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Bioactive Components in Moringa Oleifera: Phytochemical Screening, Gc-Ms Profile, in-Vivo and in-Silico Components Against Anti-Depressant Activity

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KEYWORDS
Antidepressant, Moringa oleifera, Docking, *In-vivo*, *In-silico* studies

ABSTRACT:

Introduction: Moringa oleifera (moringaceae) has been known since ancient times for its rich medicinal properties. Using both in vivo and in silico research, the effects of the ethanol extract of Moringa oleifera leaves (EOL) on depression were assessed.

Objectives: The goal of the current research was to evaluate Moringa oleifera's (MO) antidepressant efficacy using a mouse model

Methods: Female albino Wistar rats were divided into five groups, taken for the study and dosed once with 2000 mg/ kg of the extract of EOL are administrated orally gavage. The antidepressant activity was evaluated using forced swim test (FST) and tail suspension test (TST).

Results: The in-silico studies included molecular docking against GPCR and PI3K protein. The results of in vivo studies showed that both AC (0.10, 0.20 and 0.40 g/kg, p.o.) and imipramine (15mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in FST model and the outcomes demonstrated that both MO (100,200,400 mg/kg, p.o.), imipramine (15 mg/kg, i.p.) significantly shortened the duration of immobility time in a dose-dependent way in TST model. The in-silico studies results are showed best docking score against the targeted compound respectively.

Conclusions: Moringa olifera showed antidepressant activity since it reduced the immobility in both FST and TST. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression. Pretreatment with Moringa olifera, also significantly increased the SOD levels and Catalase with simultaneous decrease in LPO levels in rat brain, suggesting its strong antioxidant activity. Since oxidative stress is reported, plays important role in depression, the antioxidant activity of Moringa olifera may contribute to antidepressant activity.

1. Introduction

Of the affective disorders, which are diseases of mood rather than thought or cognition, depression is the most prevalent. It ranges from mild illness that borders on normalcy to a severe (psychotic) sadness that is accompanied by delusions and hallucinations. Bipolar and unipolar are the two varieties [1]. Reactive depression, unipolar depression, which is typically non-familial (approximately 75% of cases), obviously linked to stressful life events, and accompanied by symptoms of agitation and anxiety. About 25% of individuals exhibit a recognizable pattern that is unconnected to outside stressors and has a somewhat different symptomatology. These patients are sometimes referred

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to as having endogenous depression [2]. Although there is less proof that antidepressant medications exhibit appreciable selectivity between these diseases, this distinction is made on a clinical basis. A less common form of depression that causes mania and depression to alternate over a few weeks is bipolar depression, which typically manifests in early adulthood. Although there is a strong hereditary tendency, neither genetic linkage studies of afflicted families nor comparisons of affected and non-affected people have revealed the identity of any particular gene or genes.

The goal of the current research was to evaluate Moringa oleifera's (MO) antidepressant efficacy using a mouse model. MO, sometimes referred to as drumstick, is a member of the Moringaceae family. It is consumed and used medicinally in India. It is extensively grown across the globe. MO has qualities that include cholesterol reducing, anti-inflammatory, antibacterial, antifungal, anti-ulcer, and antioxidant. It is also anti-diabetic. MO includes calcium, magnesium, potassium, iron, oleic, palmitic, and stearic acid, flavonoids, saponins, glycoside, gum, and protein, according to chemical analysis[3,4]. The leaves have shown strong anti-inflammatory and antioxidant properties, which may be used to treat depression caused by inflammation or OS.

2. Objectives

This study used mice that had received dosages of ethanolic extract of MO (MOE) or with fluoxetine to assess the effects of MO-induced treatment with the conventional antidepressant drug [5,6,7], fluoxetine, in a range of depression-related tests.

3. Methods

Collection and Authentication of Plant Materials

Moringa olifera aerial parts were gathered and authenticated.

Extraction of Plant Materials

The plant is grinded into a coarse powder with the help of suitable grinder.

GCMS Profile and Preliminary Phytochemical Screening

The extracted phytochemicals of M. Oleifera leaves were analyzed using GC-MS is fitted with an Agilent GCMS column (30 m, 0.25 mm) made of 5% diphenyl and 95% dimethyl polysiloxane. Electron spray ionization is utilized. Flow rate: 0.8 mL/min. An injection volume of was 1 µl. The relative percentage amount of each component was estimated by comparing its average peak area to the overall area, using the GC-MS software for spectra and chromatogram analysis.Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, and Flavonoids and Moringa olifera extract were analyzed using phytochemical screening as per the standard methods [8].

Animals

The Oral acute toxicity test of the extracts was determined prior to the experimentation on animals according to the OECD (Organization for Economic Co-operation and Development) guidelines no 423. Female Albino wistar rats (130-140 g) were taken for the study and dosed once with 2000 mg/kg of the extract. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. 10 No mortality was observed till the end of the study revealing the 2000 mg/kg dose to be safe. Thus, 1/5, 1/10 and 1/20 doses of 2000 mg per kg i.e. 100 mg/kg and 200 mg/kg 400mg/kg were chosen for subsequent experimentation. Five-week-old, healthy adult male rats weighing between 40 and 60 grams on average were chosen. Four animals per cage are kept in a temperature-controlled room (27 0C ±3 0C) with a 12:12 hour light/dark cycle. Nine animals are permitted for a seven-day period to acclimatize to their environment. They are given conventional food and unrestricted water. Animal Ethics Committee was consulted beforehand in order to obtain approval to conduct the study.

Acute toxicity studies [9]

Acute oral toxicity test of the extracts, determined prior to the experimentation on animals according to the OECD guidelines no 423. Female Albino wistar rats (130-140 g) were taken for the study and dosed once with 2000mg per kg of the extract. The treated animals were monitored for 14 days to observe general clinical signs and symptoms as well as mortality. No fatality was detected till the end, indicating that the 2000 mg/kg dose is safe. So, 1/5, 1/10, and 1/20 doses of 2000 mg/kg, i.e. 100mg/kg and200mg/kg 400mg/kg were chosen for subsequent experimentation.

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In-Vivo models of Depression employed in the study

1. Forced swimming test (FST)

2. Tail suspension test (TST)

1. Forced swimming test (FST)

The procedure was described by Porsolt et al. (1978) was used. Swimming sessions were conducted by placing rats in individual glass cylinders (45 cm high×20 cm in diameter) containing (25±2 °C) water 38 cm deep, so rats could not support themselves by touching the bottom with their feet.2 swimming were performed between 12:00 h and 19:00 h, a 6-minute exam was administered 24 hours after the first 15-minute pretest.

Doses were given once daily for 7days. On the 7th day rats were subjected to15 min pretest. After 15 minutes in the water, the rats were removed, allowed to dry in heated enclosure (32°C) before being restored to their original cages. They were again placed in the cylinder 24h later and the immobility period was measured during the course of a 6 min test. Floating behavior during this 6 min period had been found to be reproducible in different groups of rats.

2. Tail suspension test (TST)

The “tail suspension test” has been described by Steru et al. (1985), facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and in escapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants diminish rats' immobility following active and unsuccessful escape attempts while hung by their tails. Doses are given once daily for 7 days. Rats are hung on the edge of a table 50 cm above the floor, adhesive tape placed approximately 1 cm from the tip of their tails holds them together. This is done on the seventh day, one hour after the test and standard medications were given. Immobility time was recorded throughout a 6-minute period. When an animal hung passively without moving its body, it was deemed motionless.

Molecular Docking studies

The CB-Dock2 (Cavity-detection guided Blind Docking) server(http://clab.labshare.cn/cb-dock/php/index.php; accessed 21 February 2022) Using a novel curvature-based cavity identification approach, Liu et al. (2020) anticipate binding sites for a given protein and compute their centers and sizes. The server works in conjunction with Auto Dock Vina and has been carefully optimized to achieve a success rate of over 70 % in the models developed. To conduct the analysis, protein files in pdb format, ligands in sdf format were employed, and five potential coupling cavities emerged. The one with the lowest binding energy was chosen based on the Vina value obtained. The ligands and the proteins were then visualized with the Licorice and Surface options respectively. The color of the ligand and the proteins was configured by elements.

Statistical Analysis

Results, expressed as mean values ± S.E.M. One-way analysis of variance was used for the statistical analysis. (ANOVA). If the overall P-value was found statistically significant ($P<0.05$)

4. Results

% Yield value of ethanolic Extract from Aerial Parts of Moringa olifera was determined to be 26.1%.

Acute toxicity studies

As per (OECD) draft guidelines 423 Female albino rats were administered Moringa olifera and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for toxicity study. Individual animals are monitored once every 30 min following medication, then every 24 hours for the next 14 days. In all the cases, no death was observed within 14 days. Overallresults suggested the LD50 value as 2000 mg/kg. For the purpose of antidiabetic findings, the therapeutic dose was therefore determined to be 1/10th and 1/20th, or 0.10 g/kg and 0.20 g/kg, of the lethal dose.

1. Forced Swim Test (FST)

The results (Table. 1) demonstrated that both AC (0.10, 0.20 and 0.40 g/kg, p.o.) and imipramine (15mg/kg, i.p.) mitigated the immobility time duration in the FST

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model significantly and in dose-dependent ways. Post-hoc analysis revealed that MO (0.10, 0.20, and 0.40 g/kg) and Imipramine (IMP) treated groups differed significantly ($p<0.001$) from vehicle treated group (Fig.1).

Table 1: Effect of MO and Imipramine (IMP on forced swim test (FST) in rats.

Group no	Treatment (dose in mg/kg)	Immobility period (sec) Mean± SEM
I	Control (0.3% CMC) +FST	136.3±7.3
II	Moringa olifera (100mg/kg, p.o.) +FST	125.2±10.3
III	Moringa olifera (200mg/kg, p.o.) +FST	98.5±8.6*
IV	Moringa olifera (400mg/kg, p.o.) +FST	82.0±7.4*
V	Imipramine (15mg/kg, i.e.) +FST	70.1±5.0*

Each column represents mean ± S.E.M. of immobility period (sec), n = 6. * = $p<0.001$ compared to control.

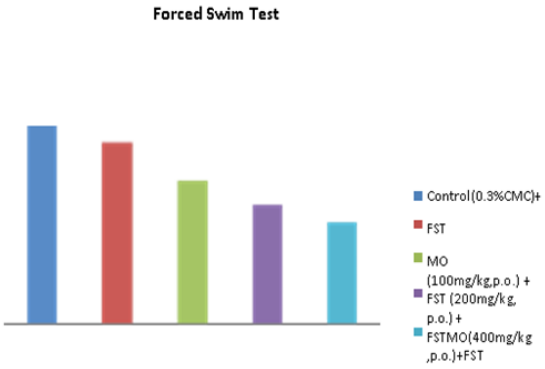

Figure 1: Effect of MO (100, 200 and 400 mg/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on forced swim test (FST) in rats. Each column represents mean ± S.E.M. of immobility period (sec), n=6. *=$p<0.001$compared to control.

2. Tail Suspension Test (TST)

The results (Table. 2) showed that both MO (0.10, 0.20 and 0.40 g/kg, p.o.) and imipramine (15 mg/kg, i.p.) mitigated the immobility time duration in the TST model significantly and in dose-dependent ways. Post-hoc analysis showed that the CS (0.10, 0.20 and 0.40 g/kg) and IMP treated groups were significantly different ($p<0.001$) from the vehicle treated group (Fig.2).

Table.2. Effect of MO and Imipramine (IMP) on tail suspension test (TST) in rats

Group no.	Treatment (dose in mg/kg)	Immobility period(sec)
I	Control (0.3%CMC) +TST	135.2±10.3
II	Moringa olifera (100mg/kg, p.o.) +TST	110.6±10.2a
III	Moringa olifera (200mg/kg, p.o.) +TST	98.4±9.3a
IV	Moringa olifera (400mg/kg, p.o.) +TST	80.3±7.1a
V	Imipramine (15mg/kg, i.p.) +TST	63.4±5.5a

Each column represents mean ± S.E.M. of immobility period (sec), n = 6. a = $p<0.001$compared to control.

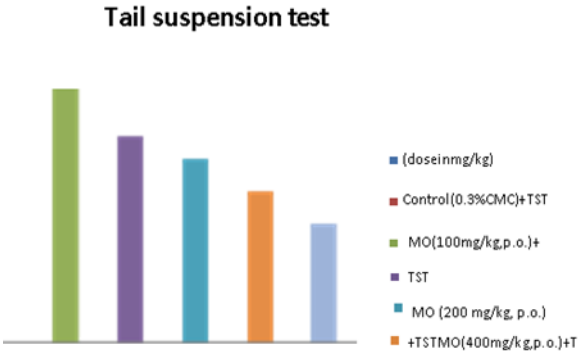

Figure 2: The Effect of MO (0.10, 0.20 and 0.40 g/kg, p.o.) and Imipramine (IMP; 15 mg/kg) on tail suspension test (TST) in rats. Each column represents mean ± S.E.M. of immobility period (sec), n=6.a=$p<0.001$in contrast to control.

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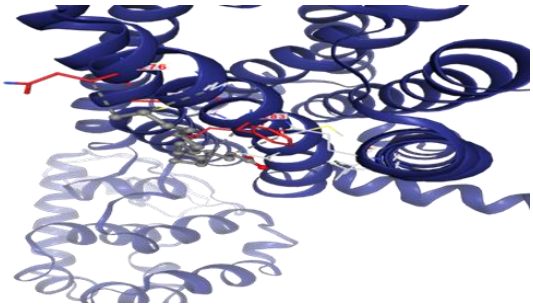
In Silico Docking Studies

All ligands and proteins were prepared using CB DOCK 2. The selected eight ligands were individually docked against the two proteins GPCR and PI3K. The best binding score of ligands 9,12,15-octadecatrienoic acid, (Z, Z, Z) -7.8, Propionic acid, 2-methyl-octyl ester -6.3 for to GPCR and respectively ligands 9,12,15-octadecatrienoic acid, (Z, Z, Z) -6.7, 1,3-benzenediol, 2-methyl- -5.8 for to PI3K. These four ligands presented the highest bonding affinity for both GPCR and PI3K (Table 3).

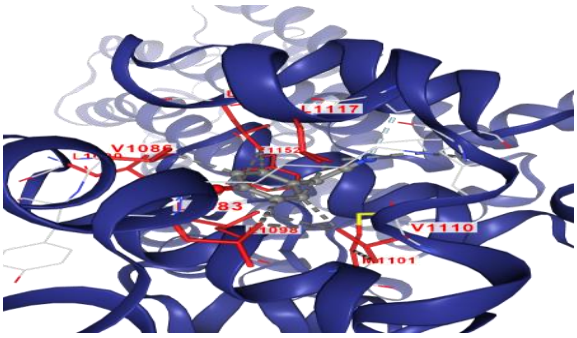
S.no	Compounds	Docking score GPCR (5zty)	Docking score PI3K(5itd)
1	9,12,15-octadecatrienoic acid, (Z, Z, Z)	-7.8	-6.7
2	Benzene acetonitrile, 4 hydroxy	-6.3	-5.6
3	Propionicacid,2-methyl-octyl ester	-6.1	-5.4
4	1,2,3-propanetriol,1-acetate-	-4.4	-4.7
5	1,3-propanediol,2-methyl-2-(hydroxymethyl)	-4.4	-4.5
6	Ethanamine, N-ethyl-N-Nitroso-	-4.1	-4.1
7	1,2,3-propanetriol,1-acetate-	-2.3	-5.8
8	Dihydroxyacetone	-3.6	-4.1

Figure 3: The docking complexes of GPCR with (a) 9, 12, 15-octadecatrienoic acid, (Z, Z, Z), (b)Benzene acetonitrile, 4 hydroxy. The docking complexes of PI3K (c)9,12,15-octadecatrienoic acid, (Z, Z, Z), (d)



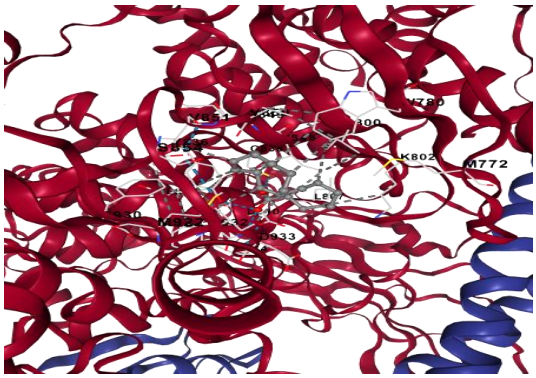
(a)

Chain A: TYR25 MET26 ILE27 PHE87 CYS89 SER90 PHE91 ASN93 PHE94 HIS95 GLY99 ASP101 PHE106 LYS109 ILE110 VAL113 THR114 PHE117 GLU181 LEU182 PHE183 PRO184 TRP194 TRP258 VAL261 MET265 LYS278 PHE281 ALA282 SER285 CYS288



(b)

Chain A: TYR25 VAL86 PHE87 SER90 PHE91 VAL92 PHE94 HIS95 PHE106 LYS109 ILE110 VAL113 THR114 PHE117 LEU182 PHE183 PRO184 TRP194 TRP258 VAL261 LEU262 MET265 PHE281 ALA282 SER285 CYS288



(c)

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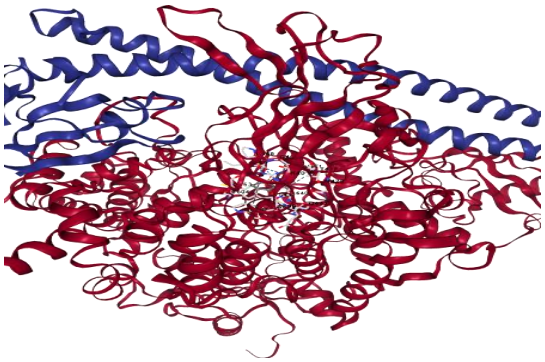
Chain A: MET772 SER774 PRO778 TRP780 GLU798 ILE800 LYS802 ASP805 LEU807 ASP810 LEU814 TYR836 CYS838 ILE848 GLU849 VAL850 VAL851 ARG852 ASN853 SER854 HIS855 THR856 SER919 MET922 PHE930 ILE932 ASP933 PHE934



(d)

Chain A: ASP133 GLU135 VAL136 HIS362 GLY363 GLY364 ALA399 ARG401 TRP424 ASN426 ASN428 PRO449 ASN457 PRO458 ILE459 GLY460 VAL461 THR462 GLY463 CYS604 ASN605 PRO607 LYS640 TYR641 GLU642 GLN643 TYR644 LEU645 LYS678 THR679 VAL680 SER681 GLN682 ARG683 LEU1006 GLY1007 SER1008 GLY1009 MET1010 LEU1013 GLN1014

5. Discussion

Herbal Shizandra chines is, Thea sinensis, Uncaria tomentosa, Valeriana officinalis, and Withaniasomnifera are among the drugs used to treat depression [10, 11]. The numerous toxicological studies have established its safety for human consumption [12]. They were found with rich source of minerals, essential fatty and amino acids, vitamins –especially vitamin B12 and antioxidant pigments such as carotenoids [8]. The forced swim test and tail suspension trials revealed antidepressant activity after 7 days of being given with MO at dosages of 0.10, 0.20, and 0.40 g/kg. The FST is the tool most widely used for assessing antidepressant activity preclinical. Drugs used in depression, this model's widespread use are partly due to its ease of use, consistency across laboratories, and ability to identify extensive selection of antidepressants [13]. Most clinically active antidepressants work in the FST, although neuroleptics and anxiolytics have distinct effects [14]. In FST, MO significantly reduced immobility period suggesting anti-depressant activity and the activity was comparable to the reference drug IMP [15]. TST is widely used as a dependable animal model of depression to screen new antidepressants [16].

MO considerably shortened the immobility length during tests of tail suspension and forced swimming, showing antidepressant action [17]. Furthermore, behavioural investigations show that this activity may be related to a facilitative influence on the serotonergic, noradrenergic, and dopaminergic systems. Good docking score towards the active binding sites of GCPR and PI3Kincluding strong binding interactions. We propose that the possible mechanism could be used for further studies.

6. Conclusion

The results from the present study confirm the antidepressant activity of Moringa olifera since it reduced the immobility in both FST and TST. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression. Pretreatment with Moringa olifera, also significantly increased the SOD levels and Catalase with simultaneous decrease in LPO levels in rat brain, suggesting its strong antioxidant activity. Since oxidative stress is reported, plays important role in depression, the antioxidant activity of Moringa olifera may contribute to antidepressant activity. Results from behavioralexperiments indicate that the antidepressant effects of Moringa olifera possibly due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

References

1. Anderson I.M., 1998. SSRIs versus tricyclic anti-depressants in depressed in patients: a meta-analysis of efficacy and tolerability, Depress Anxiety. 7:11–7.
2. Manji H.K, Drevets W.C, Charney D S., 1998. The cellular neurobiology of depression, Nat Med. 7, 541-547.
3. Bhoomika R, Babita G, Goyal R (2007). Phyto-pharmacology of Moringa Oleifera Lam an overview, Nat Prod Radiance. 6, 347–5.
4. Porsolt R.D, Bertin A, Jalfre M., 1977. Behavioral despair in mice: A primary screening test for

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JCHR (2024) 14(3), 1095-1101 | ISSN:2251-6727

antidepressants. Arch Int Pharmacodyn Ther. 229, 327–36.

5. Butterweck V, Christoffel V, Nahrstedt A, Petereit F, Spengler B, Winterhoff H., 2003. Step by step removal of hyper for in and hypericin: activity profile of different hyper in cum preparations in behavioral models, Life Sci. 73, 627–639.
6. Aquila D.P.S, Peana A.T, Panin F, Grixoni C, Cossu M, Serra G., 2003. Reversal of anti-depressant induced dopaminergic behavioral super sensitivity after long-term chronic imipramine withdrawal, Eur J Pharmacol. 458 (1–2), 129–34.
7. Deakin J.F.W, Graeff F.G, Guimaraes F.S., 1992. 5-HT receptor subtypes and the modulation of aversion. Marsden C.A, Heal D.J editors. Central serotonin receptors and psychotropic drugs. Oxford: Blackwell. Scientific Publications. 147–74.
8. Morpurgo C., 1968. Aggressive behaviour induced by large doses of 2-(2, 6-dichloro phenyl amino) -2 imidazoline hydrochloride (ST 155) in mice, Eur J Pharmacol. 3(4), 374–7.
9. Machado D.G, Bettio L.E.B, Cunha M.P, Capra J.C, Dalmarco J.B, Pizzolatti M.G, et al., 2009. Anti-depressant-like effect of the extract of Rosmarinus officinalis in mice: involvement of the mono aminergic system, Prog Neuro psycho pharmacol. Biol. Psychiatry. 33, 642–50.
10. Arutla S, Arra G.S, Prabhakar C.M, Krishna D.R., 1998. Pro and anti-oxidant effects of some anti-leprotic drugs in-vitro and their influence on super oxide dismutase activity, Arzneim. -Forsch J Drug Res. 48, 1024.
11. Baker G.B, Dewhurst W.G., 1985. Biochemical theories of affective disorders. Dewhurst W G, Baker GB (Eds), Pharmacotherapy of affective disorders. Croom Helm, Beckenham.
12. Morcos N.C., Bems M and Henry W.L., 1988. Phycocyanin: laser activation, cytotoxic effects, and uptake in human atherosclerotic plaque, Lasers Surg Med, 8(1), 10-7.
13. Rao J.S, Ertley R.N, Lee H.J, De Mar Jr J.C, Arnold J.T, Rapoport S.I, Bazinet R.P., 2007. N−3 poly unsaturated fatty acid deprivation in rats decreases frontal cortex BDNF via a p38 MAPK-dependent mechanism, Mol. Psychiatry. 12, 36–46.
14. Xia X, Cheng G, Pan Y, Xia Z.H, Kong L.D., 2007. Behavioral, neurochemical and neuroendocrine effects of the ethanolic extract from Curcuma longa L. in the mouse forced swimming test. J Ethnopharmacol. 110, 356–63.
15. Peng WH, Lo KL, Lee YH, Hung TH, Lin YC., 2001. Berberine produces anti-depressant-like effects in the forced swim test and in the tail suspension test in mice, Life Sci. 81: 933–8.
16. Bilici M, Efe H, Koroglu M.A, Uydu H.A, Bekaroglu M, Deger O., 2001. Anti-oxidative enzyme activities and lipid peroxidation in major depression: alterations by anti-depressant treatments, J Affective Disorders. 64, 43–51.
17. Maes M, Meltzer H.Y., 1995. The serotonin hypothesis of major depression. In: Bloom FE, et al, editor. Psychopharmacology: the third generation of progress. New York: Raven Press 993–1044.