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ANTIDEPRESSANT ACTIVITY OF THE HERBAL EXTRACT, KHAMIRA GAOZABAN AMBRI JADWAR OOD SALIB WALA

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ABSTRACT

Khamira Gaozaban ambri jadwar ood salib wala (KGA) is a traditional medicine in Southeast Asia, used as anxiolytic, antiepileptic and nervine tonic. We have evaluated its role as an anti-depressant agent in animal models of stress. We evaluated an anti-depressant activity by forced swim and marble burying method. 96 NMRI mice were randomly divided into Control group which received saline, a standard group which received Imipramine and two test groups which were given two doses 86 mg/kg and 170 mg/kg doses of KGA with different models of treatments. Each group consisted of 6 animals irrespective of sex. Results show pronounce anti-depressant effect both in acute (One day) and sub-acute (10 days) treatment with CMS (chronic mild stress) model and one day and 15 day treatments in marble burying test. Concluding results suggest strong anti-depressant activity of KGA in different treatment patterns.

Keywords: Khamira Gaozaban ambri jadwar Ood Salib wala (KGA); Depression; Marble burying test; Forced swim test.

INTRODUCTION

Stresses are increasing day by day, in spite of advancements and comforts in life. It is an essential feature of life and is becoming one of the major causes of a number of diseases like Diabetes Mellitus, [1] Hypertension, [2] Conversion disorders,[3] Anxiety and Depression .[4] Physical or Psychological stress activates the hypothalamus - hypophyseal system, to cope up with stressful situations .[5]

Prolonged stress causes atrophy of hippocampus, amygdala and prefrontal cortex due to excessive and sustained release of glucocorticoids. [6] Atrophy of the hippocampus is also associated with decreased amount of BDNF (Brain derived neurotrophic factor) which is responsible for neuronal plasticity including neurogenesis[7] and mood stability. [8] Treatment of stress disorders by administration of anxiolytic and antidepressant agents are common. The target of antidepressant agents are increased synthesis of BDNF via enhancement of monoamine neurotransmitters in the hippocampus and prefrontal cortex. [7]

Khamira Gaozaban Ambri Jadwar Ood Salib Wala (KGA), which is a product of Hamdard Laboratories (Waqf) Pakistan, has been used for over hundred years as a nervine tonic, anxiolytic and anticonvulsant. According to Said [9] the formulation consists of 19 constituents. In this study we have evaluated the anti-depressant activity in animals.

MATERIALS AND METHODS

Experimental animals: We used NMRI mice irrespective of sex weighing 20-30 grams throughout the experiments from the Dr. HMI Institute of

Pharmacology and Herbal Sciences which follow standard internationally accepted guidelines about animal care. We conducted all the experiments between 9.00-13.00 hrs.

Drugs and chemicals: We purchased Khamira Gaozaban Ambri Jadwar Ood Saleeb Wala which is a product of Hamdard Laboratories of Pakistan from the local Hamdard Outlet in Karachi, Imipramine HCl (5 mg; Novartis) from the local market and normal saline (NaCl; Extra pure USP-BP) from Scharlar S.A. LaJota 86-08016- Barcelona, Spain.

Behavioral procedures:

Forced Swim test: Porsolt and coworkers [10] introduced this method which is the measure of level of learned helplessness produced by forcing the rodents to swim. A cylinder of 26 x 19 cm filled with fresh water every time up to 15 cm height.

Marble Burying Test: Defensive (Marble) Burying is the behavior in rodents in response to aversive/ unconditioned stimuli.[11] The test consists of placing of 20 glass marbles evenly (Fig 1) spaced in five layers of four.[12]

Experimental Protocol:

Acute (one day) treatment of different doses of KGA for its antidepressant activity: Four groups of 26 animals, 6 animals in each group treated with control (Saline), positive control (Imipramine, 15 mg/kg) and treated (KGA 86 and 170 mg/kg) groups. We forced the animals to swim (pretest swim session for 10 minutes). Animals removed after 10 minutes, dried with the towel and returned to their home cages. Mice were again forced to swim 24 hrs later in the similar condition for 5 minutes,[13] which was the test session.[14] In all the treatment groups the treatment was given 23, 5 and 1 hour before the test session.[15]

Effect of Sub-acute (10 day) treatment of different doses of KGJ for its antidepressant activity in depression like model: Preparation of depression model mice was according to the protocol followed by Ito [16] using forced swim test twice with a 10 day interval and Chronic Mild stress (CMS) with slight changes. Briefly we forced the mice swim individually for 10 minutes. After 10 minutes animals were allowed to dry and returned to their home cages. CMS-1 on the fourth day was tilting of the cage more than 30° from horizontal for 48 hours. CMS-2 on the 7th day pouring 200 ml of water on sawdust bedding of the cage for 24 hours and after one day gap CMS-3 i.e. deprivation of animals from food for 24 hours. [17] At the end of 10^{th} day we forced animals to swim again for 5 minutes, where the duration of immobility was observed. Oral treatment once daily according to scheme 1 was given by the intragastric gavage tube. We calculated volume of the drug according to the weight of the animal.

One Day and 15 days treatment of different doses of KGJ in marble burying test: A group of 30 mice received saline, Imipramine (15 mg/kg) and KGA (86 and 170 mg/kg doses) by oral gavage tube and one hour after the dosage we placed the animals individually in cages with glass marbles for 30 minutes in case of acute treatment.

In case of sub-acute treatment, we gave treatment with the same drugs to separate group of animals for fifteen days. We placed the animals one hour after the last dose in the cages with glass marbles. The total number of marbles buried, were counted at the end of 30 minutes.

Statistical analysis: All the results analyzed by one way ANOVA followed LSD (Least significant difference) using computer software "statistical product selective solution" (SPSS-19) version 19. All the results were considered significant when $p < 0.05$.

RESULTS

Immobility test for One day treatment: One way ANOVA revealed significant differences between groups, $F(4, 25) = 5.82$, $p = 0.002$. A post Hoc comparison using Fisher LSD test revealed that groups receiving 86 mg/ kg of KGA and 15 mg/kg of Imipramine showed a significantly greater reduction in the immobility ($p < 0.005$) as compared to other two treatment groups that is 170 and 340 mg/kg groups of KGA that were not significantly different from the placebo group (Figure 2). Antidepressant activity of 86 mg/kg KGA group show highly significant ($p = 0.001$) antidepressant effect which is comparable to 15 mg/kg dose of Imipramine ($p = 0.002$). Higher doses like 170 mg/kg ($p > 0.05$) and 340 mg/kg ($p > 0.05$) of KGA are not effective and lower dose is more effective in single day treatment.

Immobility test in 10 day treatment with chronic mild stress: One way ANOVA revealed significant differences between groups, $F(4, 25) = 5.74$, $p = 0.002$. Post hoc analysis using LSD tests showed least significant difference between 86 mg/kg ($p = 0.003$), 170 mg/kg ($p = 0.018$) & 340 mg/kg doses ($p = 0.000$) of KGA and 15 mg/kg dose of Imipramine showing comparable results ($p = 0.002$) (figure 3).

On 10 day treatment along with chronic mild stress modal all three doses of KGA showed anti-depressant activity with Imipramine.

Marble burying test One hour after single treatment: One way ANOVA for Marble burying after single treatment showed no significant difference between groups F (4, 25) = 1.373, ns. Only 86 mg/kg dose showed significant result t = 2.39 ($p < 0.05$) (Figure 4). Even Imipramine at 15 mg/kg dose single treatment did not show significant result because standard effect of anti depressant agents is seen after two weeks of administration. 86 mg/kg dose on the other hand showed response after single administration.

Marble burying test after 15 days' treatment: Marble burying test after fifteen days' treatment revealed a significant difference between groups. F (4, 25) = 5.57, p = 0.002 using One way analysis of variance. Post hoc analysis using LSD showed significant reduction of Marble burying by 86 mg/kg dose of KGA ($p = 0.02$) and 15 mg/kg dose of Imipramine ($p = 0.01$) compared to placebo groups and other doses (170 and 340 mg/kg) of KGJ showed non- significant results ($p > 0.05$) (Figure 5).

DICUSSION

Target of Anti-depressant treatment is increased mono-amine neurotransmission which provides negative feedback to HPA axis. Cortisol released because of activation of HPA axis leads to depressed BDNF (Brain derived neurotrophic factor) formation, leading to decreased neurotrophic support and brain atrophy. [6]The Major outcome of which is decreased neuronal densities in the hippocampus and prefrontal cortex leading to depression. [7] Depression can also be precipitated by radical injury [18] mostly the stress induced behavioral depression.

Khamira Gaozaban Ambri Jadwar Ood Salib Wala is an herbal tonic which is in use for over a hundred years. The present study was designed to study its anti-depressant effects as its new indication.

According to Deussing,[17] forced swim test is the behavioral model which shows response with a number of antidepressant agents. The effect of single day treatment on forced swim behavior showed significant 30.35% fall in immobility time ($p < 0.005$) by 86 mg/kg dose. Imipramine HCl proved its anti-depressant activity by decreasing the immobility time by 27.46% ($p < 0.005$).

In case of sub-acute treatment depression was produced by a series of chronic mild stresses (CMS) along with the forced swim test. [16, 17] Stressful experiences contribute in pathogenesis of depression in human beings. So to produce depression like symptoms, animal model of chronic mild stress is generated to produce human depression like symptoms.[19] Subacute (10 days) treatment showed significant decrease in immobility time by 86 mg/kg (19.3 % fall) and 340 mg/kg doses (25.5 % fall) of KGA thus proving strong potential to relieve depression. In comparison Imipramine decreased immobility by 21% ($p < 0.005$) at 15 mg/kg dose.

Marble burying is used to assess the anxiolytic ,[20] antidepressant[21] and antipsychotic[22] activities. Here this paradigm is used as an anti-depressant model. Marble burying behavior was tested in acute and sub-acute (15 days) treatment. In acute treatment 86 mg/kg dose showed a 30 % fall in marble burying ($p < 0.05$). In case of Sub-acute treatment fall was 33.71% and of Imipramine was 38.55% ($p < 0.05$). Conventional anti-depressant agents show their effect two weeks after administration. However, in single day marble burying where only a single dose was given Imipramine did not show its fall in marble burying whereas 86 mg/kg dose of KGA showed 21% fall, thus showing its strong anti-depressant potential, from the start of treatment.

Major theory to treat depression is to target monoamine levels in the synapse. [23] When monoamine levels are increased they decrease the activity of the HPA axis and increase the transcription of BDNF. As neurotrophic supply is continued the symptoms of depression start ameliorating. But because the process of transcription takes a little time, anti-depressant action is seen in fifteen days. Further research is necessary to prove the exact mode of action of Khamira Gaozaban Ambri Jadwar Ood Salib Wala, as anti-depressant potential is obvious from the results presented above.

CONCLUSION

To conclude, KGA have strong anti-depressant potential in all four experimental paradigms, especially its role in marble burying test with single treatment. Further mechanism of action elucidation is necessary and is in progress.

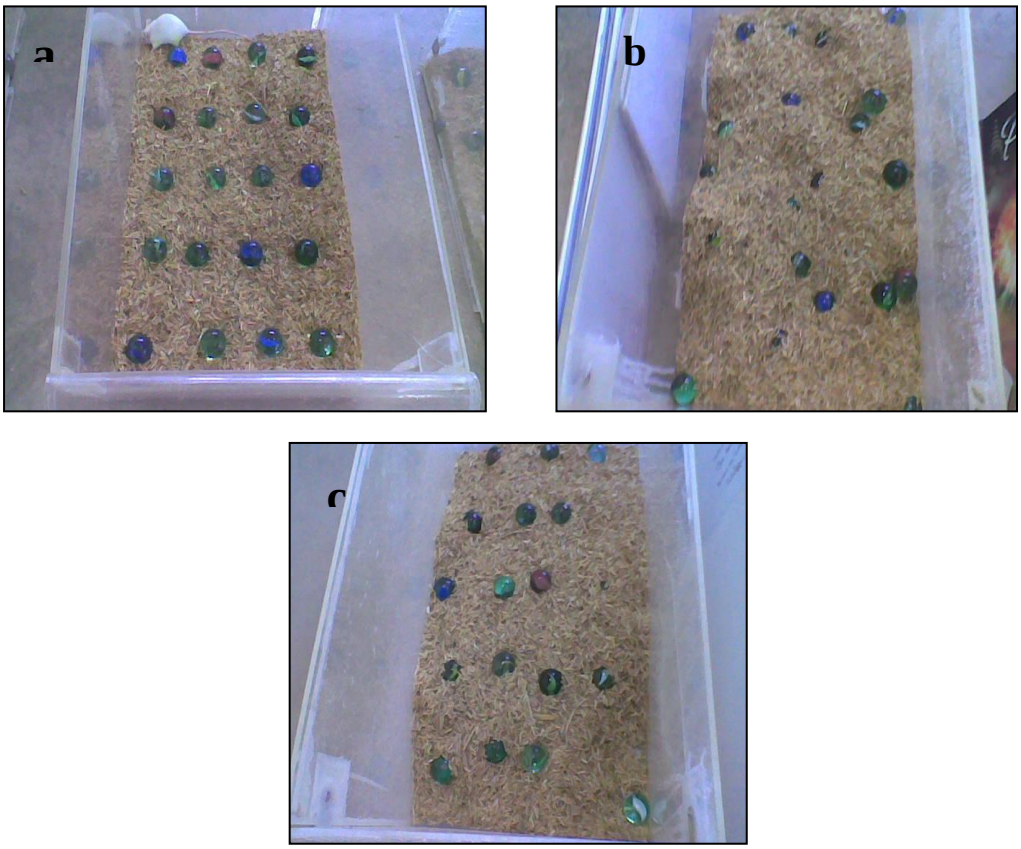


Figure 1:Marble burying experimental paradigm (a) Beginning of the experiment with arrangement of marbles (b) End of experiment. Saline control shows complete derangement of the arrangement after one hour (c) End of experiment with test drug KGA.

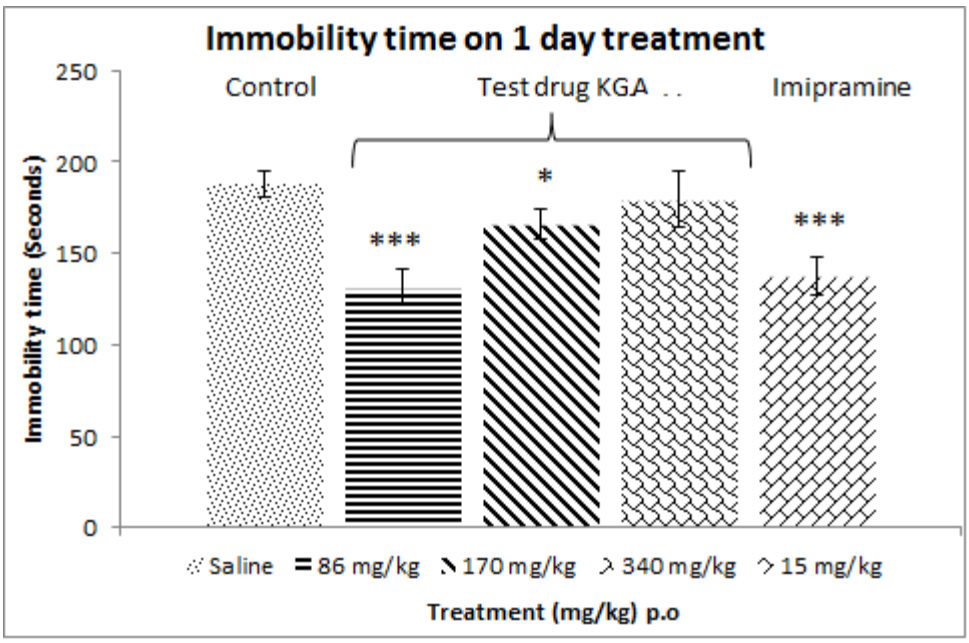


Figure 2:Effect of single day (thrice a day) treatment of different doses KGA on immobility time in forced swim test

Results are shown as average ± S.E.M; * = $p < 0.05$; *** = $p < 0.005$; n = 6 for each group

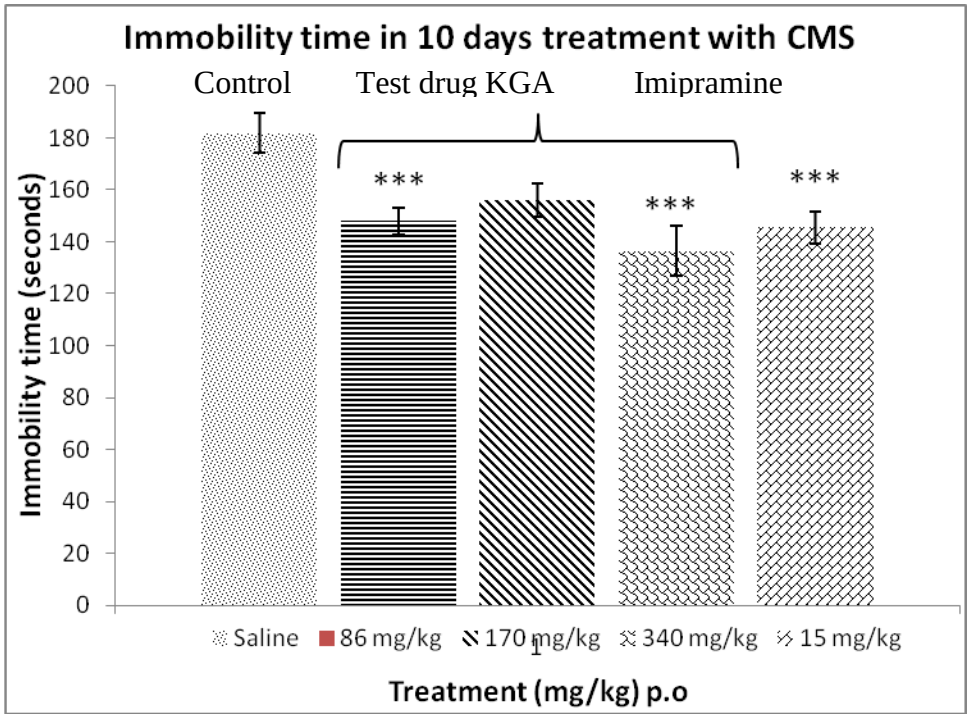


Figure 3:Effect of 15 days treatment of different doses of KGJ on immobility time in forced swim test with chronic mild stress model

Results are shown as average ± S.E.M; *** = $p < 0.005$; n = 6 for each group

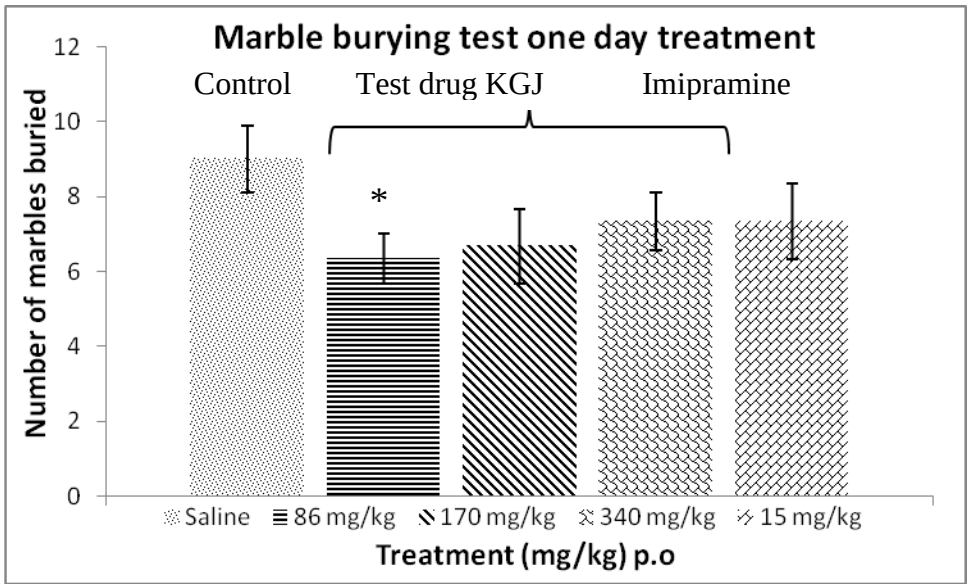


Figure 4: Effect of one day treatment of KGA on marble burying behavior of mice

Results are shown as average ± S.E.M; * = $P<0.05$; n = 6 for each group

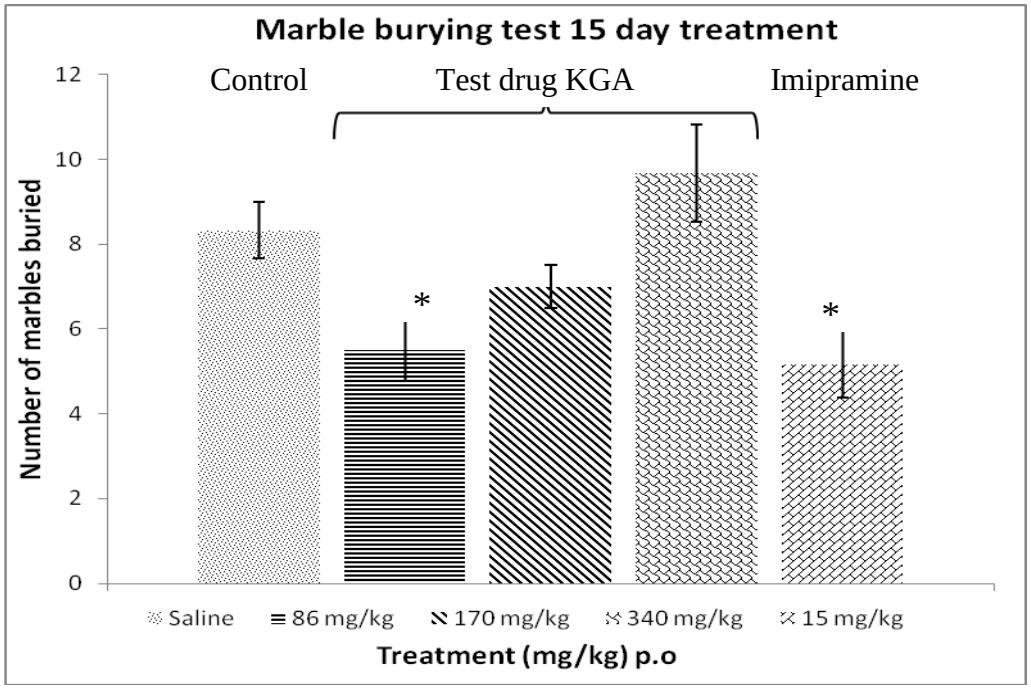
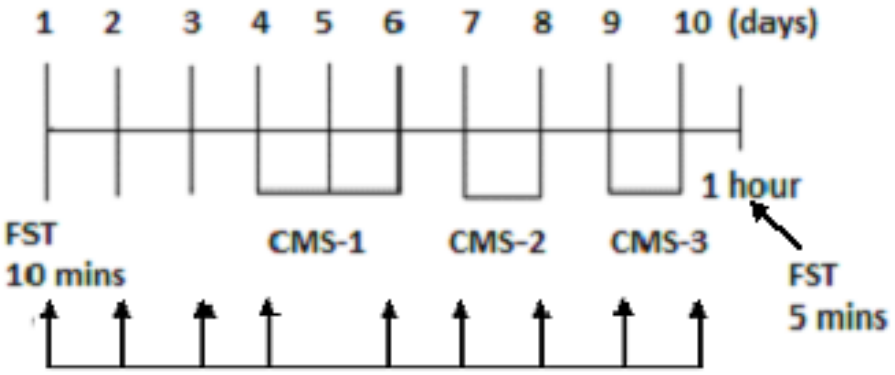


Figure 5: Effect of one day treatment of KGA on marble burying behavior of mice

Results are shown as average ± S.E.M; ***** = P<0.05; n = 6 for each group



Scheme 1: Schedule for drug administration in the depression like model mouse

REFERENCES

1. Surwit R, Schneider MS, Feinglos MN, Diabetes Care, 1992; 15(10): 1413-22.
2. Kulkarni S, O'Fatrell I, Erasi M, Kochar MS, Wisc Med J, 1998; 97(11): 34-8.
3. Sayeed MN, Ahmed S, Arshad N, J Coll Physicians Surg Pak, 2005; 15(8): 489-92.
4. Alfonso J, Pollevick GD, van der Hart MG, Flügge G, Fuchs E, Frasch ACC, Eur J Neurosci, 2004; 19 (3): 659–66.
5. Zielger DR, Herman, JP, Integr Comp Biol, 2002; 42(3): 541-51.
6. McEwans BS, Metabolism, 2005;54(5): 20 - 3.
7. Castren E, Voiker V, Rantamaki T, Curr Opin Pharmacol, 2007; 7(1): 18 - 21.
8. Duman R, Montaggia L, Biol Psychiatry, 2006; 59(12): 1116-27.
9. Said M, Preparation of Khamira Gaozaban Ambri Jadwar Ood Salib wala. Hamdard Pharmacopeia of Eastern Medicine. 1970. 2nd Imprint, pp124.
10. Porsolt RD, Le Pichon M, Jalfre M, Nature, 1977; 266: 730-2.

11. Coleta M, Batista MT, Campos MG, Carvalho R, Cotrim MD, de Lima TCM, da Cunha AP, Phytother Res, 2006, 20: 1067-73.
12. Liesbeth ASB, Laurent B, Agnes LA, Ronan D, Adrian N-T, Behav Pharmacol, 2008; 19(2): 145-52.
13. Norte MCB, Cosentino RM, Lazarini CA, Phytomedicine, 2005; 12: 294-8.
14. Yu ZF, Kong LD, Chen YJ, J Ethnopharmacol, 2002; 83: 161-5.
15. Mendes FR, Mattei R, Carlini EL, Fitoterapia, 2002; 73: 462-71.
16. Ito N, Nagai T, Yabe T, Nunome S, Hanawa T, Yamada H, Phytomedicine, 2006; 13: 658-67.
17. Deussing JM, Drug Discov Today: Disease Models, 2006; 3(4): 375-83.
18. Ng F, Berk M, Dean O, Bush AI, Int J Neuropsychopharmacol, 2008; 11(6): 851 – 76.
19. Schweize MC, Henniger MSH, Sillaber I, PLoS ONE, 2009; 4(1): e4326.
20. Almeida ER, Afr J Pharmacy and Pharmacol, 2008; 2(5): 95-100.
21. Hedlund PB, Sutcliffe JG, NeurosciLett, 2007; 414(3): 247-51.
22. Nicolas LB, Kolb Y, Prinssen EPM, Eur J Pharmacol, 2006; 547(1-3): 106-15. 23.
23. Delgado PL, J. Clin Psychiatry, 2004; 65 (Suppli 4): 25 – 30.