# ANTIDEPRESSANT ACTIVITY OF AQUEOUS EXTRACT OF FRUITS OF *EMBLICA OFFICINALIS* IN MICE.

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**ABSTRACT:** Depression is a widespread psychiatric disorder affecting around 5% of the population. Furthermore, it is difficult to predict which patient will respond to any given treatment. In the traditional systems of medicine, many plants and formulations have been used to treat depression for thousands of years. *Emblica officinalis* (EO) contains tannic acid as its main ingredient and this compound has been shown to have non-selective mono-amine oxidase activity. Therefore, the present study was undertaken to evaluate the antidepressant potential of acute and chronic administration of EO in forced swim test (FST) and tail suspension test (TST). Inbred adult male Swiss Albino mice weighing 25-30g were used in the study. Standard drug (imipramine) and test drug (EO) were suspended in 1% gum acacia. The vehicle (10ml/kg, p.o), imipramine (10mg/kg, p.o) and EO (0.8mg/kg, 2mg/kg, 4mg/kg, p.o. respectively) were administered 1hour prior to acute study. In chronic study, all drugs were given for 10 days and the last dose was given 1hour before the experiment. Duration of immobility was noted in both the models. In our study, both imipramine and EO significantly reduced the duration of immobility in both experimental models as compared to the animals in the control group. The antidepressant activity of EO was comparable to that of standard drug imipramine. The results of the present study indicate the potential for use of EO as an adjuvant in the treatment of depression.

**Keywords:** Forced swim test, Tail suspension test, *Emblica officinalis,* Depression

## INTRODUCTION

Depression is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia. The prevalence of depression in general population is estimated to be around 5%. At present 121 million people are estimated to suffer from depression. An estimated 5.8% of men and 9.5% of women experience a depressive episode in their lifetime with suicide being one of the most common outcome of depression (WHO 1998, Stahl SM, et.al., 1998, Richelson E, et.al., 2001).

Despite the development of new molecules for pharmacotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated in many patients. Although the currently prescribed molecules provide some improvement in the clinical condition of patients, it is at a cost of having to bear the burden of their adverse effects (Tripathi KD 2008, Hardman JG, et.al., 2007). Furthermore, it is difficult to predict which patient will respond to any given treatment. It has been reported in earlier studies that only two out of three patients responds to any given antidepressant treatment, and of these, one would probably have responded to placebo alone (Walker R, et.al., 1999). Along with the classical theory of decrease in the neurotransmitter levels in the brain leading to the pathogenesis of clinical depression, recent studies have also shown the involvement of oxidative stress in the phenomenon (Sarandol A, et.al., 2007, Ibrahim E, et.al., 2007).

Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders (Tripathi KD, et.al., 2008, Sembulingam K, et.al., 1997). On one hand these agents have a less adverse effect profile, and on the other hand they have been shown to be comparable in efficacy to their synthetic counterparts. *Emblica officinalis* (EO) Gaertn, commonly known as Amla, is a number of small genus Emblica belongs to the family Euphorbiaceae. All parts of the plant are used for medicinal purpose. The fresh or the dry fruit used in traditional medicines for the treatment of diarrhea, jaundice and inflammations (Deokar AB 1998). The pulp of the fruit is smeared on the head for head ache and dizziness (Perry LM 1980). In our earlier study, the aqueous extract of EO has shown significant anticataleptic and antioxidant activity in mice (Sudhakar P, et.al., 2009). EO fruits mainly contains tannins and vitamin C like substances in abundance and their chemical constituents include gallic acid, ellagic acid, emblicanin A, emblicanin B, Punigluconin and some 10-12 flavanoids (Nadkarni Ak, et.al., 1992). The aqueous extract of the EO fruits contain 30.0% tannins and 10.0% Gallic acid (estimation and purity of active principles were done by the Quality Control Laboratory, M/s. Natural Remedies, Bangalore, lab reference no.0505211). EO fruit is an important constituent of “Triphala”, an Ayurvedic formulation known for its rejuvenating properties.

From our laboratory, we have already reported, antianxiety (Gopalakrishna HN, et.al., 2006), anticataleptic and antioxidant (Nair V, et.al., 2007) properties of the polyherbal formulation NR-ANX-C, anticataleptic, antioxidant and anxiolytic activity (Sudhakar P, et.al., 2010) of EO in experimental models. *Emblica officinalis* (EO) is one of the important constituents of this polyherbal formulation(NR-ANX-C). Based on our previous studies that show the involvement of a central action of this formulation we have investigated the antidepressant activity of EO fruit aqueous extract (EO) using two behavioural models for screening antidepressants; FST and TST in mice.

## MATERIALS & METHODS

### Animals

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Kasturba Medical College, Manipal University, Mangalore, India. Adult male Swiss Albino mice weighing 25-35 gm from our breeding stock were used in this study. The animals were housed at 24±2 ºC with 12:12 h light and dark cycle. They had free access to food and water *ad libitum*. The animals were acclimatized for a period of 7 days before the study. The study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

### Drugs and chemicals

The standard antidepressant drug imipramine(M/s.Alkem Ltd.Mumbai) was obtained from our institutional pharmacy. The test drug EO was standardized and provided by M/s.Natural Remedies Pvt. Ltd., Bangalore. The extract was yellowish-brown in colour and on HPLC analysis was found to contain tannic acid 30.00% and gallic acid 10.00% as the main constituents. Pytochemical analysis of the dried powder of EO gave positive results for tannins, alkaloids, carbohydrates, polyphenols and amino acids. All drugs were dissolved/suspended in 1% gum acacia (vehicle) (Sudhakar P, et.al., 2007).

### Experimental design

On the day of the experiment, the animals were divided randomly into control and experimental groups (n=6). Group 1 received the vehicle, 1% gum acacia(10ml/kg) and served as the control group, groups 2, 3 and 4 received the test drug (EO) in doses of 0.8, 2.0 and 4.0mg/kg, and group 5 received the standard drug imipramine (10mg/kg) *per orally*. Drugs/vehicle was administered to the animals 60 minutes prior to the behavioural evaluation in acute study. For chronic study, a new set of animals were used. They were grouped as in acute study and were administered the drugs/vehicle for a period of 10 days. Behavioural evaluation was carried out 60 minutes post drug/vehicle administration on $10^{th}$ day. The antidepressant activity of the test drug was evaluated using the following experimental models of depression TST and FST:

**Tail suspension test (TST):** The method described by *Steru, et. al.* was used in our study (Steru L, et.al., 1985). The animals were hung by the tail on a plastic string 75 cm above the surface with the help of an adhesive tape. The duration of immobility was observed for a period of 8 minutes. The duration of immobility was recorded during the last 6 minutes of the observation period. Mice were considered to be immobile only when they hung passively and were completely motionless.

**Forced Swim Test (FST):** The method described by *Porsolt, et. al.* was used in our study (Porsolt RD, et.al., 1977). Each animal was placed individually in a 5 liter glass beakers, filled with water upto a height of 15 cm and were observed for duration of 6 minutes. The duration of immobility was recorded during the last 4 minutes of the observation period. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface. The water was changed after each test.

**Statistical analysis**

The mean±S.E.M. values were calculated for each group. The data were analysed using one-way ANOVA followed by Dunnet's multiple comparison test. P< 0.05 was considered to be statistically significant.

**RESULTS**

**Tail suspension test (TST):** Results were given in table 1. A significant (P<0.01) decrease in the duration of immobility was seen with the standard drug imipramine and EO in all the tested doses as compared to the control (group 1). In both acute and chronic study EO in doses of 2 and 4mg/kg produced a greater decrease in the duration of immobility as compared to the standard drug imipramine.

**Table 1: Effect of EO on immobility time in the Tail Suspension Test (TST) using mice**

| Group (Drug Treatment) | Duration of Immobility (sec) | |
|---|---|---|
| | Acute Study | Chronic Study |
| Group 1 (1% gum acacia 10.0 ml/kg)) | 233.16±11.33 | 211.66±16.44 |
| Group 2 (EO 0.8 mg/kg) | 171.50±04.87** | 168.83±07.11* |
| Group 3 (EO 2.0 mg/kg) | 139.16±12.88** | 141.37±05.98** |
| Group 4 (EO 4.0 mg/kg) | 161.33±03.55** | 153.33±12.36** |
| Group 5 (Imipramine 10.0 mg/kg) | 163.66±06.27** | 157.00±05.50** |

Test solutions were administered orally 60 min prior to the test. Values represented mean±S.E.M. (n=6), *P<0.05, **P<0.01 vs. control (group 1).

**Forced swim test (FST):** Results were given in table 2. A significant (P<0.01) decrease in the duration of immobility was seen with the standard drug imipramine and all tested doses of EO as compared to the control (group 1). In acute study, EO in a dose of 2mg/kg was more efficacious than imipramine in reducing the duration of immobility. However in chronic study, EO in both the higher doses tested (2 and 4mg/kg) was more efficacious than imipramine.

**Table 2: Effect of EO on immobility time in the Forced Swim Test (FST) using mice**

| Group (Drug Treatment) | Duration of Immobility (sec) | |
|---|---|---|
| | Acute Study | Chronic Study |
| Group 1 (1% gum acacia10.0 ml/kg)) | 119.17±4.34 | 122.83±06.61 |
| Group 2 (EO 0.8 mg/kg) | 75.50±8.21** | 85.83±05.68** |
| Group 3 (EO 2.0 mg/kg) | 41.89±6.53** | 54.83±03.97** |
| Group 4 (EO 4.0 mg/kg) | 67.50±5.03** | 55.00±11.37** |
| Group 5 (Imipramine 10.0 mg/kg) | 57.33±9.81** | 66.33±03.59** |

Test solutions were administered orally 60 min prior to the test. Values represented mean±S.E.M. (n=6), *P<0.05, **P<0.01 vs. control (group 1).

## DISCUSSION

Mood disorders are one of the most common mental illnesses, with a lifetime risk of 10% in general population. Prevalence of depression alone in general population is estimated to be around 5% with suicide being one of the most common outcomes (WHO 1998, Stahl SM, et.al., 1998, Richelson E, et.al., 2001). Most of the drugs that are currently being used in the treatment of depression have adverse effects that affect the quality of life of the patient. This leads to patient's non-compliance to medication, which further complicates the problem (Tripathi KD 2008, Hardman JG, et.al., 2007). Ayurveda mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders (Sembulingam K, et.al., 1997) and are claimed to have a better side-effect profile than conventional drugs. *Emblica officinalis* (EO) fruit is an important constituent of "Triphala", an Ayurvedic formulation known for its rejuvenating properties. From our laboratory we have already reported the central actions of a polyhrebal formulation NR-ANX-C (Gopalakrishna HN, et.al., 2006, Nair V, et.al., 2007), which contains EO as one of the main constituents.

In the present study we have evaluated the antidepressant activity of EO in TST and FST. The development of immobility when rodents are suspended by their tail during TST and when they are placed in an inescapable cylinder of water during FST reflects the cessation of their persistent escape-directed behavior. Conventional drugs reliably decrease the duration of immobility in animals during these tests. This decrease in duration of immobility is considered to have a good predictive value in the evaluation of potential antidepressant agents (Porsolt RD, et.al., 1977).

In the present study, EO in the highest dose tested (4mg/kg) was superior to imipramine in both the experimental models in both acute and chronic study. Exact mechanisms underlying the antidepressant action cannot be concluded at the moment due to the presence of large number of phytochemicals in the EO. 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 (Dar A & Khatoon S, et.al., 1998). Another possible mechanism of action is the attenuation of oxidative stress produced during depression, by the polyphenols and tannic acid present in EO.

*Emblica officinalis* fruit is widely used in the Indian subcontinent and is known to be safe on chronic consumption. In our study, the antidepressant efficacy of EO was found to be superior to that of the standard drug imipramine. We believe that EO has the potential to be used as an adjuvant in the treatment of depression and other mood disorders. Further studies may help to elucidate the possible mechanisms of action of *Emblica officinalis*.

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