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Assessment Of Antidepressant Effects Of Clopidogrel And Spirulina In Mice Via The Tail Suspension Test

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To date, the search for novel pharmacotherapy from various medicinal plants and their constituents for psychiatric illnesses has significantly progressed. The present study was undertaken to evaluate the antidepressant potential of spirulina & clopidogrel in different animal models like Tail suspension Test (TST).In this study spirulina & clopidogrel, at two dose (150,300 mg/kg & 75,150 mg/kg) respectively, and standard imipramine stock solution prepared with Sodium-CMC (15 mg/kg) were administered on test day to separate groups of Wistar rats and albino mice. From these two animal models it was found that, spirulina & clopidogrel showed significant reduction in the duration of immobility in TST at both dose levels in dose dependent manner and the effect of high dose i.e.300 of spirulina & 150 mg/kg of clopidogrel was compared with imipramine. spirulina & clopidogrel also showed significant antidepressant effect compared to imipramine preparation, study confirmed that spirulina & clopidogrel increase the neurotransmitter levels of NA, DA, 5HT suggesting neurotransmission modulation is a probable mechanism of action. spirulina & clopidogrel possesses significant antidepressant activity.

Keywords: Antidepressant activity, spirulina & clopidogerl, imipramine , Tail suspension test, Serotonin, Noradrenalin, Dopamine.

Introduction:

Depression is one of the most common psychiatric disorders. It is characterized by feelings of intense sadness, helplessness, worthlessness, and impaired functioning (Sally Roach, 2008). The primary clinical manifestations of major depression are significant depression of mood and impairment of function. Some features of depressive disorders overlap those of the anxiety disorders, including panic-agoraphobia syndrome, severe phobias, generalized anxiety disorder, social anxiety disorder, posttraumatic stress disorder, and obsessive-compulsive disorder. Extremes of mood also may be associated with psychosis, as manifested by disordered or delusional thinking and perceptions that often are congruent with the predominant mood. (Goodman and Gilman's et al., 2008). Many neurodegenerative and neuropsychiatric diseases, including depression, have been linked to oxidative stress, a pathological condition arising when physiological oxidative actions by reactive oxygen species are no longer balanced by antioxidative defences. The brain is very vulnerable to

oxidative processes both because it consumes about 20% of the oxygen utilized by the body and because it contains great amounts of polyunsaturated fatty acids severely prone to peroxidation, which produces cell degeneration and death. Major depression is accompanied by a decreased antioxidant status and by induction of oxidative and nitrosative (IO&NS) pathways. Antidepressant drugs like Trimipramine, Amoxapine, Fluoxetine having variable side effects such as sleep disturbances, problems with sexual functioning, temporary weight loss, blurred vision, difficulty urinating, weight gain or loss, muscle twitches, increased heart rate, low blood pressure, dizziness and sedation, and insomnia. (Goodman and Gilman's et al., 2008) Since on one hand the synthetic drugs having variable side effect as mention above while on the other hand free radical species are responsible for neuropsychiatric disorder such as depression.

Spirulina is a type of cyanobacteria, also known as blue-green algae, and it has a unique structure that sets it apart from other microorganisms. clopidogrel is an antiplatelet medication that is commonly used to reduce the risk of blood clot formation in individuals with certain cardiovascular conditions. The primary mechanism of clopidogrel involves its activation and inhibition of platelet function. Here's a detailed explanation of the mechanism of clopidogrel.

It was hypothesized spirulina & clopidogrel suggest that, spirulina has neuroprotection as well as antioxidant activity. This may be useful in the treatment of depressant. Hence, present study was undertaken to evaluate the antidepressant activity of spirulina & clopidogrel various animal model.

Material and method:

Animals:

- The animals were housed at a temperature of 25 ± 2^0C and relative humidity of 45-55% under 12:12 light: dark cycle. The animals had free access to standard pellet diet All the experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Modern Institutional animal ethical committee,
  [Approved Protocol No.IAEC/**01/2022/02**].

Drugs and chemicals:
Test drug, spirulina & clopidogrel
Standard drug, Imipramine tablet (Cipla, India)
Vairous Reagents,
Thiobarbituric acid, O-pthalaldehyd, Sodium sulphate, Sodium carbonate, n-butanol (Research Lab Fine chemical Industries, Mumbai, India)
Trichloro acetic acid, DTNB, Phosphoric acid, Phosphate buffer, 0.1N HCL, Heptane, Iodine solution, Ethanol, Acetic acid, Na- CMC (Research Lab Fine chemical Industries, Mumbai, India)

Instruments:

Name of Instruments	**Manufacturer**
Animal Housing cages (Polypropylene)	B.I.K Industries, Mumbai

Animal weighing electronic balance	-
Chemical weighing balance	-
Professional quartz timer	-
Deep Freezer	BLUESTAR Ltd
Micro centrifuge RM-12C	REMI Instruments
Spectrofluorimeter	ELICO, India
Tissue Homogenizer RQ-127A	REMI Instruments
UV-Visible Spectrophotometer V-530	JASCO Instruments

Preparation and administration of drugs:
Preparation of test drug spirulina & clopidogrel **solution:**

spirulina & clopidogrel to be administered in a two dose (150,300 mg/kg & 75,150 mg/kg) respectively, p.o. body weight of animals. Dose was prepared by dissolving in 1% sodium carboxy - methyl cellulose (Na-CMC) to prepare the stock solution. The different doses were prepared by further diluting the stock solution.

Storage of test drug solution:
Test drug was stored in cool and dry place away from sunlight. Fresh drug solution was prepared for work of each day. The solution were kept in airtight, amber colored bottle and stored at room temperature until ready for use.

Volume and route of test drug administration:
The volume of drug solution to be administered was calculated based upon the body weight of animals by p.o. route.

Selection of dose of spirulina & clopidogrel
The dose for the spirulina & clopidogrel was selected on the basis of the reported LD_{50} values and as the doses used in previous studies.
LD_{50} values

Species	**Route**	**LD_{50}**
mice	Oral	150,75 &300,150 mg/kg respectively of both drugs

In the previous study spirulina & clopidogrel administered orally at two dose (150,300 mg/kg & 75,150 mg/kg) respectively a day. Hence, on the basis of this of previous study, the dose for the study of spirulina & clopidogrel was selected as two dose (150,300 mg/kg & 75,150 mg/kg) respectively (Jun-Shik Choi et al.,2005)

Preparation of Impramine solution:
Impramine tablet was purchased from market brand .The concentration of Impramine solution to be administered was 10 mg/kg, p.o. body weight in rat and mice accordingly. Dose was

prepared by dissolving in 1% sodium carboxy - methyl cellulose (Na-CMC) to prepare the stock solution. The different doses were prepared by further diluting the stock solution

Storage of Impramine solution:
Impramine was stored in refrigerator (2-8ºC). Fresh Impramine solution was prepared for each day work. The solution was kept in airtight, amber colored bottle and stored until ready for use.

Volume and routes administration of Impramine solution: (Badhe et al., 2010)
The volume of Impramine solution to be administered was calculated based upon the body weight of animals. In case of intraperitoneal administration, the volume administered did not exceed 1 ml/kg body weight. In the screening method for forced swim test and tail suspension test in Rat Morin Hydrate and Impramine by oral route for both acute and chronic study.

PHARMACOLOGICAL STUDIES:

ANIMALS:
Species: Male wistar rat (150-200gm)

Tail suspension test in mice (with minor modifications) (Dan Zhou et al., 2010, Vogel et al., 2002).

Purpose & Rationale:
The "tail suspension test" has been described by Steru et al. (1985) as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioural despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail.

Procedure:
Albino mice weighing 18–25 g were used preferentially. Four Group are prepared each group contain Six Animal. Groups of 6 animals were treated with the test compounds or the vehicle by oral route 60 min prior to testing or continue for 2 weeks. For the test the mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 5 minutes. Mice were considered immobile when they hang passively and completely motionless for at least 1 minute.

I. Normal control group :-
Animals were treated with 1% Na CMC (5 ml/kg) orally, the animals was then assessed for depression on 7^{th} day and at the end of 14^{th} day.

II. Impramine treated group:-

Impramine (10 mg/kg) orally for 2 weeks, the animals was then assessed for depression on 7th day and at the end of 14th day.

Spirulina (150) & clopidogrel (75) treated group:-
Spirulina (150 mg/kg) & clopidogrel (75 mg/kg) orally for 2 weeks, the animals was then assessed for depression on 7th day and at the end of 14th day.

III. Spirulina (300) & clopidogrel (150) treated group:-
Spirulina (300 mg/kg) & clopidogrel (150 mg/kg) orally for 2 weeks, the animals was then assessed for depression on 7th day and at the end of 14th day.
Evaluation: The value of each parameter was compared with standard and normal group.

Parameters to be determined
I. Behavioral Parameter (Vogel et al., 2002).
a. Duration of Immobility

Statistical Analysis:
The Arithmetic mean ± SEM values were calculated for each experiment. The results were analysed using one-way ANOVA followed by Dunnett's test or Student's 't' test.

Evaluation of Antidepressant Activity of spirulina & clopidogrel in Tail suspension test in mice (Chronic on 7th day):

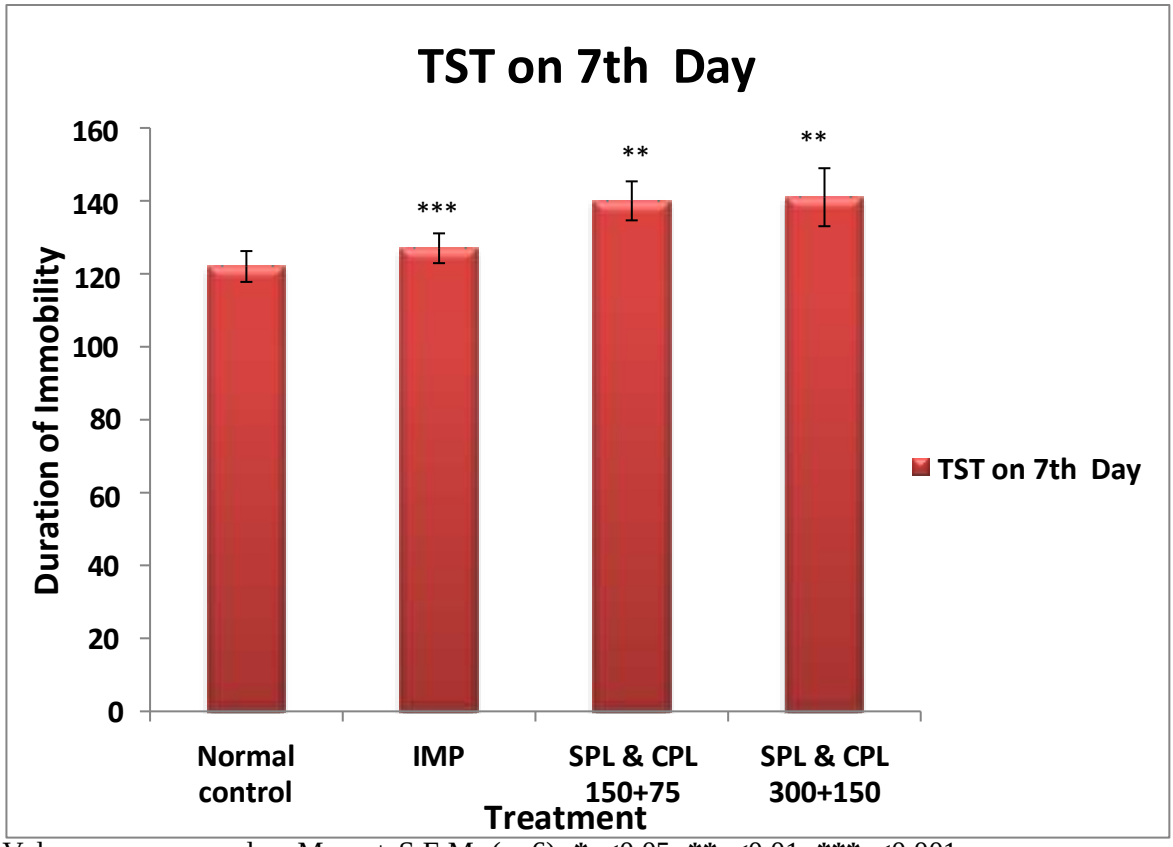
Effect of spirulina & clopidogrel in Tail suspension test in mice (Chronic on 7th day). Administration of Imipramine (15mg/kg, p.o.) and spirulina 150 mg/kg, 300 mg/kg & clopidogrel 75 mg/kg, 150 mg/kg respectively showed significant effect with*******p<0.001 and ******p<0.01 respectively as compared to normal control group.

Table 6.6 Tail suspension test (Chronic on 7th day)

Group	Treatments Mg/kg	Duration of immobility (Seconds) (Mean ± SEM)
I	Normal Control (Vehicle only)	122 ± 4.22
II	Imipramine (15)	127 ± 4.08***
III	spirulina & clopidogrel (150 +75)	140 ± 5.34**
IV	spirulina & clopidogrel (300 +150)	141 ± 7.96**

Values are expressed as Mean ± S.E.M, (n=6). *****p<0.05, ******p<0.01, *******p<0.001 as compare with haloperidol induce control (###) group, Using one-way ANOVA followed by Dunnett's test.

Fig Tail suspension test in mice (Chronic study results on 7th day)



Values are expressed as Mean ± S.E.M, (n=6). *p<0.05, **p<0.01, ***p<0.001 as compare with normal control group, Using one-way ANOVA followed by Dunnett's test.

Evaluation of Antidepressant Activity of spirulina & clopidogrel in Tail suspension test in mice (Chronic on 14th day):

Administration of Imipramine (15mg/kg, p.o.) showed significant effect on duration of immobility with**p<0.01 as compare with normal control group.

Administration of **spirulina** (150,300 mg/kg, p.o.) & **clopidogrel** (75,150 mg/kg, p.o.) showed significant effect on Immobility with *p<0.05, **p<0.01 respectively as compared to normal control group.

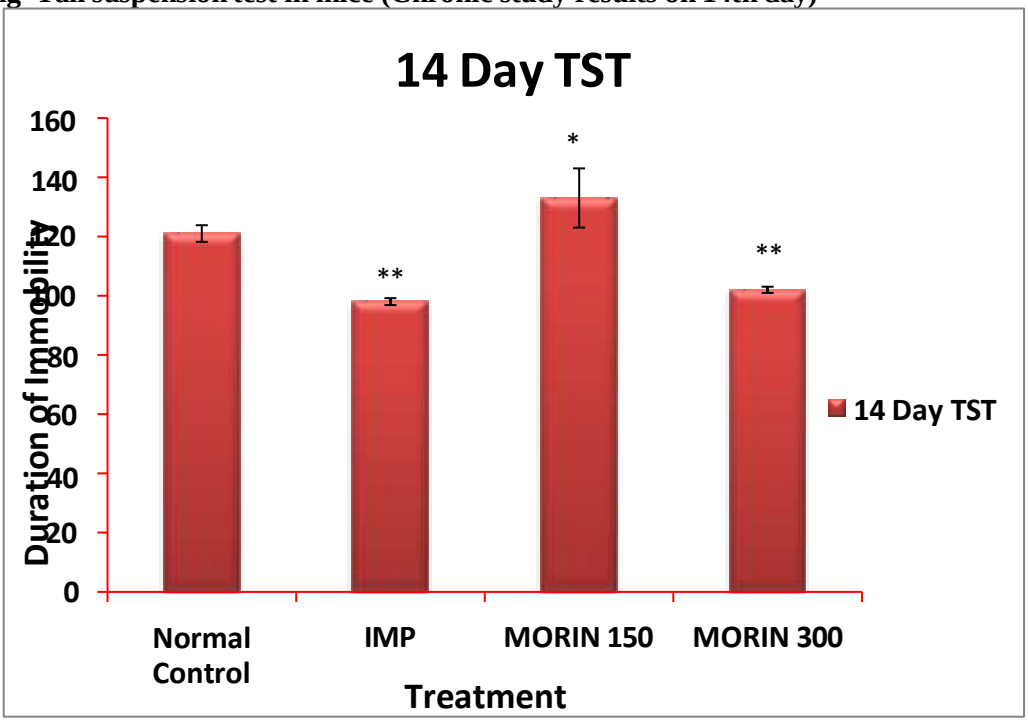
Table - Tail suspension test (Chronic on 14th day)

Group	Treatments Mg/kg	Duration of immobility (Seconds) (Mean ± SEM)
I	Normal Control (Vehicle only)	122± 2.8
II	Imipramine (15)	98± 1.16**
III	spirulina & clopidogrel (150 +75)	133± 9.93*
IV	spirulina & clopidogrel	102 ± 1.04**

	(300 +150)	

Values are expressed as Mean ± S.E.M, (n=6). *p<0.05, **p<0.01, ***p<0.001 as compare with normal control group, Using one-way ANOVA followed by Dunnett's test.

Fig Tail suspension test in mice (Chronic study results on 14th day)



Values are expressed as Mean ± S.E.M, (n=6). *p<0.05, **p<0.01, ***p<0.001 as compare with normal control group, Using one-way ANOVA followed by Dunnett's test.

Discussion:-

Depression is one of the most common psychiatric disorders. It is characterized by feelings of intense sadness, helplessness, worthlessness, and impaired functioning (Sally Roach, clinical Pharmacology, 2008). The primary clinical manifestations of major depression are significant depression of mood and impairment of function.Depressive disorders are pathologically characterised by decreasing the neurotransmitters levels in the brain. There is numbers of biochemical processes that are involved in pathogenesis and progression of neuropsychological disorders. The concept of oxidative stress and antioxidants may be directly or indirectly involved in the pathogenesis of depression (Bilici et al., 2002; Lucca et al., 2009). Clinical features of major depressive disorder (MDD) are trouble sleeping or excessive sleeping, a dramatic change in appetite, often with weight gain or loss, fatigue and lack of energy, feelings of worthlessness, self-hate, and inappropriate guilt, extreme difficulty concentrating, agitation, restlessness, and irritability, feelings of hopelessness and helplessness, recurring thoughts of death or suicide (Stephen, 2009). Mood disorders comprise

a various disabilities, including major depressive disorder (unipolar depression), bipolar disorders.

The pathological hallmarks of depressive disorders are depletion of neurotransmitters such as nor adrenaline, dopamine and serotonin in brain. Depressive disorders can be caused due to various endogenous or exogenous factors (Goodman et al., 2001).

Current Antidepressant drug are Selective serotonin reuptake inhibitors (SSRIs) like Fluoxetine, Fluvoxamine, Tricyclic antidepressants (TCAs) like Imipramine, Amoxapin and Monoamino oxidase inhibitors (MAOIs**)** like Moclobemide, Clorgyline and they have side effects/ limitations are Nausea, vomiting, diarrhoea, restlessness, agitation, sleep disturbances, problems with sexual functioning, headaches and temporary weight loss. Blurred vision, dry mouth, constipation, difficulty urinating, changes in sexual desire or ability, weight gain, muscle twitches, increased heart rate, low blood pressure and dizziness and sedation (Tripathi, 2004).

Structurally, spirulina is composed of tiny, spiral-shaped, single-celled organisms. These organisms belong to the cyanobacteria family, which are photosynthetic bacteria capable of producing energy from sunlight.because of their broad spectrum pharmacological activities and extensive biological effects. The individual spirulina cells are cylindrical in shape, forming long, thin filaments. These filaments are typically coiled or spiral-shaped, which is where the name "spirulina" is derived from. Spirulina is a nutrient-dense food, rich in vitamins, minerals, antioxidants, and essential fatty acids. Some studies have shown that certain nutrients, such as omega-3 fatty acids and B-complex vitamins, may have positive effects on mood and mental health. Consuming a balanced diet that includes nutrient-rich foods like spirulina may help support overall mental well-being. Spirulina contains phycocyanin, a potent antioxidant compound that can help reduce oxidative stress in the body. Oxidative stress is associated with inflammation and can contribute to mood disturbances and depressive symptoms. By reducing oxidative stress, spirulina may have a positive impact on mood.also Spirulina contains compounds that may have neuroprotective properties, which means they can help protect the brain from damage and support brain health. These effects may contribute to better mental well-being.(sajjad moradi et al., 2021).

platelet serotonin (5-HT) receptor in depression. Considered are studies of 2A receptor binding, and 5-HT-induced platelet activation and aggregation. 5-HT receptor density tends to increase in 2A depression, although this more clearly relates to suicidality than depression,their for clopidogrel is an antiplatelet drug and this reduced platelet activation and aggregation

Therefore Spirulina & clopidogrel were used in current studies to explore its antidepressant effect in experimental animal models.

TST is based on the observation that rodents (almost always mice although gerbils and rats have been used (Varty et al., 2003; Chermat et al., 1986). After initial escape-oriented movements, develop an immobile posture when placed in an inescapable stressful situation. In the case of the TST the stressful situation involves the hemodynamic stress of being hung in an uncontrollable fashion by their tail whereas in the FST mice are placed in a cylinder filled with water (Thierry et al., 1986). The test is usually quite short, 6 min, and the amount of time they spend immobile is recorded either manually or through an automated device (Steru et al., 1985). Acute antidepressant treatments decrease these immobility scores. An obvious

advantage of this test is its ability to detect a broad spectrum of antidepressants irrespective of their underlying mechanism it is inexpensive, methodologically unsophisticated method.

Administration of Imipramine on 7th day and 14th day (15mg/kg, p.o.) showed significant decreased in duration of Immobility as compared to normal control group. Administration of Spirulina & clopidogrel (150+75 mg/kg, p.o.) and Spirulina & clopidogrel (300+150mg/kg, p.o.) on 7th day and 14th day showed significant decreased in duration of Immobility as compared to normal control group.

The present neuro-behavioral studies shown that Spirulina & clopidogrel, a herbal & synthetic combination having antidepressant effects in experimental animal models of depression.

References:

1. Ana Maria Mendoza-Wilson a, Hisila Santacruz-Ortega b, Rene R. Balandran-Quintana.,2011 Relationship between structure, properties, and the radical scavenging activity of morin ,Journal of Molecular Structure 995 (2011) 134–141.

2. Anisman, H. Cascading effects of stressors and inflammatory immune system activation: Implications for major depressive disorder. J Psychiatry Neuroscience. (2009) 3, 4-20.

3. Aronson, S.C., Depression: A Treatment Algorithm for the Family Physician, Virginia E. Ayres, Hospital Physician. (2000) 21-44.

4. Badhe, S.R., Badhe, R.V., Ghaisas, M.M. Evaluation of antidepressant activity of anacyclus pyrethrum root extract, International journal of green pharmacy. (2010) 79-82.

5. Baluchnejadmojarada, Mehrdad, R., Mehdi M., Neuroprotective effect of silymarin in 6-hydroxydopamine hemi-parkinsonian rat: involvement of estrogen receptors and oxidative stress tourandokht neuroscience letters. 480,(2010) 206–210.

6. Bilicia, M., Hasan, E., Arif, K.R.L.M., Hu seyin A.U., Bekaroglua, M., Deger, O., Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments, Journal of Affective Disorders. 64, (2001) 43–51.

7. Carroll, B.J., Cassidy, F., Naftolowitz, D. Pathophysiology of hypercortisolism in depression. Acta psychiatrica Scandinavica, (2007) 90-103.

8. Chien-Yun Hsiang a, Shih-Lu Wu a, Tin-Yun Ho.,Morin inhibits 12-O tetradecanoylphorbol-13-acetate-induced hepatocellular transformation via activator protein 1 signaling pathway and cell cycle progression. Life Sciences, Vol. 57,(2005) 51-56

9. Cooper, J., Bloom, F., Roth, R., The biochemical basis of neuropharmacology. 7th edition. New York: Oxford University Press,Life Sciences.(1997)

10. Dinesh, D., Praveen, K.G.,Inhibition of MAO- A and GABA: Probable mechanism for antidepressant like activity of nardostachys jatamansi DC. In mice. Indian journal of experimental biology, (2008) 212-218.

11. Duman, R.S., Pathophysiology of depression: the concept of synaptic plasticity. European Psychiatry.17, Supplement 3, (2002) 306-10.

12. Emerit, J., Edeas, M., Bricaire, F., Neurodegenerative diseases and oxidative Stress. Biomedicine & Pharmacotherapy 58, (2004) 39–46.

13. Fava, G.A., Emanuela, O., The mechanisms of tolerance in antidepressant action. Progress in Neuro-Psychopharmacology & Biological Psychiatry.(2010)

14. Fuchs, E., Boldizsa´r, C., Maarten, H.P., Kole, Thomas M, Paul J., Lucassen, Alterations of neuroplasticity in depression: The hippocampus and beyond. European neuro psychopharmacology. 14, (2004) S481–S490.

15. Goodman, Gilman's, Manual of pharmacology and therapeutics. McGraw hill, (2008) 280-300.

16. Gonzalo, V.P., Herlinda, B.J., Javier, Vela´zquez, M., Antidepressant effects of nicotine and fluoxetine in an animal model of depression induced by neonatal treatment with clomipramine, Progress in Neuro-Psychopharmacology & Biological Psychiatry. 29, (2005) 39– 46.

17. Guadarrama, C.G., Alarcon-Aguilarb, Lezama-Velascoc, F.J., Vazquez-Palacios, G., Bonilla-Jaimed, H., Antidepressant-like effects of Tagetes lucida Cav. in the forced swimming test. Journal of Ethnopharmacology. 120,(2008) 277–281.

18. Hui Xie a,1, Ma-cheng Yan b , Di Jin c, Jia-jia Liu , Min Yu , Dong Dong , Cheng-cheng Cai , Sheng-Li Pan., Studies on antidepressant and antinociceptive effects of ethyl acetate extract from Piper laetispicum and structure–activity relationship of its amide alkaloids.(2011)

19. Ingram, RE, Scott, W., Siegle, G., Depression Social and Cognitive Aspects. Oxford Textbook of Psychopathology. Oxford: Oxford University Press. (1999) 203-26