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Systematic Evaluation of Neuropharmacological Depressant Activities on Central Nervous System (Cns) Using Methanolic Extract of Digera Muricata (L.)Mart.in Albino Mice

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ABSTRACT

The aim of this study was to systematically evaluate the neuropharmacological profile for methanolic extract Digera muricata (MEDM) in Swiss albino mice and belongs to family Amaranthaceae for various models. Preliminary phytochemical evaluation and acute toxicity values were also carried out and LD50 was found to be 200 mg/kg by ip route with different doses such as 50, 100 and 200 mg/kg (i.p.). The MEDM administered results produced reduction in spontaneous motor activity, exploratory behaviour and motor coordination and prolonged different standards sleeping time. It also decreases the rate of all the different standards induced mortality in mice. The present obtained investigations for CNS were suggested that the extract may possess depressant principles with potential neuroleptic properties and contains some active principles, which may be sedative in nature.

KEYWORDS Digera muricata, CNS, different standards and Sleeping

INTRODUCTION

Depression is a chronic, recurring and potentially life threatening illness that affect up to 20% of the population across the globe. This disease is one of the top ten causes of morbidity and mortality worldwide and represents a high cost to countries economy. The main symptom of depression is characterized by a pervasive low mood, feeling of helplessness, loss of interest and loss of pleasure in most of the usual activities. Antidepressant medications represent the best-established treatment for major depressive disorder, they have adverse effects that can compromise the therapeutic treatment and also provide an opportunity for alternative remedies based on natural products (Fournier *et al.,* 2010). Natural products, on the contrary, are complex and often contain many potentially active substances and even the ethical imperative for further scientific study on medicinal plants. There are also large numbers of herbal medicines whose therapeutic potential have been assessed in a variety of animal models. These studies have provided useful information for the development of new pharmacotherapies from medicinal plants for use in clinical psychiatry for the treatment of depression (Zhang, 2004).

The medicinal plants were used to treat various central nervous disorders including depression, epilepsy, and psychosis is on the increase worldwide (Kinjal Chauhan *et al.,* 2011). Plant kingdom has remained a target of search for new drugs and lead compounds by multinational drug companies and research institutes (Mahendran *et al.,* 2011). The World Health Organization (WHO) supported programs designed to use medicinal plants more effectively in traditional health care systems especially in developing countries, where they are readily available, easily affordable, and already integrated into the people's cultures. Moreover the synthetic drugs are very expensive to develop since, for the successful development of a new product usually costs in the range of 0.5 to 5 million dollars. On the contrary, many medicinal herbs constitute the cornerstone of traditional medicinal practice worldwide. These herbs are relatively cheap and available of drugs and the structural diversity of their component molecule makes a valuable source of novel lead compounds. It is therefore, essential that efforts should be made to introduce new medicinal plants to develop drugs which are cheaper, safer and more effective. Plants still represent a massive untapped source of structurally novel compounds that might serve as lead for the development of novel drugs (Almeida *et al.,* 2001). The *Digera muricata* (L.) Mart. is a wild edible herb used by village people. It is popularly known for herbal remedy for various ailments. In our early publication of phytochemical and antimicrobial activities by Pratima and Sundar (2010) and antioxidant activities (Sundar Mety, 2011) antidepressant activities of aqueous extract (Sundar Mety *et al.,* 2014). The leaves are used for treatment of diabetic (Jagatha and Senthilkumar, 2011). But the scientific basis for its medicinal use especially for boiled root infusion given to mother after child birth to increase lactation purpose is to be evaluated. Therefore, the present study was undertaken to assess systematically evaluate the Neuropharmacological activities of *Digera muricata*. The objective of this study was to systematically evaluate the possible neuroleptic potential of this widely used plant in the management of neuropsychiatric disorders.

MATERIALS AND METHODS

The experimental plant samples of *Digera muricata* (L.) Mart. was collected from Humnabad Taluka in Bidar District, Karnataka, India. The plant was identified with the help of the Flora of Presidency of Madras (Gamble, 1958) and the Flora of Gulbarga district (Seetharam *et al.,* 2000). The voucher specimens were deposited in the Botany department herbarium, Gulbarga University, Gulbarga (HGUG).

EXTRACTION

The shade dried plant materials were powdered using mixer grinder and subjected to Soxhlet extraction using petroleum ether, chloroform, methanol, ethanol and distilled water. The obtained extracts were condensed and used for preliminary

phytochemical tests by adopting standard methods and it was published in our earlier publication[7].

ANIMALS

Swiss albino mice (20-25 g) of either sex were purchased from the Animal Research Branch of the Luquaman College of Pharmany, Gulbarga,Karnataka, India. They were maintained under standard nutritional and environmental conditions throughout the experiment. All procedures described were reviewed and approved by the institutional animal ethics committee (IAEC). The experiments were done in an isolated and noiseless room. The animals were deprived of food 12h before experimentation.

DRUGS AND CHEMICALS

As a standard reference chemicals like Phenobarbiton, diazepam, imipramine and chlorpromazine, normal saline solution were purchased from Hi-Media Pvt. Ltd.

ACUTE TOXICITY TEST

The methanolic and aqueous extracts of *Digera muricata* were tested for acute toxicity in mice by following the procedure of OECD423 Acute Classic method. The experimental design was approved by the Institutional Animal Ethical Committee. To determine acute toxicity of MEDM and AEDM in animals were administered intraperitonealy with different doses (50, 100, 200mg/kg BW) to different groups of mice and observed for the sign of behavioral and mortality for 72 hours.Acute toxicity of the extract was estimated using mice. They were distributed into seven groups consisting of six mice in each group as shown in Table-1. The numbers of death in each group within 72h were recorded. The LD_{50} was estimated from the graph of probity against log dose of the extract.

Table-1: should be here

Table-1: shows that acute toxicity in mice with different doses		
Groups	Doses mg/kg of B.W	Administrated Drugs
Group-I	4ml	distilled water control
Group-II	50	MEDM
Group-III	100	MEDM
Group-IV	200	MEDM
Group-V	50	AEDM
Group-VI	100	AEDM
Group-VII	200	AEDM

NEUROPHARMACOLOGICAL ACTIVITIES OF *D. MURICATA*IN ALBINO MICE

In the present study the *Digera muricata* was chosen for systematically evaluation of neuropharamacological activities because, it is evident from the studies that the local edible plant *Digera muricata* exhibited the maximum antioxidant activity in methanol, aqueousand ethanolextracts when compared to other extracts (Sundar and Pratima, 2011). The healthy adult animal studies were carried out by taking Swiss albino mice weighing about 20-25gm from inbred colony and were divided into six groups containing six animals each as shown in Table-2.

SPONTANEOUS MOTOR ACTIVITY (SMA)

Spontaneous motor activity was recorded using Actophotometer (Techno LE3806, India). Activity was automatically recorded after treatment with respective doses at every 0 hrs upto 8 hours. The experiments were repeated at an interval of 1, 2, 4 and 8 hours, for a period of 8 hours. Results of the treated groups were compared with those of control groups at each time interval (Amos *et al,* 2001) as shown in Table-2.

EXPLORATORY BEHAVIORAL PATTERN

The study was carried out using wooded board measuring 40x40cm with 16 evenly spaced holes (Perez *et al.,* 1998) as shown in Table-2.

MARBLE-BURYING TEST (MBT)

This test consisted of a Plexiglas cage of 23X17X14 cm with a smooth lid punctured by small ventilation holes. The floor was covered with a 5 cm layer of sawdust and 20 glass marbles were placed in contact with each other in the centre of the maze (Broekkamp *et al.,* 1986). After one hour the index of depressant/ antidepressant effect were recorded and calculated and groups as shown in Table-2.

TAIL SUSPENSION TEST (TST)

The Swiss albino mice were weighed 20-25 gm and used preferentially[16], for the test, the mice were suspended on the edge of the shelf 60cm above platform by thread placed approximately 1cm from the tip of the tail. The duration of immobility is recorded for a period of 5 minutes mice are considered immobile when they hang passively and completely motionless for 1 min and groups as shown in Table-2.

Table-2; should be here

Table-2: shows that induced extract and drugs using different standards		
Groups	Doses mg/kg of B.W	Administrated Drugs
Group-I	4ml	distilled water control
Group-II	3mg /kg,	phenobarbiton
Group-III	4 mg /kg, and	diazepam
Group-IV	3 mg /kg and	imipramin
Group-V	3 mg	chlorpromazine
Group-VI	200	MEDM

STATISTICAL ANALYSIS

All the data obtained were expressed as mean ± standard error. Differences in means were estimated by means of ANOVAs (Tukey) using Graph pad instat software 3.10 version. Results were considered significant at $P < 0.05$ and graphs were represented using Microsoft excel 2010.

RESULTS AND DISCUSION

ACUTE TOXICITY AND GENERAL BEHAVIORAL STUDY

The extract did not produce any toxic symptoms of mortality up to the dose level of 200 and 150mg/kg body weight in mice and hence the drugs were considered to be safe for further pharmacological screening. According to the OECD-423 guidelines for acute oral toxicity the LD_{50}dose of 200 and 150mg/kg of AEDM, MEDM. While conducting the toxicity studies animals were observed continuously for neuropharmacological general behavioral changes and significant reduction of spontaneous locomotor motility, drowsiness and remarkably quite were behavior.

In the present investigation, the acute toxicity of *Digera muricata* (L.) Mart. was recorded upto1000mg/kg. the present observation suggested that, 200mg/kg and 150mg/kg of body weight (b.w.) of mice. However, the AEDM possessed significant ($p<0.01$) reduction in blood glucose level when compared to MEDM at dose of 150 and 200 mg/kg.

CNS BEHAVIORAL ACTIVITIES OF *DIGERA MURICATA*IN MICE

1. SPONTANEOUS MOTOR ACTIVITY (SMA)

It has been a growing trend in research of phytomedicine as an alternative step towards effective treatment of neurobiological disorders and its related complications. The alternative systems of medicines have paved much to investigate the effectiveness of the medicinal plants for neuropharmacological basis of treatments. The present investigation undertaken to analyze and check the potentiality of the drug exhibiting level for CNS depressant locomotor activity in mice using actophotometer. Decrease in the locomotor activity was found with MEDM ($p<0.01$ and 0.05) from 1st hour upto 8th hr. However, decrease in motor activity was retained by the mice in all the standards except diazepam and control for 1st hour upto 8thhr. The MEDM (200mg/kg b.w) had significant at $p<0.05$ for depressant activity when compared with standard except chloropromazine treated (3mg/kg b.w) and control groups (Fig-1). Our present investigation supports the previously reported in methanolic extract by Sneha Anarthe and Sanjay Chaudhari (2011) was agreed with our current obtained results.

2. EXPLORATORY BEHAVIOR PATTERN (EBP)

In the present investigation, the exploratory behaviors pattern of albino mice administered with MEDM was studied. The exploratory behavior activity was observed significant in MEDM at 200mg/kg. The sleeping time increase in albino mice induced by all the standards and samples (MEDM) which is used as standard reference drug and test sample in the present studies. It was interestingly observed and recorded that the MEDM extract (200mg/kg b.w) had significant at p<0.05 for depressant activity when compared with standard diazepam treated group (3mg/kg b.w) (Fig-2).

Similarly published research work by Shammy Sarwar *et al.,* (2014) have measure of the level of excitability of the CNS and this decrease may be closely related to sedation resulting from depression of the central nervous system. The extracts significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to 5th observation period (120 min). Both hole cross and open field tests showed that the depressing acting of the extracts was evident from the 2nd observation period in the test animals at the doses of 200 & 400 mg/kg body weight. Maximum depressant effect was observed from 3rd (60 min) to 5th (120 min) observation period. From the result this is observed that, *Clitoria ternatea* Linn. has CNS depressant activity by using both open field & hole cross tests, which is comparable to the reference drug Diazepam at doses of 1 mg/kg and Sutradhar, and Choudhury, (2012), Md. Amran Howlader and Mahmudul Alam (2011).

3. MARBLE BURYING TEST (MBT)

These results of marble burying test was significant with MEDM at 200mg/kg and the sleeping time increase in albino mice induced by all the reference drugs which are used as standard reference drug in the present study. The marble burying test there was a significant diminution of the object burying reflex only at the highest dose of MEDM extract (200mg/kg b.w; Fig-3) test samples significant level p being <0.05, <0.01 or 0.001. Relatively at specific time interval depending upon the mode of action exhibited by the test sample, variation in burrowing nature of animal depending upon the potentiality of the test samples was recorded. All the groups were compared with normal control and standard reference drugs administered to albino mice (Fig-3).

Regardless, the marble burying test is still quite useful for assessing psychotropic drugs (Nicolas *et al.,* 2006; Li *et al.,* 2006; Bruins *et al.,* 2008). For example, in this study, we repeated the marble burying test 20 times for each set of mice, and the number of buried marbles did not change. This lack of habituation provides reliability and validity to experiments having a within-subject design. The marble burying test may have other advantages in psychopharmacological studies, but further study is required to elucidate these advantages.

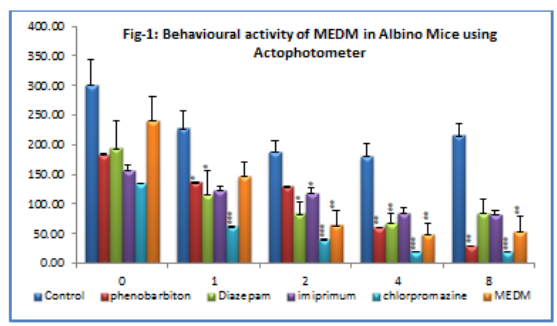
4. TAIL SUSPENSION TESTS (TST)

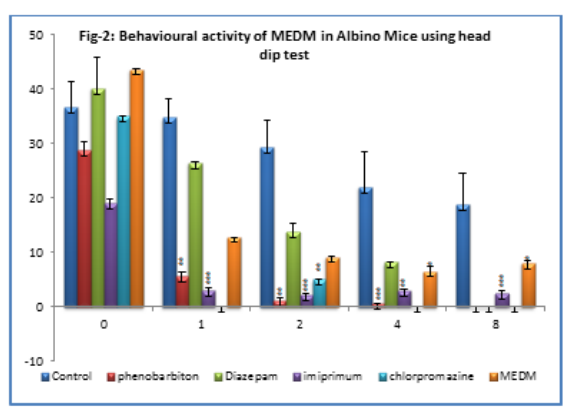
Finally the experiment was extended to observe the different behavioral activity using automatic tail-suspension test allow as a fast and reliable screening of the psychotropic properties (anti-depressants, sedatives) of drugs. Basically, the measuring principle is based on the energy developed by mice trying to escape from their suspension. During the test, the movements of the mice were analyzed in terms of force, energy and power developed over time. In the present investigation, the MEDM of 150mg/kg (b.w) doses were studied to update our study with response to *Digeramuricta* plant. The methanolic extract had more significant increase in immobility time at p<0.01 when compared to control which indicated as CNS depression while compared with standard reference drugs indicates CNS depressant effect in albino mice was dose dependent (Fig-4).

Our present investigation were agreed with earlier reports were also observed in other plant extracts by Sutradhar and Choudhury, (2012), Khatun *et al.,* 2011, **Khairnar Pranit (2010),** Daniele *et al.,* (2008) have obtained the results depicted in pre-treatment of mice with the inhibitor of serotonin synthesis PCPA (100 mg/kg, i.p., once a day for 4 consecutive days) significantly prevented the decrease in the immobility time elicited by rutin (0.3 mg/kg, i.p.). The underlying principle measuring the lack of active coping behavior is identical in the tail suspension test, but their variability in response to certain depressant and antidepressants indicates potentially different substrates and neurochemical pathways mediating performance in these tests. These issues may underlie the observed behavioural differences.

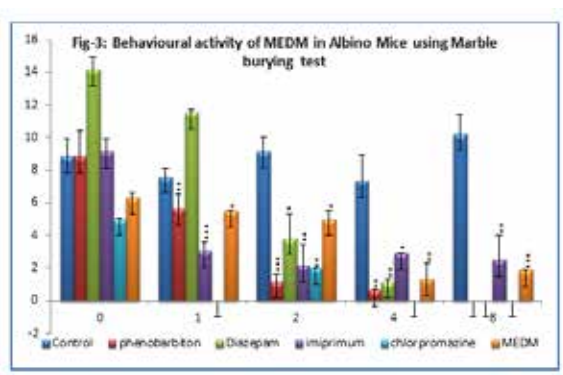
Conclusion

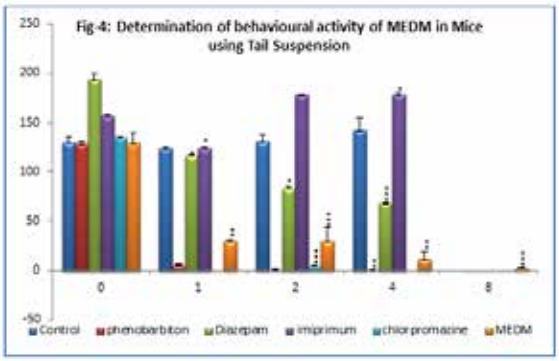
In conclusion, it could be suggested that the crude ethanolic extract of *Digera muricata* surely possess central nervous system depressant activities. Based on the results of the present study, we conclude that the methanol extract possesses strong analgesic and CNS depressant activity in dose dependent manner. However, further studies are necessary to examine under lying mechanisms of analgesic and CNS depressant effects and to isolate the active compound (s) responsible for these pharmacological activities.





Results are expressed as Mean±SE (n=6). Data processed by Graph Pad Instant by Tukeyo Method test *** p<0.001 significant when compared to control group. ** p<0.01, * p<0.05 significant when compared to standard groups.





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