Original Research Article

Evaluation of psychopharmacological and neurosafety profile of Swas Kas Chintamani Ras (SKC) in Swiss-Webster mice

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Abstract
Objectives: Swas Kas Chintamani Ras (SKC) is an ayurvedic preparation indicated for respiratory diseases. Our study was aimed to determine the psychopharmacological and neurosafety profile of SKC.

Materials and Methods: Psychopharmacological effects and neurosafety profile of this drug were determined by nine complementary test methods namely, open field, locomotor activity, hole cross, hole board test, elevated plus maze, staircase, forced swimming test, and rotarod test. Male mice (Swiss-Webster strain, 20-40 g body weight) bred in the Animal House of the Department of Pharmacy, Jahangirnagar University, were used for the pharmacological experiments.

Results: The drug decreased total ambulation and movement in the central region and standing up behavior and lowered emotional defecation. The drug also made the mice to take a shorter time to come out of the cage. Also, animals spent less time in open arm and the movement in the closed arm and locomotors reduced (p=0.003), where a number of rearing (p=0.04) behaviour indicating possible anxiolytic activity. Also, no signs of antidepressant activity were observed among SKC-treated group.

Conclusion: We concluded that our drug showed no neurotoxic effect and it also showed some beneficial neuropharmacological properties.

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Introduction
Ayurvedic system of medicine is the most ancient medicine system originated in India about 3000 years ago (Ramakrishna et al., 2006). Most of the primitive traditional methods of healing such as Tibetan, Chinese and Greek medicine have been influenced by Ayurvedic medicine (Mandell et al., 2014; Dash, 1984; Dastur, 1960; Mishra and
Chandra, 2010). In Indian sub-continent, nearly 80% of the population is reported to use Ayurveda and medicinal plants to help meet their primary health care needs. Ayurveda can help maintain health in a person by maintaining the individual body's mind and spirit in perfect equilibrium with nature (Nadkarni, 1976; Verma, 1991; Sadhana et al., 2012). A well-known herbal medicine is Swas Kas Chintamani Ras, which is available as tablet formulation and used for treatment of heart diseases, lung diseases, diabetes, cough, cold and other respiratory diseases. It helps to improve strength and immunity (Mishra and Chandra, 2010; Nadkarni, 1976). This herbal preparation contains heavy metal ingredients, due to which it is recommended to only be taken under strict medical supervision (Verma, 1991; Sadhana et al., 2012). The tablets are normally 125 – 250 mg and taken once or twice a day, before or after meal or taken as directed by an Ayurvedic practitioner. It is also advised to be used along with long pepper and honey and is administered for a period of one month normally. This medicine has traditionally been administered along with a water decoction of wheat. Many companies promote this product as Chintamani Ras with GOLD. This product contains purified and processed mercury, purified and processed sulphur, purified and processed silica, iron bhasma, tin bhasma, purified asphaltum, gold bhasma, silver bhasma, leadwort, Eclipta alba and Terminalia arjuna. If gold bhasma is not included, it can not be called Chintamani Ras (Hebbar BAMS, 2015). Thus, self-medication of this medicine may be dangerous since it contains heavy metal ingredients. That is why patients are advised to take this medicine at corrected doses, for a limited duration of time and under close supervision of a doctor. Over-dosage may cause severe poisonous effects. It should not be prescribed during pregnancy and lactation and for children (Hebbar BAMS, 2015).

Studies on behavioral patterns are carried out to get a clear picture of the effect of the drugs by investigation of the pattern of behavior and emotional defecation of the animals (Boissier and Simon, 1964). Ayurvedic medicine has a good safety profile (Ernst, 2002). But, a recent study has reported that heavy metal content of the Ayurvedic preparations (e.g. lead) exhibits numerous toxicity (Keen et al., 1994). The safety profile of most of the Ayurvedic medicine preparations has not yet been completely investigated though studied drugs contain heavy metals requiring research to be carried out in this regard. As Ayurveda is becoming available in the international market with the goal of reaching herbal access for each and every part of world, elucidation of safety profile of Ayurvedic drugs is needed validate their use. After reviewing the current literature, we found that no research has been executed to validate claims of Swas Kas Chintamani Ras (SKC) as a whole aggregate for psychopharmacological activities. Hence, the present study examines psychopharmacological and neurosafety profile of SKC in Swiss-Webster mice, which was done as per the recommended approach in Ayurveda. If found to be effective, SKC may be considered a beneficial therapeutic adjuvant or for a candidate for prevention of psychopharmacological disorder.

Materials and Methods

Collection of the ayurvedic formulation

For evaluating psychopharmacological and neurosafety profile of SKC, it was collected from Sree Kundeswari Aushadhalaya Ltd, Chittagong, Bangladesh.

Experimental animal

Male mice (Swiss-Webster strain, 20-40 g body weight) bred in the Animal House of the Department of Pharmacy, Jahangirmagar University, were used for
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the pharmacological experiments. They were kept in cages (30 × 20 × 13 cm) and soft wood shavings were employed as bedding in the cage. Animals had free access to standard laboratory food and tap water ‘ad libitum’ and were maintained under the natural day-night cycle. They were fed with “mouse chow” (prepared according to the formula developed at BCSIR, Dhaka). Before starting an experiment, the animals were carefully marked on different parts of their body, which was later used as an identification mark for a particular animal so that the response of a particular mouse prior to and after the administration could be noted separately.

Doses Used In Different Experiments
For Open Field test 100, 200 and 400mg/kg body weight (BW), for locomotor test 100, 200 and 400mg/kg BW, for hole cross test 100, 200 and 400mg/kg BW, for hole board test 100, 200 and 400mg/kg BW, for elevated plus maze test 100, 200 and 400mg/kg BW, for staircase test 100mg /kg BW, for forced swim test 100mg/kg BW, and for rotarod test 100,200 and 400 mg/kg BW were used.

Psychopharmacological activity test
The open field test
In this experiment, the method developed by Gupta (1971) was employed (Gupta et al., 1971). The floor of an open field of half square meter was divided into a series of squares, each alternately colored in black and white. The apparatus had a wall of 40 cm. The number of squares, traveled by the animal, was recorded for a period of two minutes. All studies were carried out between 8 a.m. and 5 p.m.

Locomotor activity in mice
The Ugo Basile model no. 47420 Activity Cage, is great value to record spontaneous co-ordinate activity of mice (in groups of two) and measure variations in this activity with respect to time. The 47420 multiple activity cage package comprises an electronic unit 7441 and an I.R. Beam Cage, which consists of an animal cage of clear Perspex, 40×40cm, designed with two sets of emitter/sensor arrays for horizontal and vertical activity. This set-up can accept up to 5 additional cages, for a total of 6. The Electronic Units incorporate a graphic display, a thermal printer and a serial port RS232 for direct connection to the PC using the software Cat. 52050. The graphic display presents all available commands. The operator sets the experiment configuration via the keyboard located below the display. The activity data are displayed at pre-set intervals and printed/routed to the computer according to the selected configuration. The data can be customized by adding animal & experiment numbers, gender, etc. Also, 7441 is provided with an internal memory, capable of storing the data of several experiments, to be unloaded to the PC later. All studies were carried out between 8 a.m. and 5 p.m.

Hole cross test
In this experiment, the method of Takagi et al (1971) was employed (Takagi et al., 1971). In a (30 × 20 × 14 cm), a hole of 3 cm in diameter at a height of 4.5 cm from the floor, was made on the dividing wall. Spontaneous movement of the animals through the hole from one chamber to the other was counted for a period of two hours. The observation was conducted 30, 60, 120 and 240 min after oral administration of test drugs and was compared with control animal administered with normal saline. All studies were carried out between 8 a.m. and 5 p.m.

Hole board test
The Hole Board test has been conceived to study the behavior of the mouse confronted with a new environment (head plunging stereotype) according to the method devised by Boissier, Simon and Lwoff (Boissier and Simon, 1964). This

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experiment was carried out using the following method of Nakama et al, 1972 (Nakama et al., 1972). A total of 16 holes, each 3 cm in diameter, were presented to each mouse in a flat space of 25 square centimeters. Each of the animals was transferred carefully to one corner of the field and the number of ambulation (expressed as the number of holes passed), head dipping and number of fecal boluses excretion was recorded for a period of 2 minutes at 30 minutes before as well as 30, 60, 120 and 240 minutes after the treatment and compared with the control animals administered with distilled water (Nakama et al., 1972). All studies were carried out between 8 a.m. and 5 p.m.

Elevated plus maze test

The elevated plus-maze, a modification of the method used by Lister (1987) (Lister, 1987), consisted of two open arms (30 x 5 x 0.5 cm) and two closed arms (30 x 5 x 15 cm) with an open roof, arranged in a way that two pairs of identical arms were opposite to each other. Arms emerged from a central platform (5 x 5 cm), and the entire apparatus was raised to a height of 50 cm above the floor level. The maze was constructed from black plexiglass. Mice were administered with test compound and placed individually in the center of the maze, facing one of the open arms. The number of entries into both open and enclosed arms and the amount of time spent in the open arms was recorded. Each test lasted for 5 min and each mouse was tested only once. The apparatus was cleaned between each test. The test compounds were administered orally (10 mg/kg) 60 min before the test to groups of 12 mice. In each experiment, a control group received only distilled water. The treatments were randomized, and the observer was unaware of the treatment given to each group (blind method) (Simiand et al., 1984). All studies were carried out between 8 a.m. and 5 p.m.

Staircase test

The apparatus consisted of a white PVC enclosure with a five-step staircase. The box is placed in a room with constant lighting, isolated from external noise, and thermostatically controlled. Native male mice weighing 21± 3 g were used in these studies. The day before the test, the animals were randomly divided into groups of 12 mice in plastic cages. All animals used for a single experiment were placed at the same height in the animal house. They were transferred to the laboratory at least 1 hr before the start of the test. Each animal was used only once. The animal was placed singly on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears were counted during a 3-min period. A step was considered "climbed" only if the mouse had placed all four paws on the step. The number of steps descended was not taken into account in order to simplify the observations. After each test, the box was rapidly cleaned to eliminate any olfactory cue which might affect the next animal’s behavior. Experimental drugs were administered orally (10 mg/kg) 60 min before the test to groups of 12 mice. In each experiment, a control group received only distilled water. The treatments were randomized, and the observer was unaware of the treatment given to each group (blind method) (Simiand et al., 1984). All studies were carried out between 8 a.m. and 5 p.m.

Forced swim test

The most widely utilized animal model of antidepressant action is the forced swim test (FST). The traditional version of this test was developed by Roger Porsolt and colleagues (Porsolt et al., 1977) and comprises exposing mice to a 15-min swim 24 h before a 5-min test exposure in 15–18 cm of 25°C water. Following an initial period in which the rat produces escape-directed behaviors, it will adopt an immobile posture, which is believed to reflect either a failure to show a persistent escape-directed behavior or a passive behavior to cease active forms of coping with the stressful stimuli. A wide range of clinically effective antidepressants has been shown to increase the time that the rat spends in active escape behaviors. All
Finally, at all three doses (100, 200, and 400 mg/kg), SKC showed a gradual increase in emotional defecation in open field test at 240 min and differences were found to be statistically non-significant (p>0.05) when compared to the corresponding control group (Table 4).

Table 1. The effect of SKC (100, 200 and 400 mg/kg) on total ambulation in the open field test.

| Conc. (mg/Kg) | Group | Ctrl (n=6) | SKC (n=6) | 95% confidence interval |
|---------------|-------|------------|-----------|-------------------------|
| 100           | Min 0 | 82.50±9.54 | 88.00±11.19 | -38.270 to 27.270       |
|               | Min 30| 54.00±18.03| 64.50±16.52| -64.946 to 43.946       |
|               | Min 60| 33.66±9.51 | 22.33±6.38 | -14.184 to 36.850       |
|               | Min 120| 37.50±13.01| 19.83±14.14| -35.208 to 50.541       |
|               | Min 180| 29.83±14.14| 12.83±5.07 | -16.460 to 50.460       |
|               | Min 240| 21.17±6.25 | 16.00±7.13 | -15.958 to 26.292       |
| 200           | Min 0 | 20.67±6.69 | 34.50±15.69| -51.853 to 24.186       |
|               | Min 30| 9.33±1.81  | 25.83±12.07| -47.466 to 14.466       |
|               | Min 60| 19.67±13.04| 12.17±9.23 | -28.113 to 43.113       |
|               | Min 120| 20.00±8.69 | 8.17±4.08  | -10.806 to 34.472       |
|               | Min 180| 11.00±6.34 | 16.33±9.04 | -29.948 to 19.281       |
|               | Min 240| 13.17±6.05 | 7.17±3.18  | -9.941 to 21.941        |
| 400           | Min 0 | 16.33±7.77 | 22.00±11.72| -37.001 to 25.668       |
|               | Min 30| 4.67±1.46  | 8.50±7.02  | -21.277 to 13.610       |
|               | Min 60| 1.67±1.67  | 6.33±2.88  | -12.086 to 2.753        |
|               | Min 120| 9.17±4.53  | 20.00±8.03 | -31.387 to 9.720        |
|               | Min 180| 18.17±7.71 | 10.83±5.37 | -13.614 to 28.281       |
|               | Min 240| 20.67±11.68| 4.83±2.47  | -10.762 to 42.428       |

F.N: NS= Not Significant.

Table 2. The effect of SKC (100, 200 and 400 mg/kg) on the center ambulation in the open field test.

| Conc. (mg/Kg) | Group | Ctrl (n=6) | SKC (n=6) | 95% confidence interval |
|---------------|-------|------------|-----------|-------------------------|
| 100           | Min 0 | 2.67±1.08  | 0.17±0.17  | -0.284 to 5.284         |
|               | Min 30| 0.33±0.33  | 0.50±0.50  | -1.505 to 1.172         |
|               | Min 60| 0.17±0.17  | 0.17±0.17  | -0.525 to 0.525         |
|               | Min 120| 0.17±0.17 | 0.50±0.34 | -1.180 to 0.513         |
|               | Min 180| 0.17±0.17 | 0.00±0.00 | -0.261 to 0.595         |
|               | Min 240| 0.00±0.00 | 0.17±0.17 | -0.595 to 0.261         |
| 200           | Min 0 | 0.00±0.00  | 0.00±0.00  | 0.000 to 0.000          |
|               | Min 30| 0.00±0.00  | 0.33±0.33  | -1.190 to 0.523         |
|               | Min 60| 0.33±0.33  | 0.00±0.00  | -0.523 to 1.190         |
|               | Min 120| 0.00±0.00 | 0.00±0.00 | 0.000 to 0.000          |
|               | Min 180| 0.33±0.33 | 0.33±0.33 | -1.050 to 1.050         |
|               | Min 240| 0.33±0.33 | 0.00±0.00 | -0.523 to 1.190         |
| 400           | Min 0 | 0.00±0.00  | 0.00±0.00  | 0.000 to 0.000          |
|               | Min 30| 0.00±0.00  | 0.00±0.00  | 0.000 to 0.000          |
|               | Min 60| 0.17±0.17  | 0.00±0.00  | -0.261 to 0.595         |
|               | Min 120| 0.17±0.17 | 0.17±0.17 | -0.525 to 0.525         |
|               | Min 180| 0.17±0.17 | 0.00±0.00 | -0.261 to 0.595         |
|               | Min 240| 0.17±0.17 | 0.00±0.00 | -0.261 to 0.595         |

N.B: NS= Not Significant.
Table 3. The effect of SKC (100, 200, and 400 mg/kg) on the standing up behavior in the open field test.

| Conc. (mg/kg) | Group   | Ctrl (n=6) | SKC (n=6) | 95% confidence interval |
|---------------|---------|------------|-----------|-------------------------|
| 100           | Min 0   | 0.33±0.33  | 0.67±0.67 | -1.994 to 1.327         |
| 200           | Min 0   | 0.33±0.33  | 0.67±0.67 | -1.994 to 1.327         |
| 400           | Min 0   | 0.33±0.33  | 0.67±0.67 | -1.994 to 1.327         |

N.B: NS= Not Significant.

Table 4. The effect of SKC (100, 200 and 400 mg/kg) on the defecation in the open field test.

| Conc. (mg/kg) | Group   | Ctrl (n=6) | SKC (n=6) | 95% confidence interval |
|---------------|---------|------------|-----------|-------------------------|
| 100           | Min 0   | 0.17±0.17  | 0.17±0.17 | -0.52 to 0.525          |
| 200           | Min 0   | 0.17±0.17  | 0.17±0.17 | -0.52 to 0.525          |
| 400           | Min 0   | 0.17±0.17  | 0.17±0.17 | -0.52 to 0.525          |

N.B: NS= Not Significant.

SKC-treated mice exerted locomotor activity at the 4th and 5th hour at the dose of 100 mg/kg, at the 3rd, 4th and 5th hour at the dose of 200 mg/kg and at the dose of 400 mg/kg SKC showed locomotor activity from the 1st hr to end of the 5th hour (Table 5). All three doses of SKC exerted a decreased at the 6th hour which was significantly different from that of the control group (p<0.05). Again 200 and 400 mg/kg of SKC showed significant increases in locomotor activity (p<0.05) at 195 and 60 min, respectively. Mice treated with SKC 100, 200 and 400 mg/kg showed increases in the motor activity with no domino effect when study carried out at hole cross board (Table 6). But, all of the results were non-significant different from the control group (p>0.05). An interesting significant (p<0.05) difference was found between SKC 100 mg/kg and the control group where total ambulation in the hole board test was decreased at the 1st hr of the experimental period. Overall decreases in head dipping activity were observed at all three doses. However, all of the results were found statistically insignificant (p>0.05) while compared with control group. SKC-treated group showed a significant (p<0.01) increase in emotional defecation at 200 mg/kg from 30 min to 120 min (Table 7).

Table 5. The effect of SKC (100, 200 and 400 mg/kg) at the 1st to the 6th hr of the locomotor test.

| Conc. (mg/kg) | Group   | Ctrl (n=6) | SKC (n=6) | 95% confidence interval |
|---------------|---------|------------|-----------|-------------------------|
| 100           | Hr1     | 22.92±3.51 | 21.93±3.85 | -5.259 to 11.255        |
| 200           | Hr1     | 40.78±3.76 | 38.92±3.09 | -8.976 to 12.686        |
| 400           | Hr1     | 50.89±2.9 | 50.41±1.15 | -3.834 to 4.808         |

N.B: Here, Hr stands for hour, * indicates p<0.05.

All three doses non-significantly (p>0.05) decreased emotional defecation and overall increased effect in emotional defecation throughout the experimental period in comparison to the control group (Tables 8 and 9). At 200 mg/kg, SKC showed an increase in the time spent in the

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open arm from 120 min to the end of 240 min. At 400 mg/kg, SKC-treated male mice did not exhibit any interesting results rather decreasing effect (Table 10). SKC at all three doses (100, 200, and 400 mg/kg) exerted overall decrease in total movement in close arm on elevated plus maze test in comparison to the respective control group. At the dose 100mg/Kg, exceptions were being observed at 180 min and 240 min. But all of the results were found statistically insignificant (p>0.05) while compared with control group (Tables 11).

Table 6. The effect of SKC (100, 200 and 400 mg/kg) in the hole cross test.

| Conc. (mg/Kg) | Min 0 | Min 30 | Min 60 | Min 120 | Min 180 | Min 240 |
|---------------|-------|--------|--------|---------|---------|---------|
| 100           | 0.00±0.00 | 2.00±0.93 | 3.83±1.37 | 5.00±1.06 | 2.83±1.22 | 3.17±0.10 |
| 200           | 1.50±0.50 | 3.17±1.19 | 2.00±0.86 | 2.83±1.51 | 2.00±1.17 | 3.67±0.32 |
| 400           | 0.83±0.30 | 2.83±0.52 | 2.00±0.75 | 3.50±0.72 | 2.17±0.54 | 2.00±0.75 |

N.B: NS= Not Significant.

Table 7. The effect of SKC (100, 200 and 400 mg/kg) in the total ambulation of hole board test.

| Conc. (mg/Kg) | Min 0 | Min 30 | Min 60 | Min 120 | Min 180 | Min 240 |
|---------------|-------|--------|--------|---------|---------|---------|
| 100           | 19.00±4.02 | 20.83±4.88 | 23.83±4.07 | 21.67±3.22 | 18.00±3.18 | 23.17±7.03 |
| 200           | 18.00±4.02 | 20.83±4.88 | 23.83±4.07 | 21.67±3.22 | 18.00±3.18 | 23.17±7.03 |
| 400           | 18.00±4.02 | 20.83±4.88 | 23.83±4.07 | 21.67±3.22 | 18.00±3.18 | 23.17±7.03 |

N.B: ** shows p<0.01 (highly significant).

Table 8. The effect of SKC (100, 200 and 400 mg/kg) in the head dipping of hole board test.

| Conc. (mg/Kg) | Min 0 | Min 30 | Min 60 | Min 120 | Min 180 | Min 240 |
|---------------|-------|--------|--------|---------|---------|---------|
| 100           | 1.00±0.63 | 1.00±0.45 | 1.00±0.45 | 1.00±0.45 | 1.00±0.45 | 1.00±0.45 |
| 200           | 0.67±1.42 | 1.83±1.32 | 1.83±1.32 | 1.83±1.32 | 1.83±1.32 | 1.83±1.32 |
| 400           | 0.50±0.34 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |

N.B: NS= Not Significant.

Table 9. The effect of SKC (100, 200 and 400 mg/kg) on the emotional defecation of hole board test.

| Conc. (mg/Kg) | Min 0 | Min 30 | Min 60 | Min 120 | Min 180 | Min 240 |
|---------------|-------|--------|--------|---------|---------|---------|
| 100           | 0.17±0.17 | 0.17±0.17 | 0.33±0.33 | 0.33±0.33 | 0.33±0.33 | 0.33±0.33 |
| 200           | 0.67±0.42 | 1.50±0.62 | 1.00±0.52 | 0.33±0.33 | 0.33±0.33 | 0.33±0.33 |
| 400           | 0.67±0.42 | 1.50±0.62 | 1.00±0.52 | 0.33±0.33 | 0.33±0.33 | 0.33±0.33 |

N.B: NS= Not Significant.

SKC 100mg/kg (male mice) group decreased both the locomotor (p<0.001) and the number of rearing (p<0.01) in the staircase test, as compared to the corresponding control group. It can be suggested that SKC has mild anxiolytic activity (Figure 1).

In the force induced swimming test, experimental group treated with SKC 100mg/kg showed an overall increase in immobile phase compared to their corresponding control group (Figure 2).
The rate of increasing immobile phase was much greater at the 24\textsuperscript{th} hr than 2\textsuperscript{nd} hr after drug administration. At the end of 24\textsuperscript{th} hr, we found an increase in immobile phase which was statistically highly significant (p<0.01). In the rota rod test, male mice treated with SKC 100, 200 and 400 mg/kg revealed an overall decrease in total fall and data obtained was comparable to the respective control group with an exception at the dose level of 100mg/kg at min 240 (Table 12).

Table 10: The effect of SKC (100, 200 and 400 mg/kg) on the time spent in open arms.

| Conc. (mg/Kg) | Group | Ctrl (n=6) | SKC (n=6) | 95% confidence interval |
|---------------|-------|------------|-----------|-------------------------|
| 100           | Min 0 | 2.67±2.29  | 0.50±0.50 | -3.056 to 7.389         |
|               | Min 60| 9.00±4.27  | 4.00±2.58 | -6.125 to 16.125        |
|               | Min 120| 3.67±2.65 | 0.50±0.50 | -3.640 to 9.973         |
|               | Min 180| 1.00±0.68 | 1.17±0.74 | -2.425 to 2.092         |
|               | Min 240| 5.17±4.25 | 0.83±0.54 | -5.204 to 13.870        |
| 200           | Min 0 | 0.00±0.00  | 0.00±0.00 | 0.000 to 0.000          |
|               | Min 60| 2.00±2.00  | 0.00±0.00 | -3.141 to 7.141         |
|               | Min 120| 5.00±5.00 | 1.00±1.00 | -2.991 to 1.991         |
|               | Min 180| 0.67±0.67 | 0.00±0.00 | -1.047 to 2.380         |
|               | Min 240| 3.00±2.16 | 2.00±2.00 | -5.559 to 7.559         |
| 400           | Min 0 | 0.83±0.83  | 0.50±0.50 | -1.832 to 2.498         |
|               | Min 60| 0.00±0.00  | 0.00±0.00 | 0.000 to 0.000          |
|               | Min 120| 2.00±0.93 | 0.00±0.00 | -393 to 4.393           |
|               | Min 180| 4.17±3.60 | 0.83±0.83 | -4.900 to 11.567        |
|               | Min 240| 6.50±3.36 | 0.00±0.00 | -2.147 to 15.147        |

N.B: NS= Not Significant.

Table 11. The effect of SKC (100, 200 and 400 mg/kg) in the total movement in close arms.

| Conc. (mg/Kg) | Group | Ctrl (n=6) | SKC (n=6) | 95% confidence interval |
|---------------|-------|------------|-----------|-------------------------|
| 100           | Min 0 | 10.50±1.43 | 10.50±2.86| -7.127 to 7.127         |
|               | Min 60| 7.50±2.46  | 5.00±1.59 | -4.027 to 9.027         |
|               | Min 120| 4.83±1.68 | 2.83±1.10 | -2.487 to 6.487         |
|               | Min 180| 3.00±0.89 | 4.16±1.72 | -5.689 to 3.355         |
|               | Min 240| 5.67±1.47 | 8.17±2.55 | -9.061 to 4.061         |
| 200           | Min 0 | 4.17±1.30  | 4.83±1.83 | -5.676 to 4.343         |
|               | Min 60| 6.67±1.40  | 3.83±1.22 | -1.318 to 6.985         |
|               | Min 120| 6.83±2.31 | 2.83±1.22 | -1.833 to 9.833         |
|               | Min 180| 6.00±1.15 | 5.67±0.84 | -2.852 to 3.519         |
|               | Min 240| 6.83±0.70 | 6.00±1.77 | -3.735 to 5.402         |
| 400           | Min 0 | 6.17±1.14  | 4.33±1.89 | -3.084 to 6.571         |
|               | Min 60| 2.67±0.76  | 1.83±0.31 | -1.129 to 2.796         |
|               | Min 120| 4.50±1.65 | 3.00±0.93 | -2.717 to 5.717         |
|               | Min 180| 5.00±2.17 | 2.67±1.26 | -3.264 to 7.930         |
|               | Min 240| 4.00±1.24 | 2.67±1.28 | -2.638 to 5.305         |

N.B: NS= Not Significant.

Figure 1. Tabular presentation of the effect of SKC (100 mg/kg) on the Stair Case Test utilizing male mice. F.N: * indicates P<0.05 (Significant), ** indicates P<0.01 (Highly Significant) and *** indicates P<0.001 (Very Highly Significant).

Figure 2. Graphical presentation of the effect of SKC (100 mg/kg) on the Forced Induced Swimming Test utilizing male mice.

N.B: ** shows P<0.01 (Highly Significant)


**Discussion**

In the present study, Swas Kas Chintamani Ras (SKC) was evaluated for the psychopharmacological and neurosafety properties. Despite intensive efforts made to discover novel psychiatric drugs for psychotic and anxiety disorders over the past two decades, unfortunately, all drugs have shown marked side effects. In this respect, Ayurvedic medications could be an attractive candidate as therapeutic strategies for treatment of these conditions (Calixto et al., 2000; Fisher et al., 1994). Reduction in the locomotor activity indicates CNS depressant property of a drug. SKC increased locomotor activity at 4th hr at 100 mg/kg but when doses increased, this effect was only observed at the very beginning of the experimental period. All doses of SKC increased interest in crossing the hole but decreased ambulation and head dipping activity which supporting neuro safety status of this drug.

Concerning psychopharmacological effects of the drug, mice spent less time to come out of the cage, but only at min 60, they spent much longer duration than any other examined dose. They also spent shorter time in open arm and had less movement in the closed arm and locomoters, where the number of rearing behavior indicating possible anxiety or anxiolytic activity. EPM test is one of the most frequently used animal models in behavioral psychopharmacology for screening drugs with potential anxiolytic effects (Wall et al., 2000). In general, reduction or increase in the number of entries and times spent in the open arms induced by a given substance, had been regarded as good indicators of its anxiogenic or anxiolytic effects, respectively (Pellow et al., 1985). The present findings reveal that administration of SKC could exhibit the anxiolytic-like effect in this paradigm. This may be due to modulation of GABA receptors by SKC. During this study, SKC did not produce any considerable changes in the elevated plus maze model. The exposure of mice to an elevated and open maze induces an exploratory cum fear drive which results in anxiety (Handley and McBlane et al., 1993; Kannan et al., 2011). Anxiolytic substances are act by ameliorating the open arm exploration, decreasing anxiety, as well as increasing the number of entries into the open arm. The SKC successfully showed such potentials at both doses demonstrating that the plant at the studied doses possesses anxiolytic activity. The result of stair case test revealed that treatment with SKC significantly decreases locomotor activities and the number of rearing behavior indicating possible anxiolytic activity. No anti-depressant activity was observed in forced swimming test among SKC-treated group. When mice are forced to swim in an inescapable situation, they tend to become immobile after initial vigorous activity. The immobility reflects a state of lowered mood in which the animals give up hope of finding an exit and resign to the experimental situation. This absence of immobility has been described as a symptom of “behavioral despair” (Karolewicz et al., 2001). Also, SKC showed significant decreases in immobility time enforcing antidepressant activity of the plant extract, which is opposite to the effect shown by classical antidepressant drugs like fluoxetine (Porsolt, 1977; Willner et al., 1990). Several previous studies have supported the present findings (Borsini et al., 1988; Yonko et al., 1984; Novas et al., 1988). In the present, we aimed to examine whether metals present in the formulation induce any neurological or psychological toxicity or not. But fortunately, not only we found no neurotoxicity following administration of this Ayurveda formulation, but also we found some medicinal activities.

Our results confirmed that SKC possesses significant antidepressant, antipsychotic and anxiolytic activity. The results are encouraging to pursue further studies to discover the underlying mechanisms and also to isolate and
characterize responsible bioactive molecule(s). The result of this present study may be create better pathway to generate preparation for drugs with neuro safety and alternative pathway to reveal other medical value of any established herbal preparation. Thus, further research must be devoted towards determination of the qualitative and quantitative composition of SKC and isolation of biologically active compounds with full elucidation of precise mechanisms of activity. With regard to the currently available medical treatments for psychiatric disorders, the results obtained from the present research seem to be important because not only anxiolytic effects were observed but also antidepressant activity was also shown by SKC. These behavioral effects were supported by some other previous findings treating mice with classical antidepressant drugs. Moreover, SKC did not modify the spontaneous locomotor activity of the animals, therefore, it is probable that these effects are not mediated by stimulation of general motor activity.

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Conflicts of interest
None declared

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