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EVALUATION OF ANTIDEPRESSANT ACTIVITY AQUEOUS EXTRACT OF FRUITS OF *EMBLICA OFFICINALIS* (AMLA) IN ALBINO MICE

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ABSTRACT

Depression is a disorder of the brain. There are a variety of causes, including genetic, environmental, psychological and biochemical factors. Currently, the available anti-depressant agents are associated with unwanted side effects and have their own limitations. An increasing number of herbal products have been introduced into psychiatric practice, as alternative or complementary medicines. Aim of the study is to evaluate the antidepressant activity of amla (*Emblica officinalis*) in albino mice and compare it with imipramine. Animal study was carried out in mice using two animal models – Tail suspension test and Forced swimming test. Results were analyzed by factorial repeated measure ANOVA and multivariate statistical tests. Amla at the above doses significantly reduced the immobility time in both the tests compared to control ($p < 0.05$).The reduction in duration of immobility at the dose of 4mg/kg was comparable to imipramine. The aqueous extract of fruits of amla (*Emblica officinalis*) has significant antidepressant activity in animal models of depression.

Keywords: Amla (*Emblica officinalis*), Anti-depressant, Forced swimming test, Tail suspension test, Imipramine

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INTRODUCTION

Depression is considered as an affective disorder characterized primarily by change of mood. It is associated with significant socioeconomic problems, morbidity and mortality. The prevalence of major depression in the general population is estimated at 5% in world population. Prevalence ranges from 9% in ambulatory medical patients to 30% in hospitalized patients. According to the World Health report approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment. This amounts to 12.3% of the global burden of disease and

expected to rise to 15% by 2020. Major depressive disorder (MDD) is a mental disorder common in psychiatric practice wherein a patient presents with at least one of two major symptoms, constant sadness or anhedonia, accompanied by at least five secondary symptoms for at least two weeks. The secondary symptoms include feelings of worthlessness, difficulty in concentrating, changes in diet and sleep patterns. It is a relapsing, remitting illness having greater than 40 percent rate of recurrence over a two-year period. It must be distinguished from normal grief, sadness, disappointment, and the dysphoria or demoralization associated with medical illness and from bipolar disorder in which depression alters with hypomania or mania. The condition is often under diagnosed and frequently under treated. Depression results from a combination of multiple etiologic factors genetic, biochemical, psychodynamic and socio-environmental. The children of a depressed person are at a higher risk for depression. Monozygotic twins have a higher concordance rate (46%) than dizygotic siblings (20%). Biochemical factors include decrease in the level of neurotransmitters like nor-epinephrine and serotonin in the brain. Crucial life events, particularly the death or loss of a loved one or an emotional trauma can precede the onset of depression. Various drugs are available for the treatment of depression. They include monoamine oxidase inhibitors, selective and non-selective monoamine reuptake inhibitors and selective serotonin reuptake inhibitors. These medications work by normalizing the levels of neurotransmitters, notably serotonin and nor-epinephrine. Approximately two-thirds of the depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing. More over these drugs have unusual side effects like – MAOIs – insomnia, hypotension, anorgasmia, weight gain, hypertensive crisis, tyramine cheese reaction. TCAs – anti-cholinergic side effects (dry mouth, tachycardia, constipation, urinary retention and blurred vision), sweating, tremor, postural hypotension, cardiac conduction delay, sedation, weight gain. SSRIs headache, nausea, other gastro intestinal effects, jitteriness, insomnia and sexual dysfunction.6The medical need for newer, better-tolerated and more efficacious treatments remains high. Ayurveda, an alternative system of medicine, practiced widely in India, uses a number of plants (whole/parts) for treatment of variety of diseases. *"Medhya rasayana"* are a group of medicines *(Centella asiatica, Acorus calamus, Jatamansi, Clitoria ternatea, Baccopa monnieri, Withania somnifera, Celastrus panniculatus, Guduchi and areca)* in *Ayurveda,* known to act on the nervous system and psychiatric conditions. Charaka states: "A single drug may have many applications owing to its diverse actions just as a man is able to perform various actions" Many popular Ayurvedic drugs such as *Ashwagandha, Bramhi,Guduchi, Katuka, Shatavari,* etc. have multifarious properties ascribed to them. Obviously, their molecular targets are shared by many cell systems and cell membrane components such as phospholipase A2, phospholipase C, adenyl cyclase and cAMP, adenosine receptors, eicosanoids, ion channels and neuroreceptors, dopamine, serotonin, norepinephrine (NE), gamma-aminobutyric acid (GABA), etc. Amla is used in traditional Indian medicines for centuries for the treatment of vitiated conditions of "Tridosha", diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, inflammations, anemia, emaciation, hepatopathy, jaundice, strangury, diarrhoea, dysentery, hemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and greyness of hair. The fruits are sour, astringent, bitter, acrid, sweet, cooling, anodyne, ophthalmic, carminative, digestive,

Stomachic, laxative, alterant, aphrodisiac, rejuvenative, diuretic, antipyretic and tonic. It is an important constituent of "Triphala", an Ayurvedic formulation known for its rejuvenating properties. Amla juice is an effective hypolipidemic agent and can be used as a pharmaceutical tool in hyperlipidemic subjects. Anti-oxidant property of the fruit extract is demonstrated in several models.9 since the depressive disorders are having a huge impact on our lives, it is worth evaluating the alternative forms of medicines which can be used for its treatment. So in this study, an effort was made to investigate the antidepressant effect of aqueous extract of fruits of amla (Emblica officinalis) and its comparison with tricyclic antidepressant imipramine in animal models of depression (1-5).

MATERIALS AND METHODS

Swiss albino mice weighing around 20 g – 40 g of either sex *were* divided into five treatment groups and orally administered with gum acacia (control), imipramine 15mg/kg (standard), amla 2mg/kg and 4mg/kg (test drugs) and combination of imipramine and amla. Total of 60 animals were used. Each group contained 6 animals. Duration of immobility was observed for 4 minutes of total 6 minutes period in tail suspension test and in forced swimming test on separate set of animals on 1st day, 8 th day and 15 th day.

Forced Swimming Test

Group 1: Received 0.1 ml/10 g of gum acacia orally (Control), Group 2: Received 15 mg/kg imipramine orally (Standard), Group 3: Received 2 mg/kg of aqueous extract of fruits of amla orally, Group 4: Received 4 mg/kg of aqueous extract of fruits of amal orally, Group 5: Received 4 mg/kg of aqueous extract of fruits of amla and 15 mg/kg of imipramine orally.

Sixty minutes after administration of the test compounds, the male mice were placed in plexiglass cylinder/ plastic tub containing 9 cm of water maintained at 25°C. After allowing one minute for acclimatization, immobility of each mouse was rated every 30s from second minute onwards for another five minutes. An animal was judged to be immobile whenever it remained floating passively in water in a slightly hunched but upright position, its nose just above the surface. After ten observations, mean values and standard deviations in each treatment group were calculated. Standard drug imipramine 15mg/kg chosen as positive control. The animals were forced to swim in a glass cylinder measuring 25cm height, 12cm diameter containing water at room temperature to a depth of 15cm. After an initial 2 minute period of vigorous activity, each animal assumed a typical immobile posture. The mouse was considered immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during next 4 minutes of total 6 minute test. After 6 min mouse was taken out, dried with a towel. The water is changed after each test because urine and the other chemicals released by the first mouse will affect the swimming pattern of the next mouse. Each animal was used only once. Groups 1 to 5 were used for this model. The procedures were conducted after 1 hour of administrating the drug orally to animals. Duration of immobility was measured in controls and animals treated with various doses of a test drug or standards. Compared with the immobility score of the control group, percent reduction was calculated (6-8).

Statistical Analysis

Tail Suspension Test (TST)

Group 6: Received 0.1 ml/10 g of gum acacia orally (Control), Group 7: Received 15 mg/kg imipramine orally (Standard), Group 8: Received 2 mg/kg of aqueous extract of fruits of amla orally, Group 9: Received 4 mg/kg of aqueous extract of fruits of amla orally, Group 10: Received 4 mg/kg of aqueous extract of fruits of amla and 15 mg/kg of imipramine orally.

Mice were treated with the test compounds or the vehicle orally 30 minutes prior to testing. For the test the mice were suspended on the edge of a shelf 58 cm above a tabletop 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 to be immobile when they hung passively and completely motionless. Animals were suspended upside down on a metal rod at a height of 55 cm from the ground with the help of an adhesive tape placed approximately 1 cm from the tip of the tail Initially the animals tried to escape by making vigorous movements but when unable to escape became immobile. The animal was considered immobile when it did not show any movement of body and hanged passively. The total duration of immobility was noted during last 4 minutes of 6 minute period. Each animal was used only once. Groups 6 to 10 were used for this model. The procedures were conducted after 1 hour of administrating the drug orally to animals. Duration of immobility was measured in controls and animals treated with various doses of a test drug or standards. Compared with the immobility score of the control group, percent reduction was calculated (9, 10).

It is carried out by using following tests: Shapiro Wilks Normality test- to test normality assumption, Repeated measure plot, Factorial repeated measure ANOVA, Multivariate statistical tests – Wilks'Lambda, Pillai Trace, H-L Trace- to test for the vector means are significant or not, Greenhouse-Geisser (G-G) Epsilon– to test compound symmetry assumption in repeated measure analysis, Huynh-Feldt (H-F) Epsilon- to test sphericity assumption in repeated measure analysis, THETA test. For all the tests a 'P' value of 0.05 or less was considered for statistical significance.

RESULTS AND DISCUSSION

The present study evaluated the antidepressant activity of aqueous extract of fruits of amla (*Emblica officinalis*) in two different animal models of depression, tail suspension test and forced swim test. Both these methods are widely used for screening antidepressant drugs. There is a significant correlation between the potency of antidepressants in both forced-swim and tail-suspension tests and clinical potency of the drugs. These tests are quite sensitive and relatively specific to all major classes of antidepressants like tricyclics, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs) and atypical antidepressants. It has been argued that tail suspension is less stressful than forced swim test and has greater pharmacological sensitivity.

Forced Swimming Test Model

Table -1, 2 and 3 shows the Univariate and Multivariate Repeated Measures Analysis Between Groups on Forced Swimming Test Model.

On day 1: There were no significant differences in duration of immobility among different groups.

On day 8: When compared to day 1 there were reduction of duration of immobility in groups 2, 3, 4 and 5, but there were no significant differences in duration of immobility among different groups.

On day 15: When compared to day 1 and day 8 (Graph1) there were reductions in duration of immobility in groups 2, 3, 4 and 5.

Table -1 Univariate and Multivariate Repeated Measures Analysis Between Groups on Day 1

GROUPS	GI	G2	G3	G4	G5
G1	-	NS	NS	NS	NS
G2	NS	-	NS	NS	NS
G3	NS	NS	-	NS	NS
G4	NS	NS	NS	-	NS
G5	NS	NS	NS	NS	-

Table -2 Univariate and Multivariate Repeated Measures Analysis Between Groups on Day 8

GROUPS	G1	G2	G3	G4	G5
G1	-	S	S	HS	HS
G2	NS	-	NS	NS	NS
G3	NS	NS	-	NS	NS
G4	NS	NS	NS	-	NS
G5	NS	NS	NS	NS	-

Table -3 Univariate and Multivariate repeated measures analysis between groups on Day 15

GROUPS	G1	G2	G3	G4	G5
G1	-	ES	ES	ES	ES
G2	NS	-	NS	NS	NS
G3	NS	NS	-	NS	NS
G4	NS	NS	NS	-	NS
G5	NS	NS	NS	NS	-

NS- Not Significant (P>0.05), S- Significant (P<0.05), HS-Highly Significant (P<0.01),
ES-Extremely Significant (P<0.001)

Tail Suspension Test Model

Table-4,5 and 6 shows Univariate and Multivariate Repeated Measures Analysis between Groups on tail suspension method.

On day 1: There were no significant differences in duration of immobility among different groups.

On day 8: When compared to day 1 there were reduction of durations of immobility (Graph 2) in groups 7, 8, 9 and 10, but there were no significant differences in duration of immobility among different groups.

On day 15: When compared to day 1 and day 8 (Graph2) there were reductions in duration of immobility in groups 7, 8, 9 and 10.

Table-4 Univariate and Multivariate repeated measures analysis between groups on Day 1

GROUPS	G6	G7	G8	G9	G10
G6	-	NS	NS	NS	NS
G7	NS	-	NS	NS	NS
G8	NS	NS	-	NS	NS
G9	NS	NS	NS	-	NS
G10	NS	NS	NS	NS	-

Table-5 Univariate and Multivariate repeated measures analysis between groups on Day 8

GROUPS	G6	G7	G8	G9	G10
G6	-	NS	NS	NS	NS
G7	NS	-	NS	NS	NS
G8	NS	NS	-	NS	NS
G9	NS	NS	NS	-	NS
G10	NS	NS	NS	NS	-

Table-5 Univariate and Multivariate repeated measures analysis between groups on Day 15

GROUPS	G6	G7	G8	G9	G10
G6	-	HS	NS	HS	NS
G7	NS	-	NS	NS	NS
G8	NS	NS	-	NS	NS
G9	NS	NS	NS	-	NS
G10	NS	NS	NS	NS	-

NS- Not Significant (P>0.05), S- Significant (P<0.05), HS-Highly Significant (P<0.01), ES-Extremely Significant (P<0.001)

CONCLUSION

Comparison of reduction in duration of immobility between 1st day, 8th day and 15th day showed that the reduction of immobility in different groups were more significant on 15th day compared to 8th and very less on 1st day. This showed that the aqueous extract of fruits of amla (*Emblica officinalis*) on chronic administration of higher doses has antidepressant effect almost similar to standard drug like imipramine. To sum up, the present study has shown that the aqueous extract of fruits of amla (*Emblica officinalis*) at a dose of 2 mg/kg, 4 mg/kg and combination significantly reduced the duration (time) of immobility of animals as compared to the control in both tail suspension test and forced swim test of depression, showing that in both the doses, it has significant anti depressant activity. Both the tests showed consistent results in terms of reduction in the duration of immobility. Exact mechanisms underlying the antidepressant action cannot be concluded at the moment due to the presence of large number of phytochemicals in the *Emblica officinalis*. However, the antidepressant activity may be attributed to the presence of tannic acid (30.00%), gallic acid (10.00%), polyphenols, flavanoids and ascorbic acid in the extract. Tannic acid has been shown to be a non selective inhibitor of monoamine oxidase, thereby increasing the levels of monoaminergic neurotransmitters in the brain. Chronic use of gallic acid has been shown to have a neurotropic action on

the hypothalamus. Another possible mechanism of action is the attenuation of oxidative stress produced during depression, by the polyphenols and tannic acid present in *Emblica officinalis*. *Emblica officinalis* extract (EOE) and quercetin has been reported to possess cytoprotective effects on account of its antioxidant activity by reducing lipid peroxidation and also significand ~ scavenges superoxide as well as inhibits its generation Aqueous extract of fruits of amla (*Emblica officinalis*) has been found to be potent antioxidants *in vitro*. Thus the present work though of preliminary in nature suggests that the *Emblica officinalis* extract has good antidepressant activity. Further elaborate research work involving more numbers of animals and different experimental models of antidepressant activity are needed to elucidate the exact molecular and biochemical mechanism of action to develop more effective compound.

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