Evaluation of the Antidepressant Like Effect for Some Natural Supplements Against Reserpine Induced Behavioral Depression in Mice

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Abstract: Background; the aim of this study was conducted in a trial to evaluate a model of antidepressant like effect, with fewer side effects and have superior efficacy. This might be achieved through combining two natural antidepressant supplements, omega-3 fatty acids (ω_3, 400mg/kg) and B-vitamins [folic acid (B_9), 3mg/kg) and cyanocopalamine, (B_{12}),1.5mg/kg)] then comparing its antidepressant like effect either against the classical antidepressant drug fluoxetine (FLU, 30mg/kg) or by a combine of each of them with a subthreshold dose of fluoxetine (8mg/kg). Open field, forced-swimming test (FST), tail-suspension test (TST), were performed to assess the potential antidepressant-like effect of this combine and to study the mechanisms by which the combine exert the antidepressant-like action in adult reserpinized mice. As well as the levels of catecholamine in hippocampus and cortex of mice brain, and the levels of eicosapentanoic acid (EPA), docosahexanoic acid (DHA) and homocysteine (Hcy) in serum, hippocampus and cortex of mice were determined, Results of this study showed that: 1- Intraperitoneal injection (ip) of 10 mg/kg reserpine for 2 weeks induced depression in mice through increasing the immobility time in TST, FST, and increasing the Hcy levels in all tested tissues. As well as decreasing the levels of catecholamine, omega-3, and decreasing the climbing and swimming time in FST. 2- Oral administration of the combined treatment markedly reduced the time of immobility in FST and TST and their effects are somewhat similar to that produced by the active dose of fluoxetine. While the effect of the individual treatment in FST partially reversed reserpine-induced depression. 3- The reduction in immobility produced by B-vitamins and FLU in FST was through increasing swimming behavior, suggesting that these supplements may act in the serotonergic system. This effect was augmented by the act of their combination. 4- The reduction effect produced by omega-3 was through increasing mainly climbing and partially swimming behaviors, suggesting that it may act mainly in the noradrenergic or dopaminergic system and to a lesser extend with serotonergic system. 5- The reduction produced by combining omega-3 plus B-vitamins was through increasing both swimming and climbing behaviors suggesting that this combination may act in both the serotonergic, noradrenergic and dopaminergic systems. 6- The behavioral data are in parallel and supporting the results of biochemical analysis showing; maximum elevations in the levels of monoamines, omega-3 as well as reduction in the levels of Hcy were achieved by omega-3 and B-vitamins combination. Conclusion: This study provides evidences that co-administration of omega-3 plus B-vitamins possesses potent antidepressant-like activity and this antidepressant-like action, as obvious in FST, may involve different transmitter systems. To better understand these actions, future studies are needed.
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Key words: depression, reserpine, omega-3, B12, folic acid, fluoxetine, catecholamine, open field test, forcing swimming and tail suspension tests.

1. Introduction

Depression is generally defined as the feeling of being ‘down’ or ‘blue’ for many days. It is one of most significant major public health problems. Major depression is characterized for symptoms at the psychological, behavioral and physiological levels (Lijian et al., 2011). Reserpine is an indole alkaloid antipsychotic and antihypertensive drug (Henry and Scherman, 1989) because of its numerous side-effects, it is rarely used today. Depression by reserpine occurs at any dose and may be severe enough to lead to suicide (Quetsch, 1959). Reserpine almost irreversibly blocks the uptake and storage of serotonin, norepinephrine and dopamine into synaptic vesicles by inhibiting the Vesicular Monoamine Transporters (VMAT), so it can precipitate depressive-like symptoms in humans and rodents (Mao and Huang, 2005). Antidepressants are commonly prescribed for depression and other affective disorders. Despite the advances in the treatment of depression with selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs), restrictions on their use have caused controversy and off-label prescription a risk. Therefore, the natural alternatives to antidepressant medication form were

considered as one of the safest options of medication (Lijian et al., 2011). Nutrition has been implicated as one of the causes and risk factors for depression. A few nutrients have been studied directly for their antidepressant properties, both to treat and prevent depression, as well as related conditions (Boult et al., 1999). Omega-3 fatty acids have been proposed as a treatment for depression, often suggested to be combined with other treatments; others used it as a monotherapy (Liperoti et al., 2009). There were several different independent lines of evidence suggesting that omega-3 fatty acids play a role in depression, and that the theory of omega-3's role in depression was biologically plausible. Epidemiol-ogical studies linking low fish consumption (the primary source of omega-3) to increased rates of depression (Liperoti et al., 2009). Evidence suggests that low levels of omega-3 fatty acids are correlated with depressive symptoms during pregnancy and after delivery (Levant, 2010). Omega-3 fatty acids may produce antidepressant effects due to their role in serotonin functioning (Borja-Hart and Marino, 2010). Findings in other animal models and in humans also indicate a role for ω3 PUFAs in the regulation of hippocampal brain-derived neurotrophic factor expression and function (Wu et al., 2007). Scientific research has established that depression is often associated with high levels of omega 6 fatty acids (Arachidonic acid) and low levels of omega 3 fatty acids (Kiecolt-Glaser et al., 2007).

Many foods have been scientifically studied to contain antidepressant-like quality due to their composition. The natural compounds in these foods act as precursors for serotonin production. Some of the precursors present in food are tryptophan, folic acid and Vitamin B12. Folic acid (folate) is a water-soluble B-vitamin that has been implicated in depressive disorders (Fava, 2007). Several studies regarding its role in the pathophysiology of depression shows that patient with depression often have a functional folate deficiency (Coppen and Bolander-Gouaille, 2005) and the severity of such deficiency correlates with depression severity Tiemeier et al., 2002); low folate is associated with poor antidepressant response. It has been shown to improve the treatment response to other antidepressants and has been shown to co-administrate it with sublethal doses of an antidepressant to decrease its side effect and to augment its antidepressant effect (Brocardo et al., 2009). Vitamin B12 also has an important role in depression, raised homocysteine, low folate and vitamin B12 concentration may cause brain damages, dementia and depression (Coppen and Bolander-Gouaille, 2005). From this point of view, This study was conducted in a trial to evaluate a model of antidepressant like effect without/or with little side effects and with superior efficacy, through combining two natural antidepressant supplements, omega-3 and B-vitamins (B9 and B12) and to study the mechanism by which the combine exert the antidepressant-like action in reserpinized mice.

2. Materials and Method

Animals

Male albino mice 25 ± 5g were used. The animals were brought from laboratory animal breeding of National Organization for Drug Control and Research (NODCAR), Giza, Egypt. Mice were kept under strictly hygienic conditions, fed on a standard basal diet, and allowed free access to drinking water. Before the beginning of the experiment, animals were allowed to adapt to the environment for 2 weeks. The experimental protocols were approved by the NODCAR's Institutional Ethical Guidelines for Animal Care and Usage.

Materials

Reserpine, folic acid, B12 and fluoxetine hydrochloride were purchased from Sigma Co. USA. Omega-3 fish oil was purchased from Egyptian local market, Sedico.

B12 and folic acid were freshly prepared before administration dissolved in water and given orally; reserpine was injected *i.p* in 5% Dimethyl sulfoxide (DMSO), while Omega 3 was given orally dissolved in 0.5% Carboxymethyle-cellulose, (CMC). The tested doses were equivalent to the daily human dose.

Induction of depression: All mice (except 12 mice were left as negative control group) were injected *i.p* by 10 mg/kg/B.wt of reserpine for two weeks, then the negative control and reserpinized mice treated daily for 4 weeks as follows:

G1, Negative control group (C), fed on basal diet and orally administrated with 0.5% of CMC and injected *i.p* with 5% DMSO (as vehicles).

G2, Positive control group (Res), administrated and injected with vehicles.

G3, Omega-3 fish oil treated group (ω_3), orally administrated 400 mg/kg/B.wt omega-3 fish oil (contains 120mg of DHA & EPA).

G4, B-vitamins treated group (B_{9+12}), orally administrated a mixture of 3mg /kg/B.wt folic acid (B_9) and 1.5mg/kg/B.wt B_{12}.

G5, Fluoxetine treated group (FLU), orally administrated 30mg/kg/B.wt FLU.HCl

G6, Omega3+fluoxetine treated group (ω_3+Flu), orally administrated as in G3 with a subthreshold dose (8mg/kg/B.wt) of FLU.HCL.

G7, B-vitamins+fluoxetine treated group (B_{9+12}+ Flu), orally administrated as in G4 with a subthreshold dose (8mg/kg/B.wt) of FLU.HCL.

G8, Omega-3 and B-vitamins combined treated group (ω_3+B_{9+12}), administrated a combined treatment as in G3+4.

At the end of the treatment schedule, animals were subjected to behavioral studies including; Open field test (for studying locomotors activity), forcing swimming test and tail suspension test (for studying antidepressant activity), then they were killed by sudden decapitation. The brain was rapidly and carefully excised and then dissected on dry ice glass plate to separate hippocampus and cortex. Tissues were homogenized in iced 70% methanol, centrifuged and the supernatant was separated. Blood samples were collected, left to coagulate and serum was separated. The separated serum and supernatant of homogenate tissues were processed for the biochemical analysis which included: monoamines transmitters (serotonin, noradrenaline ND, and dopamine, DA) were determined in both hippocampus and cortex by using HPLC according to the method of Pagel et al., (2000), serum and tissue concentrations of homocysteine was determined by HPLC method of Jayatilleke and Shaw, (1993) and omega 3 PUFA (EPA, DHA) were determined in serum and tissues by using GC according to the methods of Xu et al., (1994).

Open field Test: Locomotors activity was measured in the open-field test. One hour after the pre last treatment, all subjects were tested in the open-field apparatus. This test was used to rule out the possibility that a reduction in the immobility time in the FST is due to an increase in the locomotors activity of mice (false positive antidepressant effect). The mice were placed individually in an apparatus consisted of a square arena (50 cm x 50 cm), with a 30-cm high, opaque, white wall. The floor was divided into equal squares. The motion path of the mouse was continuously traced manually. The mice were individually placed in the center of the arena and the number of squares crossed (ambulation) over 3 minutes was measured. The open field was cleaned with a water-alcohol (10%) solution before behavioral testing to avoid possible bias due to odors and/or residues left by mice tested earlier. (Saillenfait and Vannier, 1988).

Forced Swimming Test: The procedure was done as described by Cryan et al., (2002). Mice were placed in clear glass cylinder (diameter 18cm, height 28 cm) containing 15 cm deep water for 6 min. the duration of the immobility was recorded during the last 5 min of the 6 min trial. Immobility time was used as an index of depressive behavior. Immobility, i.e., when the mice remained floating in the water, making only the necessary movements to keep their heads above water, and the active behaviors include: swimming, i.e., when the animal made active swimming motions; and climbing, i.e., when they made vigorous movements with their forepaws in and out of the water, were video-taped and scored (Detke and Lucki, 1996). The water was changed for each mouse after test session to avoid influence of alarm substance. Following the test, the animals were dried in a warm enclosure (30.0 ±1.0 °C).

Tail suspension test: A cord of about 50 cm in length was stretched between two metal tripods to which the mouse was attached by the tail with sticky tape. Mice were considered immobile when they hung passively and completely motionless, the time of immobility was video-taped and scored (Steru et al., 1985).

Statistical analysis: Data are expressed as mean ± S.E. All the data were analyzed using one way analysis of variance (ANOVA) followed by determination of least significant difference (LSD) for multiple comparison test. P-values≤ 0.05 was considered significant.

RESULTS

Effect of treatment on catecholamine function

The data in table (1a&b), showed that the i.p injection of reserpine (10mg/kg/B.wt) for 2 weeks induced a significant decrease ($P<0.05$) in catecholamine levels in both cortex and hippocampus. Administration of ω_3, B-vitamins and their combinations were significantly restored ($P<0.05$) the monoamine levels in hippocampus area (except DP level that can partially ($P> 0.05$) restored by B-vitamin and FLU). The data showed that the amelioration effect of the combined treatments on the levels of catecholamine has more pronounced effect than that did by the individually one and that ω_3+ B_{9+12} combination gave the marked effect. In cortex area the levels of monoamines differ according to the type of treatment. B-vitamin treatment and their combinations could restored ($P<0.05$) NE level significantly but ω3 treatment and their combinations significantly restored ($P<0.05$) DP level, while serotonin level was markedly increased ($P<0.05$) by all treatments. FLU treatment could restore serotonin and NE in both two areas and failed to restore DP.

Effect of treatment on omega-3 fatty acids (DHA, EPA) content in serum and brain area: The present study showed that after reserpine injection, only DHA contents of serum, hippocampus and cortex were significantly decreased ($P<0.05$), and this effect was significantly restored by ω_3 treatment and their combinations. The data also showed that treatment with B-vitamins and their combinations significantly ($P<0.05$) restored DHA in cortex and hippocampus and partially in serum, while treatment with FLU could not restored this level significantly in all tested tissues (table 2a).

The effect of treatment on Hcy content in serum, hippocampus and cortex: The data implicated in table (2b) showed that *i.p* injection of reserpine increased (P<0.05) the level of Hcy content in serum and both two brain areas (hippocampus, cortex) in comparison with that observed in control group. They also showed that administration of B-vitamins and their combinations decreased this level significantly (P<0.05) in both serum and the two brain areas in comparison to reserpine treated group .While this level was partially restored by FLU, ω3 and ω3+FLU treatment.

Effect of treatment on open field test: As shown in figures (1, 2) and table (3), the mice in control group showed normal activity, they moved about actively crossing 32 squares/3min; whereas the reserpine-treated mice were depressed and crossed only 17 squares/3min. These differences were statistically significant (P < 0.05). There was no any significant difference in the number of squares crossed between reserpinid mice and all treated groups.

The effect of treatment on tail suspension and forced swimming tests: Table (3) and igures (1, 3) show the effects of reserpine, and tested materials on the duration of immobility in the forced swim and tail suspension tests. Both the individual and combined treatment reduced the duration of immobility that increased by reserpine injection. This effect was clearer in TST than in FST. Where in FST, the significant reduction effect (P<0.05) was shown only in the combined treatment. Similar effects were observed for treating the mice with classical antidepressant FLU at the dose of 30 mg/kg and this effect was more pronounced in TST (29% reduction) than that observed in FST (20% reduction).The data in table (3) and figure (4) also demonstrated that the reduction effect by B-vitamins and their combination was through increasing swimming activity (P<0.05) without affecting the climbing activity. While the reduction effect caused by ω3 and their combinations treatment was through increasing (P<0.05) the affect on both swimming and climbing behaviors, in parallel to that observed in reserpine treated group.

Table (1): The effect of omega-3, B-vitamins (B_{9+12}), fluoxetine and their combined treatment on the level of catecholamine in a) hippocampus, b) cortex, of adult reserpinized mice after 4 weeks of treatment.

a)

Animal group n= 12	Hippocampus catecholamine (ng/g)		
	Noradrenaline	Dopamine	Serotonin
C	305.45 ± 7.23	235.11 ± 6.98	140.00 ± 2.15
Res	243.65 ± 5.76*	201.47 ± 8.53*	67.74 ± 1.75*
Flu	284.74 ± 9.51#	211.98 ± 12.66	113.22 ± 4.77#
ω_3	271.14 ± 11.64#	223.88 ± 5.14#	87.74 ± 2.34#
B_{9+12}	277.55 ± 8.10#	208.22 ± 4.35	91.37 ± 3.11#
ω_3+ Flu	281.08 ± 20.08#	225.11 ± 14.45#	93.14 ± 7.14#
B_{9+12}+Flu	281.74±15.11#	220.00 ± 20.74#	111.89 ± 6.08#
ω_3+ B_{9+12}	291.05 ± 10.01#	222.43 ± 5.38#	122.47 ± 2.12#,a

b)

Animal group n= 12	Cortex catecholamine (ng/g)		
	Noradrenaline	Dopamine	Serotonin
C	268.87 ± 7.45	373.35 ± 7.46	167.14 ± 2.84
Res	231.56 ± 5.78*	289.46 ± 11.24*	112.76 ± 2.32*
Flu	247.32 ± 3.74#	294.45 ± 8.76	132.32 ± 4.33#
ω_3	234.71 ± 8.53	320.00 ±16.74#	124.74 ± 3.43#
B_{9+12}	246.00 ± 3.67#	311.42 ± 17.43	124.11 ± 4.14#
ω_3+ Flu	241.34 ± 11.28	318.11 ± 6.85#	127.14 ± 7.14#
B_{9+12}+Flu	261.74 ± 10.14#	314.00 ± 15.14	141.89 ± 11.08#
ω_3+ B_{9+12}	252.34 ± 6.47#	332.02 ± 6.46#	128.84 ± 4.34#

Data expressed as mean of 12 mice ± SE, multiple comparison test was done by using ANOVA followed by LSD, Significant different between C and Res groups: *P<0.05, between Res and the treated groups: #P<0.05, between FLU and the treated groups: aP<0.05.

Table (2): The effect of omega-3, B-vitamins (B_{9+12}), fluoxetine and their combined treatment on the level of a) Omega-3 (EPA, DHA), b) homocysteine, in serum, hippocampus and cortex of adult reserpinized mice after 4 weeks of treatment.

a)

Animal group n= 12	Hippocampus ω_3 (g/100g TFA)		Cortex ω_3 (g/100g TFA)		Serum ω_3 (g/dl TFA)	
	EPA	DHA	EPA	DHA	EPA	DHA
C	5.45 ± 0.30	6.11 ± 0.36	5.11 ± 0.24	5.87 ± 0.43	4.22 ± 0.19	9.78 ± 0.38
Res	5.22 ± 0.34	4.48 ± 0.34*	5.00 ± 0.14	4.14 ± 0.27*	4.05 ± 0.22	8.54 ± 0.34*
Flu	5.12 ± 0.43	5.00 ± 0.38	5.14 ± 0.49	4.89 ± 0.19$^{\#}$	4.24 ± 0.40	9.11 ± 0.51
ω_3	5.43 ± 0.46	6.17 ± 0.48$^{\#}$	5.22 ± 0.44	5.11 ± 0.34$^{\#}$	4.44 ±0.34	11.28 ±0.74$^{\#}$
B_{9+12}	5.31 ± 0.43	5.76 ± 0.37$^{\#}$	5.13 ± 0.37	5.11 ± 048$^{\#}$	4.11 ± 0.25	8.98 ± 0.38
ω_3+ Flu	5.49 ± 0.22	6.67 ± 0.64$^{\#,a}$	4.98 ± 0.22	5.24 ± 0.26$^{\#}$	4.52 ± 0.23	11.97 ±0.82$^{\#}$
B_{9+12}+Flu	5.21 ± 0.36	5.74 ± 0.21$^{\#}$	4.78 ± 0.41	5.00 ± 0.14$^{\#}$	4.19 ± 0.17	9.11 ± 0.88
ω_3+ B_{9+12}	5.37 ± 0.26	7.15 ± 0.34$^{\#,a}$	5.12 ± 0.12	5.19 ± 0.34$^{\#}$	4.78 ±0.14$^{\#}$	11.79 ±0.34$^{\#}$

b)

Data expressed as mean of 12 mice ± SE, multiple comparison test was done by using ANOVA followed by LSD. Significant different *between* C and Res groups: *P<0.05, *between* Res and the treated groups: $^{\#}$P<0.05, between FLU and the treated groups: ^{a}P<0.05.

Animal group n= 12	Hippocampus Hcy (nmol/g)	Cortex Hcy (nmol/g)	Serum Hcy (ug/ml)
C	0.96 ± 0.11	1.17 ± 0.077	8.24 ± 0.44
Res	1.38 ± 0.12*	1.44 ± 0.092*	9.87 ± 0.46*
Flu	1.26 ± 0.091	1.28 ± 0.084	9.68 ± 0.64
ω_3	1.29 ± 0.11	1.24 ± 0.14	9.02 ± 0.80
B_{9+12}	1.14 ± 0.081$^{\#}$	1.12 ± 0.12$^{\#}$	8.59 ± 0.34$^{\#}$
ω_3+ Flu	1.28 ± 0.094	1.21 ± 0.078	9.90 ± 0.67
B_{9+12}+Flu	1.18 ± 0.16	1.08 ± 0.076$^{\#}$	8.66 ± 0.44$^{\#}$
ω_3+ B_{9+12}	1.11 ± 0.060$^{\#}$	1.04 ± 0.080$^{\#}$	8.74 ± 0.28$^{\#}$

Table (3): The effect of omega-3, B-vitamins (B_{9+12}) fluoxetine and their combined treatment on the animal behaviors in open field, TST and FST of adult reserpinized mice after 4 weeks of treatment.

Data expressed as mean ± SE, multiple comparison test was done by using ANOVA followed by LSD.

Animal group	Open Field (n=12) Ambulation (no of squares crossed	TSA (n=6) Immobility time in second	FST (n=6) Immobility time in second	FST (n=6) Swimming time in second	FST (n=6) Climbing time in second
C	24.64 ± 1.87	150.67 ± 8.98	110.18 ± 9.12	169.89 ± 12.40	20.16 ± 2.07
Res	17.22 ± 2.40*	270.22 ±11.85*	188.42 ±12.44*	106.92 ± 7.00*	4.44 ± 0.40*
Flu	16.54 ± 1.55	192.87 ±12.54$^{\#}$	150.14 ± 11.55$^{\#}$	144.54 ± 8.14$^{\#}$	4.78 ± 0.25
ω_3	18.29 ± 2.11	240.00 ± 9.11$^{\#}$	163.04 ± 10.11	128.00 ± 9.11	7.19 ± 0.51$^{\#}$
B_{9+12}	17.88 ± 2.85	229.05 ± 11.13$^{\#}$	160.17 ± 12.53	134.83 ± 12.15$^{\#}$	4.88 ± 0.34
ω_3+ Flu	16.18 ± 1.04	224.43 ± 10.28$^{\#}$	154.60 ± 11.74$^{\#}$	141.78 ± 10.84$^{\#}$	5.08 ± 0.28
B_{9+12}+Flu	17.09 ± 2.16	197.33 ± 14.96$^{\#}$	148.67 ± 9.96$^{\#}$	147.18 ± 12.16$^{\#}$	4.58 ± 0.36
ω_3+ B_{9+12}	18.64 ± 2.60	207.54 ± 12.40$^{\#}$	149.78 ± 8.60$^{\#}$	141.17 ± 8.60$^{\#}$	6.98 ± 0.30$^{\#}$

Significant difference between C and Res groups: * P<0.05; between Res and the treated groups: #p<0.05.

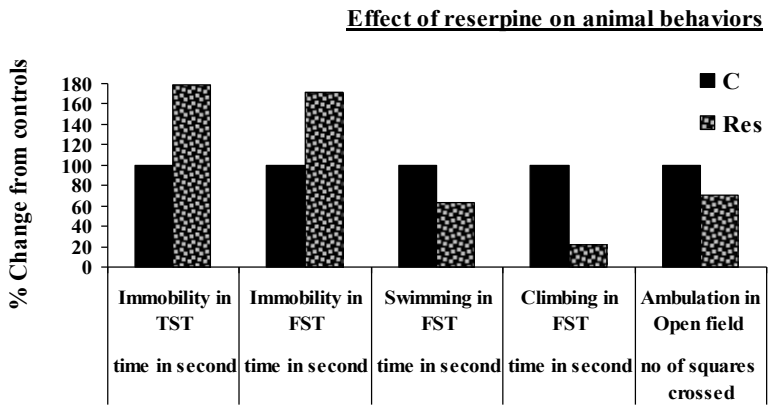

Figure 1: Effects of two weeks treatment with reserpine injection (10mg/kg) on the adult mice behaviors in TST, FST, and open field test. Columns represent % Changes from control group. Reserpine increased the immobility in both two tested, while swimming, climbing (in FST) and ambulation (in open field) were decreased.

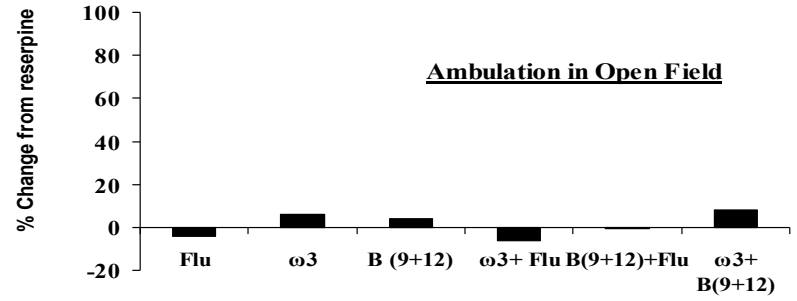

Figure 2: Effects of treated materials (ω_3, B-vitamins, Flu and their combines) on the number of squares crossed in the open-field test in mice. Columns represent % Changes from reserpine group. All drugs were administered 1h before open field test. There is no any change in motor activity by the tested materials.

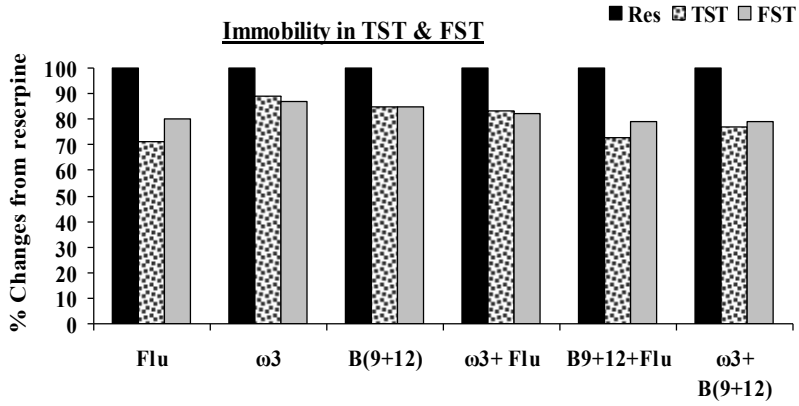

Figure 3: Effects of treated materials (ω_3, B-vitamins, Flu and their combines) on the immobility time in the TST and FST in mice. Columns represent % Changes from reserpine group. All drugs were administered 1h before the tests. All treatment reduced immobility time and showed somewhat similar responses with both tests except FLU treatment that showed potent response with TST.

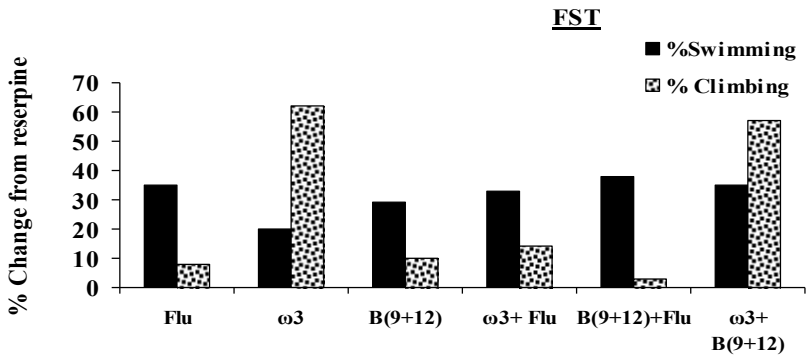

Figure 4: Effects of treated materials (ω_3, B-vitamins, Flu and their combines) on the swimming and climbing time in the FST in mice. Columns represent % Changes from reserpine group. All drugs were administered 1h before the tests. Swimming behavior was increased by all the treated materials but partially with ω_3 treatment, while climbing behavior was markedly increase by ω_3 and $\omega_{3+}B_{(9+12)}$ treatment.

DISCUSSION

In recent years, depression has become recognized as a major public health problem. It is estimated that in Egypt approximately 8-9% of the population have some symptoms of depression, pointing out that the proportion of depressed Egyptian people increases in the countryside more so than in urban areas (Mohamed, 2009). Understanding how to prevent and treat depression is therefore an urgent subject. In this study reserpine was used as an animal depression model. The daily i.p injection (10 mg/kg) of reserpine for two weeks induced a significant decrease in catecholamine levels in the cortex and hippocampus. Reserpine is a sympatholytic drug that depletes catecholamine in peripheral nervous tissues and in the brain (Bao et al., 2006), it can irreversibly inhibit the vesicular uptake of monoamines, including noradrenaline, dopamine and 5- hydroxytrytamine which depletes monoamines in the brain, and produces depression-like syndrome in animals (Bourin et al., 1983; Lijian et al., 2011). These syndromes can be antagonized by major classes of antidepressant drugs. Many types of antidepressant drugs, such as tricyclic antidepressants and selective serotonin-reuptake inhibitors (SSRIs), as well as antidepressant herbal medicines like St. John's wort, are used to treat depression. However, most of the synthetic drugs are not without side effects (Chambers et al., 2006). Furthermore, disturbances of the drug-metabolizing enzyme systems were revealed with St. John's wort (Bolandghamat et al., 2011) and thus, the search for new natural antidepressants with fewer side effects is the role of this study.

Omega-3 fatty acids, monoamines and depression

In the present study, treating mice with omega-3 fatty acids, either individually or in combined (either with B-vitamins or with subthreshold dose of FLU) for 4 weeks, antagonized reserpine-induced depression by increasing the lower levels of catecholamine in both hippocampus and cortex. Polyunsaturated fatty acids (PUFAs) are a component of the phospholipids that form the membranes of all cells. In the membrane, PUFAs serve as precursors for signaling molecules, activate transcription factors and nuclear receptors, and influence the physicochemical properties of the membrane, thus affecting the function of membrane-bound proteins such as receptors and ion channels. In the brain, *omega*-3 PUFA, DHA; is the most abundant, representing about 15% of all lipids in that tissue, it accumulates in the brain and sufficient DHA is necessary for optimal brain development and neuronal function (Davis et al., 2010).

Many studies are investigating a possible relationship between omega-3 compounds and mood disorders, specifically depression. The first study by Edwards et al., (1998) investigated a relationship between omega-3 levels and depressed patients. It was found that omega-3's are less concentrated in red blood cell membranes when compared to healthy controls, postulating a similar relationship with cerebral amounts of omega-3. Hibbeln (2002) compiled data exemplifying a negative trend in DHA content in breast milk and post-partum depression across 23 countries. Able et al., (2009) results stated that omega-3 deficient diets lead to less 5HTconcentration and precursor TPH-2 mRNA in cerebral structures, supporting a causal relationship between ω3 and serotonin. Belzung et al., (2008) broadened this subject by investigating various monoamines in association with omega-3 diets and found that NE, 5HT, and DA related compounds (HVA, DOPAC) were restored in the frontal cortex and hippocampus. Decreased serotonergic function plays a central role in the theories of the pathogenesis of depression. Studies in animals and humans indicate that various aspects of the serotonin system are affected by ω3 PUFA status. In animal studies, adult female rats with a diet-induced decrease in brain DHA content of about 25% initiated after adulthood had decreased concentrations of serotonin in the frontal cortex (Levant et al., 2008). Similarly, rats fed an omega-3 PUFA-deficient diet from birth, which produced brain DHA levels 61% lower than controls as a result of inadequate accumulation during postnatal development, exhibited decreased midbrain expression of tryptophan hydroxylase, the enzyme that synthesizes serotonin, and increased serotonin turnover in the prefrontal cortex (McNamara, et al., 2009). Conversely, ω3 PUFA-supplemented diet reversed decreases in brain serotonin levels in mice subjected to unpredictable chronic mild stress (Vancassel et al., 2008). In humans, the density of platelet serotonin transporter binding, another marker of depression and suicide, was also correlated with plasma DHA levels (DeVriese et al., 2004). Thus, many of the serotonergic alterations associated with low dietary or tissue ω3 PUFAs are consistent with those observed in depression. Although the monoamine theory of depression focuses on serotonin and norepinephrine, the CNS dopamine systems also appear to play a role in the disease. Decreased dopaminergic function, particularly of the mesolimbic system, appears to underlie anhedonic behavior associated with depression in several animal models (aan het Rot et al., 2009). Reserpine induced animal depression leads to diminished dopamine levels and may induce a depressive syndrome as well as dopamine receptor-blockers (Clausius et al., 2009). Variation in diet and tissue ω3 PUFA content in other animals models also results in alterations in the CNS

dopamine systems, but these effects vary considerably depending on the magnitude of the change and the point in development when the manipulation was made (Levant, et al., 2010).

B-vitamins, monoamines and depression:

Treating mice with B-vitamins (folic acid and B12), either individually or in combined (either with omega-3 or with subthreshold dose of FLU) for 4 weeks, antagonized reserpine-induced depression by increasing the lower levels of catecholamine in both hippocampus and cortex. Both low folate and low vitamin B12 status have been found in studies of depressive patients, and an association between depression and low levels of the two vitamins is found in studies of the general population (Chen et al., 2005). People with either low blood levels of the B-12, folic acid, or high blood levels of the amino acid homocysteine, (functional marker of both folate and vitamin B12 deficiency) are both more likely to be depressed and less likely to get a positive result from anti-depressant drugs. Studies of Coppen & Bailey (2000) compare antidepressant effect of folic acid itself, a cheap vitamin with no side-effects, with other antidepressants, these studies show that more patients treated with folate experienced a reduction in their Hamilton Rating score of greater than 50% after ten weeks compared to those on antidepressants. Brocardo et al., (2008) have shown that the antidepressant like effect elicited by folic acid is mediated by an interaction with the serotonergic (5-HT1A and 5-HT2A/2C receptors) and noradrenergic systems. There are several mechanisms by which folate may affect CNS pathways implicated in the depressive disorders. Biopterin, which is dependent on L-methylfolate for synthesis, serves as an essential co-factor for converting phenylalanine to tyrosine, and for hydroxylation of tyrosine and tryptophan to yield dopamine, norepinephrine, and serotonin. Tyrosine and tryptophan hydroxylases are the rate-limiting steps in the synthesis of these monoamine neurotransmitters (Donald, et al., 2009). Folate is also involved in formation of S-adenosyl methionine (SAMe), which serves in the CNS as the sole methyl donor. Furthermore the deficiency of vitamin B12 may lead to different brain damages and depression (Najam et al., 2011). Vitamin B12 is required to synthesize SAMe which is involved in the synthesis of certain neurotransmitters and catecholamine and is also involved in the brain metabolism.

Low serum folic acid levels have been associated with a delayed onset of clinical improvement during treatment with fluoxetine in depressed disorder patients (Papakostas et al., 2005). Thus, augmenting antidepressant drugs with folic acid for treatment resistant major depression is a pharmacological option in treating human depression (Fava, 2007). The aforementioned findings may explain our results since the antidepressant-like actions produced by either folic acid or omega-3, plus sub-effecting dose (8mg/kg) of fluoxetine (an antidepressant drug that impinges on the serotonergic pathway) have the same, if not more, efficacy produced by the effective dose of FLU (30mglkg). The efficacy of the combination folic acid or omega-3, plus fluoxetine in producing antidepressant actions can be explained by modifications in the serotonergic pathway (Resler et al., 2008).

Homocysteine and depression

Vitamin B12 and folic acid was used to convert homocysteine to SAMe - a source of methyl-groups. Without sufficient B-vitamins, blood homocysteine levels rise contribute to cerebral vascular disease and neurotransmitter deficie-ncy, both which can lead to depression. A potential mechanism for the homocysteine effect on transmitters is by inhibition of the enzyme necessary to catalyze the methylation reactions between the catecholamines and SAMe (Folstein et al., 2007). The aforementioned findings are in consistent with the present study, which showed increase levels of homocysteine in reserpinized mice that exhibited lower levels of catecholamine and these levels were antagonized by both B-vitamins and omega-3 administrations and this effect was augmented by the combination of both of them. Also the data showed an inverse relationship between the level of omega-3 and homocysteine in both serum and two brain areas. The relation between omega-3 and homocysteine comes from a new meta-analysis of the scientific evidence. they suggested that supplements of omega-3 fatty acids are associated with lower levels of homocysteine, an amino acid linked to increased risks of heart disease and dementia (Huang et al., 2011), data suggest that the increased rates of cardiovascular disease in patients with depression may be the result of one or more physiological factors that predispose a patient to both depression and cardiovascular disease. Omega-3 fatty acid deficiency and elevated homocysteine levels are considered the two possibly related factors that may have a causal relation with both depressive disorders and cardiovascular disease (Severus et al., 2001). The mechanism for omega-3 to reduce homocysteine may be related to a B-vitamin-dependent enzyme called cystathionine-gamma-lyase (CSE). Data from various animal and in vitro studies suggests that omega-3s may affect the genes responsible for CSE activity: CSE catalyzes the conversion of cystathionine, an intermediate in the conversion of Hcy to cysteine (Huang et al., 2011).

Behavioral tests and depression

Although there is debate regarding the extent to which subhuman species can experience depression,

several rodent models have been proven highly reliable as drug screens for the prediction of antidepressant efficacy. Among these tests, the forced swim test and tail suspension test are perhaps the most validated (Cryan et al., 2005) and is sometimes also used as a putative rodent model of depression. Either FST or TST is an aversive stressful situation that generates behavioral despair, i.e., immobility. In both TST and FST, the amount of time spent immobile was decreased in both omega-3 and B-vitamins treatments than in reserpine treated mice. The combinations of folic acid or omega-3, plus sub-effecting dose (8mg/kg) of fluoxetine also induced an additive effect in both tests. Consistent with these findings, mice treated six weeks feeding with a PUFA-enriched diet induced behavioral changes in the FST, the TST and the Novelty-Suppressed Feeding Test (Venna et al., 2009). Moreover, more than 5 weeks supplementation with a PUFA blend containing 70% alpha-linolenic acid induced antidepressant-like effects in the FST with an increase in both swimming and climbing behaviors, as well as the combination of a shorter duration of PUFA supplementation with a low dose of imipramine also induced an additive effect in the FST (Venna et al., 2009). Conversely, adult rats fed an omega-3 PUFA-deficient diet beginning at weaning, which resulted in brain DHA levels 36% lower than controls, exhibited more time immobile in the forced swim test (DeMar et al., 2006). Concordant with Lafourcade et al., (2011) explored the behavioral consequences of the omega-3-deficient diet in mice that spent greater time in forced swim test, and that nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions. Similar effects have been reported in the tail suspension test, another rodent antidepressant drug screen (Venna et al., 2009). Furthermore, adult male mice that were treated with folic acid administered by both oral and intra-cerebroventricular route produced an antidepressant-like effect in FST and in TST and that this effect appears to be mediated by an interaction with the serotonergic (5-HT1A and 5-HT2A/2C receptors) and noradrenergic systems. In order to prove that the reduction of immobility time was not caused by the stimulation of motor activity, all groups were submitted to the open-field field test. Some authors claim that the open field test does not involve aversive stimulation (Crawley, 1985). However, the open field test can be used as an animal model of anxiety (Treit and Fundytus, 1988). Many researchers use it as a complement to the FST to avoid false positive results that can be obtained in the FST. The administration of all tested materials resulted in any significant changes in locomotors function, indicating that antidepressant effect found in the FST was not bases on the stimulation of locomotors activity. However, in present study, reserpine-induced decrease in locomotion (number of squares crossed) in open-field test as compared to control group. This data are concurrent to the previous studies that have shown reserpine to decrease locomotion in mice and other animals. (Yan et al., 2004). The hypomotility of reserpine may due to its monoamine theory of depression that leads to depleted monoamine in brain, as a consequence, hypothermia and hypomotility are observed. (Lijian et al., 2011). Forcing swimm-ing test, in this study, was followed the technique designed by Detke et al. (1995). In this technique not only immobility is evaluated, but also active behaviors such as swimming and climbing were evaluated. This modification to the FST provides a more refined analysis of a purely behavioral assay and is used to distinguish drugs having a common therapeutic outcome, but acting on distinct neurotransmitter systems. Accordingly, antidepressant drugs with predominant noradrenergic or dopaminergic-elevating effects reduce immobility by increasing climbing behavior. Conversely, antidepressant drugs with predominant serotonin-elevating effects reduce immobility by increasing swimming behavior (Detke and Lucki, 1996). The data obtained for fluoxetine, and for folic acid reduced immobility by increasing swimming behavior, suggesting that these drugs may act in the serotonergic system. Similarly, the serotoninergic action of folic acid was estimated by the study of Detke and Lucki (1996). While the study of (Brocardo et al., 2009) suggests that folic acid's effects in the FST involve serotonergic, noradrenergic, glutamatergic and opioid systems. They speculate that the effect of folic acid in the FST may act in different transmitter systems in multiple brain sites. The present data showed that antidepressant like activity of omega-3 treatment reduced immobility by increasing mainly climbing and partially swimming behaviors, suggesting that these drugs may act in the noradrenergic or dopaminergic system in addition to serotonergic system. In contrast when combining omega-3 with FLU, it showed to reduce immobility by increasing swimming behavior, hence omega-3 either found to augment FLU action or the action of FLU overcome that produced by omega-3. The interesting findings in this study showed that when omega-3 combining with B-vitamins it showed reduction in immobility by increasing both swimming and climbing behaviors, suggesting that this combination may act in both the serotonergic, noradrenergic and dopaminergic systems. Thus, it is tempting to speculate that the superior antidepressant-like actions produced by this combination in FST may involve different transmitter

systems. In addition, the behaviors of this combination strengthen the biochemical results that showed maximum elevation in the levels of monoamines, omega-3 as well as further reduction in Hcy levels. Thus this study provides evidences that co-administration of omega-3 with B-vitamins possesses potent antidepressant-like activity. Its antidepressive mechanism might be related to balance the function of central monoaminergic nervous system through influences in different transmitter systems. To better understand these actions, future studies are needed.

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