

Evaluation of anxiolytic and antidepressant effect of different dosage forms of the *Guduchi*

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Abstract

Background: In the field of psychopharmacology many of ayurvedic dosage forms are being researched for their anxiolytic and antidepressant effect. *Guduchi* is a well-known *Medhya Rasayan* well explained in Ayurveda classics. **Aim:** To evaluate the anxiolytic and antidepressant effect of three dosages forms of *Guduchi* (*Guduchi Satva*, *Ghana* and *Churna*) using open field test, elevated plus maze test in anxiety and behavioral despair test in depression. **Materials and Methods:** Adult Charles-Foster albino rats of either sex divided into five groups which were given carboxymethyl cellulose, lorazepam 1 mg/kg (standard anxiolytic) imipramine 10 mg/kg (standard antidepressant), *Guduchi Satva* 112.5 mg/kg, *Ghana* 45 mg/kg and *Churna* 180 mg/kg, respectively, for 22 days. **Statistical Analysis:** A statistical analysis was done by one-way ANOVA test followed by Tucky and Kramer multiple comparison tests using Graph Pad Prism 6. $P < 0.05$ was considered significant. **Result and Conclusion:** *Guduchi Satva* and *Ghana* significantly reversed the sub-acute stress-induced alterations in behavioral parameters in all the tests. *Guduchi Satva* and *Ghana* as compared to *Guduchi Churna* found to be having anxiolytic and antidepressant activity in experimental animals in behavioral parameters such as rearing, grooming, and immobility period. Thus, these formulations can be used in prevention and treatment of anxiety and depression.

Key words: Antidepressant, anxiolytic, *Churna*, dosage forms, *Ghana*, *Guduchi satva*

INTRODUCTION

Now-a-day, Hurry-Worry-Curry pattern of lifestyle leads to stress and make the person more susceptible to the diseases like anxiety and depression. Stress has a significant impact on causation of these two diseases.^[1] There are ample references for psychoneuropharmacological effects of *Guduchi* (*Tinospora cordifolia*).^[2] *Guduchi* is very well-known "*Medhya Rasayana*."^[3] In general, *Guduchi* is administered in a *Kwath* (Decoction) or *Swaras* (expressed juice) forms, but there are always certain problems which are being faced by patients as well as physicians due to non-availability of plant, stability and unpalatability of products.

Therefore, we had designed three dosages forms of *Guduchi* namely *Guduchi Satva*, *Ghana* and *Churna* to avoid above referred problems and search a dosage form which could be more effective in lesser dose.

MATERIALS AND METHODS

Materials

Dosages forms

The raw material of the test formulation was procured from Banaras Hindu University (BHU) campus in January 2012. Moreover, authenticated in Dravyaguna Department in BHU, Varanasi. Fresh *Guduchi* stem was used to prepare *Satva* and *Ghana* and dried stem was used to prepare *Churna*. *Guduchi Satva*, *Ghana* and *Churna* are prepared as per classical methods. A voucher specimen (RS-08-2008) of

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test formulations were kept in the museum of Rasa Shastra Department.

Animals

Total 60 adult Charles-Foster albino rats of either sex (150-200 g) were subjects for this study. Animals were procured from the Central Animal House of Institute of Medical Sciences, BHU. They were housed in groups of six in colony cages at ambient temperature of $25 \pm 2^\circ\text{C}$ and 50-60% relative humidity with 12 h light/dark cycle. They had free access to pellet chow (Brook Bond, Lipton, India) and water. Animals were exposed only once to every experiment. The experiment was performed after approval from the Institutional Ethics committee (2011-12/385) and the principle of laboratory animal care (NIH) Publication No. 86-23. Received 1985) guidelines were followed throughout.

Dose selection and schedule

The human dose of *Satva* is 1250 mg/day,^[4] *Churna* is 2 g/day and that of *Guduchi Ghana* is 500 mg/day.^[5] Considering adult human dose, the dose for the experimental study was calculated by converting the human dose to animal dose based on the body surface area ratio using the table of Paget and Barnes.^[6] Dose thus calculated as for *Satva* 112.5 mg/kg/day, *Ghana* 45 mg/kg/day and *Churna* 180 mg/kg/day.

Study design

Animals were divided into five groups of six animals each, comprising of both male and female in each group. Group A received carboxymethyl cellulose (0.1%) served as vehicle control; Group B received standard drug lorazepam 1 mg/kg, (Ativan, Wyeth Pharma India) for anxiolytic study and standard antidepressant imipramine 10 mg/kg (Dapsonil, Nicolas Piramal India, Ltd.) for anti-depressant activity. Group C, D and E received *Satva* 112.5 mg/kg, *Ghana* 45 mg/kg and *Churna* 180 mg/kg, respectively.

Methods

Different sub-acute variable stresses were induced in rats one by one continuously for 1 week. The rats of stress groups were subjected to the following seven types of stresses in the following order, i.e., 24 h light rearing, 24 h dark rearing, 24 h starvation, 24 h crowding, 24 h isolation, 24 h immobilization stress + 2 h immobilization and cold saline injection (1 ml once) to maximize the unpredictability on the nature of the stressors and time of delivery.

Open field test

An open field apparatus similar to that of Bronstein and modified by Jaiswal was used to study the open field exploratory behavior in rats.^[7,8] Animals were given the dose of drugs 45 min prior to the experiment. Each animal was centrally placed in the apparatus for 5 min and the following behaviors were noted. Ambulation (number of squares

crossed by the animal), immobility period (period for which animal remained immobile), rearing (number of times the animal stood on the hind limbs), grooming (number of times the animal made these responses *viz.* grooming of the face, licking/washing and scratching the various parts of the body, fecal pellets (number of fecal excreted during the period).

Elevated plus maze test

The plus maze consists of two opposite open arms, (50 cm × 10 cm) crossed with two opposite enclosed arms of the same dimension with walls 40 cm high. The arms were connected with a central square (10 cm × 10 cm) to give the apparatus a plus-sign appearance.^[9] The maze was kept elevated 50 cm above the floor in a dimly lit room. Animals were given the dose of drugs 45 min prior to the experiment. The rats were placed individually on the central square of plus maze facing an enclosed arm. The time spent and the number of entries made by the rats during the next 5 min, on the open and enclosed arm was recorded.

Behavioral despair test

The rat was placed in a cylinder (45 cm × 20 cm) containing water up to 38 cm height of the cylinder water ($25 \pm 2^\circ\text{C}$) so that the rat could not touch the bottom of the cylinder with its hind limb or tail rats also could not climb over the edge of the chamber.^[10] Two swim sessions were conducted, an initial 15 min pre-test, followed by the 5 min test 24 h later. Drugs were administered after pre-test. The period of immobility (remained floating in water without struggling and making only those movements necessary to keep its head above water) during 5 min test period was noted.

Statistical Analysis

A statistical analysis was done by one-way ANOVA test followed by *post-hoc* Tucky AND Kramer multiple comparison tests using Graph Pad Prism 6. $P < 0.05$ was considered significant.

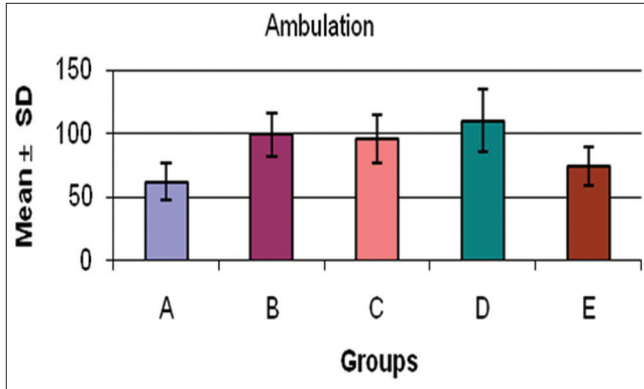
RESULTS

Data pertaining to the open-field exploratory test shows significant ambulation in standard ($P < 0.05$), *Satva* ($P < 0.05$), and *Ghana* groups ($P < 0.001$) as compared to control group [Graph 1]. More significant in *Ghana* group ($P < 0.001$) there was a significant difference between *Ghana* and *Churna* group ($P < 0.05$). For the immobility period [Graph 2] there was significant difference between standard and control group ($P < 0.01$) also between *Ghana* and control group ($P < 0.05$) also between standard and *Churna* group ($P < 0.01$). A significant difference was found between *Ghana* and *Churna* group ($P < 0.05$). In rearing [Graph 3] standard and *Ghana* were found significant over the control ($P < 0.01$). Standard found significant over the *Churna* ($P < 0.05$). There was significant difference between *Ghana* and *Churna* group

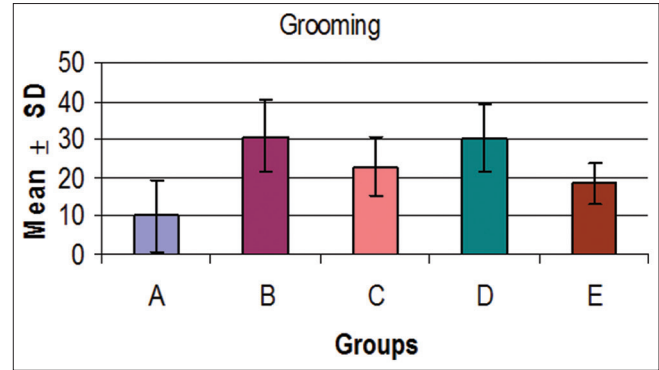
($P < 0.05$). There was significant difference for grooming [Graph 4] between standard and control group and between *Ghana* and control group ($P < 0.01$). No significant difference was found between standard and *Satva*, *Ghana* and *Churna* groups. *Satva* and *Churna* are nearer to standard. Fecal pellet [Graph 5] shows no significant variations among the groups

but was more in control and *Churna* group as compared to standard, *Satva* and *Ghana* group.

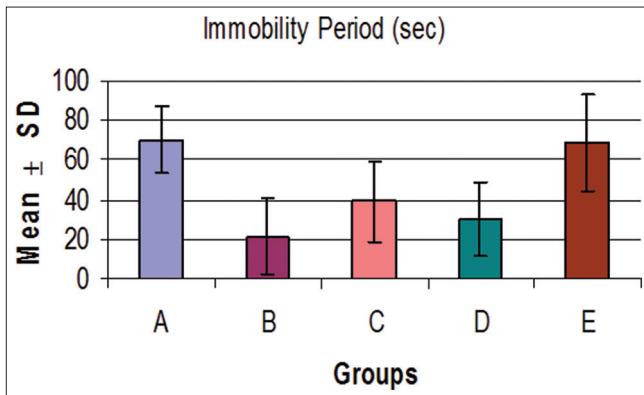
Data pertaining to elevated plus maze test shows significant increase in number of entries [Graph 6] in open arm both in standard (lorazepam) ($P < 0.01$) and *Satva* ($P < 0.05$) treated



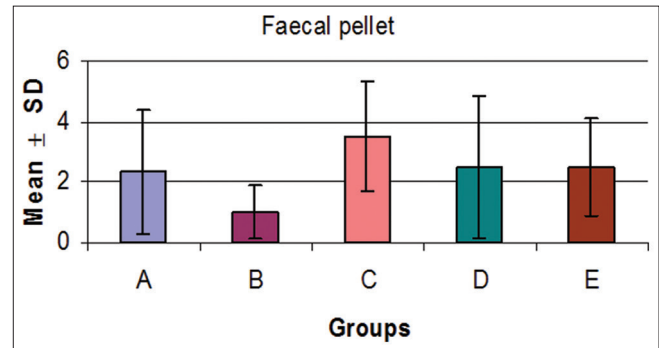
Graph 1: Effect of *Guduchi Satva*, *Ghana*, *Churna* on ambulation (number of squares crossed) in open-field test in rats (values are mean \pm standard deviation values), A - Control, B - Lorazepam, C - *Satva*, D - *Ghana*, E - *Churna*



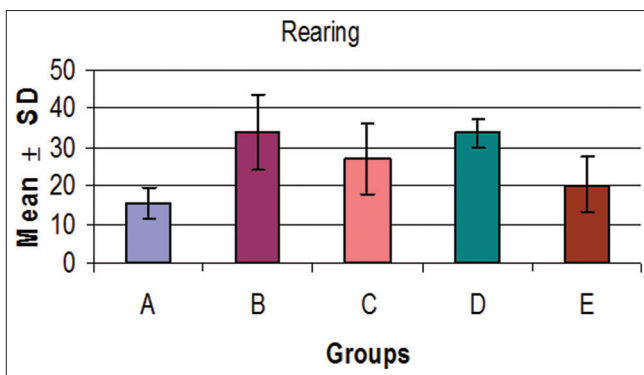
Graph 4: Effect of *Guduchi Satva*, *Ghana*, *Churna* on number of grooming in open-field test in rats (values are mean \pm standard deviation values), A - Control, B - Lorazepam, C - *Satva*, D - *Ghana*, E - *Churna*



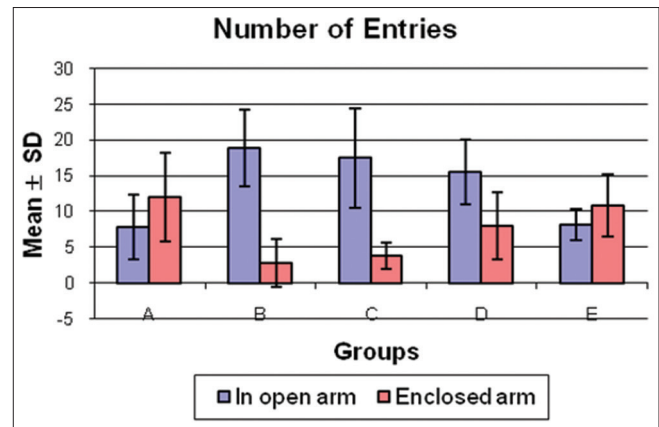
Graph 2: Effect of *Guduchi Satva*, *Ghana*, *Churna* on immobility period (seconds) in open-field test in rats (values are mean \pm standard deviation values), A - Control, B - Lorazepam, C - *Satva*, D - *Ghana*, E - *Churna*



Graph 5: Effect of *Guduchi Satva*, *Ghana*, *Churna* on number of fecal pellet in open-field test in rats (values are mean \pm standard deviation values), A - Control, B - Lorazepam, C - *Satva*, D - *Ghana*, E - *Churna*



Graph 3: Effect of *Guduchi Satva*, *Ghana*, *Churna* on number of rearing in open-field test in rats (values are mean \pm standard deviation values). A - Control, B - Lorazepam, C - *Satva*, D - *Ghana*, E - *Churna*



Graph 6: Effect of *Guduchi Satva*, *Ghana*, *Churna* on elevated plus maze behavior test in rats: Number of entries (values are mean \pm standard deviation values), A - Control, B - Lorazepam, C - *Satva*, D - *Ghana*, E - *Churna*

group as compared to control group and significant decrease in number of entries in open arm in *Churna* group as compared to standard ($P < 0.01$) and *Satva* group ($P < 0.05$). *Ghana* group is nearer to *Satva* group. *Satva* proves more significant. There was significant decrease in number of entries enclosed arm both in standard (lorazepam) ($P < 0.01$) and *Satva* ($P < 0.05$) group as compared to control group and significant increase in no. of entries enclosed arm in *Churna* group.

There was significant increase in spent time [Graph 7] in open arm in standard (lorazepam) ($P < 0.001$) *Satva* ($P < 0.01$) and *Ghana* ($P < 0.05$) group as compared to control group and significant decrease in time spent enclosed arm in *Churna* ($P < 0.001$), *Ghana* ($P < 0.01$) and *Satva* ($P < 0.05$) Group as compared to standard group. *Ghana* group is nearer to *Satva* group.

There was significant decrease in spent time enclosed arm in standard (lorazepam) ($P < 0.01$) and *Satva* ($P < 0.01$) and *Ghana* ($P < 0.01$) groups as compared to control group and increase in time spent enclosed arm in *Churna* ($P < 0.05$) group as compared to the standard group. *Churna* group significantly spent more time than and *Satva* ($P < 0.05$) and *Ghana* group as compared to standard ($P < 0.05$) group. *Ghana* group is nearer to *Satva* group.

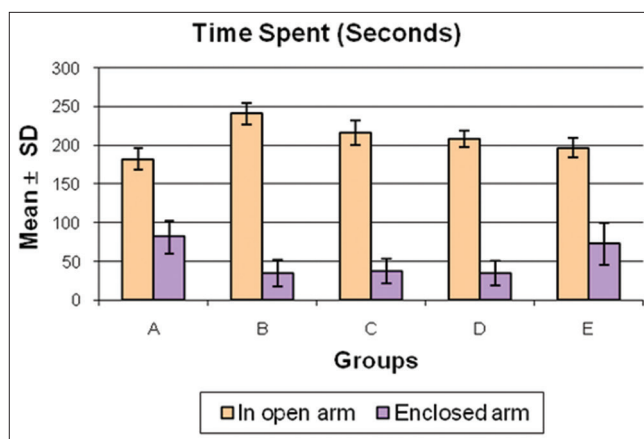
The ratio of open/closed arm [Graph 8] suggest that the ratio of number of entries was significant in lorazepam ($P < 0.001$) *Satva* ($P < 0.05$) and *Ghana* ($P < 0.05$) as compared to control group. No significant difference found in *Churna* and Control group.

The ratio of open/closed arm suggest that the ratio of time was significant in lorazepam ($P < 0.001$) *Satva* ($P < 0.05$) and *Ghana* ($P < 0.05$) as compared to control group. No significant difference was there in *Churna* and control.

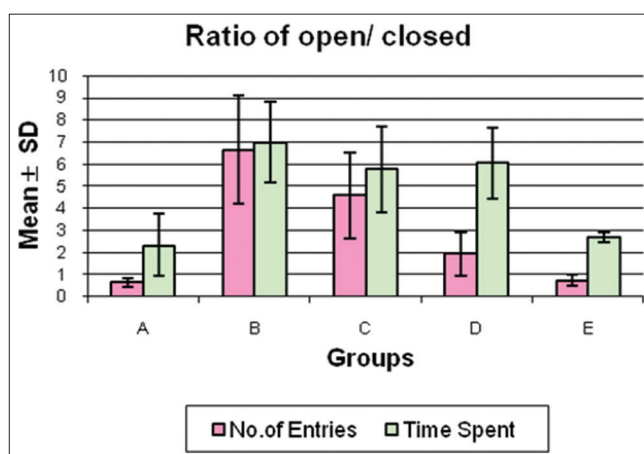
Data pertaining to the behavioural despair shows [Graph 9] immobility period was decrease significantly in *Ghana* group ($P < 0.01$) and standard (imipramine) group ($P < 0.01$), *Satva* group is nearer to *Ghana* group. Immobility period was found increase in *Churna* group as compared to the standard (imipramine) group.

DISCUSSION

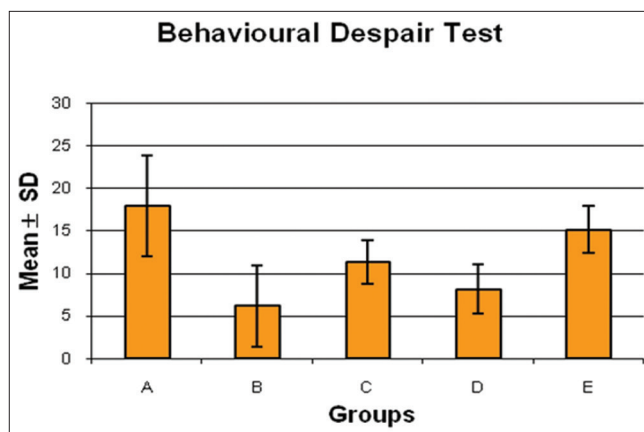
Psychopharmacology is the study of drug-induced changes in mood, sensation, thinking, and behavior.^[11] It encompasses a wide range of substances with various types of psychoactive properties.^[12] Recent researches suggest that animal models are valid for studying the antidepressant effects of drug because of strong resemblances between stressed animals and depressed humans^[13] in terms of changes in various body functions, such as, the regulation of endocrine systems, learning and memory, histology of certain parts of the brain, behavior and others.



Graph 7: Effect of *Guduchi Satva*, *Ghana*, *Churna* on elevated plus maze behavior test in rats: Time spend in seconds (values are mean \pm standard deviation values), A - Control, B - Lorazepam C - *Satva*, D - *Ghana*, E - *Churna*



Graph 8: Effect of *Guduchi Satva*, *Ghana*, *Churna* on elevated plus maze behavior test in rats: Ratio of entries and time spent (values are mean \pm standard deviation values), A - Control, B - Lorazepam, C - *Satva*, D - *Ghana*, E - *Churna*



Graph 9: Effect of *Guduchi Satva*, *Ghana*, *Churna* on immobility period in seconds in behavioral despair test in rats (values are mean \pm standard deviation values), A - Control, B - Imipramine, C - *Satva*, D - *Ghana*, E - *Churna*

Thus, in the open field test when the animals were taken from their cage and placed in a novel environment they express their anxiety and fear by decreasing ambulation and exploration, freezing, rearing and grooming behavior and increase in defecation due to heightened autonomic activity. These behavioral changes are attenuated by classical anxiolytic and augmented by anxiogenic agents.^[14] Reduced number of ambulation, grooming and rearing and increase in immobility time suggested the propensity towards the anxiety and listlessness. *Guduchi Satva* and *Ghana* significantly reversed the sub-acute stress-induced alterations in behavioral parameters in open field test [Graph 1-5]. This suggests the anxiolytic effect of *Guduchi Satva* and *Ghana*.

Elevated plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the gamma-aminobutyric acid (GABA)-benzodiazepine complex.^[15] Rodents have a natural aversion for high and open spaces and prefer closed arms therefore, spend a greater amount of time in the closed arm. When exposed to the novel maze allay, the animals experienced an approach-avoidance conflict, which was stronger in the open arms as compared to closed arms. The decreased aversion to the open arms was the result of an anxiolytic effect expressed by an increased number of open arm entries [Graph 6], and time spent in the elevated plus maze, and the decreased time spent on the central platform was another indication of a reduced decision-making behavior. Pre-treatment with *Guduchi Satva* and *Ghana* significantly increased the latency of first entry to closed arm and significantly increased the time spent in open arm [Graph 7]. This indicates the anxiolytic effect of *Guduchi Satva* and *Ghana*. Benzodiazepines act by facilitating inhibitory GABAergic.^[16] *Guduchi Satva* and *Ghana* may modulate the GABAergic system *Guduchi Satva* and *Ghana* significantly reversed the sub-acute stress-induced alterations in behavioral parameters in elevated plus maze test. This suggests the anxiolytic effect of *Guduchi Satva* and *Ghana*.

Antidepressant effect on forced swimming model is valid for a broad spectrum of antidepressants mainly including tricyclics and monoamine oxidase (MAO) inhibitors, which significantly decrease immobility time in forced swimming test (FST).^[17] Immobility is thought to reflect either a failure to persist in escape directed behavior after persistent stress, or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli.^[18] Several antidepressants reduce the immobility after forced swimming present study, *Guduchi Satva* and *Ghana* significantly decreased.^[19] In the immobility time of rat in FST and was comparable with standard antidepressant drug imipramine [Graph 9]. The observed effect may be attributed to blockage of 5-HT reuptake or MAO inhibition.

Investigations on the open field behavior and elevated plus maze paradigm according to accepted tenets showed that stressed animal were anxiety prone. *Guduchi Ghana* and *Satva* has significantly prevented stress-induced anxiety.

Guduchi Ghana and *Satva* being the most and *Guduchi Churna* being the least significant among them. This is in accordance with the earlier reported anxiolytic, activity of *Guduchi*.^[20] *Guduchi Satva* and *Ghana* treated rats reversed the stress-induced increased immobility period in the behavioral despair test. *Guduchi Ghana*, *Satva* being the most and *Guduchi Churna* being the least significant among them. This is in accordance with the earlier reported, antidepressant activity of *Guduchi*.^[21]

With proven stress-attenuating^[22] activity in addition, *T. cordifolia* has several properties generally associated with adaptogenic,^[23] antioxidant properties.^[24] It is also known to possess beneficial effects on learning, stress and memory.^[25] Earlier studies on formulation find it as an immunomodulator.^[26] Thus, the observed anxiolytic and anti-depressant profile of *Guduchi Satva* and *Ghana* may be attributed to one or more bioactive principles present in this drugs. The exact mechanism of action of the drug needs to be evaluated by further extensive studies.

CONCLUSION

Guduchi Ghana and *Satva* is most potent dosage form as compared to *Guduchi Churna* to validate anxiolytic and antidepressant action of *Guduchi* in pharmacological experimental model performed in this research work. The findings also confirm the ayurvedic claim that this plant should always used in the fresh form. The behavioral observations further add to the existing knowledge that these drugs may be used in stress-induced emotional disturbances. However, further studies are needed to elucidate the neurohumoral mechanism involved in the observed behavioral manifestations cause by the *Guduchi* under no-stress and stress condition.

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