Evaluation of anxiolytic and anticonvulsant potential of 
*Mirabilis jalapa* ethanolic extracts on standardized rat models

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ABSTRACT

Anxiety and Convulsion are the most prominent, crippled and cruel neurological diseases in recent times. There are many indigenous plants have beneficial properties to treat mental disease and psychic complaints. Evaluation of anxiolytic and anticonvulsant activity of leaf and root ethanolic extracts of *mirabilis jalapa* in rat models were carried out using standardized experimental methods. The dried leaves and root was macerated with ethanol separately and administered the discern dose of 200 mg/kg p.o. and 400mg/kg p.o. from each extract and employed in Elevated Plus Maze test (EPM) and Open field test (OFT) with 2mg/kg i.p of Diazepam as a standard drug to assess the anxiolytic activity. Maximal electroshock induced convulsion (MES) (Phenytoin-20mg/kg i.p as a standard drug) and Pentylenetetrazol (PTZ) induced seizures analyzed where Diazepam (5mg/kg) i.p as a standard drug to assess the anticonvulsant activity. Substantial changes in all tested activities EPM, OFT in anxiety model and MES, PTZ in anticonvulsant model were observed. The results revealed that ethanol leaf extract (400 mg/kg p.o.) was more impetus due to the high amount of flavonoid, phenolic compounds, steroids, terpenoid contents possess tremendous anti-anxiety whereas the ethanol root extract (400 mg/kg p.o.) and ethanol leaf extract (400 mg/kg p.o.) produce significant anticonvulsant potential effect compared to the control and standard drug treatment group. This study suggested that the plant *M. jalapa* is a much more active compound consistent medicinal plant to derive a potent drug against anxiety and convulsion.

Keywords: Anxiolytic, Convulsion, Mirabilis jalapa, Pentylenetetrazol (PTZ) Induced seizures, Electroshock induced convulsion.

INTRODUCTION

Anxiety disorders are among the most frequent mental disorders encountered in clinical practice [1]. These affect one-eighth of the total population worldwide, and have become a very important area of research interest in psychopharmacology [2]. Various psychodynamic, psychoanalytic, behavioral, cognitive, genetic and biological theories have been proposed to explain the etiology and pathophysiology of anxiety disorders[3]. These represent a heterogenous group of disorders, perhaps with no single unifying etiology. These are said to be Bio-Psycho-Social factors that contribute to anxiety disorders [4]. In addition to the high prevalence, anxiety disorders account for major expenditure for their management [5] and anxiety disorders have a substantial negative impact on eminence of life [6].

Now a days, only very few effective synthetic anti-anxiety medicines are existing in the market and clinical practice. Furthermore, anxiety patients are faced with multifarious difficulties associated with the currently approved anti-anxiety drugs. The demand of herbal medicines is increasing due to their wide application and therapeutic efficacy with least side effects. There is a need for novel medications for the treatment of psychiatric diseases because benzodiazepines have various drawbacks, including an increased rate of dependence, tolerance, abuse liability, psychomotor impairment, and potentiation of other CNS depressant drugs. Therefore, the development of new Anti-Epileptic Drugs that can modulate seizures through modifying the underlying pathology is a need of hour. Various plants have been investigated for their anxiolytic effects [7], *Mirabilis jalapa* Linn. of Nyctaginaceae family is extensively used as a medicinal
plant in almost all folklore remedies around the world for treating various diseases. However, studies have evidenced its antibacterial, antiviral, antioxidant, anti-diabetic, anti-inflammatory, anti-stress and many other activities. Phytochemical analysis of leaf and root parts of *M. jalapa* revealed the presence of potent secondary metabolites in sufficient extent.

Literature survey reveals the traditional use of natural products as anxiolytics and anticonvulsant for the management of neurological disorders and which is gaining a lot of interest. In due regard, we have undertaken present herbal drug research aimed to investigate the *M. jalapa* for the anxiolytic and anticonvulsant activity because which is previously reported for muscle relaxant, antistress and antioxidant property.

**Materials and Methods**

**Chemical reagents and its dilutions**

Pentylenetetrazole (PTZ, sigma Chemical Co.), Phenytoin Sodium (Epsolin- Zyus Cadila Healthcare Ltd.), Diazepam (Valium, Roche) and Control animals received equal volume of the appropriate vehicle (0.5% of CMC) orally. Extract and standard drug were suspended in 0.5% carboxyl methyl cellulose prepared freshly each day of the experiment.

**Collection and Authentication**

The whole fresh plants material of *Mirabilis jalapa* Linn. were collected from Coimbatore, Tamilnadu in the month of August – September and authenticated by the botanist Dr. P. Revathi, PG & Research Department of Botany at Kongunadu Arts and Science College, Coimbatore-641 029, India. The specimen submitted and identified as *Mirabilis jalapa* belonging to the family Nyctaginaceae.

**Extraction of *Mirabilis jalapa* leaves and roots**

The fresh leaves and roots were collected and washed under running tap water and dried in shade. Dried leaves and roots were powdered and 500 g of coarse powder was defatted with petroleum ether, this helps in removal of colouring materials like chlorophyll. The defatted marc was placed in thimbles made up of cellulosic filter paper extracted with Ethanol solvent in Soxhlet apparatus at a temperature not exceeding 60°C for 72 h. The extractive value of *Mirabilis jalapa* ethanolic leaf and root was obtained to be 8 and 7 %w/w respectively. The extract was concentrated under reduced pressure in rotary vacuum evaporator to yield a thick viscous mass substances. The viscous mass was allowed to dry in porcelain dishes, and stored for further preliminary phytochemical and pharmacological investigations [8].

**Experimental Dose Selection and its management**

Healthy adult male Wister albino rats weighing 180-200g will be selected and maintained at standard experimental condition. The animals fed with standard rodent pellet purchased from Sri Venkateshwara Enterprises Ltd, Bangalore and water ad libitum. The animals were fasted for 12 hours prior to the experiment with free access to only water. The procedures in this study were conducted after obtaining due clearance from the Institutional Animal Ethical Committee (JKKMMRAFCP/IAEC/2020/002). Ethanolic leaves and
The root extract of *Mirabilis Jalapa* 200mg/kg and 400 mg/kg body weight doses were selected based on the literature reference \(^9,^{10}\).

**Screening of Anxiolytic Activity**  
*(I) Elevated Plus-Maze Test*

The apparatus was made of Plexi glass and consisted of two open arms (15 cm x 5 cm x 1 cm) and two enclosed arms (15 cm x 15 cm x 5 cm). The arms extended from a central platform (5 x 5 cm\(^2\)) forming a plus-sign with like arms opposite each other which was elevated 60cm from the floor. Male Wister rats weighing 180- 200g were grouped into six (n=6). Four groups (III, IV, V, VI) were treated with the extracts (200and 400 mg/kg p.o), other groups (II) treated with Diazepam 5mg/kg and the last grouped administered with 0.5% of CMC to serve as control (I) and 1 hour after oral treatment with the extract and vehicle, rat were placed individually in succession in the central platform of the maze for 5 minutes and their behavioral parameters like number of entries and time spent in open arms were recorded. An arm entry was defined when all four paws of the rat were in the arm \(^{11}\).

**Open Field Test**

The study was conducted according to the method described by Brown *et al.* \(^{12}\) with some modifications. The apparatus was made up of plywood measuring 72cm × 72cm × 36cm. On the top of OFT apparatus fix camera to ensure that the mouse under investigation is visible to the observer. The floor, made of cardboard, was divided into 16 equal squares (18cm × 18cm) with blue marker and a central square drawn with brown marker. Here, the cardboard was covered with a transparent Plexiglas. The animals were divided into six groups and Diazepam is a standard drug as mentioned in the previous experiment. Thirty minutes later, each mouse was placed individually at the corner of the arena and its behavior monitored for 5min. The number of squares crossed and number of rearing each rat was recorded. The apparatus was wiped between observations with 70% ethyl alcohol and allowed to dry to remove any olfactory cue \(^{13}\).

**Screening of Anticonvulsant Activity**

*(i) Maximal electroshock-induced seizure test*

Animals were divided in six groups comprising of six animals each and were fasted overnight with *water ad libitum*. Extract was suspended in 0.5% carboxyl methyl cellulose 60 min prior to administration and induction of MES. First group was administered with vehicle 0.5% CMC, and was considered as control. As explained earlier, the grouped animals administered with standard drug Phenytoin (20mg/kg) i.p and extracts 60 min. prior to the induction of MES. The animals were applied with a supramaximal electrical stimulus of 150mA for 0.2Sec through the ear electrodes \(^{14}\). The animals were observed for various phases of MES induced seizures duration of tonic hind limb flexion (THLF), tonic hind limb extension (THLE), clonus and stupors were noted. The decrease in the duration of extensor phase was taken as an index of antiepileptic activity of the extracts \(^{13}\).

**Pentylenetetrazole-Induced Seizure Test**
Diazepam (5mg/kg po) i.p; administered for the standard group. After 1 hour and 30 minutes of treatment with drugs orally and intraperitoneally respectively, each rat was administered pentylenetetrazole, 60 mg/kg intraperitoneal. The treated animals were placed individually in clear plastic observation chambers (15cm x 15cm x 15cm) which was placed on a large plain glass elevated above the floor (80cm) and a mirror placed behind the glass at an angle of 45° to the glass on the floor to enable clear and complete view of the animals analyses includes onset of Clonus convulsion, onset of Tonic convulsion and number of animals survived.  

Statistical Analysis  
Results are expressed as mean ± SEM. Statistical differences in the mean were calculated using one-way ANOVA followed by Tukey – Kramer’s post hoc test for significance level of p<0.05, p<0.01, p<0.001 using Instat Version 3.0 software.

Results and Discussion

Extract yield of Mirabilis jalapa

The main objective of this work is to develop potent agent having no or less side effects from indigenous plants for the therapeutic management of the conditions like anticonvulsant and anxiolytic activity was undertaken. The resultant extracts were initially filtered with Muslin cloth before using Whatman filter paper No. 1 (Maidstone, United Kingdom). The filtrate was concentrated in vacuum at 45°C using a rotary evaporator which gave a greenish black residue of 40gm from 500gm leaf powder that corresponded to a yield of 8% and dark brownish semi-solid (35gm) was extracted from 500gm of root powder that yields 7% of extract. The extracts were stored in desiccators for further physicochemical, phytochemical and pharmacological studies.

Phytochemical Evaluation

The phytochemical evaluation results revealed the presence of biologically active secondary metabolites such as flavonoids, Steroids, Phenols, glycoside, Alkaloid (Table 1) implicate major anticonvulsant response. Quercetin is one of the flavonoids shows anticonvulsant effects and efficient agent in spatial memory impairment and neuronal death induced by repeated cerebral ischemia.  

Evaluation of Anti-anxiety activity

Elevated Plus Maze (EPM) Model Experiment:

In the EPM, control (0.5 % CMC used as vehicle) treated male rats had a tendency to prefer to stay in the closed arm for a longer period of time than in the open arm. Treatment with diazepam (5mg/kg) increased the number of open arm entries, and time spent in the open arm (14.91 ± 1.10 sec. and 259.10 ± 1.40) suggests that diazepam produces anxiolytic activity, which is in accordance with the earlier findings. Mirabilis jalapa extract produced a dose dependent increase in time spent in open arm along with an increase in number of open arm entries (Table 2; Figure 1). Comparably Mirabilis jalapa leaves extract’ (400 mg/kg) anxiolytic effects was significant to that of diazepam 5 mg/kg as 13.31 ±1.12 number of entries and 241.57 ± 1.27 sec. time spent into the open arm.

Table 1: Qualitative phytochemical analysis of Mirabilis jalapa extracts
| Constituents | Test | Ethanolic root extract | Ethanolic leaf extract |
|-------------|------|------------------------|------------------------|
| Alkaloid    | 1)Meyer’s test | + | + |
|             | 2)Hager’s test | + | + |
|             | 3)Wagner’s test | + | + |
|             | 4)Dragandruff's test | + | + |
| Carbohydrate| 1)Fehling test | + | + |
|             | 2)Molisch test | + | + |
| Protein     | 1)Millon’s test | + | + |
|             | 2)Biuret test | + | + |
| Glycosides  | a)Cardiac glycoside | - | - |
|             | b)Anthraquinone glycoside | + | + |
|             | c)Saponine glycoside | + | + |
| Flavanoids  | 1)Shinoda test | + | + |
|             | 2)Lead acetic test | + | + |
| Tannin      | 1)Gelatin test | + | + |
| Steroids    | 1)Liberman-burchard reaction | + | + |
|             | 2)Salkowski reaction | + | + |
| Phenols     | 1)Ferric chloride test | + | + |
| Gums and mucilage | - | - |

+(ve) and +(ve) Indicates Presence and Absence of Chemical constituents

Table: 2 Anti-anxiety activity of *Mirabilis jalapa* using Elevated plus-maze Test
| Group | Treatment | No. of Entries in Open arm | Time Spent in Open arm (Sec) |
|-------|-----------|----------------------------|-----------------------------|
| I     | Control (0.5% CMC) | 3.23 ± 1.72 | 14.50 ± 1.61 |
| II    | Diazepam (5mg/kg) | **14.91 ± 1.10*** | **259.10 ± 1.40*** |
| III   | MJRE (200mg/kg) | 06.40 ± 1.55* | 197.34 ± 1.93** |
| IV    | MJRE (400mg/kg) | 12.36 ± 1.24*** | 211.53 ± 2.44*** |
| V     | MJLE (200mg/kg) | 11.31 ±1.32*** | 215.38± 1.66*** |
| VI    | MJLE (400mg/kg) | **13.31 ±1.12*** | **241.57 ± 1.27*** |

Values are presented as mean ± SEM, where (n=6), *p<0.05, **p<0.01, ***p<0.001 compared to control.

Open Field Test

The number of squares crossed and Number of rearing each rat was recorded. The number of times (frequency) the subject crosses the grid lines on the grid floor insert with all four of its paws and Frequency of rearing behavior where the subject stands on its hind paws were observed. In general, unsupported rearing (subject does not rest its front paws on the walls) is a better measure of anxiety (Figure 1). In the open field test (OFT), diazepam and extract (Mirabilis jalapa leaves and root 200 and 400 mg/kg p.o.) treated rat showed significant increase in the number of rearing and number of squares crossed during 5-min interval as compared to vehicle-treated control group. Whereas, anxiolytic effects of Mirabilis jalapa leaves extract (400 mg/kg) was comparable to that of diazepam 5 mg/kg (Table 3).

Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing flavonoids, alkaloids, phenolic compounds and tannins possess activity against many CNS disorders [18]. Investigations on the phytochemical screening of Mirabilis jalapa revealed the presence of alkaloids, glycosides, steroids, tannins, phenolic compounds and flavonoids. It is possible that the mechanism of anxiolytic action of title plant could be mediated by these phytochemicals [11, 13].

Evaluation of Anticonvulsant Activity

Maximal Electro Shock Induced Seizures Experiment:

Phenytoin blocked the MES induced seizures in the dose of 20 mg/Kg b.w. in all the Wister albino rats. There was significant increase in the time taken for the onset of Tonic Hind Limb Extension (THLE) in the dose dependent manner with all the doses of Ethanolic extract of Mirabilis jalapa leaves and root and there was significant reduction in the duration of Tonic Hind Limb Extension (THLE) in the dose of 400 mg/Kg leaves extract of Mirabilis jalapa (Figure 1 & Table 4).

Table: 3 Effect of Mirabilis jalapa using Open field test in rat
### Table: 4 Anticonvulsant Effect of *Mirabilis jalapa* by MES induced seizures method

| Groups | Treatment | Duration of Tonic Hind Limb Flexion (Sec.) | Duration of Tonic Hind Limb Extension (Sec.) | Clonus (Sec) | Stupor (Sec) |
|--------|-----------|-------------------------------------------|---------------------------------------------|--------------|--------------|
| I      | Control   | 12.60±1.25                                | 15.88±0.35                                  | 12.36±1.29   | 9.38±0.88    |
| II     | Phenytoin (20mg/kg)i.p | **3.12±0.54***                           | **4.63±1.72***                              | **4.65±0.95*** | **3.98±1.35*** |
| III    | MJRE (200mg/kg). | 10.24±1.20*                              | 12.28±1.19<sup>NS</sup>                    | 9.31±1.10**  | 7.81±1.10**  |
| IV     | MJRE (400mg/kg)  | **8.12±1.54***                           | **07.75±1.01***                             | **6.65±0.85*** | **5.43±1.74*** |
| V      | MJLE (200mg/kg). | **7.88±0.45***                           | **08.21±1.25***                             | **7.23±0.98*** | **4.90±1.25*** |
| VI     | MJLE (400mg/kg)  | **4.58±0.33***                           | **5.84±3.01***                              | **4.87±1.19*** | **4.11±0.54*** |

Values are presented as mean ± SEM, where (n=6), *p<0.05, **p<0.01, ***p<0.001 compared to control, NS: Statistically not significant

The present study shows that the Ethanolic extract of leaves and roots of *Mirabilis jalapa* may function in a similar manner to Phenytoin, it is possible that the anticonvulsant effects might be due to inhibition of Ca<sup>2+</sup> currents or blockade of glutamatergic neurotransmission mediated by N-methyl-d-aspartate (NMDA) receptor<sup>[19]</sup>, although the specific receptor interactions were not evaluated. Further evaluation of *Mirabilis*
*Mirabilis jalapa* is needed to establish its exact mechanism of action. However, we conclude that the ethanolic extract of *Mirabilis jalapa* is a potent anticonvulsant drug.

**Pentylenetetrazole Induced Seizures Experiment**

Pentylenetetrazole produced tonic seizures in all the animals used. A dose of 200 mg/kg of Ethanolic extract of roots of *Mirabilis jalapa* protected 50% of the animals against seizures and did not affect the onset (latency) of seizures to any significant extent. Ethanolic extract of roots (400 mg/kg) and 200 mg/kg of Ethanolic extract of leaves of *Mirabilis jalapa* at the dose of protected only 83.33% of the animals against seizures and 400 mg/kg of Ethanolic extract of leaves of *Mirabilis jalapa* protected 100% of the rat against seizures and increased the latency of the seizures (Figure 1 & Table 5). *In vitro* and *in vivo* studies indicated that flavonoids may pass the blood-brain barrier and have many effects on the central nervous system [20].

The present study shows that the Ethanolic extract of leaves and roots of *Mirabilis jalapa* may function in a similar manner to BZD, it is possible that the anticonvulsant effects might be due to significant glycineric and GABAergic potentiating mechanisms. The *Mirabilis Jalapa* extracts might be inducing the release of these neurotransmitters and thus inhibiting the convulsions [14]. However, we conclude that the ethanolic extract of *Mirabilis jalapa* is a potent anticonvulsant agent.

**Table: 5 Anticonvulsant Effect of *Mirabilis jalapa* using PTZ Induced Convulsion**

| Group | Treatment | Onset of Clonus convolution(s) | Onset of Tonic convolution(s) | No. of animals survived | % Protection |
|-------|-----------|-------------------------------|-------------------------------|-------------------------|--------------|
| I     | Control+PTZ (60 mg/kg) | 35.20±3.34 | 70.10±5.19 | 0/6 | 0.0 |
| II    | Diazepam(5mg/kg)+ PTZ (60 mg/kg) | ND | ND | 6/6 | 100 |
| III   | MJRE(200mg/kg)+ PTZ (60 mg/kg) | 53.43±1.98** | 90.29±1.23** | 3/6 | 50.00 |
| IV    | MJRE(400mg/kg)+PTZ (60 mg/kg) | 109.16±4.45*** | 161.19±1.72*** | 5/6 | 83.33 |
| V     | MJLE(200mg/kg)+ PTZ (60 mg/kg) | 105.42±6.14** | 199.11±2.61*** | 5/6 | 83.33 |
| VI    | MJLE(400mg/kg)+ PTZ (60 mg/kg) | 198.58±2.56*** | ND | 6/6 | 100 |

ND-Not Detected; Values are presented as mean ± SEM, where (n=6), *p<0.05, **p<0.01, ***p<0.001 compared to control

Pentylenetetrazole have excitatory effects associated GABA antagonism that induces seizures in
One of the pathophysiological mechanisms of epileptic seizures is an imbalance between excitatory and inhibitory amino acids in the brain. Therefore, many of the antiepileptic drugs are used to ameliorate this imbalance. Glutamate and γ-amino butyric acid (GABA) are the major excitatory and inhibitory neurotransmitters in the central nervous system, respectively. Literature suggested that quercetin may contribute γ-amino butyric acid (GABA) and glutamate receptors balance.

Anxiety is a severe psychiatric disease with high impact on the public health. The present study is the first evidence of the antianxiety and anticonvulsant properties of the leaves and root of Mirabilis jalapa ethanolic extract which were effective against tonic seizure, and generalized seizure or myoclonus; these results envisage the implicate major anticonvulsant response of the plant is due to the presence of more concentrated flavonoids, phenolic chemical constituents. Mirabilis jalapa possesses muscle relaxant activity, also supportive evidence of showing anxiolytic and anticonvulsant effects. A proper identification of medicinal properties and their scientific evaluation provides with much superior relief than the contemporary practice of medicine. Further study is required for isolation and identification of active constituents and to confirm exact mechanisms.

Conclusion
A number of ethnomedicinal plants have been found as potent neurobehavioral substances and those could be served as alternatives to modern medicine. The results of the present investigation are significant and encouraging towards the goal for future utilization and standardization of Mirabilis jalapa plant. The preliminary phytochemical studies were carried out in the ethanolic extract of Mirabilis jalapa leaves and roots, observed the significant presences of alkaloids, carbohydrates, flavonoids, phenols, steroids, glycosides and tannins. However, on the basis of our in vivo experiment results, the higher doses (400 mg/kg b.wt.) of ethanolic leaves and root extraction of Mirabilis jalapa showed significant anxiolytic and anticonvulsant activity. The present study is the first evidence of the antianxiety and anticonvulsant properties of Mirabilis jalapa. It is concluded that neuroprotective effects of Mirabilis jalapa might be due to the presence of tannins, phenolic compounds and flavonoids. This evaluation also suggested that further study is required for isolation, identification of active constituents and to confirm exact mechanisms in order to find an effective drug against anxiety and convulsion.

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*Figure 1: Anti-anxiety and Anticonvulsant Evaluation of M. jalapa*
a) Control group animal in closed arm

b) *Mirabilis jalapa* extract treated group animal in open arm

c) *Mirabilis jalapa* extract treated animal in Open Field

d) Anticonvulsant activity animal observed in Observation Chamber