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### Evaluation of anti-depressant activity of ethanolic extract of *Verbascum thapsus* in albino mice

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#### ABSTRACT

In the present study the obtained results were compared with standard drugs and it was observed that methanolic extract produces 43.12% age yield. The Preliminary phytochemical studies were performed and chemical components of extracts were identified that the leaf extracts for alkaloids, flavonoids and tannins. The extracts doses were identified by using acute toxicity studies as recommended by experts in O.E.C.D guidelines and determined for their activity. The optimum safe concentration was determined to be up to 2000 g/kg, since no mortality and abnormal toxicity was observed at this dose and it was finalised that selected dose of leaf extracts are 100, 200 and 400 mg/kg as per body weight and the extracts were given by oral administration (p.o.). Neuropharmacological activity was carried out by performing various experimental models like general behavioural test, locomotor activity (Actophotometer), anxiolytic activity by Elevated Plus Maze (EPM) and Light-Dark model transition (LD), antidepressant activity like Forced Swim Test (FST) and antioxidant activity by DPPH model (in-vitro method). The methanolic leaf extracts of *Verbascum Thapsus* showed moderate activity in general behaviour at the dose of 200 and 400 mg/kg compared with Diazepam (2 mg/kg). The Methanolic leaf extracts of *Verbascum Thapsus* at the dose of 200 and 400 mg/kg showed significant (\*\* $p < 0.05$ ) increase locomotor stimulator activity activity when compared with Diazepam. The antidepressant activity was carried out by FST. The results were observed that the Methanolic leaf extract of *Verbascum Thapsus* showed a significant (\*\* $p < 0.05$ ) decrease in the mmobility period at dose of 400 mg/kg when compared with that of Fluoxetine, a standard drug. The leaves extract of *Verbascum Thapsus* evaluated for anxiolytic activity in mice using EPM and LD. The mice treated with erbasicum Thapsus leaf extracts at the dose of 400 mg/kg, showed significantly ( $p < 0.05$ ) increased the time spent in the open arm and in the total number of entries in the EPM and also increased the time spent in the light area and decreased the time spent in the dark area with respect to control and diazepam (2 mg/kg). Leaf extracts of MK and NN were showed to possess a reduction in DPPH radicals and this can be observed by decreases in absorbance at 517 nm. In the present study Methanolic leaf extracts of *Verbascum Thapsus* had showed a significant (\* $p < 0.05$ ) at 160 and 320  $\mu\text{g/ml}$  and the  $\text{IC}_{50}$  values were found to increase respectively with the increasing concentrations of reference standard, Ascorbic acid.

The antiradical activity possess due to the presence of phenolic compounds in the leaf extracts. Due to the presence of more percentage of alkaloids, flavonoids, tannins and phytosterols, shows better neuropharmacological activity and can be inferred that the contents in the extracts are responsible for its activity. The hydroalcoholic leaf extracts of *Verbascum thapsus* was found to possess good antioxidant activity, which supports its neuropharmacological activity, maintaining the quality, safety and efficacy of the fixed dose and also establishing scientific evidences to satisfy the aim and objectives of the study.

**Keywords:** *Verbascum thapsus*, Anti-oxidant, Neuro-pharmacological, anxiolytic, Anti stress and locomotor activity.

## INTRODUCTION

Plants, herbs and medicaments based upon them are used for human health care from time immemorial. The patient acceptability and demand of the herbal medicines from natural sources are more because there are with least or no side effects and have similar or better therapeutic activity and are often best and their easy availability from nature. The medicinal herbs are at most significant in treating ailments of neurology. In this study, scientifically established attempts were carefully considered to evaluate the neuropharmacological activity of two authenticated herbs against various induced neuronal disturbances and alteration in behavioural. Identification of the plants has been done using expertise of natural product scientists and extracted the leaves of *Verbascum Thapsus* with methanol for this experimental study to establish their neuropharmacological activity as per their ethnopharmacological claim. In today's stressful world, anxiety and depression are extremely common, dramatic and debilitating multifaceted disorders for which effective treatment strategies for the patients are inadequate due to the complexities of the ailment. Further, there is a rise in number of people suffering from age-associated dementia and cognitive disorders, which cause terrible reduction in the quality of the sufferer. There are other Central Nervous System (CNS) related maladies such as obsessive compulsive disorders and epilepsy, whose treatment and cure is still in infancy. The management and treatment of these CNS disorder is a vexing problem due to the complexities of the disorders as well as the limitations of the allopathic medications that are currently in the market. Hence, the need of the hour is to explore the utilities of medicinal plants for treating various ailments. The immense potential benefits that these herbal can offer us need to be exploited [1]

## MATERIALS AND METHODS

### Drugs

Standard drug will be received as a gift sample from manufacturers will be used in this study, all other chemicals used will be of analytical grade. [2]

### Collection of raw material

Leaves of *Verbascum thapsus* will be collected from the surrounding gardens. The sample will be identified and authenticated by the botanist. Fresh leaves will be cleaned and shade dried at room temperature. [3]

### Extraction of *Verbascum thapsus*

The powdered materials will be extracted with 70% Methanol by Soxhlet's extraction method. The extracts will be concentrated using rotary flash evaporator and percentage yield of the same will be recorded. Finally the extract will be used for qualitative phytochemical analysis and to evaluate Antidepressant activity. [4]

### Qualitative Phytochemical Analysis

The crude extracts thus obtained will be subjected for preliminary phytochemical analysis using standard procedures described in the literature. [5]

### Plant Material authentication

The plant *Verbascum thapsus* was collected from Sri Venkateshwara University Tirupati, India. The plant was authenticated by Dr.Madhava Chetty, Department of Botany and voucher specimen of the plant were preserved at institute herbarium library. [6]

### Preparation of leaves extract

Fresh leaves are collected, washed to remove adhered dirt, rinsed with distilled water, blotted and dried in shade. The shade-dried specimens were powdered in a mixer. This powder was used for

solvent extraction. About 200 g of the powdered material was subjected to Soxhlet extraction using 200 ml solvent ethanol. This cycle was repeated many times, over hours or a few days, until the colour of the solvent in the siphon of the soxhlet faded away. The extracts were concentrated under reduced pressure and preserved in refrigerator until further use. At the end of the hot extraction process each extract was filtered. The filtrate was concentrated and the solvent was recovered using rotary evaporator. The extracts were then kept in desiccators to remove remaining moisture, if present, and finally stored in air tight containers at 4°C for further use. [7]

### Acute toxicity testing

Studies were carried out in order to check the toxic effects of the extracts. The study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines no 425. Rats were used for this purpose. The animals were fasted overnight, providing only water, after which the extract was administered to the respective groups orally at the dose level of 2000 mg/kg body weight by gastric intubation and the groups were observed continuously for 24 hrs for behavioral, neurological and autonomic profiles, and then at 24 hrs and 72 hrs for any lethality. The animals were further observed for toxic symptoms for 14 days. According to the guidelines if mortality is observed in 2 or 3 animals, then the dose administered is assigned as a toxic dose. If mortality is observed in one animal, then the same dose is repeated again to confirm the toxic dose. If

mortality is not observed at all, the extract is considered as non-toxic. Alternatively, the toxicity test is started with a dose of 100 mg/kg body weight and repeated for further other doses such as 250, 500, and 1000 and finally 2000 mg/kg body weight. [8]

### Antioxidant activity of Extracts (In-vitro method)

#### DPPH radical scavenging activity

DPPH radical scavenging activity was measured using the method of Cotelle with some modifications. In 3 ml of reaction mixture containing 0.2 ml of DPPH (100 µM in methanol) and 2.8 ml of test solution, at various concentrations (5, 10, 20, 40, 80, 160 320 µg/ml) of the extracts of MK and NN was incubated at 37°C for 30 min absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. [9]

#### Screening of Antidepressant activity

For depression study, the animals are divided randomly in six groups with six mice per group as follows

- Group-I: Vehicle control (0.5% CMC)
- Group-III: Animals treated with Standard drugs (given in the table No.4.1)
- Group-IV: Animals treated with Ethanolic extract (low dose)
- Group-V: Animals treated with Ethanolic extract (high dose) [10]

Table no 4.1

| Sl.no. | Standard Drug | Methods                                       | Dose     |
|--------|---------------|---|----------|
| 1.     | Diazepam      | Actophotometer, Elevated Maze, light and dark | 1 mg/kg  |
| 2.     | Fluoxetine    | FST and TST                                   | 20 mg/kg |

## RESULTS

The results obtained from different evaluation parameters are presented below systematically:

### Percentage yield of the extracts of *Verbascum thapsus*

The obtained results were compared and it is observed that methanolic extract produces more percentage yield than that of aqueous extract from both the plants. But the extracts of *Verbascum thapsus* produce 43.12% yield.

**Table: 5.1. Percentage yield of the extracts of both the plants**

| Sl.No. Extract            | (vt) % yield |
|---------------------------|--------------|
| Methanolic . Extract (ME) | 43.12        |

The values shown above in the table no. 5.1 extracts yield were calculated established scientific formula with respect to the raw material used as referred in practical manual.

### Phytochemical Screening Methods of *Verbascum thapsus*

The obtained extracts were carefully characterized for determination of various phytochemical constituents. The obtained results are summarized in Table: 5.2 below.

**Table: 5.2. Phytochemical Composition of methanolic extracts of *Verbascum thapsus***

| Chemical constituents    | MEVT |
|--------------------------|------|
| Alkaloids                | +    |
| Carbohydrates            | +    |
| Glycosides               | -    |
| Flavanoids               | +    |
| Phytosterols             | +    |
| Saponins                 | --   |
| Phenolic Compounds       | +    |
| Tannins                  | +    |
| Proteins and Amino acids | --   |

“+” indicates presence and “-“indicates absences of the phytochemical constituents

The evaluated results in above table explains that phytochemical constituents such as alkaloids, Flavanoids, Phenolic compounds and tannins were present in methanolic extract of *Verbascum thapsus*

### Acute Toxicity Study

To determine the safe dose, animals were treated with different doses and were kept for observation as per established OECD guidelines. The results of the observation are noted in Table: no. 5.3 below.

**Table: 5.3. Toxicity signs and symptoms**

| PARAMETER                | MEVT 2000 mg/kg |
|--------------------------|-----------------|
| Righting reflex          | --              |
| Corneal Reflex           | --              |
| Clinical abnormalities-- |                 |
| Death (24 hrs)           | --              |
| Death (1-7 days)         | --              |

The above table explains keenly observation of different behavioural parameters. The result shows no significant change. Finally it is confirm the selected dose is safe and efficacious one, after a through established scientific experimentation for a period of seven days. *Verbascum thapsus* were found to be safe till a dose of 2000 mg/kg (2 g/kg) since no mortality and abnormal toxicity was observed at this dose. According to OECD guidelines<sup>100</sup>, with maximal safe dose can be selected for the study. Hence the different dose selected such as 100, 200

and 400 mg/kg for the study. Extracts were given by oral administration (p.o.). The extracts were suspended in 0.5% w/v carboxy methyl cellulose (CMC).

### Pharmacological Screening for *Verbascum thapsus* leaves extracts

In-vitro method of antioxidant activity for *Verbascum thapsus* leaves extracts. The test compounds have been reported to show high radical scavenging activity against the DPPH free radical

generating system. The antiradical activity of test compound and Ascorbic acid against DPPH was shown in Table and the IC<sub>50</sub> values were found to increase with respect to the concentrations of reference standard, Ascorbic acid. The results are given in Table: 5.4 below and represented in Figure 5.1. The leaf extracts of MEVT and Ascorbic acid shows antiradical activity against the DPPH free radical generating system. It was found that the radical-scavenging activities of all the extracts increased with increasing concentration. The

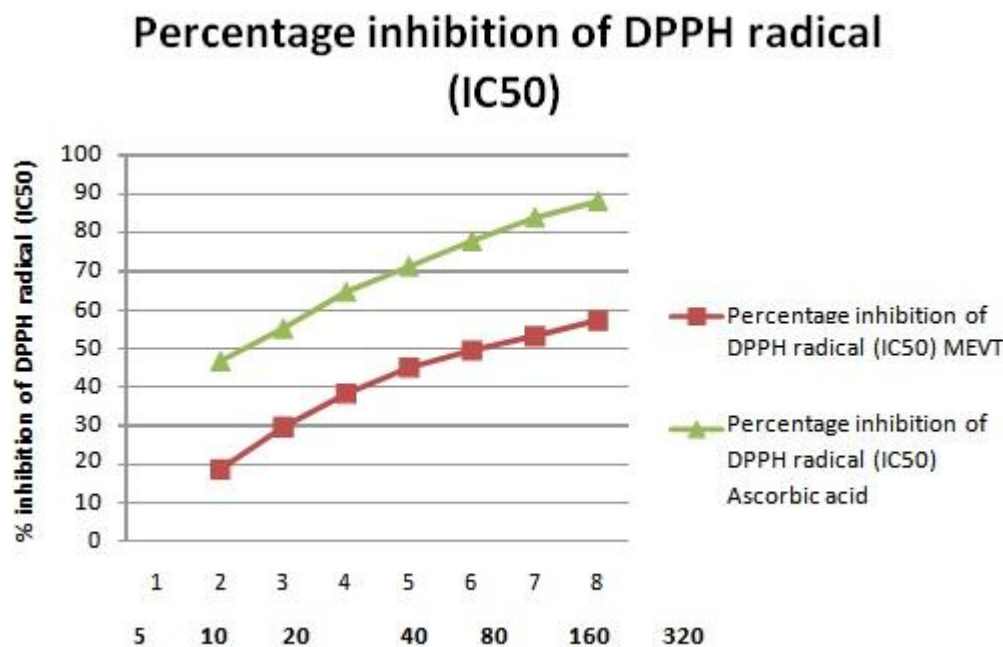
Methanolic leaf extracts of VT increase in concentrations significantly (\*p<0.05) higher radical scavenging activity. The effective free radical scavenging activity of MEVT (58.2±2.91) were founds at 320 µg/ml and also showed the antioxidant activity at 160 µg/ml. The radical activities assay that were showed the results clearly indicate the free radical scavenging activity of MEVT and this activity is comparable with standard drug, Ascorbic acid.

**Table No: 5.4:- In-vitro method of antioxidant activity of *Verbascum thapsus* leaves extracts**

| Conc. of drugs (µg/ml) | Percentage inhibition of DPPH radical (IC <sub>50</sub> ) |               |
|------------------------|---|---------------|
|                        | MEVT  | Ascorbic acid |
| 5                      | 19.6±1.80   | 47.6±0.48     |
| 10                     | 20.5±2.30   | 56.15±0.65    |
| 20                     | 39.2±3.15   | 65.6±0.48     |
| 40                     | 39.9±2.72   | 70±1.33       |
| 80                     | 48.4±2.57   | 77.8±0.82     |
| 160                    | 50.1±3.84*  | 84.9±1.1      |
| 320                    | 58.2±2.91*  | 89.1±0.51     |

Values are expressed as (Mean ± SEM), n= 6, All groups were compared with standard group and significance shown by \*p<0.05. Statistically analyzed

by one- way analysis of variance (ANOVA) followed by Dunnett’s test.



**Fig: 5.1. In-vitro concentration dependent percentage inhibition of DPPH radical by test compounds and ascorbic acid (Values expressed as Mean ± SEM).**

### Effect of *Verbascum thapsus* leaves extracts on General behavioural profile

The results of this experiment were presented in Table: 5.5 below. Spontaneous activity, awareness and alertness were evaluated by placing a mouse in a bell jar. Touch responses was noted when the animal was touched with a forceps, Pain response were graded when a small artery clamp was attached to the base of tail. The extracts of MEVT shown

spontaneous activity, alertness and awareness; sound, touch and pain responses at dose of 100, 200 & 400 mg/kg and there was showed a moderately degree of inquisitive behaviour. When compared to that of reference standard drug Diazepam. However, the standard drug Diazepam caused very strong depression responses when compared with all extracts of MEVT.

**Table: 5.5. Effect of *Murraya koenigii* leaves extracts on General behavioural profile in mice**

| Group No. | Group Name  | Dose      | Spontaneous | Alertness | Awareness | Sound Response | Touch Response | Pain Response |
|-----------|-------------|-----------|-------------|-----------|-----------|----------------|----------------|---------------|
| 1         | 2% Tween 80 | 10 ml/kg  | -           | -         | -         | -              | -              | -             |
| 2         | MEVT        | 100 mg/kg | -           | -         | -         | -              | -              | -             |
| 3         |             | 200 mg/kg | -           | -         | -         | -              | -              | -             |
| 4         |             | 400 mg/kg | -           | -         | -         | -              | -              | -             |
| 5         | Diazepam    | 1 mg/kg   | ++++        | ++++      | ++++      | ++++           | ++++           | ++++          |

Depression levels: -- active; +, slight depression, ++, moderate depression, +++, strong depression, +++++, very strong depression, n = 6.

Effect of Methanolic extract of *Verbascum thapsus* leaves extracts on locomotor activity using Actophotometer The Locomotor activity of each mouse was recorded individually for 10 min using Actophotometer, which enables the animal to move

across a light beam to be recorded as a locomotion count. The results are presented in Table: 5.6 and Figure: 5.2 below. Statistical analysis shows a significant increase in the locomotor activity in all the extracts of MEVT at the doses of 400 mg/kg dose levels shown highly significant (\*p<0.05) effect as locomotor stimulating activity compared with Diazepam.

**Table: 5.6. Effect of MEVT leaves extracts on locomotor activity**

| Group No. | Treatment  | Dose      | Counts / 10min |              |
|-----------|------------|-----------|----------------|--------------|
|           |            |           | Basal activity | After dosing |
| 1         | 2% Tween80 | 10 ml/kg  | 91.2±9.7       | 90.5±11.2    |
| 2         | MEVT       | 50 mg/kg  | 90.2±5.1       | 117.1±6.2*   |
| 3         |            | 100 mg/kg | 92.1±11.0      | 131±9.2*     |
| 4         |            | 200 mg/kg | 92.4±5.3       | 143.6±8.4**  |
| 5         | Diazepam   | 1 mg/kg   | 125.4± 10.2    | 85.3± 6.8*   |

Values are expressed as (Mean ± SEM), n= 6, All groups were compared with Normal control group \*p<0.05 and standard group and significance

shown by \*p<0.05. Statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test.

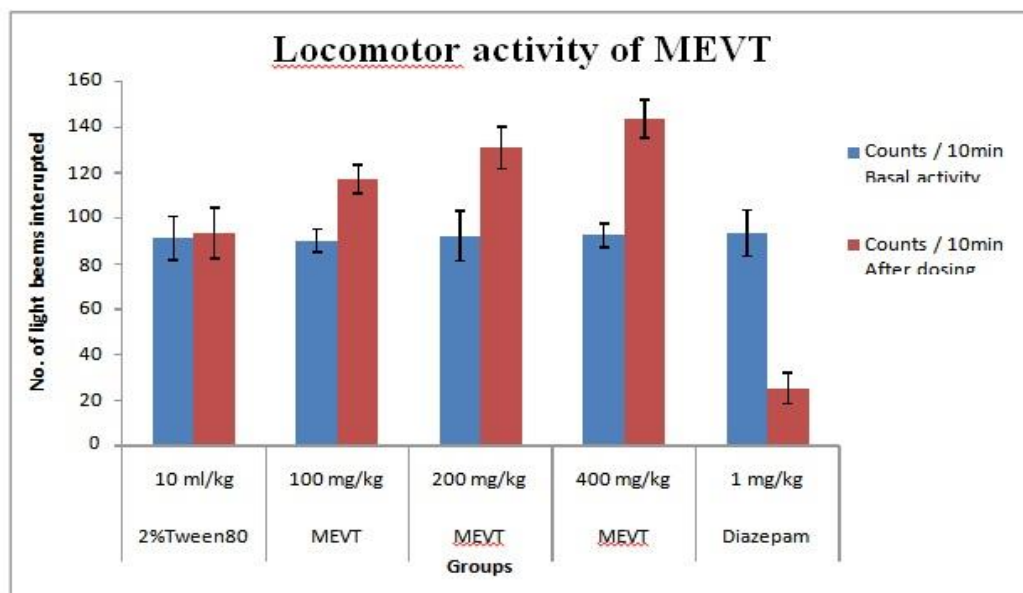


Fig: 5.2. Effect of *Verbascum thapsus* leaves extracts on locomotor activity (Values expressed as Mean  $\pm$  SEM)

**Effect of *Verbascum thapsus* leaves extracts on immobility period in Forced swim test**

Depression was produced by forcing the animal to swim individually in a glass jar containing fresh water of 15cm height and temperature was maintained at 25°C. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering drugs to the groups. The antidepressant activity was expressed as reduction in the immobility time among the control, standard and animals treated with test drug.

The results of this study are shown in Table 5.7 and Figure 5.3 below. the treatment of all extracts of MEVT showed moderately decreases in the immobility time at dose of 100, 200 and 400 mg/kg, but the extracts of MEVT were showed more significantly (\*p<0.05) decrease in the immobility time at the dose of 400 mg/kg and at these prominent, considerable doses only changes in the swimming behavior was observed when compared with control and Fluoxetine. Similarly the Fluoxetine (20 mg/kg) treated group was also showed a significant decrease in the immobility time when compared with control group.

Table: 5.7. Effect of MEVT on immobility period in Forced swim test

| Group No. | Treatment  | Dose      | Immobility period (Sec) |                    |                   |
|-----------|------------|-----------|-------------------------|--------------------|-------------------|
|           |            |           | Pre-treatment           | Post-treatment     |                   |
|           |            |           |                         | 7 days             | 14 days           |
| 1         | 2% Tween80 | 10 ml/kg  | 151.2 $\pm$ 9.3         | 147.0 $\pm$ 11.2   | 148.3 $\pm$ 9.7   |
| 2         | MEVT       | 100 mg/kg | 150.3 $\pm$ 15.2        | 138.6 $\pm$ 11.5*  | 130.4 $\pm$ 12.4* |
| 3         |            | 200 mg/kg | 153.1 $\pm$ 12.8        | 140.4 $\pm$ 9.4*   | 125.5 $\pm$ 10.6* |
| 4         |            | 400 mg/kg | 152.2 $\pm$ 10.5        | 138.4 $\pm$ 11.8** | 115.3 $\pm$ 9.6** |
| 11        | Fluoxetine | 20 mg/kg  | 154.1 $\pm$ 10.1        | 132.3 $\pm$ 9.3*   | 109.2 $\pm$ 10.6* |

Values are expressed as (Mean  $\pm$  SEM), n= 6, All groups were compared with Normal control group \*p<0.05 and standard group and significance

shown by \*p<0.05. Statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s test.

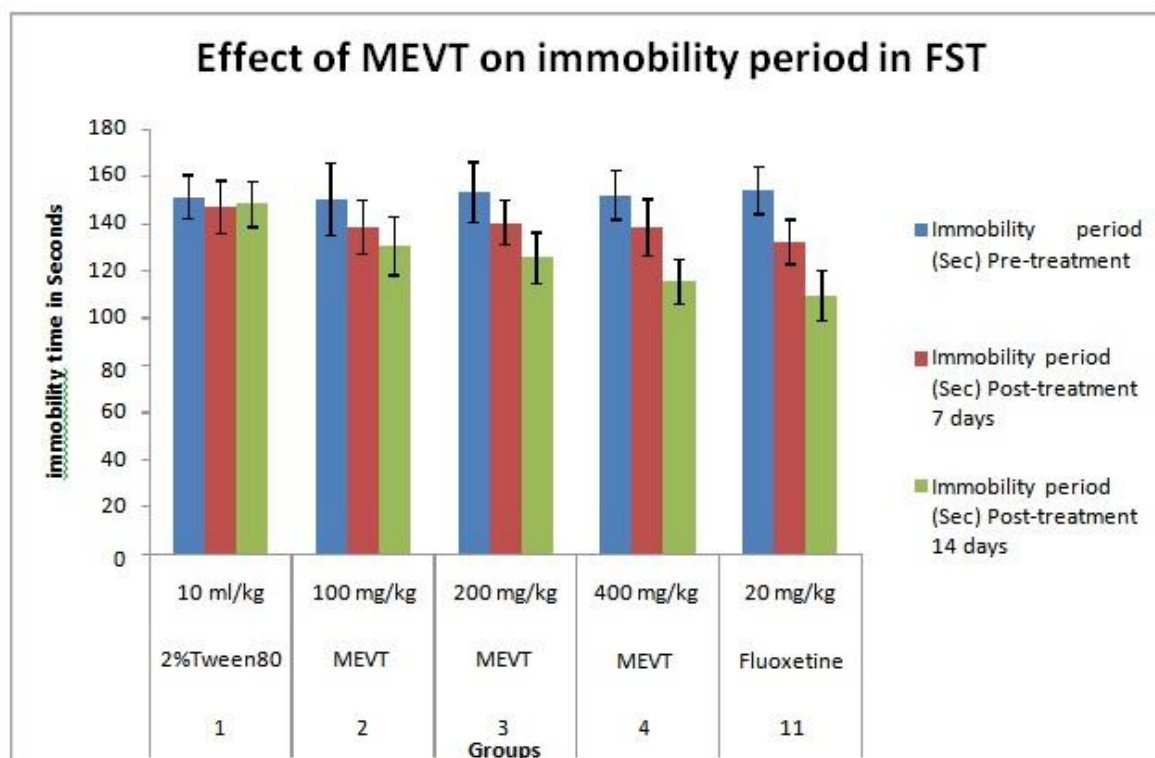


Fig: 5.3. Effect of MEVT leaves extracts on immobility period in FST (Values are expressed as Mean ± SEM)

Effect of *Murraya koenigii* leaves extracts on immobility period Tail suspension test The total duration of immobility induced by tail suspension was measured and was recorded during a 6 min. period. Changes in the immobility duration were studied after administering. The antidepressant activity was expressed as the reduction in duration of immobility time among the control, standard and tested groups.

The results of this study are shown in Table: 5.8 and Figure: 5.4 below. A significant (\*p<0.05)

decrease was observed in the immobility period at 200 mg/kg dose of MEVT when compared with that of Fluoxetine. But in the treatment of all extracts of MEVT there was only moderate decrease in the immobility period at 100, 200 and 400 mg/kg dose levels when compared to that of control and Fluoxetine, similarly, Fluoxetine treated animals were (20 mg/kg), as expected, showed a significant decrease in the immobility period when compared with the control group.

Table: 5.8. Effect of MEVTs on immobility period in Tail suspension test

| Group No. | Treatment  | Dose      | Immobility period (sec) |                |              |
|-----------|------------|-----------|-------------------------|----------------|--------------|
|           |            |           | Pre- treatment          | Post-treatment |              |
|           |            |           |                         | 7 days         | 14 days      |
| 1         | 2% Tween80 | 10 ml/kg  | 154.2±10.2              | 158.0±9.8      | 156.3±10.7   |
| 2         | MEVT       | 100 mg/kg | 152.3±12.5              | 147.5±10.5*    | 140.2±11.8*  |
| 3         |            | 200 mg/kg | 154.1±10.8              | 143.1±11.9*    | 131.2±10.5*  |
| 4         |            | 400 mg/kg | 150.3±15.9              | 131.2±10.6**   | 118.1±12.4** |
| 5         | Fluoxetine | 20 mg/kg  | 153.1±10.4              | 118.3±10.2*    | 101.2±10.3*  |



Values are expressed as (Mean ± SEM), n= 6, All groups were compared with normal control group \*p<0.05 and standard group and

significance shown by \*p<0.05. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett’s test.

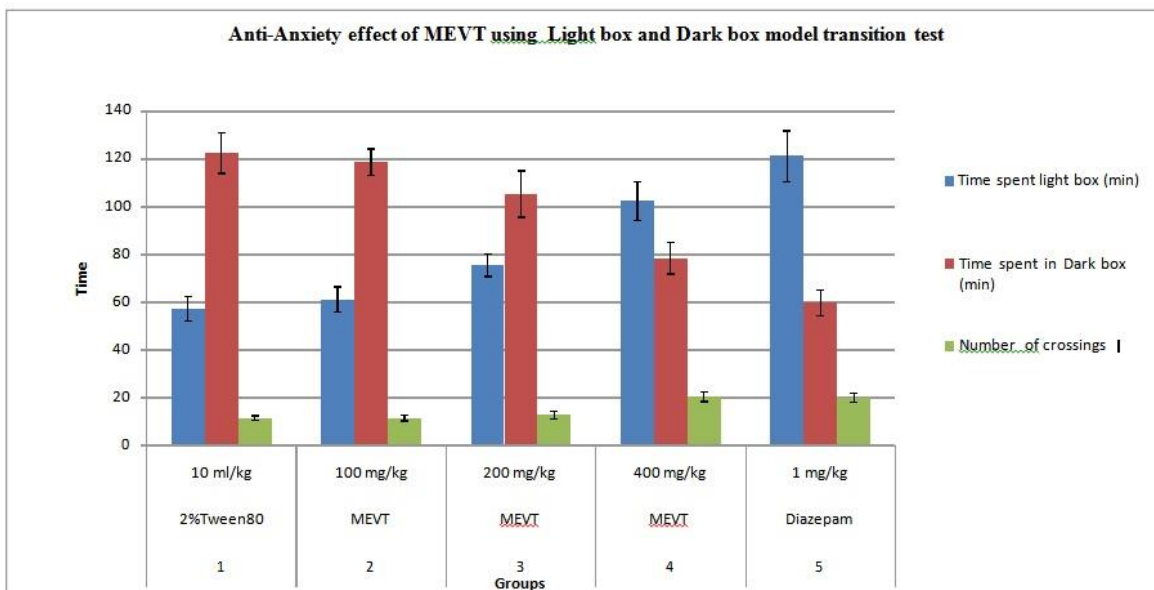


Fig: 5.4. Effect of MEVT on immobility period in Tail suspension test

Effect of MEVT on number of entries and time spent in Elevated Plus Maze method of Anxiety. The total time spent in the two closed quadrants, which have black walls (20cm high) and two open quadrants, was recorded for 5 min. The total duration of time spent in the open quadrant is thought to negatively reflect anxiety, which indicates anti-anxiety activity. The obtained results were presented in Table: 5.9 and Figure: 5.5 below. In this study, the extract of MEMK and HEMK were showed a significant (\*p<0.05) increase in the time

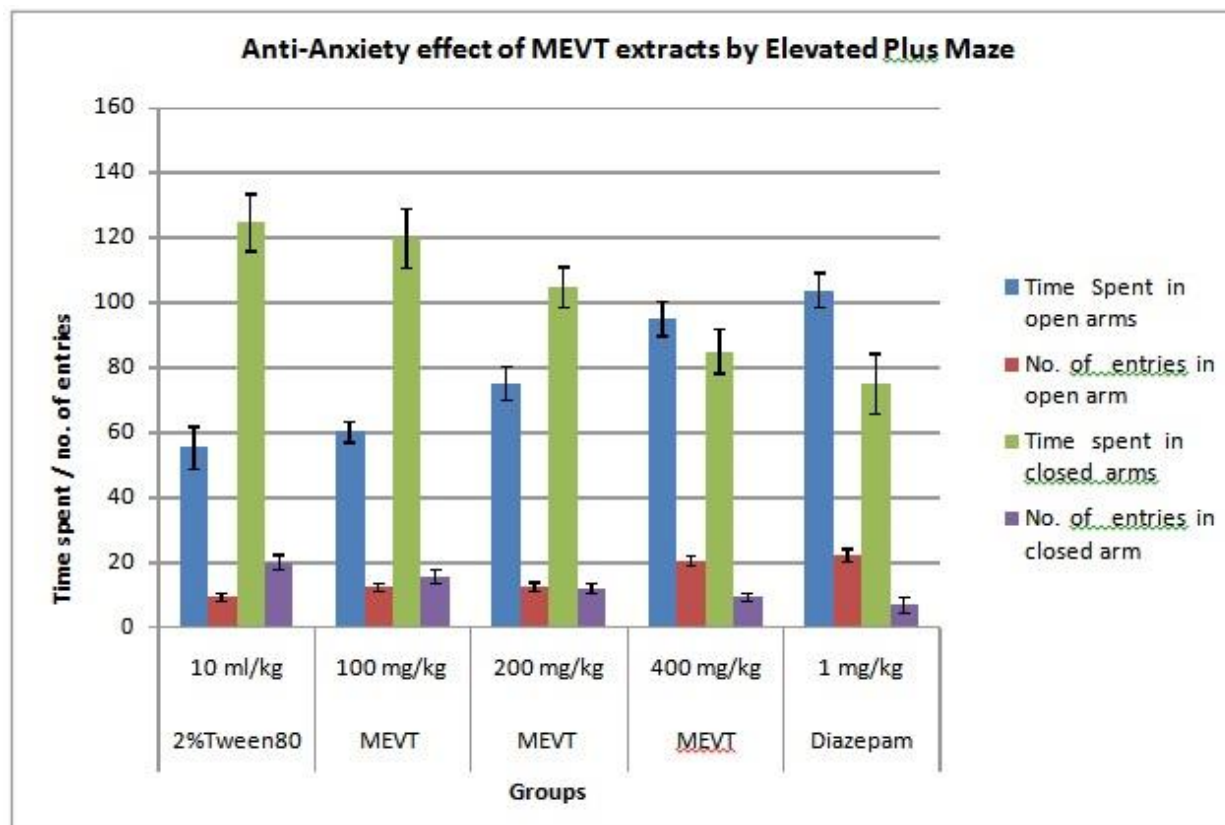
spent and number of entries into the open arm at the dose of 200 mg/kg and decrease in the time spent and number of entries in to the closed arm when compared with the control, which was received 2% tween 80. But the extract of MEVT at 400 mg/kg dose level, when compared with that of the Diazepam, both extracts were exhibited equipotent to Diazepam, a standard drug. The extracts MEVT at (100, 200 mg/kg) were shown moderate anxiety activity, in comparison with the control.

Table: 5.9. Anti-Anxiety effect of MEVT extracts by elevated plus maze method.

| Group No. | Treatment  | Dose     | Time Spent in open arms | Number of entries in open arm | Time spent in close arms (min) | Time Spent in closed arms |
|-----------|------------|----------|-------------------------|-------------------------------|--------------------------------|---------------------------|
| 1         | 2% Tween80 | 10 ml/kg | 55.3± 6.4               | 9.2± 1.2                      | 124.7±8.8                      | 20± 2.4                   |
| 2         |            | 100      | 60.2±3.2*               | 12.3±1.1*                     | 119.8±9.2*                     | 15.7±2.2*                 |
| 3         | MEVT       | 200      | 75.2±5.1*               | 12.5±1.2*                     | 104.8±6.2*                     | 11.9±1.5*                 |
| 4         |            | 400      | 95.1±5.4**              | 20.3±1.5**                    | 84.9±6.7**                     | 9.2±1.2**                 |
| 5         | Diazepam   | 1 mg/kg  | 103.8±5.4*              | 22.2±1.9*                     | 75.2±9.2*                      | 6.8±2.5*                  |

Values are expressed as (Mean ± SEM), n= 6, All groups were compared with Normal control group \*p<0.05 and standard group and significance shown

by \*p<0.05 Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett’s test.



**Fig: 5.5. Anti-Anxiety effect of MEVT extracts by elevated plus maze method**

Effect of MEVT on Light Dark model transition test the animals were placed individually in the illuminated part of the cage. The following parameters were recorded during the test session of 5 min such as total no. of crossings between the light and dark area, total time spent in the illuminated part of the cage, time spent in the dark part of the cage.

The obtained results were presented in Table: 5.10 below and represented in Figure: 5.6. In this study, the mice was treated with 2% tween 80, it was showed less time spent in the light area and more time spent in the dark area. It is observed that the treated mice with MEVT extracts at dose of

400 mg/kg and Diazepam (2 mg/kg) shows a significant increase time spent in the light area and decrease time spent in the dark area. The extracts MEVT shown significant reduce time spent in the light area and increase time spent in the dark area at the dose of 100, 200 and 400 mg/kg. The MEVT extracts (400 mg/kg) were exhibited significant increase the number of crossing into the light area as well as dark areas, when compared to that of Diazepam, a standard drug. The lower dose (100 mg/kg) of extract of MEVT did not increase the number of crossing into the light area when compared with Diazepam.

**Table: 5.10. Effect of MEVT extracts on number of crossing and time spent Light box and Dark box in light and dark model transition test**

| Group No. | Treatment | Dose      | Time spent lighted box (min) | Number of crossings | Time spent in Dark box (min) |
|-----------|-----------|-----------|------------------------------|---------------------|------------------------------|
| 1         | 2%Tween80 | 10 ml/kg  | 67.4± 5.1                    | 11.4± 1.0           | 112.6±8.6                    |
| 2         | MEVT      | 100 mg/kg | 61.2±5.2*                    | 11.5±1.2*           | 118.8±5.6*                   |
| 3         |           | 200mg/kg  | 70.6±4.8*                    | 12.7±1.5*           | 109.4±9.8*                   |
| 4         |           | 400mg/kg  | 102.5±8.2**                  | 20.4±2.1**          | 78.5±6.6**                   |
| 5         | Diazepam  | 1 mg/kg   | 121.2±10.6*                  | 20.0±2.01*          | 59.8±5.4*                    |

Values are expressed as (Mean  $\pm$  SEM), n= 6, All groups were compared with Normal control group \*p<0.05 and standard group and significance shown

by \*p<0.05. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett's test.

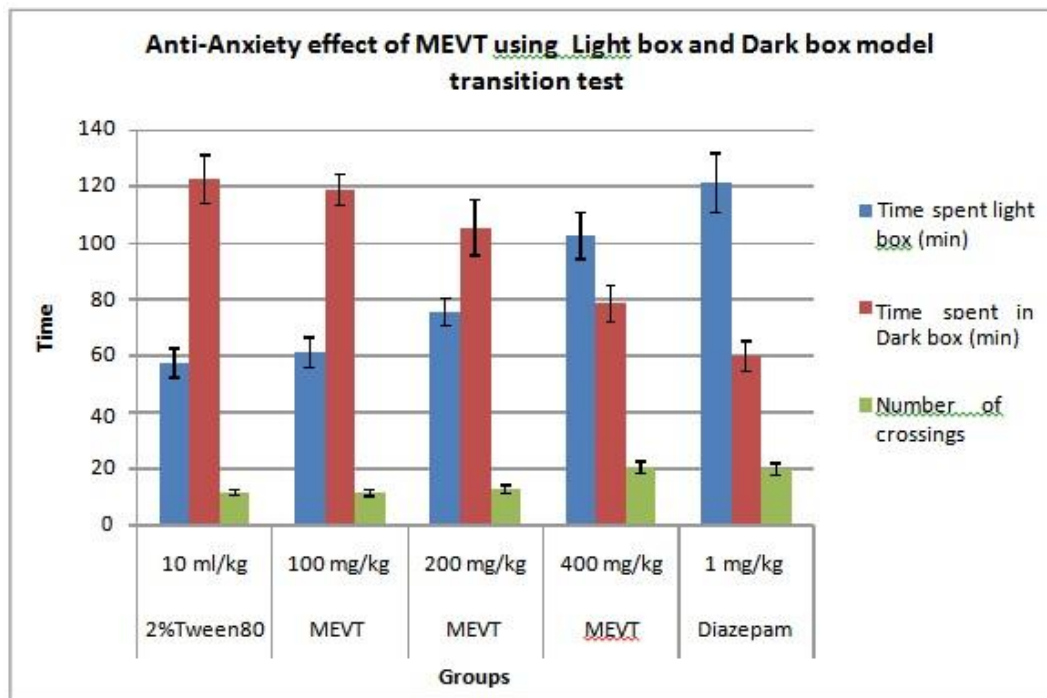


Fig: 5.6. Anti-Anxiety effect of MEVT using Light box and Dark box model transition test.

## DISCUSSION

Plants have been used in traditional medicine from time immemorial. India is perhaps the largest producer of medicinal herbs and is rightly called the “Botanical garden of the World”. Medical plants and their products are the oldest and tried as health-care products. Their importance is growing not only in developed countries but in many developing countries. The allopathic drugs are good in onset and have good therapeutic activity but side effects associated are poignant. Thus, the herbal medicines from natural sources with least (or) no side effect having similar better therapeutic activity. The herbal medicines have wide therapeutic actions and safety profile. This makes the herbal therapies to be successful.

The plants *Verbascum thapsus* Linn. leaves (Family: Scrophulariaceae) was selected. The plant have been screened for their phytochemical constituents and to investigate the extent of their neuro-pharmacological effects by extracts and using various methods.

The literature review and ethnic claims reported that the whole plant i.e. *Verbascum Thapsus* had medicinal important and used in treatment of varieties

of pupurpose such as hair tonic, antidepressant, dysenteric, diarrhoea, fungal infection, inflammation, emesis and diabetes. Each part of the plant enriched with significant pharmacological activity. The stem is used for cleaning, strengthen gums and teeth. The bark is used as hair tonic, stomachic and carminative. The leaves are used as stomachic, purgative, anti-anaemic, anti-helminthic, analgesic, antiulcer, anti-inflammatory, stimulant for hair growth and hypoglycaemic activity. Fruits are used as astringent. The roots are used as analgesic and cooling agent.

In view of above claims and facts, the present work was undertaken as a research study to prove the usage of the plants in the treatment of neuro-pharmacological disorders and neuro-psychological behaviour scientifically. The present study is designed to the presence of different phytochemical constituents and pharmacological activity of leaves extracts. Investigate the methanolic extract of *Verbascum Thapsus* was screened for neuro-pharmacological activities in rodents. The physical status and percentage yield of these plant extracts were recorded for future references. The percentage yield of the methanolic extracts extracts were

considerably good. Several attempts were taken by researchers to determine the chemical constituents present in particular plant part. Some of them have already reported regarding the current plants which are selected for present study such as *Verbascum Thapsus* is a rich source of organic compounds with diverse chemical composition. The presence of contains carbohydrates, flavonoids, alkaloids, tannins, terpenoids, steroids and glycosides in plant extracts. The leaves contain proteins, carbohydrates, fibre, minerals, oxalic acid. Fresh young leaves contain.

In order to gather scientific evidence regarding the above claim that the leaves are having specific chemicals constituents. We have followed the established procedure of specific test as reported by several practical manuals. The phytochemical study revealed that the presence of alkaloids, flavonoids, phenolic compounds and tannins, and positive results in the phytochemical investigation of ethanolic Extract of *Verbascum Thapsus* (MEVT). The results obtained suggest that the presence of carbohydrates, proteins and amino acids in all the extracts of MEVT. The extracts doses were identified by using acute toxicity studies as recommended by scientific experts with a detailed procedure in O.E.C.D guidelines<sup>100</sup> and were selected for the research. The optimum safe concentration was determined to be up to 2000 mg/kg since no mortality and abnormal toxicity was observed within 48 hrs at this dose and it was finalised that selected doses of extract for MEVT as 100, 200 and 400 mg/kg as per body weight. The animals which received the mentioned doses did not produce any significant changes in behavioural pattern and failed to elicit clinical abnormality. The investigation on these extracts were carried out with

a dose i.e. 100, 200 and 400 mg/kg b.w. p.o. considered safe.

## CONCLUSION

In the present research the methanolic extracts of *Verbascum Thapsus* was found to be safe as no toxicity was exhibited in mice up to 2000 mg/kg b.w. (p.o.) in acute toxicity studies. The methanolic extract of *Verbascum Thapsus* at the dose of 400 mg/kg exhibited significant neuro-pharmacological activity. Based on the results of the present study of different extracts on psychopharmacological tests, we conclude that the extracts 400 mg/kg possess CNS stimulant activity. Methanolic extracts showed the CNS stimulant activity in dose dependent manner. The alteration in exploratory behaviour in animals is similar to the action of other CNS agents. A significant change in motor coordination and muscle relaxant activity was also noted in animals treated with crude extract. The results all together indicates that the extract shows CNS stimulant activity.

However, further studies are necessary to examine underlying mechanisms of CNS stimulant effects and to isolate the active compound(s) responsible for these pharmacological activities. The methanolic extract of *Verbascum Thapsus* was also found to possess a marked antioxidant activity, however further research is required to develop them as safe and effective antioxidant agents. Finally it is concluded that *Verbascum Thapsus* had shown neuro-pharmacological activity, which needs a comprehensive investigation for developing them as safe and effective herbal drugs, for better patient acceptability and establish it self as a patient curly ailments against neuro-pharmacological disorder.

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