow	170.00±10.10	54.38±6.81	28.63± 8.86
W1 FxR 15%	126.00±24.00	40.83±9.84	26.67± 9.35
W1 FxR 30%	160.00±17.40	51.00±8.42	22.00±10.63
W2 FxR 30%	141.00±9.13	94.00±11.49$^{CC\wedge}$	71.50±9.14$^{CC\wedge}$
Control	153.00±16.00	109.30±4.21	9.33±5.58
IFN-α+Chow	114.00±10.80	52.67±9.21***	32.50±6.99*
IFN-α+FxR 30%	95.20±14.30*	123.00±13.11$^{\#}$	24.33±8.09
IFN-α+Flx	89.50±9.19*	168.80±11.79***$^{\#}$	21.00±7.71

Total activity= horizontal exploration + vertical exploration. Ground flaxseed was applied in the regime (FxR 15 and 30 %) for one or two weeks. IFN-α (16×10^5 IU/kg) was injected alone or from day 9 to 14 of applying FxR 30%, control group was injected with normal saline, Flx (20 mg/kg) was injected 30 min before the tests. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison tests. FxR: flaxseed regime; IFN-α: Interferon-α; Flx:Fluoxetine; FST: forced swimming test; W: week; ^{CC} p<0.01 compared with the chow group; *p<0.05, ***p<0.001 compared with control group; ^ p<0.01 compared with FxR 30% (1 w); #p<0.001 compared with IFN-α+Chow.

The percentage of weight change after a week of applying flaxseed regime 15% significantly increased (p=0.002) compared with normal chow diet, but this effect was not observed with flaxseed regime 30% even after 14 days. This indicates that various factors can affect the weight change in animals.

The locomotor test is commonly performed before most behavioral pharmacological test. As shown in Table 2, there was no noticeable difference in the locomotor activity following applying normal chow or different flaxseed regime (the values for one- and two-weeks' normal chow consumption were merged together). Co-administrating flaxseed regime 30% and IFN-α significantly reduced total activity compared with the control group (p=0.0125). This result was parallel to the standard drug fluoxetine.

Ingesting flaxseed regime 15, or 30% for one week did not reduce the immobility time during the FST (Figure 1A). Therefore, flaxseed regime 30% was administered for two weeks that significantly reduced the immobility time (84.66±7.36 s, vs normal chow 150.85±12.90 s; p=0.0014). The reduced immobility time indicates antidepressant-like effect, since immobility reflects a progress of disappointment of persistence in escape behavior (i.e. despair behavioral). This effect was also confirmed by the sucrose preference test (Table 3) that showed 80% preference for sucrose solution over water. By injecting IFN-α, the immobility time increased (154.50±11.22 s, vs control group 121.30±7.14 s; p=0.031), therefore it imposed depressive like effect that was in line with previous research [11]. IFN-α depressant activity is highly dependent on dosage and timing of the behavioral tests and administration could describe some behavioral discrepancies during studies [4]. Several mechanisms are involved in cytokines' depressive effects including alteration in the tryptophan pathway, and inflammatory effects on neuroplasticity and neurogenesis [4,17].

Table 3. The effect of flaxseed regime and flaxseed oil on sucrose preference test

Groups	Sucrose preference (%)
Control	77
1W FxR 15%	68
1W FxR 30%	65
SfO 25%	72
FxO 12.5%	84
FxO 25%	86

Percentage of sucrose preference = (sucrose consumption/ sucrose consumption+ water consumption) ×100. The chow and control results were similar thus only one is applied; n=6; FxR: flaxseeds regimen; FxO: flaxseed oil; IFN-α: interferon-α; SfO: sunflower oil

By applying the flaxseed regime 30%, the depressant-like effect induced by IFN-α was halted. The immobility time significantly reduced (92.67±11.60 s, p=0.0033 compared with IFN-α that had normal chow), the result was similar to injecting antidepressant drug fluoxetine. Similar results were reported by the sucrose preference test (Table 3) during co-administration of IFN-α and flaxseed regime 30%. The preference

increased to 90%, while the preference was marginal when IFN-α was injected alone (65%). There are reasons for flaxseed antidepressant like effects as it is a good source of nutrients. Firstly, flaxseed is rich in amino acids such as essential amino acids (threonine, methionine, valine, phenylalanine, tryptophan and lysine); some are important precursor for the synthesis of brain neurotransmitters [10]. IFN-α increases IDO activity that is an enzyme converting tryptophan to neuroactive metabolites (kynurenic acid); therefore, the 5-HT/ kynurenic acid ratio decreases [5]. A study on cancer patients receiving either IFN-α or IL-2 demonstrated decreases in tryptophan serum concentrations and the ratio of tryptophan to other amino acids (like tyrosine, isoleucine, valine), which was related to the depression severity [18]. This statement is supported by a previous study that showed soybean diet, as a good source of tryptophan, counteracted with IFN-α depression-like effects [11]. In addition, the mobile behavior during FST following flaxseed regime was quite similar to fluoxetine, supporting the possible involvement of the serotonergic system. That is, catecholamine selective antidepressants preferably increase climbing time while the SSRIs like fluoxetine mostly increase swimming time [15]. As shown in Table 2, in the flaxseed regime 30% for two weeks, group climbing ($p=0.006$) and swimming time ($p=0.008$) were significantly higher than the normal chow group. As it was consumed along with IFN-α injections, flaxseed regime 30% alike fluoxetine significantly increased the swimming time compared with animals that ingested normal chow with IFN-α ($p<0.001$).

The second assumption is that, flaxseed contains approximately 40% lipids especially ALA, that might also be considered for its benefit antidepressant effect. Many clinical studies and meta-analyses have shown that omega-3 polyunsaturated fatty acids have antidepressant effects [19,20]. Omega-3 polyunsaturated fatty acid, EPA, was effective in preventing depression in hepatitis C virus infected patients that received IFN-α therapy [21]. Therefore, the polyunsaturated fatty acid content in flaxseed regime 30% maybe a reason for preventing IFN-α depression, by anti-inflammatory effects.

Furthermore, flax contains substantially more lignans than other plants [8]. Lignans are phenolic compounds that act as both antioxidants and phytoestrogens. Lignans have neuroprotective effects that have been attributed, partly to their anti-Inflammatory, anti-oxidative, and antiplatelet aggregation properties [22]. Kynurenine the metabolite produced by IDO can cross the blood-brain barrier and break to metabolites such as quinolinic acid that is an agonist of the N-methyl-D-aspartate (NMDA) receptor; it has neurotoxic effects [23]. Honokiol, is a lignan that prevented oxidative stress in mice brain that was exposed to NMDA by restoring cellular glutathione levels and glutathione peroxidase activity [24]. Thus, the phenolic compounds of flaxseed regime could be important in preventing potential IFN-α neurotoxicity.

Excessive use of flaxseed could cause increased intestinal movements and abdominal bloat due to high fiber content leading to laxative effects [25]. Cyanogenic glycosides are nitrogen-containing secondary metabolites in different plants. Diglycosides and monoglycosides are the naturally present cyanogenic glycosides in flaxseed which can be hydrolyzed to produce hydrogen cyanide, producing acute or chronic toxicity [26]. These toxic chemicals need to be removed from flaxseeds to enhance their health benefits.

There was no noticeable difference in the locomotor activity after a week of feeding the animals with flaxseed oil compared with sham group; or injecting IFN-α, and flaxseed oil plus IFN-α compared with control (Table 4). As it is presented in Figure 1B, during FST, the immobility time of flaxseed oil treatments was significantly lower than sham (142.00±16.66 s); 78.83±13.27 s for flaxseed oil 12.5% ($p=0.0141$) and 71.00±14.68 s for flaxseed oil 25% ($p=0.0095$). The co-administration of flaxseed oil 25% with IFN-α significantly reduced the immobility time (94.17±10.12 s, vs IFN-α+chow 154.5±11.22 s, $p=0.0025$). As presented in Table 3, flaxseed oil 25% caused a high preference of sucrose solution to water that was also observed when it was concomitantly administered with IFN-α, that supported flaxseed oil antidepressant efficacy.

The activities during FST are shown in Table 4. The climbing time of flaxseed oil 25% group was significantly higher than the sham group ($p=0.031$). Co-administration of flaxseed oil 25% with IFN-α produced a meaningful change in the swimming time compared with IFN-α+chow ($p<0.001$).

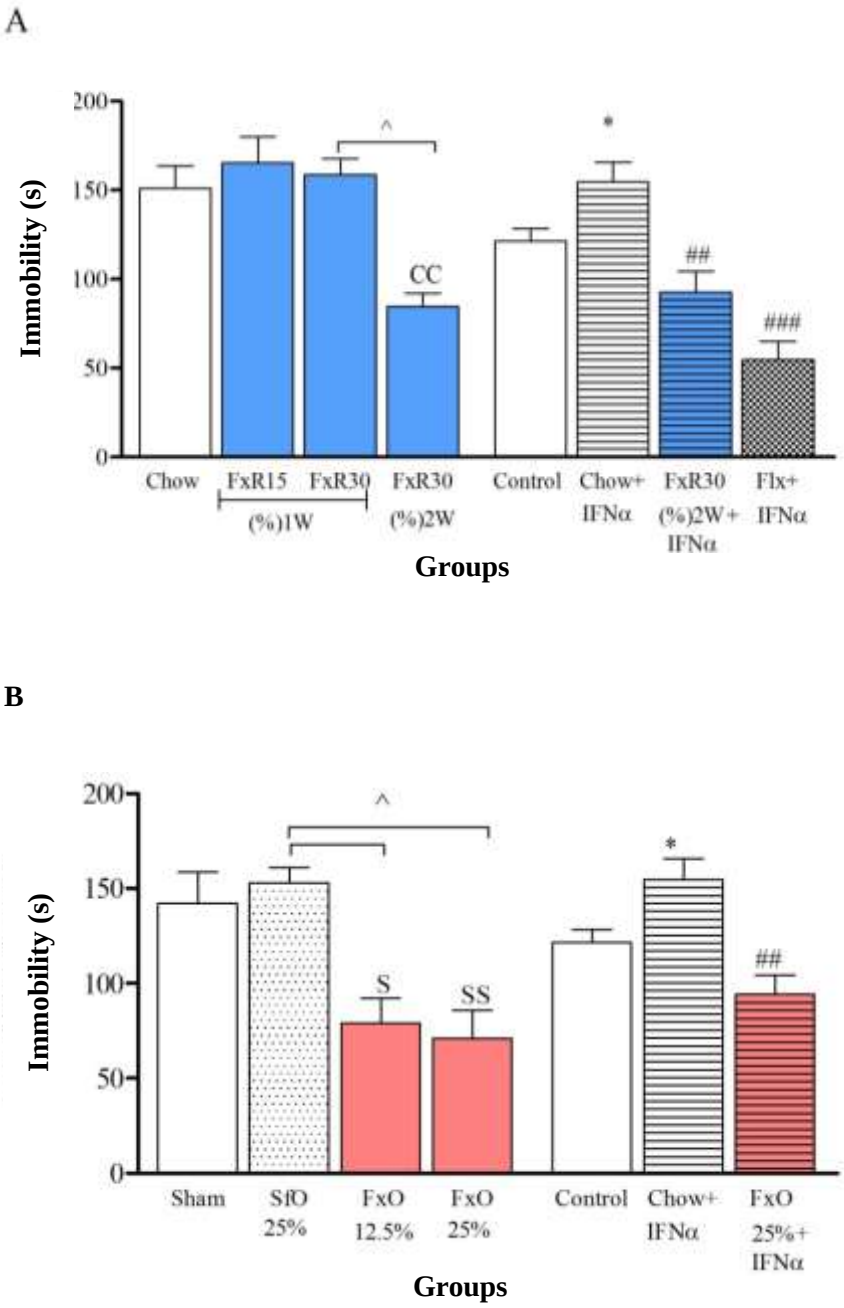

Figure 1. The effect of flaxseed on FST, A) Immobility time following administration of FxR (15 and 30 %) for one or two weeks. Flx (20 mg/kg); B) Immobility time following feeding with FxO 12.5% or 25%, or SfO 25% for a week; The sham group was fed with water. IFN-α (16×10^5 IU/kg) and control group received normal saline. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison tests (n=6); W: week; Flx: fluoxetine; FxR: flaxseed regime; FxO: flaxseed oil; SfO: sunflower oil; ^{CC} $p<0.01$ compared with chow group; ^{S} $p<0.05$, ^{SS} $p<0.001$ compared with sham groups, *$p<0.05$ compared with the control group. ## $p<0.01$, ###$p<0.001$ compared with Chow+IFN-α. ^ $p<0.01$ as shown

Feeding the animals with sunflower oil 25% did not make obvious changes in behavior during FST compared with the sham group. Flaxseed has higher value and appreciably more health benefits due to the larger variety of vitamins, minerals and natural nutrients (fibers and phenolic compounds) than flax seed oil that mainly contains ALA [8]. Flaxseed oil in a similar manner as flaxseed regime reduced the immobility time in the FST, and prevented the depression induced by IFN-α. But, the difference in the antidepressant-like potency should be considered. Flaxseed oil was

effective in lower concentration and lower ingestion period compared with flaxseed regime. Therefore, the polyunsaturated fatty acid, ALA content of flaxseed has more vital role in its mental health benefits compared with other components. Different mechanisms have been proposed for omega-3 effects against anxiety [27].

Table 4. The effect of flaxseed oil on locomotor and activity during FST

Groups (n=6)	Locomotor total activity (count)	FST swimming time (s)	FST climbing time (s)
Sham	167.80±25.49	73.33±14.49	24.67±13.59
SfO 25%	122.20±9.49	49.33±5.88	31.17±6.71
FxO 12.5%	112.00±18.95	104.70±27.26	56.50±16.80
FxO 25%	158.80±15.06	91.33±8.32	71.83 ± 7.75^{S}
Control	153.00±16.0	109.30±4.21	9.33±5.58
IFN-α+Chow	114.00±10.80	$52.67\pm9.21^{***}$	$32.5\pm6.99^{*}$
IFN-α+FxO 25%	120.50±15.26	$137.80\pm10.02^{\#}$	6.66±2.94

Total activity= horizontal exploration + vertical exploration. The sham group was fed with water and other groups by flaxseed oil (FxO 12.5% or 25%), or sunflower oil (SfO 25%), IFN-α (16×10^5 IU/kg) was injected, control group was injected with normal saline. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison tests; SfO: sunflower oil; FxO: flaxseed oil; IFN-α: interferon-α; FST: forced swimming test; ^{S} P<0.05 compared with sham group; *p<0.0 5; ***p<0.001 compared with control group; #p<0.001 compared with IFN-α+Chow.

One of the proposed mechanisms for polyunsaturated fatty acids antidepressant activity is their anti-inflammatory effect by antagonizing membrane arachidonic acid, thus reducing prostaglandin E2 synthesis and neuroprotection [28].

Phospholipase A2 and cyclooxygenase-2, are the main enzymes in the metabolism of polyunsaturated fatty acids, prostaglandin production and inflammation, therefore they are related to cytokine-induced depression and sickness behavior [29]. IFN-α has also been used as a model to study the role of inflammation in depression. Non-steroid anti-inflammatory drugs (ibuprofen, and celecoxib) were able to prevent IFN-α induced depression in mice [30]. On the other hand, the antidepressant effect was not observed by sunflower oil. It has different fatty acid profile primarily composed of polyunsaturated fatty acid linoleic acid (omega-6), and high ratio of omega-6:3 [31]. Meanwhile, there has long been concerns about the risk of an excessive intake of edible oils rich in omega-6 polyunsaturated fat acid (such as sunflower). Due to all of this evidences, anti- inflammation effect of ALA could be one reason for preventing against IFN-α depression-like effect.

However, the main drawback in this study was that since this study was a behavior pharmacology study the changes in brain neurotransmitter (5-HT, and catecholamine) level, or the serum level of inflammatory mediators were not evaluated following the administration of flaxseed regime or flaxseed oil that warrants further evaluations.

Conclusion

To our knowledge, this study for the first time proved the effectiveness of flaxseed in preventing IFN-α induced depression in mice. Flaxseed oil showed more effective antidepressant effects than flaxseed regime, ALA content of flaxseed might be promising for preventing IFN-α depression. Taken together and with the evidence that were discussed, this study supports the use of flaxseed in the diet as an effective strategy to prevent depression in high-risk groups, such as individuals taking IFN-α. Although interpretation from animal to human studies demands further cautions.

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Author contributions

Azadeh Mesripour was involved in supervision, conception, design, execution and interpretation of the study and writing and editing of the manuscript; Maryam Almasi contributed in the experiments and writing of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

ALA: α-linolenic acid; CNS: central nervous system; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; FST: forced swimming test; Flx: Fluoxetine; 5-HT: 5-hydroxytryptamine; FxR: flaxseeds regimen; FxO: flaxseed oil; IFN-α: interferon-α; IDO: indoleamine 2, 3-dioxygenase; NMDA: N-methyl-D-aspartate; SfO: sunflower oil.