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EVALUATION OF ANTIDEPRESSENT ACTIVITY OF LEONURUS CARDIACA IN WISTAR ALBINO RATS

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Abstract:

Depression is a widely prevalent form of mental illnesses worldwide. It is commonly associated with sad mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, and low energy. Leonurus cardiachas many medicinal properties, and are used in traditional medicine in the treatment of various medical conditions. This study was conducted to better understand the antidepressant activity of Leonurus cardiaca.

To evaluate the potential antidepressant activity of Ethanolic Extract of Leonurus cardiaca (EELC) leaves on depression in Wistar albino rats.

Wistar albino rats of either sex weighing 150-200g were used. Sixty rats were divided into two arms. Each arm was further divided into five groups (n=6). Drugs were given orally once daily, for ten days. Group 1 was the Control group and received saline. Group 2 received standard drug – Imipramine (15mg/kg). Group 3 received EELC (100mg/kg). Group 4 received EELC (200mg/kg). Group 5 received EELC (400mg/kg). Antidepressant potential of EELC was evaluated by submitting the Rats to Forced Swim Test (FST) and Tail Suspension Test (TST) on the first and tenth day.

The study showed significant reduction in immobility time in both Forced Swim Test and Tail Suspension Test in the EELC group when compared with the control group.

The study suggests that Ethanolic Extract of Leonurus cardiacahas anti-depressant activity and can be considered for use in therapy of depression after further testing.

Keywords: *Leonurus cardiaca, Antidepressant activity, forced swim test, Open Field Test.*

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INTRODUCTION:

Depression: It is basically acknowledged as illness with symptoms such as anxiety and sleep disturbances. It can be a persistent, recurring illness that can cause many personal suffering for individuals and their families. At present, disability caused by depression is estimated to be the fourth most important cause of worldwide loss of life years. This has resulted into a requirement of search for effective treatments, including antidepressant drugs, herbal remedies, psychotherapy and electroconvulsive shock therapy.

The neurobiology and pharmacology of depression:

I. Neurotransmitter Systems:

Within the central nervous system (CNS), the catecholamines, adrenaline, noradrenaline and dopamine forms the adrenergic systems. Out of these, few of the adrenergic neurons are radiating from the ancient limbic system and plays to role of discharging the catecholamines within the frontal cortex. Thus, the catecholaminergic pathways are claimed to be responsible for mood, alertness and stress responses. The primary neurotransmitter, which modulates the excitatory catecholamine systems of the CNS, is Serotonin. The Serotonin neurons are responsible for the control of memory, mood, sex drive and appetite.[1]

The systems of serotonin and noradrenaline are the important their main cell small bodies in brainstem areas that serve as headquarters for shipping axonal projections by the brains in specific pathways that mediate specific functions (See Figure No. 1 for an illustration of the serotonin projections and Figure No. 2 for an illustration of the noradrenergic projections).

Multiple serotonergic and noradrenergic pathways may be dysfunctional in depression, generating many different symptoms.

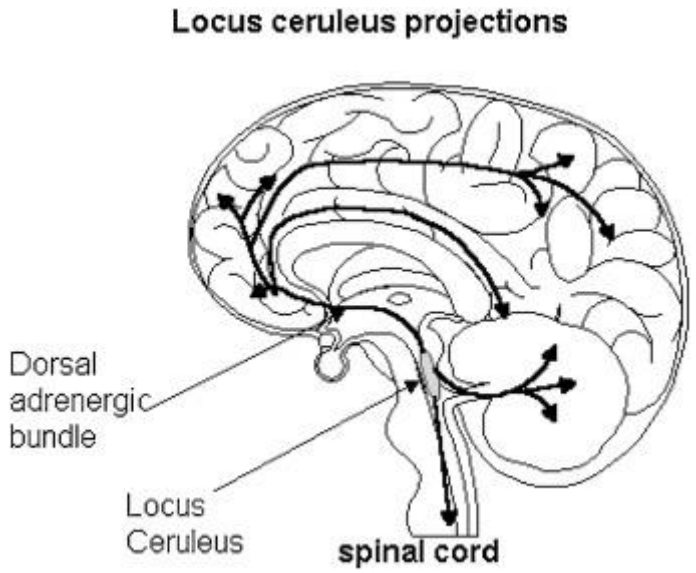

Figure: The projection of the noradrenaline system

The nucleus of the dorsal raphe projects the serotonin system and the raphemagnus. The serotonin receptors (5-HT) have been identified into various sub-types with the 5-HT1 and 5- HT2 sub-types being of greater interest in psychiatry. The most important of the 5-HT1 subclass is 5- HT1A which is concentrated in the hippocampus and raphe. The release of this 5 – HT from presynaptic neurons is modulated by this autoreceptor. The 5 - HT2 rceptors occur in high concentrations in the frontal cortex and nucleus accumbens. [2]

II. Hypotheses of Depression:

Several hypotheses of the biological determinants of depression have emerged over the past century. The most important of these and the implications thereof are reviewed below. Today it is generally accepted that depression is not necessarily due to a shortage of one vital brain neurotransmitter, but rather to a disruption in the equilibrium between different regulatory systems.

A. The Biogenic Hypothesis of depression:

The most common characteristic of depression as claimed by monoaminergic hypothesis are a result of inadequate concentration of serotonin and noradrenaline in the synaptic clefts of the neurons in

the brain.[3] This hypothesis has evolved to consider the possibility that depression may be the result of a deficiency in signal transduction from the monoamine neurotransmitter to its postsynaptic neuron, even with normal levels of neurotransmitter and receptor being present. Emerging theories that link genetic and environmental risk factors for depression suggest that stress can cause depression by down regulating certain genes, resulting in less key gene products, such as the brain-derived neurotropic factor (BDNF), being produced. BDNF sustains the viability of neurons, so if the encoding gene is repressed the result may be atrophy or even apoptosis of neurons.

B. The dopamine hypothesis of depression:
The original hypothesis was formulated in the late nineteen seventies by Solomon Snyder and linked schizophrenia with dopamine (DA) activity. Later, this hypothesis was extended to include depression following the observation that many antidepressants influence the metabolism of dopamine. Following chronic antidepressant treatment, the presynaptic DA receptors become subsensitised and this gets in an enhancement of DA release. A reduction in homovallinic acid (HVA), the main metabolite of dopamine, in the cerebral spinal fluid (CSF) of depressed patients who demonstrate marked motor retardation has also been reported. Therefore, a decrease in the ratio of HVA to DA is indicative of decreased turnover of DA. This hypothesis is also supported by reports of significantly reduced dopamine turnover in depressed suicide victims.

C. The permissive hypothesis of depression:
This hypothesis emphasizes 5-HT as a neuro-modulator and its importance as a focus for antidepressant action. According to this theory, a lowered concentration in the central nervous system (CNS) of 5-HT results in an affective state regulated by NA. Decreased 5-HT and NA levels will give rise to depression. This Averages that 5-HT may act as a 'permissive' modulator of neurotransmitter function through connections between serotonergic pathways and make connections with noradrenergic and dopaminergic pathways via the associated receptors.

D. The glutamatergic N-methyl-D-aspartate hypothesis:
As per recent researches, one of the important roles involved in the mechanism of depression is dysfunction of CNS glutamatergic pathways. Many of the researches confirm that the compounds, which induce reduction in the activities at the N – Methyl – D – Aspartate receptors produce effects similar to pharmacologically active antidepressants. Hence, it is assumed that the common pathway affected by antidepressant drugs, whenever there are adaptive changes in NMDA receptor complex.

E. The kynurenine hypothesis:
This hypothesis emerges from the premise that depression arises from altered levels of serotonine (5-Hydro. Trypt.) in the mind Serotonin is a metabolite of the esential A. A. tryphtophan (TRP) and all 5-Hydro. Trypt required by the neurons in the brain is synthesized in the brain because 5- Hydro. Trypt is unable to cross the BBB. Therefore, the availability of TRP is essential for the synthesis of 5-HT in CNS. There are several factors which affect the production and transport of TRP from the blood stream into the CNS, in which deficiency of Vit. B6, Stress, escalated cortisol levels and even high doses of TRP (200m.g. of TRP).These are the factors simulating the conversion of TRP into kynurenine, which further results ito reduced TRP level. Therefore, the inhibition of liver enzyme tryptophan 2,3-dioxygenase (also known as tryptophan pyrrolase) during the first and rate - limiting step of the pathway of kynurenine would enhance circulating levels of TRP and thereby lead to increased neural production [15]

MATERIALS AND METHODS:

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal (s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols and final execution of the standardized protocol. All this requires good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

Drugs and Chemicals:
Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Table No 1: Drugs and Chemicals

S.No	*Materials*	*Company Name*
1.	Imipramine	Nicholos Piramal Ltd

Experimental animals:
Wistar albino rats 60 in number, weighing 150-200 g, of either sex, maintained under standard conditions in the Institutional animal house were used. They were housed in clean, transparent polypropylene cages in groups of six and maintained at standard laboratory temperature and humidity (40-60%) with light/dark cycle of 12:12 hours. Animals were fed commercial pelleted chow and water. The rats were allowed to acclimatize to these conditions for a week before starting the experiments. The standard drug, Imipramine hydrochloride, was obtained from Abbot Healthcare Pvt Ltd (Depsonil 25).

Wistar albino rats (150-200 g) and Swiss albino mice (18-22g) of either sex selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. All the animals were maintained under standard conditions, that is room temperature 26 ± 1°C, relative humidity 45 - 55% and12:12 h light – dark cycle. Animal studies had approval of IAEC

Plant Material Collection
The fresh leaves of Leonurus cardiacawas collected from local market. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

Preparation of plant extracts:
Preparation of Aqueous Extract:
The Leonurus cardiacaplants were washed, the leaves were shade dried and powdered. About 200 g of the dried leaf powder of Leonurus cardiacawas extracted with 99.9% Ethanol in Soxhlet extractor for about 36 hours. The ethanol was then evaporated from the mixture by placing it in a beaker and heating it over a water bath. The extract gave a yield of brownish paste like mass weighing 6g. The yield obtained was 3% w/w with respect to dried powder.

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Selection of dose for animal study
The dose considered for the experiment on rats was obtained from conversion of human dose of Leonurus cardiaca(3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats and 0.002 for mice (Ghosh 1984). Hence the calculated dose for the rats (considering human dose3 and 5 g/kg) is 200 mg/kg and for mice is 20 mg/kg. Acute toxicity was done at dose of 200mg/kg body weight

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Acute oral toxicity:
The acute oral toxicity of aqueous and alcoholic extracts of Leonurus cardiacawas determined by using rats and mice which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 200mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed up to 7days for their mortality, behavioral and neurological profiles.

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Screening for antidepressant activity:
The Ethanolic extracts of Leonurus cardiacaleaves were tested for antidepressant activity using despair swim test and tail suspension test.
Treatment
The Wistar albino rats (n=60) were divided into two arms which was further divided into five groups, each group having six Wistar albino rats. Drugs were given orally after 12 hours of fasting every day, for ten days.
The drugs were prepared and administered per oral (0.1ml/10g).
Group 1 was administered normal saline (10ml/kg).
Group 2 was given standard drug Imipramine (15mg/kg). 12
Group 3, 4 and 5 received 100mg/kg, 200mg/kg, and 400mg/kg doses of the test compound Ethanolic Extract of *Leonurus cardiaca* respectively.

For the Acute study, on day 1, one arm of 30 Wistar albino rats were subjected to Tail Suspension Test (TST), while 30 mice in the other arm were subjected to Forced Swim Test (FST), one hour after feeding the respective drugs. For Sub acute study, on day 10, the Wistar albino rats were again subjected to TST and FST, one hour after feeding respective drugs.

Procedure for antidepressant activity:
Forced swim test (fst):
The method used was as described by Porsolt et al. The rats were individually forced to swim in a vertical plexiglass cylinder (capacity: 5L, height: 50cm diameter: 18cm) containing l5cm of water maintained at temperature: 25◦C. Rats were subjected to pre-screening, which lasted for 15 minutes. 24

hours after pre-screening, the trial was performed for 6 minutes of which the first two minutes were not recorded, and the periods of immobility for the latter four minutes was measured (in seconds) with a stopwatch. Rats were considered to be immobile when they made only the bare necessary movements to stay afloat, or when they were motionless. The Rats were taken out of the plexiglass cylinder after 6 minutes. They were dried with a dry towel, and kept under a dim lamp for drying. The water was discarded after every test, and fresh water was used for the next rats.

TAIL SUSPENSION TEST (TST) :

The method used was as described by Steru et al. Antidepressants that are used in practice are able to reduce the period of immobility of rats when they try to escape when suspended by their tail. This test was a reliable screening method for antidepressants, including those involving serotonergic system. Mice ware hung on a wooden rod, 50 cm above the table, by attaching them from their tail end with the use of an adhesive tape. The first two minutes were not recorded, and the periods of immobility for the latter six minutes was recorded (in seconds) with a stopwatch. Rats were considered to be immobile only when they were motionless and not attempting to escape.

Statistical analysis:

Statistics The recorded data was entered in Microsoft Excel. The variables recorded followed normal distribution, hence, results have been expressed as mean (in seconds) ± standard error of mean (SEM). The data was analysed using one way ANOVA followed by post-hoc Dunnet's test. Probability 'p' value less than 0.05 was considered as statistically significant.

Open-field test:

For open-field test, animals were divided into four groups (n = 10 /group): control (0.9% saline), the three doses of *Leonurus cardiaca* (100, 200, 300 mg/kg) for one-week treatment. To assess the effect of *Leonurus cardiaca* on locomotor activity, mice were evaluated in the open-field paradigm (TRU SCAN Activity Monitoring Systems, Coul bourn Instruments) previously described. Animals were individually placed in a box (40 × 60 × 50 cm). The rats were not habituated to the box before the test. The mice were placed in the center and their behavior was noted immediately and continued for 4 min. The parameters such as total movements, total distance, total ambulatory move time were recorded by video camera and registered in the computer. During the interval of the test the apparatus was cleaned

RESULTS:

Antidepressant activity of *leonurus cardiaca:*

In the Acute study, on Day 1, standard drug Imipramine (15mg/kg) and test drug EELC (100mg/kg, 200mg/kg, 400mg/kg) showed significant reduction in immobility times when compared to control in both FST and TST (Table 1, Figure 1). In the Sub acute study, on Day 10, both Imipramine (15mg/kg) and EELC (100mg/kg, 400mg/kg) showed significant reduction in immobility times when compared to control in both FST and TST.

Table 1

Day 1	Tail Suspension Test	Forced Swim Test
Normal Saline	231.2(±10.08)	142.0(±1.36)
Imipramine 15mg/kg	175.0(±1.01)*	107.12(±1.10)*
EELC 100mg/Kg	166.1(±21.3)*	97.14(±2.10)*
EELC 200mg/kg	186.2(±1.01)*	113.14(±4.12)*
EELC 400mg/kg	121.0(±1.15)*	111.21(±1.11)*

- Immobility time shown in seconds as mean (± SEM),*denotes statistically significant value, # denotes statistically not significant value.

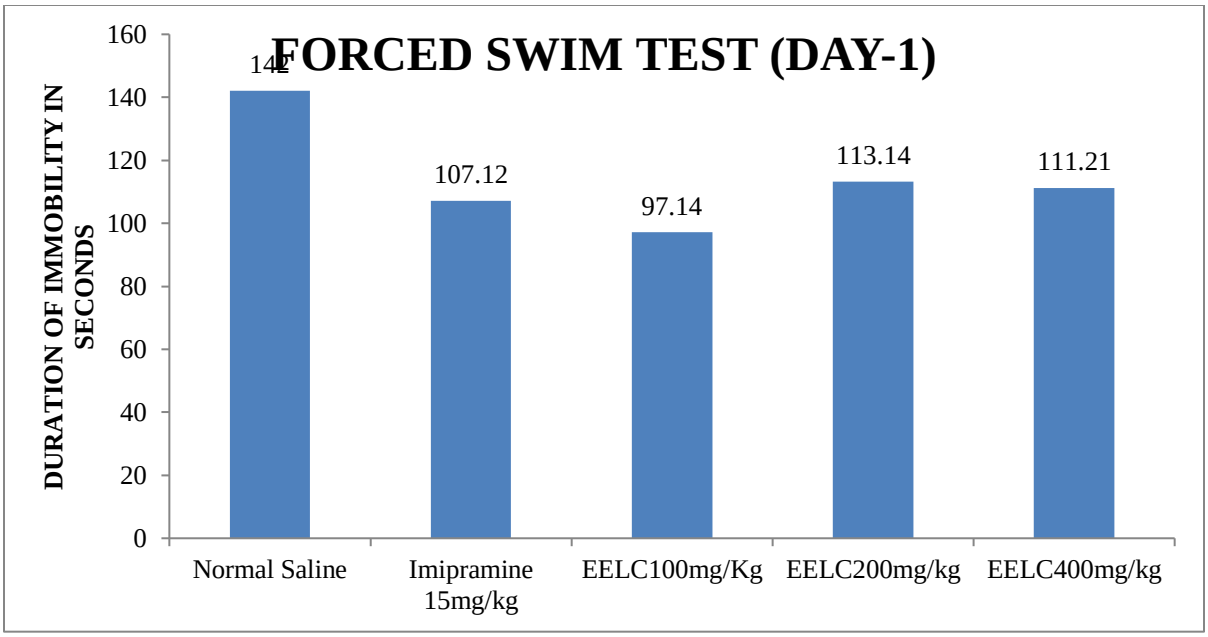

Fig: Tail Suspension Test Day-1

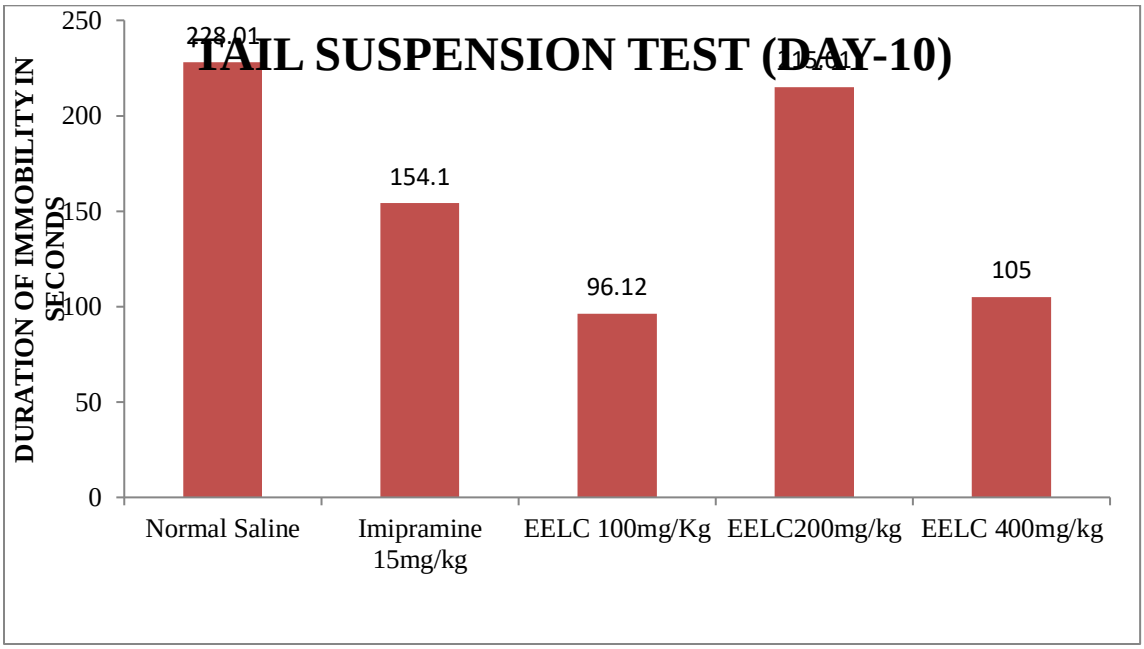

Fig: Forced Swim Test Day-1

Table 2

Day 10	**Tail Suspension Test**	**Forced Swim Test**
Normal Saline	228.01(±10.15)	150(±1.21)
Imipramine 15mg/kg	154.10(±01.24)*	94.1(±2.15)*
EELC 100mg/Kg	96.12(±5.192)*	67.142(±1.26)*
EELC 200mg/kg	215.01(±20.53)#	96.412(±12.41)*
EELC 400mg/kg	105(±12.01)*	90.251(±05.10)*

- Immobility time shown in seconds as mean (±SEM), *denotes statistically significant value, #denotes statistically not significant value.

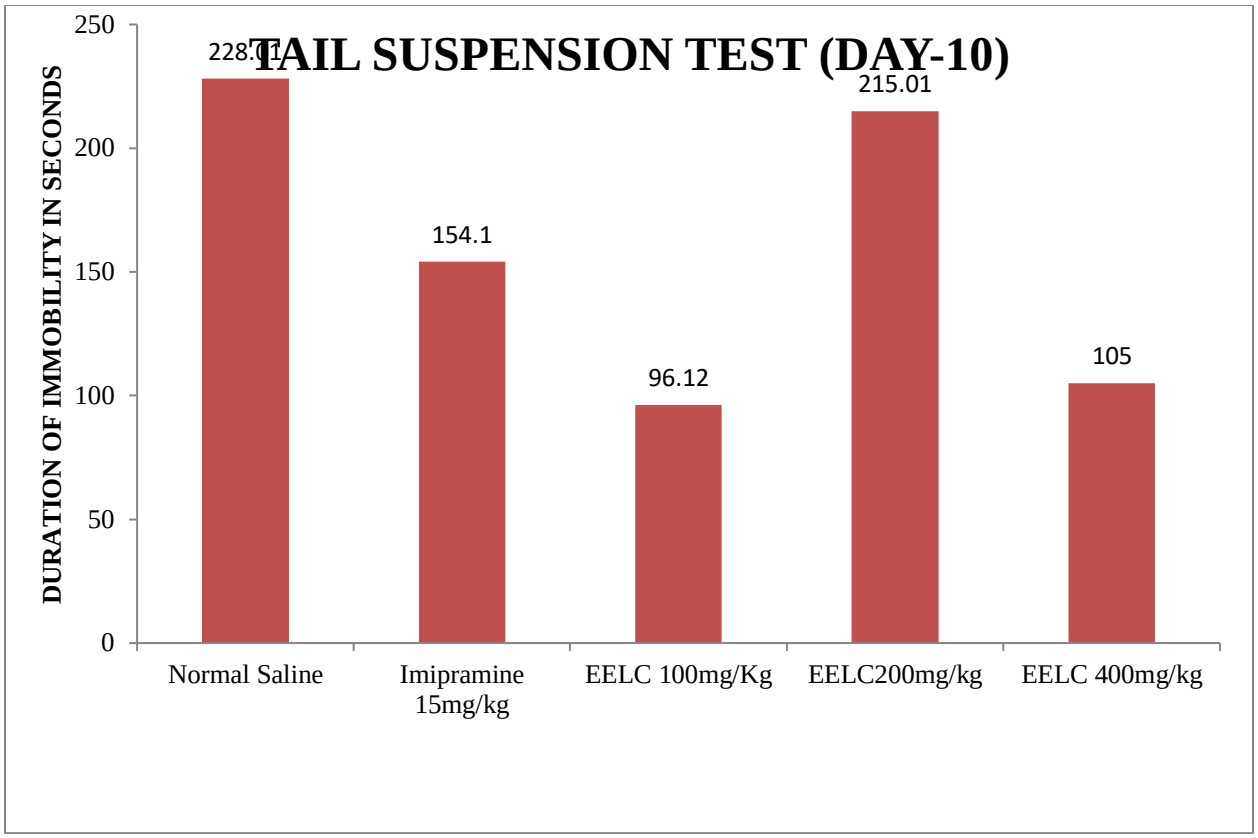

Fig: Tail Suspension Test Day-10

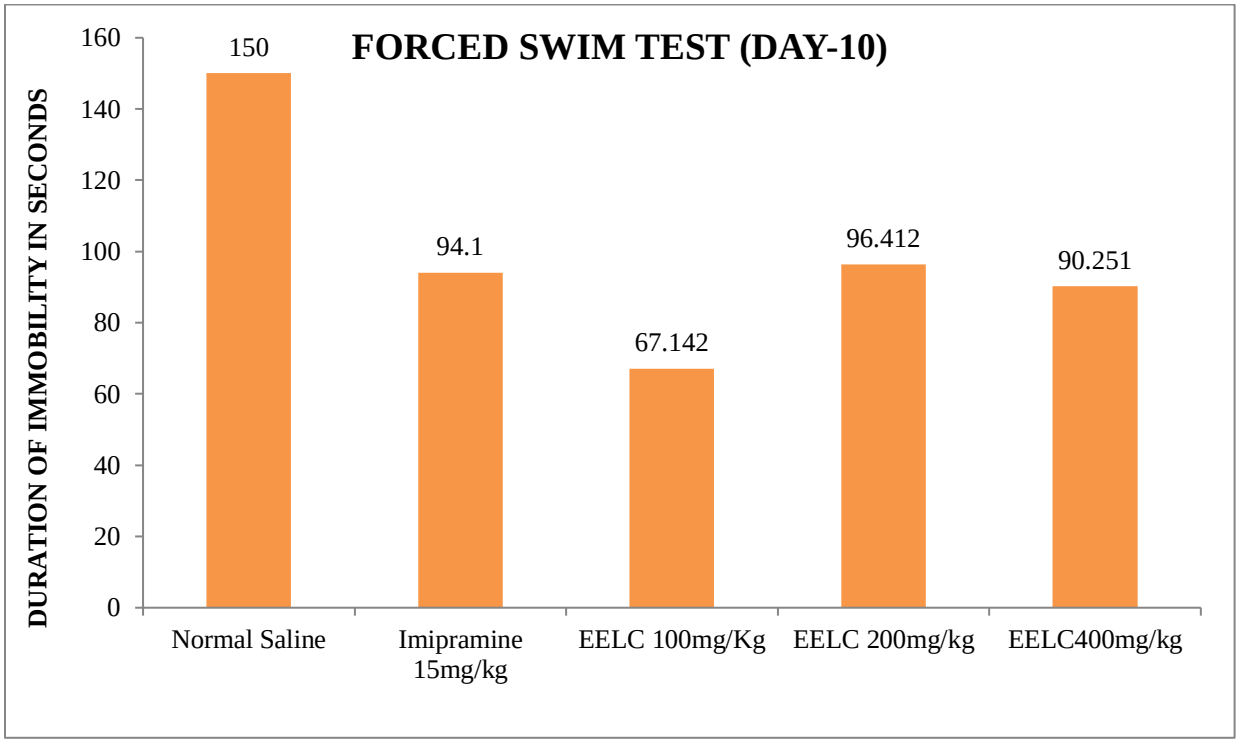

Fig: Forced Swim Test Day-10

DISCUSSION:

A previous study concluded that aqueous leaf extract of *Leonurus cardiaca* exhibited antidepressant and anxiolytic properties in forced swimming (FST) and tail suspension (TST) tests, the elevated plus maze (EPM) model and locomotor activity count.

In this study, both Imipramine and *Leonurus cardiaca* showed a reduction in immobility times in acute and sub acute study in both FST and TST. Lowest immobility times were recorded with Leonurus cardiaca at 100 mg/kg doses in most recordings, and at times, it showed comparable or even better reduction in immobility times than Imipramine in both tests in acute and sub acute study.

Imipramine inhibits nor epinephrine transporter and Serotonin transporters, increasing their availability at synaptic cleft, thereby reducing depression. The antidepressant action of *Leonurus cardiaca* is probably similar to the mechanisms of anti-depressant agents, like Imipramine, that are effective in the above screening models. Phytochemical investigations done in a study showed the presence of alkaloids, flavonoids and tannins in the extract. It is likely that the antidepressant activity seen with *Leonurus cardiaca* could be because of the above mentioned phytoconstituents.

CONCLUSION:

In the present study plant parts of *Leonurus cardiaca* have been be evaluated for antidepressant activity. As literature shows that traditionally this plant is being use in the treatment of depression. The plants materials *Leonurus cardiaca* used for the present studies were commercially procured from local market. Albino rats were used for the antidepressant activity.

The results obtained in this study suggest that Ethanolic Extract of *Leonurus cardiaca* has anti-depressant activity and can be considered for use in therapy of depression after further testing.

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