ORIGINAL ARTICLE

Evaluation of antidepressant effect of Drakshasava in Wistar rats

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Abstract

Introduction: Depression is a common clinical condition observed in all the age group. Presently available drugs are having many adverse effects. Literature suggests Drakshasava can be used in patients suffering from anxiety and depressive disorders. **Aims:** The aim of the study was to evaluate antidepressant activity of Drakshasava in experimental models. **Materials and Methods:** The antidepressant activity was studied in albino Wistar rats using models for locomotor activity with actophotometer, forced swimming test (FST), sucrose preference test (SPT), and measuring the cortisol levels. Fluoxetine (30 mg/kg) was used as standard drugs and two doses of Drakshasav were used (2 ml, 4 ml/kg). **Results:** Drakshasav produced significant decrease in duration of immobility in FST and increase in locomotor activity and sucrose drinking. Furthermore, significant decrease in the cortisol levels was observed. **Conclusion:** Drakshasav showed promising antidepressant activity in FST and SPT. It can be used as an adjuvant in the treatment of depression.

Key words: Depression, Forced swim test, Locomotor activity, Sucrose preference

INTRODUCTION

Depression is a major affective disorder, common in all the age groups and is many times underdiagnosed. It is a disorder of mood, characterized by pervasive and persistent low mood or apathy along with other symptoms such as sadness, loss of interest and pleasure, worthlessness, guilt, physical and mental slowing, melancholia, self-destructive ideation that last at least 2 consecutive weeks and is severe enough to disrupt daily activities. Often it is seen that the feelings of hopelessness and sense of guilt increases gradually leading to recurring thoughts of suicide.[1] There are some risk factors consider to be responsible for precipitating the condition such as poverty, living alone, economical dependency, staying without spouse, not being consulted for decisions, and feeling of ill have been identified in different studies.[2,3]

Depression is a common illness worldwide, with an estimated 3.8% of the population affected, including 5.0% among adults and 5.7% among adults older than 60 years.[4] Approximately 280 million people in the world have depression.[5] The Indian scenario is not very different. The population-based study from India has reported the prevalence of depression was 15.1%. India is home to an estimated 57 million people affected by depression.[6] According to the World Health Organization, Global Burden of depression is increasing at a very high rate and projected to become the leading cause of long-term disability by 2030. Scientist's projection shows that globally depression will be a second leading cause of disease burden but in low-and middle-income countries it will be third leading cause of disease burden by 2030.[7]

There is lot of research work going on worldwide in search of the effective drug for the treatment of depression. Many drugs are getting added in the list of antipsychotic drugs, currently available drugs such as antidepressants, selective monoamine oxidase inhibitors (MAOI), selective serotonin reuptake inhibitors, and specific serotonin-noradrenaline reuptake inhibitors are not free from the adverse effects.[8] Prolonged

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usage may result into variety of minor side effects which are mainly due to anticholinergic property such as dryness of mouth, mydriasis, and constipation. Generalized CNS effects such as sleepiness, fatigue, restlessness, headache and also it may produce a serious effect like increase in suicidal tendencies.[9] Approximately two-thirds of the depressed patients respond to the currently available treatments but response takes at least 2–3 weeks to appear, full benefits take still longer and the magnitude of improvement is still unsatisfactory.[10] For many patients individualized approach is required. Hence, over the period of time quality of life of an individual drastically reduces both by the disease and by the drug treatment as well.[11]

Drakshasava is a polyherbal hydroalcoholic preparation, containing draksha, kumara, dhatkipushpa, kankol, chavak, ranuk, nagpushpa, trijat, lavang, marich, piper, chitrak, jatiphal, pipalimoola sugar, etc. These compounds are claimed to have many beneficial effects on human health such as lipid lowering specifically low density lipoproteins, cardioprotective[12] diuretic,[13] antimicrobial,[14] and antioxidant effects.[15] Drakshasava is used as general health tonic. Draksha, is the important ingredient of Drakshasava, is nothing but dried fruits of Vitis vinifera[14] The *V. vinifera* is has been studied at molecular level and observed that it contains large amount of phenolic compounds. They act as the natural antioxidants and have free radical scavenging property.

Literature suggests that the patients suffering from anxiety and depressive disorders can be effectively treated with Drakshasava with minimal adverse effects. It can improve the moods of a person and reduces the symptoms like loss of interest in day to day activities, self and surrounding, decreased appetite, loss of sleep, and suicidal thoughts.[16] But no scientific studies are available on this preparation for these activities. The present study was undertaken to evaluate the antidepressant effect of Drakshasava in animal model.

MATERIALS AND METHODS

Drugs and Chemicals

Fluoxetine hydrochloride (FLUDAC®, Cadila Pharmaceuticals, Ahmedabad, India) obtained from the pharmacy and Drakshasava (Sandu brothers) was used obtained from Ayurveda medicine shop. Other chemicals were obtained from the local supplier of chemicals.

Animals

30 Wistar rats of either sex weighing180–250 g were used for the study. The animals were housed in laboratory cages and maintained under standard light/dark cycle, temperature (23 ± 2°C) and humidity maintained conditions. The animal were given free access to food and water from aqua guard. Behavioral studies were carried out during the light phase.

The animals were allocated into five groups of six animals each:

- Group 1 – Control-Saline treated
- Group 2 – Disease control-Saline treatment
- Group 3 – Treatment with Drakshasava (2 ml/kg body weight low dose p.o.)
- Group 4 – Treatment with Drakshasava (4 ml/kg body weight high dose p.o.)
- Group 5 – Treatment with Fluoxetine (10 mg/kg p.o).

Gross observation

Animals were observed for body weight, food intake, and locomotor activity daily.

Unpredictable Chronic Mild Stress (UCMS)

UCMS protocol was used for inducing depression in the rats. It consisted of chronic exposure to unpredictable mild stressors over a period of 3 weeks. All groups, except for Group 1 (Control), were subjected to UCMS. One of the following stressors was administered daily (in random order) over a period of 3 weeks:

1 h in empty water bottle (no water); food deprivation for 20 h; water deprivation for 20 h; swimming at 4°C for 10 min; 24 h high density of housing (6 rats in a cage); 24 h isolation in separate cages (1 rat in a cage); 45° cage tilt for 17 h; soiled bedding (200 ml water in 100 g sawdust bedding) for 12 h; persistent illumination (light for 24 h); tail pinch for 1 min; and inescapable shock (1.5 mA, 15 s on, 150 s off) for 10 min. Immediately after each stress session, the rats were returned to the cage and maintained in standard conditions until the next stressor was given. On the day15-Locomotor Activity, forced swim test (FST) and sucrose preference test (SPT) were performed to confirm the depression. During the last 1 week of UCMS, animals were given daily treatment of saline, Drakshasava or fluoxetine once daily as per the groups with continue exposure to the mild stress. Locomotor activity, SPT, and FST were performed after 1 h of drug dose administration on day 22. Retro-orbital blood withdrawal was done under ketamine anesthesia for cortisol estimation in all the animals.

Locomotor Activity

The locomotor activity was assessed using an actophotometer. Actophotometer operated on photoelectric cells which was connected the circuit with a display unit. Beam of light falling on the photocell from all sides, when animal moves the beam is cutoff and a count recorded. These cutoffs was counted and recorded for a period of 10 min and taken as a measure of the locomotor activity of the animal.[17]

Forced Swim Tset (FST)

Immobility time was evaluated in the FST after 2 weeks of exposure to UCMS. Rats were individually forced to swim inside a vertical Plexiglas cylinder of a standard measurement. Initially animals swim but after 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. An animal judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. This phase is measured as the immobility time. The water was changed after each test. Efforts or struggling was taken as a positive indicator for antidepressant action.[18]

Sucrose Preference Test (SPT)

Anhedonia was assessed on day 15 and at the end of UCMS protocol, where drugs were administered in the last week of the protocol. Animals were allowed to habituate to the sucrose solution before the UCMS protocol to establish baseline preference levels. To test sucrose preference, animals deprived for 18 h food and water. Animals were presented with two bottles with specific quantity, one with the 1% sucrose solution and other with water and after 24 h the quantity consumed was measured.[19] Sucrose preference was calculated according to the formula: Sucrose preference = [sucrose intake/(sucrose intake + water intake)] × 100.

Anhedonia defined as a reduction in sucrose preference relative to baseline levels. Antidepressants reduce the anhedonia.

Cortisol Level Assessment

All rats were subjected to force swimming for 25 min. At the end of the swimming session blood was collected by retro orbital puncture under ketamine anesthesia (100 mg/kg). Plasma was separated by centrifugation, the supernatant 1 ml of plasma was collected in a separating funnel to which 25 ml of chloroform was added; it was shaken for 5 min and the organic layer was collected in a beaker. This was repeated three times. The pooled organic layers were evaporated on a boiling water bath. The residue collected was dissolved in absolute methanol and the solution was filtered through a 0.22-µm membrane filter, the filtrates were injected into the HPLC system for analysis of cortisol levels.[20]

Statistical Analysis

Data represented as mean ± SD. One-way Analysis of variance was used to evaluate the data. Differences between groups were determined by *post-hoc* analysis, using Tukey's test. Statistical significance was accepted at $P < 0.05$.

RESULTS

There was no change in counts of locomotor activity of control rats over the study period. In disease control animals the count was significantly decreased up to day 15 and remained low till the 22nd day showing the depression. Drakshasav group showed significant decrease in the count on day 15, but after the drug treatment with both the doses there was highly significant increase in the counts. Results of low dose Drakshasav were comparable to the fluoxetine. High dose of Drakshasav was more effective than the fluoxetine.

Day 15 - Immobility time was almost similar in all the groups but it was significantly ($P < 0.001$) high in comparison with the control in FST.

Day 22 - FST results shows that in control group no significant change in immobility time though there was little increase observed at the end of the study. Disease control group animals remained depressed as it is seen from the immobility period before and after treatment, rather there was increase in the immobility period after treatment. Drakshasava showed significant ($P < 0.001$) decrease in the immobility period in both the doses. Effect of Drakshasava was comparable with the results of fluoxetine ($P < 0.001$).

At baseline, the choice of drinking sucrose in all the animals was 100% but after the exposure to the chronic unpredictable stress for 15 days rats of all the groups showed significant ($P < 0.001$) decrease in the sucrose preference. Rats treated with Drakshasav showed highly significant ($P < 0.001$) increase in sucrose preference compared to disease control treated rats. Fluoxitine also increased sucrose preference compared to disease control group ($P < 0.001$). High dose Drakshasav results are comparable to fluoxetine [Figure 1].

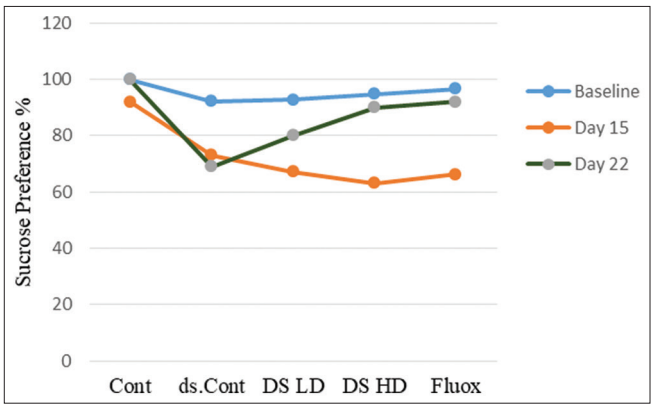

Figure 1: Sucrose preference test: Effect of drakshasav on sucrose preference. Cont: Control, ds Cont: Disease control, DSLD: Drakshasav low dose, DSHD: Drakshasav low dose, Fluox: Fluoxitine. Values are expressed as Mean ± SD, n = 6/group, $^{\$\$\$}P < 0.001$ when compared to the control. $^{***}P < 0.001$ when compared to the disease control, $^{\#\#\#}P < 0.001$ when compared to the Fluoxitine.

Cortisol levels were tested at the end of the study. It was observed that cortisol levels were significantly ($P < 0.001$) high in the disease control group. In the Drakshasav both the dose groups there was significant ($P < 0.01$) decrease in the cortisol level. Fluoxetine also reduced the cortisol levels ($P < 0.05$).

DISCUSSION

Depression is the commonest psychiatric disorder observed in all the age groups and both sexes. Population studies of children and adolescents have reported the prevalence of depression in children ranging between 0.4% and 2.5% in children and between 0.4% and 8.3% in adolescents.[21] In the elderly as well it is high and it is commonly misdiagnosed and under treated condition.[22] Many factors such as lower income, higher unemployment, and disturbed families[23] contribute in the development of depression, stress is one of the major component for the onset of neurological manifestations.[24] Depression is a significant severe hidden problem in Indian medical students and needs urgent attention and serious consideration.[25]

Pathogenesis of depression includes genetic, environmental and psychological factors and disturbances in different biogenic amines. Conventionally, the first-line treatment used is based on stabilization of the levels of key biogenic amines such as selective serotonin, norepinephrine reuptake inhibitors, and MAOI.[26] Prolonged usage of these drugs may results into variety of various side effects affects quality of life of the patients and increase in suicidal tendencies have been observed.[27] Hence, there is always a search for the effective and potent drug for the treatment of depression.

UCMS is one of the behavioral models resembling in some respects human depression.[28] Rats exposed every day to small amount of stress which was totally different from the previous and unexpected based on socio-environmental stressors.[29] Constant exposure to the stressors leads to increase in the different levels of biochemical in the brain. UCMS is a naturalistic model resembling human depression which shows anhedonia, diminished interest in, or the diminished reinforcing effects of, environmental stimuli.[30] UCMS has also shown to contribute in elucidation of the pathophysiological mechanisms of depression such as decreased neurogenesis and HPA axis alterations.[31]

Our results shows that in the locomotor activity, the number of counts of breaking the light beam on photoactometer in all the animals was significantly reduced after the chronic exposure to the stressors. Decrease in the locomotor activity correlated with the anxiety like behaviors [Figure 2]. In FST model prolonged immobility time [Figure 3] and in SPT reduced intake of sucrose water [Figure 1] was observed, which is indicative of anhedonia. Disturbance in the dopamine levels

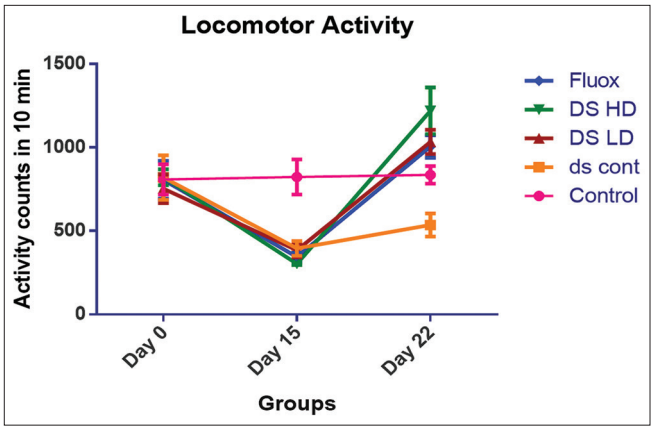

Figure 2: Effect of Drakshasava on locomotor activity in UCMS induced depressed rats. ds Cont: Disease control, DSLD: Drakshasav low dose, DSHD: Drakshasav low dose, Fluox: Fluoxitine

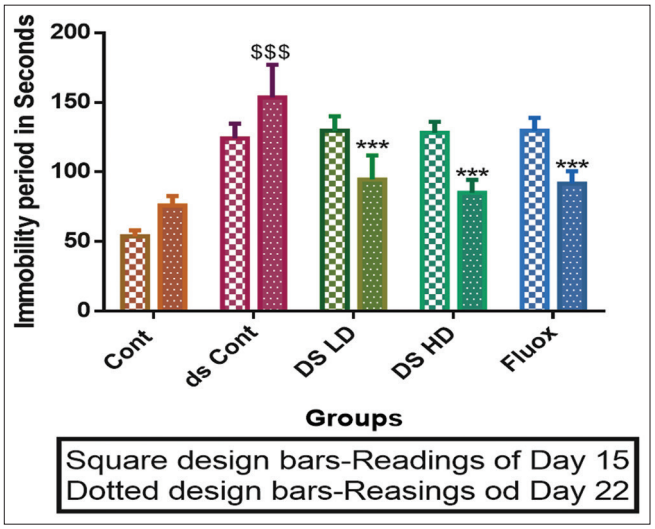

Figure 3: Effect of Drakshasav on Immobility time with FST in UCMS induced depressed rats. Cont: Control, ds Cont: Disease control, DSLD: Drakshasav low dose, DSHD: Drakshasav low dose, Fluox: Fluoxitine. Values are expressed as Mean ± SD, n = 6/group, $^{\$\$\$}P < 0.001$ when compared to the control. $^{***}P < 0.001$ in comparison with disease control

in the mesolimbic system is considered to be the basic reason for anhedonic behavior.[32]

Increase in the cortisol levels [Table 1] indicate that the animals were in the depressed state.[33] After the treatment with the Drakshasava there was highly significant increase in the locomotor count, comparable to the fluoxetine. It shortened immobility time in FST model in both the doses. Shortening of immobility time in the FST depends mainly on the increase in the 5-HT and catecholamine neurotransmission in the brain[34] and increased sucrose intake showing the elevation of the mood associated with increase in the dopaminergic activity.

Drakshasava is a mixture different herbs and these agents have marked antianxiety property. Depression is characterized by

Table 1: Effect of Drakshasav on cortisol levels in UCMS induced depressed rats

Groups	Treatment	Dose	Cortisol levels (mcg/dL)
1	Control	0.5 ml	2.48±1.13
2	Disease control	0.5 ml	5.51±0.98$^{\$\$\$}$
3	Drakshasav LD	2 ml/kg	3.60±0.95**
4	Drakshasav HD	4 ml/kg	3.41±0.45**
5	Fluoxetine	10 mg/kg	4.15±0.83$^{\$}$

LD: Low dose, HD: High dose, Values are expressed as Mean±SD, *n*=6/group, $^{\$\$\$}P$<0.001 when compared to the control, **P<0.01 when compared to the disease control, $^{\#\#\#}P$<0.001 when compared to the Fluoxitine

increase in the oxidative stress. Draksha (Grapes) is the main ingredient of drakshasava, covering of the draksha[35] contains resveratrol (RSV) which is tested for the antidepressant activity and shows its usefulness in the Alzheimer's disease[36] showing significant increase in the memory and activity as well. Ayurvedic preparation Drakshasava contains RSV in small amount,[37] since it is polyherbal medication in antidepressant effects some additional mechanisms are involved. Antidepressant effect of Drakshasava can be partly attributed to the RSV and antioxidant properties of the various ingredients in it.

In stressful condition release of glucocorticoids is the body's response to deal with it is the normal physiological phenomenon. Increase in the secretions of cortisol is a well-established aspect in the pathology of the depressive disorders.[38] Study conducted by Lee *et al*. observed significant increase in serum corticosterone levels in UCMS model[39] In our study, there was increase in the cortisol levels in all the groups when exposed to the stressors. But after the drakshasav treatment cortisol levels were significantly reduced in a dose dependent manner. Fluoxetine showed minimal effect on the cortisol levels suggestive of the efficacy of drakshasav in reducing the stress, depression, and associated manifestation. Further studies are required to evaluate the active chemical constituents responsible for the observed antidepressant activity.

CONCLUSION

Drakshasava showed positive response to all the parameters tested for antidepressant activity. It increased the locomotor activity and sucrose preference. Drakshasava reduced immobility time in FST and also showed reduction in cortisol levels. Hence, it can be an effective adjuvant in the treatment of depression for effective control on the symptoms of depression. Further studies are required to know the exact molecular mechanism of Drakshasava.

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