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Original Research Article

Evaluation of adaptogenic and antidepressant activity of hydroalcoholic leaves extract of Salacia chinensis in mice

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ABSTRACT

Aim: Study was carried out to evaluate the Adaptogenic and Antidepressant effect of Salacia chinensis leaf extract in animal model.

Introduction: Salacia chinensis is a plant species belonging to the family Celastraceae. This study was carried out to evaluate the Adaptogenic and Antidepressant effect of Salacia chinensis leaf extract in animal model. Swimming Endurance Test was used to evaluate the Adaptogenic activity in albino mice while Tail Suspension Test and Elevated Plus Maze test was used to evaluate the Antidepressant activity in albino mice. In Swimming Endurance model various biochemical parameters like blood glucose, cholesterol, triglycerides were estimated, weight of organs like liver, spleen and adrenal gland was measured in stress control and extract treated animals. Immobility as behavioural parameter was assessed in Tail Suspension Test and Elevated Plus Maze model of depression after treatment with hydroalcoholic extract of Salacia chinensis leaf at selected dose of 200 mg/kg and 400 mg/kg. Results of the study have showed significant Adaptogenic and Antidepressant activity of Salacia chinensis.

Materials and Methods: Preliminary Phytochemical Analysis (15) Salacia chinensis leaves extract was subjected to chemical tests for the identification of their active constituents.

Result: Phytochemical analysis: The results have indicated the presence of flavonoid, alkaloids, terpenoids, saponins, glycosides, carbohydrates.

Swimming Endurance Test: The biochemical estimation results obtained showed decrease in blood glucose, triglyceride, and slight increase in cholesterol level when compared to control which support the adaptogenic activity of the title plant as shown in table no: 02,03,04 and fig no: 03,04,05,06,07.

A. Tail suspension test: The duration of immobility was reduced after treated with hydroalcoholic extract of Salacia chinensis and results are significant on both high dose 400 mg/kg, p.o. ($P<0.001$) and low dose 200 mg/kg, p.o. ($P<0.01$) with that of standard Imipramine (10 mg/kg, p.o.) indicating that the extract possess significant antidepressant activity. Results depicted in table no: 05 and fig no: 08,09.

B. Elevated plus maze method: The hydroalcoholic extract of Salacia chinensis at high dose (400 mg/kg, p.o) ($P< 0.05$) significantly increased the number of entries in to open arm and total time spent in open arm as compared to control.

It shows increase in the mobility of the mice in open arm which suggest the antidepressant activity shown in table no:06 and fig no: 10,11.

Conclusion : Based on the results obtained from this study it can be concluded that the hydroalcoholic extract of Salacia chinensis leaves possess Adaptogenic and Antidepressant activity.

This beneficial effect of Salacia chinensis leaf might be due to presence of flavonoids, alkaloids and its derivatives.

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1. Introduction

1.1. Introduction to herbal medicine

Medicinal plants are an important source of new chemical entities with potential therapeutic values. More than 75% of the total population in developing countries relies on traditional medicines based on plant products.[1] In the search for new therapeutic products for the treatment of neurological disorders, medicinal plants research worldwide has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal model.[2] As we study the ways in which herbs work, we come to understand the various levels of relationship between herbs and the human body. On the most basic physical level, we can identify a plant's chemical compounds and observe the various physiological reactions elicited in the human body. The great gift of herbal medicine is that it humbly offers to us its healing power and its mystical wisdom, which we can only partially understand.[3]

1.2. Adaptogen

Adaptogens are herbs that are non-toxic, produce a nonspecific defensive response to stress, and have a normalizing influence on the body. Adaptogens help the body adapt to stress, support its normal function, and restore balance. They increase the body's resistance to physical, biological, emotional and environmental stressors.[4]

1.3. Stress

Stress is basically a reaction of mind and body against change in the homeostasis. The productive stress is called Eustress while harmful stress is called Distress. If the stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. "Stress had been coined as the "mother of all diseases". Stress is involved in the pathogenesis of a variety of diseases that includes psychiatric disorders such as depression and anxiety, immuno-suppression, endocrine disorders including diabetes mellitus, male impotence, cognitive dysfunction, peptic ulcer, hepato and nephro dysfunction, hypertension, and ulcerative colitis.[5]

1.4. Common stressor

1. **Spiritual:** A lack of purpose in life and a diseased soul
2. **Psychological:** Anger, fear, sadness, concern, anxiety, sorrow, loss, desire, significant life changes, mental disease, psychological trauma, and a heavy burden of responsibility.
3. **Nutritional:** Food allergies, nutritional inadequacies, alcoholism and drug use, diets lacking in minerals, and free radicals.
4. **Biological:** Exposure to viruses, parasites, germs, and mould
5. **Physical:** High blood pressure, surgery, trauma, severe sickness, infection, hard activity, lack of oxygen, drunkenness and drug usage, loss of sleep, chronic overstimulation, pregnancy, and having a new baby are all factors that can affect blood pressure.
6. **Chemical:** exposure to heavy metals, toxins, household and industrial chemicals, herbicides, pesticides, fungicides, insecticides, fumes, smoking, cigarettes, and synthetic medications.
7. **Environmental:** exposure to extremes in temperature, noise, barometric pressure fluctuations, altitude changes, allergens, xenoestrogens, UV light, microwaves, high-voltage electric lines, radio waves, and radiation, as well as other factors.[6]

2. General Adaptation Syndrome (GAS)

General adaptation syndrome is a theorized physiological response to stress developed by Hans Selye. This theory was developed after experimenting and observing the physiologic responses of animals to stress.[5]

2.1. General adaptation syndrome stages

1. Stage-1: Alarm Reaction
2. Stage-2: Resistance
3. Stage-3: Exhaustion

Alarm is the first stage. The body's stress response is a state of alarm when a threat or stressor is perceived or experienced. In order to trigger the fight-or-flight response, adrenaline will be created during this period. The Hypothalamic-Pituitary-Adrenal (HPA) axis is also activated, resulting in cortisol production.

Resistance is the second stage. If the stressor persists, it will be vital to try to cope with it in some way. Although the body begins to adapt to environmental stress or demands, it cannot do so indefinitely, and its resources are gradually depleted.

Exhaustion is the third and final stage in the GAS model. All of the body's resources are finally drained at this stage, and the body is unable to function normally. The symptoms of the autonomic nervous system that first appeared may return (sweating, raised heart rate etc.). Long term harm may happen if stage three is prolonged because the body and immune system are fatigue and function is compromised, culminating in decompensation. As a result, apparent ailments such as ulcers, depression, and diabetes, as well as digestive and cardiovascular problems, might occur, as well as other mental illnesses.

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2.2. Depression

Depression is one of the most prevalent and life-time threatening forms of mental illness characterized by the absence of positive impact by extreme exaggeration, lack of interest and mood disturbance, which adversely affect cognition and psychomotor function.[7,8] Sorrow and sadness are normal human emotions, everybody has these feelings but not last longer, major depression is more where it's a period of overwhelming sorrow.[9]

The burden of depression and other mental health conditions is on the rise globally. Although a number of synthetic drugs are being used as the standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment. Several drug-drug interactions can also occur. These conditions create an opportunity for alternative treatment of depression by using herbal medicine.[10]

3. Review of Literature

3.1. Botanical classification

1. Kingdom: Plantae
2. Division: Tracheophyt
3. Class: Magnoliopsida- dicots, dicotyledones
4. Subclass: Rosids
5. Order: Celastrales
6. Family: Celastraceae
7. Genus: Salacia
8. Species: S. chinensis
9. Binomial name: Salacia chinensis
10. Synonym: Chinese salacia, lolly berry, saptachakra[11,12]

Figure 1: Salacia Chinensis leaves

3.2. Vernacular name

1. Common name: Chinese Salacia, Holly-leaved, Spindle tree
2. Kannada: Ekanayaka
3. Marathi: Saptarangi
4. Sanskrit: Saptachakra, Eanayaka, Pitika
5. Telugu: Nerani
6. Tulu: Ekanayake
7. Tamil: Cuntan, Karukkuvai
8. Malayalam: Cherukoranti, Ekanayakam

Figure 2: Salacia Chinensis leaves

3.3. Distribution

Salacia chinensis (Celastraceae) is Seven species of Salacia such as S.beddomei, S.chinensis, S.fruticosa, S.gambleana, S.macrosperma, S.malabarica and S.oblongawere is native India, Sri Lanka, China, and Southeast Asia to New Guinea.[13]

3.4. Acute toxicity studies

An acute oral toxicity study for the extract was conducted in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines No. 423. It has been reported that Salacia chinensis seems to be safe at a dose level of 2000-4000 mg/kg b.w.[14]

4. Materials and Methods

Experimental animal: Male albino mice of swiss strain, weighing about 25-30grm.

Table 1: Phytochemical screenings

Test for phytoconstituents of chinebsis leaves extract	
Phytoconstituents	Salacia chinensis leaves extract
Alkaloids	+
Terpenoids	+
Saponins	+
Proteins	+
Phenol	+
Glycosides	+
Steroids	+
Tannins	+
Carbohydrates	+
(+) present	(-) absent

4.1. Methods

Preliminary Phytochemical Analysis [15]

Salacia chinensis leaves extract was subjected to chemical tests for the identification of their active constituents.

4.2. Method of collection of data

The leaves of Salacia chinensis was collected from the Indian Institute of Horticultural Research Hesaraghatta. The sample was identified and authenticated by Botanist. A herbarium specimen was preserved in the college museum for future reference. Fresh leaves were cleaned, and shade dried at room temperature.

4.3. Extraction procedure

The sample of Salacia chinensis leaves were blended to powder (100g) after that extracted with methanol by using Soxhlet's extaction method Thereafter, the extract was evaporated to dryness over water bath. Finally the concentrated crude extract was used for the study. [16]

4.4. Evaluation of adaptogenic activity

4.4.1. Swimming endurance test in mice [17]

Animal: Albino mice weighing 20-30gms

Adaptogenic activity was carried out by placing the mice individually in a transparent glass cylinder (12 cm in diameter, height 25 cm), which was filled with water to a height of 15 cm. Two swim sessions were conducted. An initial 15-min pre-test followed 24 hour later by a 6-minute test. In the pre-test session, the mice which have not yet treated was made to swim in a glass cylinder for 15 min. In the second session, each mouse received a respective dose of sample 1 hour prior to test, and was placed in the cylinders.

The following behaviors were recorded during the last 4 min:

1. Immobility: Floating in water without swimming. The mouse was considered immobile when it floats motionlessly or made only those movements necessary to keep its head above the water surface
2. Swimming: Active movements of extremities and circling in the container
3. Climbing: Active movements of forelimbs on the container wall

After 15min in the water, the mice were removed and allowed to dry in a heated enclosure (32 ℃) before returned to their home cages.

Extracts was given to mice once daily for a period of 14 days and on 15th day mean swimming time for each group was calculated, blood was collected through retro orbital blood withdrawal method under light inhalant anesthesia to estimate biochemical parameters like blood glucose, triglycerides and cholesterol.

Animals were sacrificed humanely by overdose of anesthesia and the weights of organs such as liver, adrenals and spleen were recorded after washing with alcohol.

5. Research Design

1. Group: Control treated vehicle
2. Group: Withania somnifera Standard drug (100mg/kg BW p.o)
3. Group: Leaves extract of Salacia chinensis (200mg/kg) BW p.o)
4. Group: Leaves extract of Salacia chinensis (400mg/kg BW p.o)

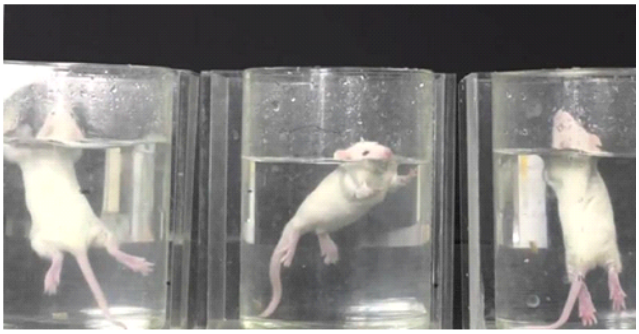

Figure 3: Swimming endurance test

6. Evaluation of Antidepressant Activity

6.1. Tail Suspension test (TST)

Albino mice weighing about 20-30 g was used.

Animals were moved from their housing colony to laboratory in their own cages and allowed to adapt to the laboratory conditions for 1-2 hours. The principle of this test is that suspending mice upside down leads to characteristic behavior immobility which resembles to human depression.

Table 2: Effect of hydroalcoholic extract of *Salacia chinensis* on swimming endurance test

Sl no	Treatment group	Dose in mg/kg, p.o	Swimming survivalime(min)
01	Control	10ml/kg	210±1.528
02	Standard (Withania somnifera)	100mg/kg	329±2.082**
03	HAESC-1(high dose)	400mg/kg	257±1.155**
04	HAESC-2(low dose)	200mg/kg	216±7.371**

HAESC:- Hydroalcoholic extract of Salacia chinensis.
Results are expressed as mean± SEM, data analyzed by using one-way ANOVA followed by Dunnett's. *P< 0.05. **P< 0.01, ***P< 0.001

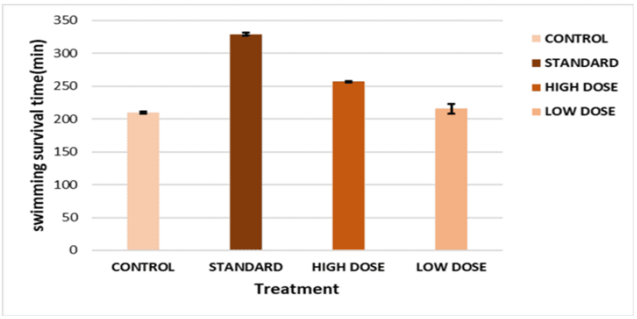

Figure 4: Effect of hydroalcoholic leaves extract of Salacia chinensis on swimming endurance test

Table 3: Effect of hydroalcoholic extract of *Salacia chinensis* on biological parameters in swimming endurance test

S.N.	Treatment group	Biological Parameters		
		Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
01.	Control (normal saline)	135±1.732	48±0.5774	54±1.528
02.	Withania somnifera	110±3.215**	58.6±1.456**	43±2.082**
03.	HAESC-1 (400mg/kg)	121.3±1.175**	53.66±1.202*	46.66±0.028*
04.	HAESC-2 (200mg/kg)	123±0.8819	43.6±1.764	44.66±1.77**

HAESC :- Hydroalcoholic extract of Salacia chinensis.
Results are expressed as mean± SEM, data analyzed by using one-way ANOVA followed by Dunnett's. *P< 0.05. **P< 0.01, ***P< 0.001

After the administration of respective sample, mice were suspended on the edge of the table 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility duration was recorded for the last 4 minutes during 6-minute period. Mice was considered immobile when they hanged passively and completely motionless

After successive 14 days of treatment with control, standard and extract drugs, the immobility was calculated and recorded. [18]

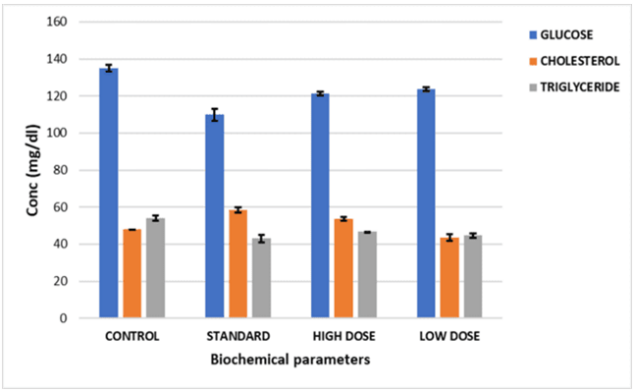

Figure 5: Effect of hydroalcoholic leaves extract of *Salacia chinensis* on biological parameters in swimming endurance test

Table 4: Effect of hydroalcoholic extracts of Salacia chinensis on organ weights in Swimming Endurance test

Sl. No.	Treatment	Organ Weights		
		Liver(g)	Spleen (g)	Adrenal gland(g)
1.	Control	1.21±0.05196	0.19±0.00577	0.0035±0.00028
2.	Withania somnifera (100mg/kg)	1.006±0.00666**	0.14±0.00577**	0.002±0.00032**
3.	HAESC-1 (400mg/kg)	1.03±0.0057**	0.156±0.0088**	0.0023±0.00015*
4.	HAESC-2 (200mg/kg)	1.15±0.0088	0.15±0.0115	0.0028±0.00017

HAESC:- Hydroalcoholic extract of Salacia chinensis.
Results are expressed as mean ± SEM, data analyzed by using one-way ANOVA followed by Dunnett's. *P< 0.05. **P< 0.01, ***P< 0.001

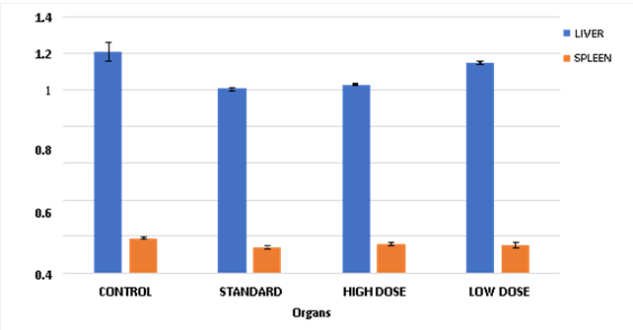

Figure 6: Effect of hydroalcoholic leaves extract of Salacia chinensis on liver and spleen weight in swimming endurance test

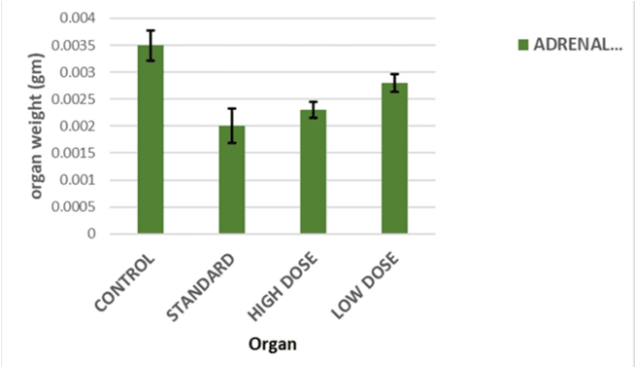

Figure 7: Effect of hydroalcoholic leaves extract of *Salacia chinensis* on adrenal gland weight in swimming endurance test

6.2. *Research design*

1. Group: Control treated with vehicle
2. Group: Standard drug (Imipramine – 10mg/kg BW p.o)
3. Group: Leaves extract of Salacia chinensis (400mg/kg BW p.o)
4. Group: Leaves extract of Salacia chinensis (200mg/kg BW p.o.)

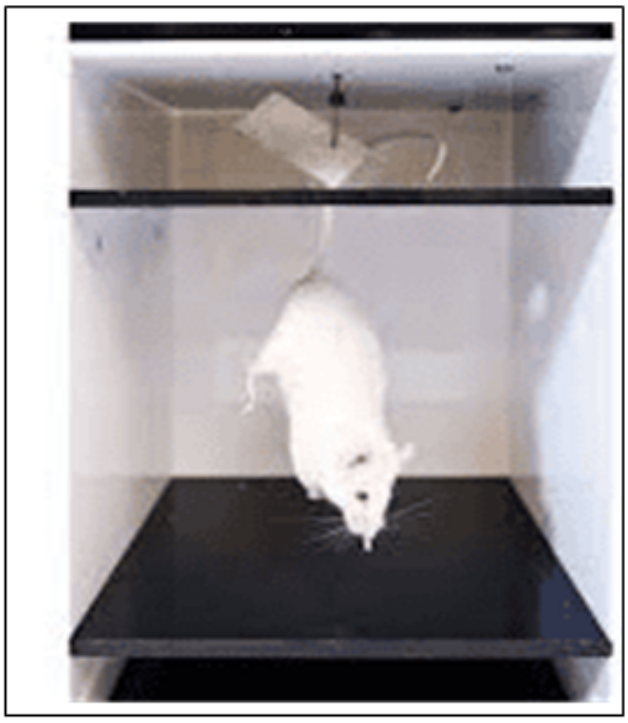

Figure 8: Tail suspension test

Table 5: Effect of hydro alcoholic extract of Salacia chinensis on tail suspension test (tst)

Sl.no	Treatment group	Dose in mg/kg (p.o)	Duration of immobility(sec)		
			1^{st} day	7^{th} day	14^{th} day
1	Control	10ml/kg	141±2.08	137±1.732	133±2.309
2	Standard (Imipramine)	10mg/kg	86±2.082**	79±309**	75±2.082**
3	HAESC-1	400mg/kg	132.6±0.6667*	104.33±1.764**	96.66±1.453**
4	HAESC-2	200mg/kg	135±0.5774	120.33±2.028	105±2.082**

HAESC :- Hydroalcoholic extract of Salacia chinensis.
Results are expressed as mean± SEM, data analyzed by using one-way ANOVA followed by Dunnett's. *P< 0.05. **P< 0.01, ***P< 0.001

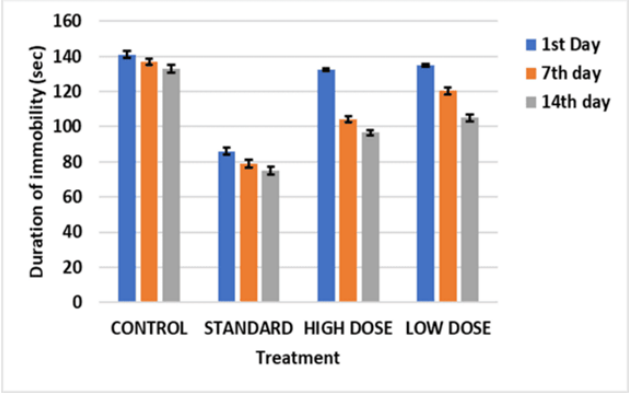

Figure 9: Effect of hydroalcoholic leaves extract of Salacia chinensis on tail suspension test

6.3. Elevated plus maze test (EPM)

This equipment made of wood and consisted of two opposite open arms, 50×10 cm (surrounded by 1 cm high Plexiglas), and two enclosed arms, 50× 10× 40 cm elevated to a height of 50 cm above the floor. The junction area of the four arms (central platform) measured 10×10 cm. The floor of the maze painted with impermeable dark epoxy resin, in order to avoid urine impregnation. [18]

6.3.1. Procedure

Animal: Albino mice weighing 20-30gm

Group 1 mice received saline served as control, group 2 received standard drug Imipramine, group 3 & 4 were treated with HAESC 200 and 400mg/kg p.o respectively 30 minutes before the experiment. After 21days of dosing period the animal's depression level was observed. Animals were freely exposed to Elevated plus maze apparatus.

Following parameters was noted for next 5 min:

1. Time spent in the open arm (s)
2. Time spent in the enclosed arm (s)
3. No of entries in open arm
4. No of entries in enclosed arm

6.4. Research design

1. Group: Control treated with vehicle
2. Group: Standard drug (Imipramine – 10mg/kg BW p.o)
3. Group: Leaves extract of Salacia chinensis (400mg/kg BW p.o)
4. Group: Leaves extract of Salacia chinensis (200mg/kg BW p.o.)

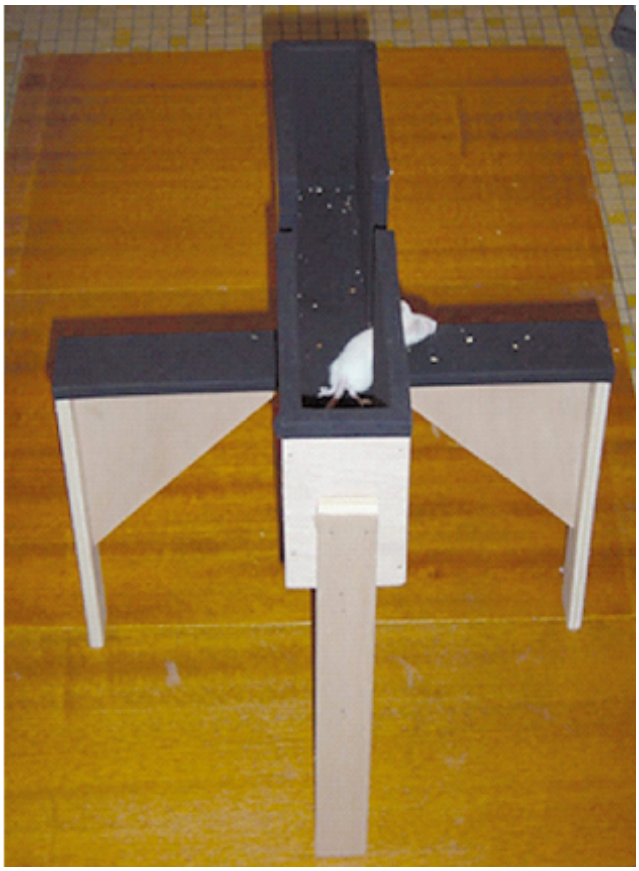

Figure 10: Elevated plus maze test

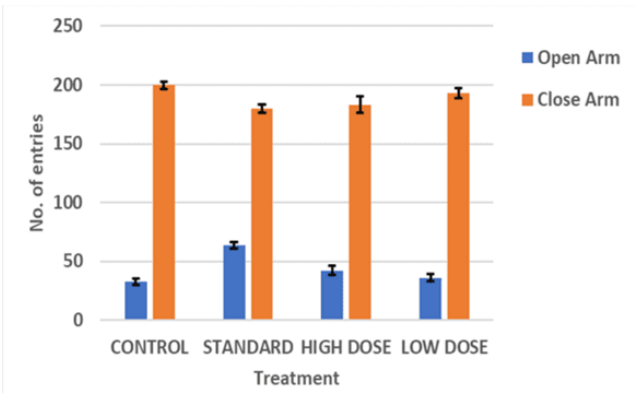

Figure 11: Effects of hydroalcoholic leaves extract of *Salacia chinensis* on Time spend in open arm & in enclosed arm

Table 6: Effect of hydroalcoholic extract of Salacia chinensis on elevated plus maze test

Sl. No.	Treatment	Time spent in Open arm (sec)	Time spent in enclosed arm (sec)	No. of Open arm entries	No. of enclosed arm entries
1.	Normal Saline	32.66±2.517	199.7±3.035	3.60±0.333	9.20±0.22
2.	Imipramine	63.73±2.969**	180.0±3.635**	7±1.155*	9.3±0.22**
3.	HAESC- 400mg/kg	42.36±4.050*	183±7.000*	8±0.5774*	10.1±0.71*
4.	HAESC-200mg/kg	36.1±3.381	193±4.583	4.66±0.8819	12.54±0.701

HAESC :- Hydroalcoholic extract of Salacia chinensis.
Results are expressed as mean± SEM, data analyzed by using one-way ANOVA followed by Dunnett's. *P< 0.05. **P< 0.01, ***P< 0.001

7. Discussion and Results

7.1. Phytochemical analysis

Successive extractions of Salacia chinensis was performed. The extract powder was greenish in colour and hygroscopic in nature.

The Salacia chinensis extract was subjected to different preliminary chemical tests to determine the chemical constituents present in the extract.

The results have indicated the presence of flavonoid, alkaloids, terpenoids, saponins, glycosides, carbohydrates.

8. Evaluation of Adaptogenic Activity

8.1. Swimming endurance test

In Swim endurance test, oral administration of HAESC at the dose of 200 mg/kg and 400mg/kg significantly increased in swimming time.

In Swim endurance test, oral administration of HAESC at the dose of 200 mg/kg and 400mg/kg significantly reduced weight of organ (i.e., Liver, spleen, Adrenal gland).

The biochemical estimation results obtained showed decrease in blood glucose, triglyceride, and slight increase in cholesterol level when compared to control which support the adaptogenic activity of the title plant as shown in table no: Tables 2, 3 and 4 and Figures 3, 4, 5, 6 and 7.

9. Evaluation of Antidepressant Activity

9.1. Tail suspension test

The duration of immobility was reduced after treated with hydroalcoholic extract of Salacia chinensis and results are significant on both high dose 400 mg/kg, p.o. (P<0.001) and low dose 200 mg/kg, p.o. (P<0.01) with that of standard Imipramine (10 mg/kg, p.o.) indicating that the extract possess significant antidepressant activity. Results depicted in Table 5 and Figures 8 and 9.

9.2. Elevated plus maze method

The hydroalcoholic extract of Salacia chinensis at high dose (400 mg/kg, p.o) (P< 0.05) significantly increased the number of entries in to open arm and total time spent in open arm as compared to control.

It shows increase in the mobility of the mice in open arm which suggest the antidepressant activity shown in Table 6 and Figures 10 and 11.

10. Conclusion and Summry

The adaptogenic and antidepressant effect of the hydroalcoholic extract of Salacia chinensis leaf was studied.

Based on the results obtained from this study it can be concluded that the hydroalcoholic extract of Salacia

chinensis leaves possess Adaptogenic and Antidepressant activity.

This beneficial effect of Salacia chinensis leaf might be due to presence of flavonoids, alkaloids and its derivatives. However, further investigation should be carried out to elucidate the exact mechanism of action.

11. Source of Funding

None.

12. Conflict of Interest

None.

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Author biography

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