Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(6); 1054-1058

ISSN: 0975-4873

Research Article

# Pharmacological Screening of *Centella asiatica, Glycerrhiza glabra, Gymnosporia montana* and Cow Urine for Controlling Side Effects of Neuroleptics as well as their Use in Various Neurological Disorders

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*Available Online: 11th October, 2015*

**ABSTRACT**

The main objective of the present investigation is to explore polyherbal containing *Centella asiatica*, *Glycerrhiza glabra*, *Gymnosporia montana* and Cow urine for its neuroleptic properties, using the tail suspension test (TST), light dark test (LDM) and elevated plus maze (EPM) test on mice. Pharmacognostic and pharmacological study of aerial part of *Centella asiatica,* root of *Glycerrhiza glabra and* Leaves of *Gymnosporia montana* was carried out. Polyherbal was found safe up to a dose of 5000 mg/kg since no mortality was observed within 48 hrs. of administration. In TST, it has shown a dose-dependent decrease in immobility time, which is an indication of its antidepressant effect, in LDM it had significantly exhibited anxiolytic activity by increasing time spent and number of crossings in light compartment. decreased the time spent in dark compartment and decreased the number of rearings in both light and dark compartments. Similarly in EPM model had significantly enhanced both number of entries and time spent in open arms and decreased in number of entries and time spent in closed arms. Phytochemical investigation revealed the presence of saponins, tannins, glycosides, carbohydrates, phenolic compounds, flavonoids. Alcoholic extracts of polyherbal were found to produce significant ($P<0.05$) neuroleptic action. In all the model, the extract at 1000mg/kg showed significant activity. Thus the result recorded with above experimental models confirms the neuroleptic action of used polyherbal extract along with cow urine.

**Keywords:** *Centella asiatica, Glycerrhiza glabra, Gymnosporia montana,* tail suspension test, light dark test, elevated plus maze.

## INTRODUCTION

Since the early human existence, many natural products came into practice for human welfare by sheer intuition or more appropriately by trial and error. In the long struggle to overcome the power fulforce to nature the human being turned to plants for food, shelter, clothing, weapons and healing. When pain or injury or disease struck, ancient people have little choice but to turn to plants. Developed empirically, by trial and error, many herbal preparations were remarkably effective[1,2].

World Health Organization (WHO) also appreciated the importance of medicinal plants for public healthcare in developing nations and evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study the potential usefulness including evaluation, safety and efficacy.

In day today life of stress and strain there is a dire need for agents having neuroprotective and neuropharmacological activity enhancing learning and memory caliber of the brain. Stress involves complex biochemical, neurological and immunological mechanisms and plays a crucial role in the genesis/progression of a variety of disease states ranging from psychiatric disorders like depression and anxiety, immunosupression, endocrine disorders including diabetes mellitus, impotency and cognitive dysfunctions.

Throughout history recorded, ethanol was and is the standard drug for treatment of feelings of discomfort, tension, anxiety and stress. Though barbiturates were dominant agents from 1900-1950 because of considerable concern about their safety lead to the search of better alteration. Moreover benzodiazepines (bdz) as anxiolytic agents have brought tremendous progress in understanding the physiological, biochemical and pathological status of the disease. However the use of tranquillizer and psychotropic drugs leads to variety of autonomic, neurologic and hematopoietic disorder, but these agents primarily relieve the symptoms and offer a palliative relief of a temporary nature. In recent years use of alternative medicine in particular, derived from plant have been increased in a number of patient with condition that affect the mind.

Here study carried out for Pharmacological Screening of *Centella asiatica, Glycerrhiza glabra, Gymnosporia*

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Table 1: Morphology of Polyherbal

| Features | Obesrevation | | |
|---|---|---|---|
| (Physical test) | *Centella asiatica* | *Glycerrhiza glabra* | *Gymnosporia Montana* |
| Nature | Coarse powder | Coarse powder | Coarse powder |
| Colour | Dark green | Pale yellow | Pale green |
| Odour | Pungent, nauseous | Faint & characteristic | Characteristics |
| Taste | Bitter | Sweet | Characteristics |
| Texture | Rough & Fibrous | Rough & Fibrous | Rough & Fibrous |

Table 2: Qualitative Standards for Polyherbal

| Parameters | *Centella asiatica*(Linn.) | *Glycerrhiza glabra* | *Gymnosporia Montana* |
|---|---|---|---|
| Total ash | 12.10 ± 0.156 | 6.33 ± 0.34 | 5.50 ± 0.12 |
| Water soluble ash | 6.73 ± 0.080 | 3.60 ± 0.044 | 2.15 ± 0.057 |
| Acid insoluble ash | 2.92± 0.159 | 2.11 ± 0.046 | 1.75 ± 0.12 |
| Moisture content | 6.77 ± 0.0088 | 5.82± 0.068 | 5.41± 0.085 |
| Aqueous extractive | 5.45 ± 0.088 | 17.45 ± 0.057 | 14.52 ± 0.078 |
| Ethanolic extractive | 8.60 ± 0.048 | 19.56 ± 0.078 | 10.52± 0.017 |
| $P^H$ | 6.7 ± 0.012 | 5.9 ± 0.034 | 6.9 ± 0.012 |

Value *(Mean ± SEM) * Value of three average reading

Table 3: Qualitative chemical analysis of various extracts

| Nature | *Centellaasiatica*(Linn.) plant extract | *Glycerrhizaglabra root extract* | *Gymnosporia Montana leaves extract* |
|---|---|---|---|
| Alkaloids | - | - | + |
| Glycosides | + | + | + |
| Flavonoids | - | + | + |
| Amino acids | - | - | - |
| Tannins &Phenolics | -,- | - , + | +,+ |
| Saponins | + | + | - |
| Carbohydrates | + | + | + |
| Steroids | - | - | - |
| Mucilage | - | - | - |

+ ve = Positive - ve = Negative

*montana* and Cow urine for their use in various neurological disorders. However, the attempt is made to check synergistic action and scientific evidence. Over all literature survey revealed that above mentioned polyherbal have been used since ancient days in Ayurvedic medical practices for the treatment of anxity, depression, parkison's disease, alzheimer's disease. So this study will provide scientific validation to this plant drugs based on traditional use or ethno botanical clue.

*Cow urine*

In Ayurveda, there are many medicines made from cow urine, milk, dung, ghee, curds. This purifies, and clears all blocks in bodily channels (shroto-shodhaka). It enhances the therapeutic actions of medicines taken along with it. It has been found to be very effective in worm infestations, skin diseases, urticaria and allergic rashes, pain abdomen due to indigestion, constipation, and ascitis etc. Chemically it Composed of Water - 95% : Urea - 2.5% : Minerals, Hormones, Salts & Enzymes - 2.5% and used as an antimicrobial agent, anti-cancer, anti-oxidant, anti-free radicals, anti leishmania effect etc.

*Need for the study*

The search for neuroleptic drugs in modern time was marked by the introduction of Benzodiazepines for the treatment of anxiety. A variety of neuroleptic drugs are flooding the world market today but a very few are relatively non-toxic and fit for long term consumption. Moreover, discontinuation of drug therapy in chronic disease conditions often leads to reappearance of symptoms[1]. A large number of synthetic drugs having side-effects are available for promoting anti-depressant and anti-anxiety activity.

## MATERIAL AND METHOD

*Collection and authentification of plant material.*

Aerial parts of all plants were collected from Pune district and authenticated by Dr. Zaware, Botanical scientist, Pune.

*Pharmacognosticevaluation*[3,4,5,6]

In the present study, Aerial parts of all plants *Centella asiatica, Glycerrhiza glabra, Gymnosporia* studied for their Macroscopical Charectristics include size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc. In Microscopic Evaluation Transverse section, Powder microscopy was performed and Observed under high magnification Power. Quality control parameters of plant material like Total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive value, Water soluble extractive value, Total moisture content, and Determination of pH carried out. Shade dried poly herbals were reduced to fine powder (# 40 size mesh)

Table 4: Anxiolytic effect of Polyherbal with elevated plus maze model in mice

| Groups | No. of Entries (counts / 5min) | | Time spent in (Total 5 min) | | | Total no. of entries |
|---|---|---|---|---|---|---|
| | Open arm | Closed arm | Open arm (sec) | Closed arm (sec) | Central Platfor (sec) | |
| Control | 5.167±0.9458 | 15.17±1.869 | 35.67±1.856 | 192.3±4.112 | 72.00±5.927 | 20.34±5.002 |
| Diazepam (3mg/kg) | 12.17±2.903$^{ns}$ | 6.333±1.202** | 106.5±1.893*** | 84.50±2.825*** | 109.0±2.671 | 18.50±2.919 |
| Test I (250mg/kg) | 8.500±1.893$^{ns}$ | 14.67±2.216$^{ns}$ | 42.33±1.944$^{ns}$ | 144.8±3.270*** | 112.8±3.420 | 23.17±3.085 |
| Test II (500 mg/kg) | 11.83±2.056$^{ns}$ | 8.500±1.176* | 109.8±1.815*** | 111.7±2.305*** | 78.50±3.722 | 20.33±1.665 |
| Test III (1000 mg/kg) | 12.00±1.949$^{ns}$ | 7.667±1.520* | 133.5±2.825*** | 119.7±2.171*** | 46.83±4.963 | 19.67±2.167 |

N=6, *p<0.05, **p<0.01, *p<0.001 when compared to control

and around 200 gm of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether (40°- 60°C), and ethahol. Finally the drug was macerated with chloroform water. Each time before extracting with the next solvent the powdered material was air dried. After the effective extraction, the solvent were distilled off, the extract was then concentrated on water bath. All the extracts were subjected to preliminary phytochemical investigation and identification of various constituents by the thin layer chromatography. Ethanol extract and aqueous extract after its preliminary phytochemical investigation was subjected to thin layer chromatography.

Polyherbal and Cow urine explored for its neuroleptics properties, using the tail suspension test (TST), light dark test and elevated plus maze test on mice. The neuroleptics activity of the extracts was compared with standard Diclofenac sodium. For the acute oral toxicity and $LD_{50}$ determination the organization for economic cooperation and development (OECD) guideline 423 was followed.

*Pharmacological Evaluation Of Polyherbal Extract*

Ethical committee clearance was obtained from IAE (institutional animal ethical committee) of CPCSEA cert. No. 461/01/C/CPCSEA

*In-vivo activity*

*Animals*

Swiss albino mice of either sex weighing between 20-30g were procured from Serum Enterprises, Pune, for experimental purpose. All the animals were acclimatized for seven days under standard husbandry conditions i.e.; room temperature of $24^{0} \pm 10^{0}$ C; relative humidity 45-55% and a 12:12h light/ dark cycle. The animals had free access to standard rat pellet diet with water provided ad libitum under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress[7].

*Elevated Plus-Maze Test in mice*

The plus-maze apparatus comprises of two open arms (16×5cm) and two closed arms (16×5×12cm) that extend from a common central platform (5×5cm). The entire maze is elevated to a height of 25cms above the floor level. Mice were placed individually in the center of the maze facing one of the enclosed arms for recording various parameters in a period of 5 min.

*Tail Suspension Test*

Depression levels of mice were examined 30 minutes after the *i.p* application of standard drug and the plant extract. Mice were suspended by their tail using adhesive tape placed approximately 1 cm from the tip of the tail attached to a wood applicator stick and hung approximately 30 cm above a table. The duration of immobility was scored manually during the test. Mice were considered immobile only when they hung passively[8].

*Light-dark model transition test in mice*

The light-dark apparatus consists of two-compartment chamber (40×60×20cm/h) comprising of a brightly illuminated area (40×40cm) and a dark area (40×20 cm) separated by a wall with a round hole (7 cm diameter) will be used. Mice were placed individually in the illuminated part of the cage and following parameters were recorded during the test session of 5 min, total no. of crossings, no. 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, no. of rearings in illuminated[9,10,11].

## RESULTS AND DISCUSSIONS

Successive extract of Polyherbal were screened for various chemical investigation and results are illustrated in Table 5.3

Qualitative chemical examinations of methanol and aqueous fruit extract srevealed the presence of Carbohydrates, Flavonoids, Steroids, Glycosides, Phenolics, Tannins and Aminoacid. Steroids was absent in aqueous extract. Mucilage was absent in both extracts.

Diazepam has long been reported for its anxiolytic activity in mice with the EPM model. In our study also, a significant anxiolytic effect was recorded with diazepam

Table 5: Anxiolytic effect of Polyherbal with Light-dark model in mice

| Treatment | No. of Crossing | Time spent in L box | Time spent in D box | No. of rearing in L box | No. of rearing in D box | No. of defecation units |
|---|---|---|---|---|---|---|
| Control | 4.167±0.600 | 95.50±5.018 | 204.5±5.018 | 6.500±0.763 | 21.33± 2.124 | 0.500± 0.341 |
| Diazepam (2 mg/kg) | 13.50±1.176*** | 219.3±3.947*** | 80.67±3.947*** | 0.6667±0.333*** | 2.500± 0.763*** | 1.167± 0.307 |
| Test I (250 mg/kg) | 6.500±0.763$^{ns}$ | 116.2±4.468** | 183.8±4.468** | 4.000±0.632* | 15.17± 0.833** | 0.6667± 0.33 |
| Test II (500 mg/kg) | 6.833±0.600$^{ns}$ | 139.8±3.655*** | 160.2±3.655*** | 3.000±0.516*** | 4.333± 0.881*** | 0.6667± 0.21 |
| Test III (1000 mg/kg) | 11.33±0.666*** | 194.5±2.405*** | 105.5±2.405*** | 1.167±0.307*** | 1.667± 0.333*** | 1.167± 0.166 |

N=6, *p<0.05, **p<0.01, *p<0.001 when compared to control

Table 6: Anti-depressant effect of Polyherbal with Tail suspension model in mice

| Treatment group | Concentration | Struggling time (in second) Mean ±SEM |
|---|---|---|
| Normal control | - | 129 ± 5.19 |
| Standard | 1 mg/kg | 266 ± 3.46 |
| Test 1 | 250 mg/kg | 210 ± 6.00 |
| Test 2 | 500 mg/kg | 244 ± 4.00 |
| Test 3 | 1000 mg/kg | 220 ± 5.29 |

as increased number of entries in to open and decreased number of entries in to closed arms and with increased time spent in open and central platform but not in closed arms. Insignificant effect was recorded with total number of entries in both the arms when compared to control. In chronic study when different doses of polyhedral i.e. 250, 500 and 1000mg/kg were administered orally daily once for seven days, it was found that lower dose (250mg/kg) increased the number of entries and time spent in the open arm, central platform and decreased the time spent in closed arm as compared to control group. Whereas medium and high doses (500 and 1000mg/kg) had increased the number of entries and time spent in the open arm, central platform and decreased the number of entries and time spent in closed arm as compared to control group and exhibited statistically significant activity.

Three different doses of Polyherbal (250, 500 and 1000 mg/kg) were subjected for anxiolytic activity using LDT model in mice. These doses when administered orally, high dose (1000mg/kg) but not the low and medium doses (250 & 500 mg/kg) had produced an increase in number of crossings and time spent in light box and decrease in the number of rearings in both light and dark compartments. High dose had statistically showed significant anxiolytic activity and Standard drug diazepam (2 mg/kg) had exhibited significant anxiolytic activity.

*Tail Suspension Test*

Three different doses of Polyherbal (250,500 and 1000mg/kg) were subjected for anti-depressant activity using TST model in mice. These doses when administered orally daily once for 7 days, high dose (1000mg/kg) but not the low and medium doses (250 & 1000 mg/kg) had produced an increased struggling time. Medium dose had statistically showed significant anxiolytic activity and Standard drug Fluoxetine (1mg/kg) had exhibited significant anxiolytic activity.

## CONCLUSION

In present study various neurological disorders like anxiety, depression, behavior changes, were evaluated in validated animal models of depression with the aim of discovering a novel agent to encompass all the problems in the existing neuroleptic therapy. To understand the treatment of various types of anxiety, it is necessary to have a detailed knowledge about anxiolytics. All anxiolytics do not act similar way to understand the pharmacology and to invent more safe and potent drugs with different screening models are very important. Nevertheless, the knowledge gathered from animal studies undoubtedly valuable therapeutically in the future studies.

Here, It can be concluded that polyherbal containing aerial part of *Centell aasiatica*, root of *Glycerrhiza glabra and* Leaves of *Gymnosporia montana* along with cow urine possess good neuroleptic action which are probably mediated via decreased influx of calcium ion or another possible mechanism is enhanced effect of the neurotransmitter gamma-amino butyric acid (GABA) at the GABAA receptors, which may have a potential benefit for the management of neurological disorders.

## ACKNOWLEDGEMENT

I gratefully acknowledge the support and generosity of The Savitribai Phule Pune University, Pune - 411 007 for the giving us an opportunity to conduct research under the Board of College and University Development Program (BCUD) with the grant of 3,00,000 /- Rs.. Without which the present study could not have been completed. I express my profound and deep sense of gratitude towards Mrs. Kinara Maheshwari, for her creative suggestions, helpful discussion, unfailing constant efforts and kind support during the project work. I would like to express my gratitude to Mr. Vivek Ingare, Mrs. Archana Yadav and Mrs. Prdnya Jagtap for

their valuable support. Thus, we have solemnized participation in this project with dedication to make it a success.

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