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**Original Article**

# Evaluation of neuropharmacological properties of a polyherbal extract

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## ABSTRACT

Many epidemiological studies conducted in India on mental and behavioural disorders report varying prevalence rates, ranging from 9.54 to 370 per 1000 population. Researchers are developing drugs to treat many different neurological disorders including pain, neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, psychological disorders, addiction and many others. The present study was aimed at evaluating the neuropharmacological properties of a polyherbal extract which was prepared by combining equal proportions of the three plants, *Catharanthus roseus*, *Calotropis gigantea* and *Jasminum malabaricum*. These plants were selected based on the evidence that they were traditionally used for treatment of mental disorders. The extracts were evaluated for neuropharmacological properties using various CNS models such as apomorphine induced stereotype behaviour, Rota rod test, Head dip test and Y-Maze test. The results revealed that the polyherbal extract possess neuropharmacological properties which may be due to the presence of important phytoconstituents present in the three extracts.

**Key Words:** Polyherbal extract, *Catharanthus roseus*, *Calotropis gigantean*, *Jasminum malabaricum*, Neuropharmacological.

## INTRODUCTION

The world health organisation estimated in 2006 that neurological disorders affect as many as one billion people worldwide and identified health inequalities and social stigma/discrimination as major factors contributing to the associated disability and suffering. [1]

*Calotropis gigantea* (family, Asclepiadaceae) has been reported as traditional medicinal plant in Ayurveda, Unani and Homeopathic system of medication for the treatment of different ailments. It has been reported that the plant parts of *Calotropis gigantea* contain various chemical constituents such as Calotropin, calotoxin, uscharin, voruscharin, uschridin, uzarigenin, syriogenin, calotonic acid and proceroside.[2] Flowers are used to treat mental disorders.[3] *Catharanthus roseus* (family, apocynaceae), formerly known as *Vinca rosea* has been reported to contain the alkaloid, alstonoine and has been used traditionally for its calming effect and its ability to reduce blood pressure.[4] It has also been reported to possess CNS depressant, antihypertensive, antitumor, cardiotonic, antihyperglycemic and cytotoxic activities.[5]

*Jasminum malabaricum* (family, Olaceae) commonly known as Malabar jasmine has been traditionally used in mental disorders.[6]

Based on the traditional claims of the three plants and the reported activities, the present study was planned to evaluate the neuropharmacological activities of the three individual extracts and a polyherbal extract prepared from the three plants.

## MATERIALS AND METHODS

**Plant materials:** The fresh flowers of *Calotropis gigantea* and the fresh roots of *Catharanthus roseus* were procured from the surroundings of Bangalore district and were authenticated. The dried flowers and leaves of *Jasminum malabaricum* was obtained from forest surrounding Tirupati and was authenticated.

**Preparation of extracts:** [7] The dried plant materials were coarsely powered and extracted for 48 hrs in batches of 250 g each, using Soxhlet extractor successively with the solvents, petroleum ether followed by methanol. The extracts so obtained were concentrated in vacuum using rotary flash evaporator and finally dried in desiccator. The

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extracts so obtained from each solvent were labeled, weighed and the yield was calculated in terms of grams percent of the weight of the powdered plant materials.

**Preparation of Polyherbal extract:** Methanolic extract of *Calotropis gigantean* (CGME), *Catharanthus roseus* (CRME) and *Jasminum malabaricum* (JMME) were taken in equal proportions and mixed thoroughly.

**Qualitative phytochemical tests [8]:** The crude extracts of each plant extracts were subjected to qualitative tests to detect the major constituents.

**Experimental animals:** Healthy Wistar rats and Swiss Albino mice of either sex, weighing between 150-200 g and 25-35 g respectively were procured from animal house of Dayananda Sagar College of Pharmacy, Bangalore, India. The animals were kept in well ventilated spacious animal house with 12 ± 1 h day and night schedule. The experiments were conducted as per the guidelines of CPCSEA and protocol approved by IAEC (Approval no. DSCP/Col/IAEC/84/12-13)

**Acute toxicity studies:** Acute toxicity studies of all extracts were performed as per OECD test guidelines. [9]

**Neuropharmacological activities:**

**Experimental design:** Animals were divided into the following groups. Each group consisted of six animals

**Group 1**: Standard (Diazepam 2 mg/kg,)
**Group 2**: Control (1%CMC)
**Group 3**: Polyherbal extract (250 mg/kg)
**Group 4**: Polyherbal extract (500 mg/kg)
**Group 5**: CGME (250 mg/kg)
**Group 6**: CGME (500 mg/kg)
**Group 7**: CRME (150 mg/kg)
**Group 8**: CRME (300 mg/kg)
**Group 9**: JMME (250 mg/kg)
**Group 10**: JMME (500 mg/kg)

**Test for motor co-ordination: Rota-rod test [10]**

Animals of group 2-10 were dosed for a period of 7 days once daily as mentioned above. On 7$^{th}$ day group 1 was administered, diazepam i.p and after 30 min animals were placed on the rotating rod. Similarly animals of group 2-10 received respective doses and after 60 min animals were placed on the rotating rod. The fall off time was noted and results were subjected to statistical analysis. Statistical significance was analyzed by ANOVA followed by Dunnet's t-test.

**Anxiolytic activity- Elevated plus maze [11]**

Animals of group 3-10 were dosed with respective extracts for a period of 7 days once daily as mentioned above. On 7$^{th}$ day animals of group 1 were administered with diazepam i.p. After 30 min all animals were placed individually at the centre of elevated plus maze and parameters such as First preference of mouse to open or enclosed arm and number of entries were noted. The results were subjected to statistical analysis. Statistical significance was analyzed by ANOVA followed by Dunnet's t-test.

**Brain corticosterone estimation: [12]**

Animals were killed by decapitation at predetermined intervals after the administration of extracts, diazepam and 1% CMC after subjecting the animals to elevated plus maze test. The brains were isolated, weighed and homogenized immediately with ice cold KCl. Brain corticosterone was estimated by fluorimetric method. For calibration curve concentrations of 0, 20, 50,100 and 200 µM/ml of corticosterone were treated identically and measured.

**Exploratory Behaviour**

**Head Dip Test: [13]**

Six Albino mice of either sex were taken in each group. To each group respective extracts was administered p.o. while control group received 1% CMC (the dose was calculated per kg of body weight) for a period of 7 days. On the last day thirty minutes after the administration of diazepam (5 mg/kg) i.p, animals were placed individually on centre of a wooden board consisting of 16 evenly spaced holes. The number of times the animal dipped their heads into the holes during 5 min was counted. Similarly animals of group 2-10 received respective doses and after 60 min the number of head dips was counted. The results were subjected to statistical analysis. Statistical significance was analyzed by ANOVA followed by Dunnet's t-test.

**Brain GABA content estimation**: [14] Animals were killed by decapitation at predetermined intervals after the administration of extracts, diazepam and 1% CMC after subjecting the animals to hole-board test. The brains were rapidly removed, blotted, weighed and taken in ice cold 5 ml trichloroacetic acid (10% w/v), homogenized and centrifuged. Brain GABA Content was measured by spectrofluorimetric method. The GABA content in homogenised brain samples was expressed in µg $g^{-1}$ of the wet brain tissue.

**Y-Maze test [15]** Six Albino mice of either sex were taken in each group. To each group respective extracts was administered p.o. while control group received 1% CMC (the dose was calculated per kg of body weight) for a period of 7 days. On the last day thirty minutes after the administration of

diazepam (10 mg/kg) i.p, animals were placed individually in Y-Maze for 3 min. The number of times a rat entered in the arm of the maze with all four feet was counted as a single entry. Similarly animals of group 2-10 received respective doses and after 60 min animals were placed individually in Y-Maze for 3 min and number of crossings were noted. The results were subjected to statistical analysis. Statistical significance was analyzed by ANOVA followed by Dunnet's t-test.

## RESULTS

**Acute toxicity studies:** All extracts administered upto dose levels of 3000 mg/kg did not produce any mortality after 24 hours and upto 14 days.

**Test for motor co-ordination: Rota-rod test:** The Polyherbal extract, CGME, CRME and JMME was evaluated for muscle relaxant activity in mice (n=6). The results revealed that CGME at doses of 250 and 500 mg/kg ($p<0.01$) body weight showed significant muscle relaxant activity compared to control (Table 1).

**Anxiolytic activity:**

**Elevated plus maze test:** The results of antianxiety activity of the polyherbal extract, CRME and JMME carried out in albino mice revealed that all extracts showed increase in percentage preference of the animals to open arm and increases the number of entries and average time spent by the mouse in the open arm with increase in doses as compared to vehicle control (Table 2). There was considerable decrease in brain corticosterone level in Polyherbal extract, CGME, CRME and JMME treated mice when compared to vehicle control, which was comparable to the standard diazepam group indicating antianxiety activity (Table 3).

**Exploratory behavioral studies:**

**Head dip test:** In head dip test the animals treated with diazepam, Polyherbal extract, CGME, CRME and JMME showed significant reduction in number of head dips when compared to vehicle control. The polyherbal extract showed significant reduction in number of head dips with a mean value of 15.16 ($p<0.01$) as compared to vehicle control with a mean value of 25.66 ($p<0.01$). The number of head dips decreased with higher dose of the extracts (Table 4). The Polyherbal extract, CGME, CRME, JMME and diazepam increased the concentration of GABA in brain compared to vehicle control indicating Antianxiety effect (Table 5).

**Y-Maze Test:** In Y-maze test, the animals treated with diazepam, Polyherbal extract, CGME, CRME and JMME showed significant decrease in number of crossings in Y-maze. There was significant reduction in number of crossings at higher doses of the extract when compared to vehicle control (Table 6).

## DISCUSSION

Phytochemical investigations revealed that CGME, CRME and JMME contain active constituents such as flavonoids, alkaloids, phenolic compounds and glycosides which are known to possess neuropharmacological properties. The results of pharmacological investigations revealed that the Polyherbal extract, CGME, CRME and JMME possess anti-anxiety properties. The Polyherbal extract, CRME and JMME did not possess muscle relaxant property which suggest that the extracts has centrally- mediated actions based on the inhibitory effects observed and not through peripheral neuromuscular blockade. CGME showed muscle relaxant activity as well as inhibitory effect which suggests that it possess both centrally- mediated actions as well as peripheral neuromuscular blockade property.[16]

The results of Elevated plus maze test revealed that the extracts possess antianxiety properties which was reflected by the decrease in corticosterone levels in the brain of the extract treated mice as compared to control.[17]

The results of Head dip test revealed the diminished exploratory behavioral profile of extracts in mice. The GABA levels in the brain were also significantly increased. Similarly in Y-Maze test the number of crossing were significantly reduced by the extracts. It may be concluded that polyherbal extract consisting of equal proportions of *Catharanthus roseus*, *Calotropis gigantea* and *Jasminum malabaricum* possess neuro-pharmacological properties in the doses used which may be attributed to the active constituents present in the extract.

## CONCLUSIONS

The results of the study revealed that the polyherbal extract, CGME, CRME and JMME possess neuropharmacological properties which may be due to the presence of important phytoconstituents. Further studies are required to elicit the mechanism of action.

**Table 1: Effect of Polyherbal extract, CGME, CRME and JMME on muscle relaxant activity- Rota rod test.**

| GROUPS/DOSE | Fall of Time (Sec ± SEM) |
|---|---|
| CONTROL | 180 ± 0.0 |
| DIAZEPAM | 24 ± 0.73** |
| POLYHERBAL-250 | 153.33 ± 16.86* |
| POLYHERBAL-500 | 180 ± 0.0ns |
| CGME-250 | 97.83 ± 5.108** |
| CGME-500 | 66.83 ± 4.43** |
| CRME-150 | 180 ± 0.0ns |
| CRME-300 | 180 ± 0.0ns |
| JMME-250 | 180 ± 0.0ns |
| JMME-500 | 180 ± 0.0ns |

All values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test. The $p<0.05$ was considered as significant; *$p<0.05$, **$p<0.01$, ns not significant as compared to Vehicle control group.

**Table No 2: Anti-anxiety activity of Polyherbal extract, CGME, CRME and JMME in albino mice using elevated plus maze**

| GROUPS/DOSE | % PREFERENCE TO OPEN ARM | TIME SPENT IN OPEN ARM (Sec ± SEM) | OPEN ARM CROSSING IN 5 MIN |
|---|---|---|---|
| CONTROL | 16.66 | 33.83 ± 6.62 | 3.83 ± 0.47 |
| DIAZEPAM | 83.33 | 162.66 ± 11.41** | 16.16 ± 1.014** |
| POLYHERBAL 250 | 50 | 97 ± 3.13** | 8.66 ± 0.95** |
| POLYHERBAL 500 | 50 | 123.83 ± 12.20** | 11.5 ± 0.76** |
| CGME-250 | 50 | 103.66 ± 6.80** | 8 ± 0.89** |
| CGME-500 | 50 | 130.83 ± 5.76** | 10.33 ± 0.66** |
| CRME-150 | 50 | 62.5 ± 2.78ns | 5.5 ± 0.76ns |
| CRME-300 | 50 | 109.5 ± 15.93** | 9.5 ± 0.76** |
| JMME-250 | 33.33 | 61.66 ± 5.43ns | 7.5 ± 0.88* |
| JMME-500 | 50 | 76.83 ± 3.80** | 9.66 ± 0.66** |

All values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test. The $p<0.05$ was considered as significant; *$p<0.05$, **$p<0.01$, ns not significant as compared to Vehicle control group.

**Table No. 3: Anti-anxiety activity of Polyherbal extract, CGME, CRME and JMME in Albino mice using elevated plus maze- Corticosterone concentration in brain.**

| GROUPS/DOSE | CORTICOSTERONE CONCENTRATION (µg/g tissue) |
|---|---|
| CONTROL | 3.23 ± 0.092 |
| DIAZEPAM | 2.26 ± 0.057** |
| POLYHERBAL-250 | 2.60 ± 0.10** |
| POLYHERBAL-500 | 2.46 ± 0.047** |
| CGME-250 | 2.77 ± 0.004** |
| CGME-500 | 2.46 ± 0.066** |
| CRME-150 | 2.89 ± 0.16* |
| CRME-300 | 2.70 ± 0.045** |
| JMME-250 | 2.88 ± 0.033* |
| JMME-500 | 2.71 ± 0.054** |

All values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test. The $p<0.05$ was considered as significant; *$p<0.05$, **$p<0.01$ as compared to Vehicle control group.

**Table 4: Effect of Polyherbal extract, CGME, CRME and JMME on exploratory behavior in Albino mice (Head Dip test)**

| GROUPS/DOSE | NUMBER OF HEAD DIPS |
|---|---|
| CONTROL | 25.66 ± 0.98 |
| DIAZEPAM | 4.33 ± 0.42** |
| POLYHERBAL-250 | 15.16 ± 2.18** |
| POLYHERBAL-500 | 9.16 ± 0.30** |
| CGME-250 | 14.16 ± 0.74** |
| CGME-500 | 10.5 ± 0.76** |
| CRME-150 | 15.5 ± 0.71** |
| CRME-300 | 10.83 ± 0.40** |
| JMME-250 | 16 ± 0.85** |
| JMME-500 | 11.33 ± 1.05** |

All values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test. $p<0.05$ was considered as significant; *$p<0.05$, **$p<0.01$ as compared to Vehicle control group.

**Table No. 5: Behavioral effect of Polyherbal extract, CGME, CRME and JMME in Albino mice- Brain GABA concentration.**

| GROUPS/DOSE | GABA CONCENTRATION (µg $g^{-1}$) |
|---|---|
| CONTROL | 268.712 ± 10.22 |
| DIAZEPAM | 946.812 ± 36.79** |
| POLYHERBAL-250 | 341.136 ± 5.58* |
| POLYHERBAL-500 | 530.329 ± 8.29** |
| CGME-250 | 321.737 ± 7.04ns |
| CGME-500 | 532.154 ± 12.13** |
| CRME-150 | 315.769 ± 6.68ns |
| CRME-300 | 475.914 ± 7.74** |
| JMME-250 | 299.34 ± 5.089ns |
| JMME-500 | 420.90 ± 21.31* |

All values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test. $p<0.05$ was considered as significant; *$p<0.05$, **$p<0.01$, ns - not significant as compared to Vehicle control group.

**Table 6: Effect of Polyherbal extract, CGME, CRME and JMME on exploratory behavior in Albino mice in Y-Maze test.**

| GROUPS/DOSE | Number of entries in 3 min ± S.E.M. |
|---|---|
| CONTROL | 16.33 ± 0.61 |
| DIAZEPAM | 2 ± 0.36** |
| POLYHERBAL-250 | 11.83 ± 0.65** |
| POLYHERBAL-500 | 8.83 ± 0.94** |
| CGME-250 | 13 ± 0.68* |
| CGME-500 | 11.16 ± 1.30** |
| CRME-150 | 12.83 ± 0.79* |
| CRME-300 | 9 ± .057** |
| JMME-250 | 13.5 ± 0.42ns |
| JMME-500 | 8.66 ± 0.55** |

All values are expressed as mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test. $p<0.05$ was considered as significant; *$p<0.05$, **$p<0.01$, ns- not significant as compared to Vehicle control group.

## REFERENCES


1) Neurological disease wikipedia free encyclopedia. https://en.wikipedia.org/wiki/Neurological_disorder (Accessed November 15, **2011**)
2) Himanshu J et al. *Calotropis gigantea* R.Br. (Asclepiadaceae): A Review. Intern J Pharm Res 2011; 3(1):10-14.
3) Madhava Shetty K, Sivaji K, Tulsi Rao K. Flowering plants of Chittor Dist, A.P. Students Offset Printers and Publishers, India, 1st ed 2008; 564.
4) http://www.tnsmpb.tn.gov.in/images/CATHARANTHUS%20ROSEUS.pdf (Accessed December 12, **2012**)
5) Madhava Shetty K, Sivaji K, Tulsi Rao K. Flowering plants of Chittor Dist, A.P. Students Offset Printers and Publishers, India, 1st ed 2008; 198.
6) Madhava Shetty K, Sivaji K, Tulsi Rao K. Flowering plants of Chittor Dist, A.P. Students Offset Printers and Publishers,India, 1st ed 2008; 192.
7) Satinder A, Karen MA. Handbook of Isolation and Characterization of impurities in Pharmaceuticals, Acedemic press, California 2003; 214-220
8) Kokate CK. Text book of Pharmacognosy, Nirali Publications, New Delhi 2007; 1-73
9) Ballantyne B et al. General and applied Toxicology, Adbridge Ed., The Macmillan press limited, London 1995; 53-56
10) Nwinyi FC, Kwanashie HO. Neuropharmacological effects of *Sorghum bicolor* leaf base extract. Res Pharm Biotechnology 2009;1:001-008.
11) Kulkarni SK. Hand book of Experimental Pharmacology 3rd edition. Vallabh Prakashan 2010.
12) Ramanathan M et al. Behavioural and neurochemical evaluation of Perment an herbal formulation in chronic unpredictable mild stress induced depressive model. Indian J Experimental Biology 2011;49:269-275.
13) Perez RMG et al. Neuropharmacological activity of *Solanum nigrum* fruit. J Ethnopharmacol 1998;62:43-48.
14) Taiwe GS et al. Antidepressant, Myorelaxant and Anti-Anxiety like effects of Nauclea latifolia Smith (Rubiaceae) roots extract in murine models. Int J Pharmacol 2010.
15) Suba V et al. Neuropharmacological profile of *Barleria lupulina* Lindl. extract in animal models. J Ethnopharmacol 2002; 81:251-55.
16) Adzu B et al. Neuropharmacological screening of Diospyros mespiliformis in mice. J Ethnopharmacol 2002;83:139-143.
17) Oliver CG, Randolph NM. Systemic hormonal and physiological abnormalities in anxiety disorders. Psychoneuroendocrinology1988;13(4):287-307.