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Research Article

Volume 1 Issue 1 *Journal of Neurology and Psychiatric Disorders*

To Evaluate the CNS Stimulant Activity Aqueous Flower Extract of *Hibiscus Sabdariff* Linn. In The Male Wister Albino Rats

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Citation: Gresamma L (2019) To Evaluate the CNS Stimulant Activity Aqueous Flower Extract of *Hibiscus Sabdariff* Linn. In The Male Wister Albino Rats. J Neurol Psychiatr Disord 1(1): 104

Received: December 22, 2018; **Published:** February 12, 2019

Abstract

Phytochemical screening of the extract shows the presence of chemical constituents like carbohydrates, glycosides, flavonoids, saponins, triterpinoids, phenolics and tannins, protiens and aminoacids, fixed oils, resins. Acute toxicity tests were performed according to the OECD guide line no.423, LD50 value was found to be 200mg/kg. CNS Stimulant activity was performed by using the locomotary activity by using the actophotometer. The extract will show the increase in the locomotory activity. From this we can conclude that the extract (*Hibiscus sabdarrifa* Linn.) shows the CNS Stimulant activity.

Keywords: *CNS Stimulant;* ***Hibiscus sabdarrifa*** *Linn; male Wistor Albino rats; Herbal plants*

Introduction

Introduction of Herbal Plants

Herbal plant is a beneficial and valuable plant that we can apply some or practically whole part of it for countless treatments. Few people utilize its part like shade dried leaves, roots, flowers, etc. for treating diseases. Few utilize its chemical compound like its extract oil for treatment or therapy. Moreover, we may also use plant herbs in cooking recipes. So, herbs have various uses but the principle purpose of using them is to regulate proper health [1].

There are various types of herbal plants and individual part of herbal plant applied is different. Herbal plants may be applied for different purposes that rely on our requirement. We may utilize them for alleviate healing like Aloe and few types of herbs and may be grown for a garden adequate of their sweet odor. Crafting with herbs may make a decent and good looking home. Artemisia is a beautiful plant herb that is simply grown in the garden. Giver King is a delightful herb that has a fresh herbal odor and dried floral like sunflower and roses or anything else are suited for our crafting.

Herbs are common in the kitchen like pepper, lemon, chilli or other condiments. Various finest cosmetics come from herbs. Approximately most of herbs are utilized for enhancement of human health. Herbs have known from centuries for therapeutic treatment. We may prepare superb fragrance and skin tonic in the soap and hair conditioner from plant herbs. Few types of herbs have been utilized in the management of acne and eczema. We may apply few types of herbs for alleviate headache and as stimulant and tonic.

Rosemary has been termed the herb of the year by the International Herb Association. In ancient Greece and Rome rosemary was considered to enhance memory. Rosemary has long been acknowledged for its therapeutic potency. If we suffer of nightmares, we could attempt keeping a sprig below the pillow of a sleeper. It may treat us. A product of Rosemary, like Rosemary tea assists in digestion but it may take time as a soothing drink to relax the nerves and induce sleep. Rosemary oil is a superb conditioning effect of the hair, assist in control of dandruff and further, be alleged and treating baldness [3-4].

CNS Stimulant Activity

Medicines that enhance both physical and mental actions are called as Central nervous system stimulants. They are applied

for treatment of narcolepsy, attention- deficit hyperactivity disorder (also termed as ADHD), and the other disorders of the central nervous system (CNS). The most usually utizedd and well-accepted central nervous system (CNS) stimulant iscaffeine (also termed as an analeptic drug). The other stimulants examples are amphetamines, like methamphetamine hydrochloride (Desoxyn) and dextroamphetamine sulfate (DextroStat, Dexedrine), and non-amphetamine drugs like, methylphenidate (Ritalin), and pemoline (Cylert). Whereas the effects of CNS stimulants are vivid, the therapeutic utilizations of these drugs are finite due to side effects caused by them.

CNS stimulants raise attention, reduce restlessness, and enhance physical coordination in persons who are suffering with ADHD, a condition where people having surprisingly more activity levels and less attention spans. The medicines can also curb impulsive and combative behavior associated with ADHD. Experts suggest that approximately 30 % of young people with ADHD are not diagnosed till middle school or later. Whereas very young children are characterized with inattentive and impulsive behavior, this particular hyperactivity sometimes quiets in teenagers to restlessness. ADHD diagnosis is solely based on guidelines suggested or fixed by the American Psychiatric Association, and there is no particular biological test to suggest the disease condition. The definite cause of ADHD is not yet known, but research findings have suggested that it affects many within a family.

The following are different stimulant drugs approved by The Food and Drug Administration in treatment of ADHD:

I. Dextroamphetamine (brand name Dexedrine and generics),
II. Methamphetamine (brand name Desoxyn),
III. Methylphenidate (brand name Ritalin and generics), and
IV. An amphetamine-dextroamphetamine combination (brand name Adderall)

Very recently, the FDA restricted the already approved stimulant pemoline (brand name Cylert), to secondary application, due to its association with liver failure.

Central nervous system stimulants could not be used to enhance alertness or in case of alternate for sleep. Even though they may initiate or induce loss of appetite and weight loss, they could not be utilized as "diet pills." Medical indication of the drug methamphetamine (Speed) as appetite suppressant is strictly for treatment of obesity only, or in treatment of anesthetic overdose [5-10]. The medication amphetamine could be avoided by people having hypertension and cardiovascular disease, and with those who are highly anxious, agitated restless, and excited.

Always intake or ingested CNS stimulants as directed. Try to avoid intake larger or more frequent doses, and do not intake the drug for longer than prescribing directions. This medical substance can be habituated if administered in large doses or over long durations. If it is compulsory to stop administering the drug, consult with the doctor who prescribed and suggested it for instructions on how to terminate. The body can take many weeks to adopt after treating with CNS stimulant has terminated [11].

The major common side effects of CNS stimulants are nervousness, restlessness, irritability, loss of appetite, sleep problems, and a false sense of healthy. Immediately after wear off of these side effects, the other side effects can occur, like drowsiness, unusual tiredness or weakness, trembling, or depression [12]. These particular side effects and after effects commonly fade away as the body adopts to the medication and do not need therapeutic treatment except that the side effects continue, or they interfere with usual activities.

Extra hazardous side effects can also occur by CNS stimulants. If breathing complications, dizzy, faintness, extreme fatigue, weakness, high fever, hives, vomiting, chest pain, irregular heartbeat, convulsions, involuntary movement, or rise in blood pressure happens, consult with the medicine prescribed doctor as quickly as possible [13].

Literature Review

Hibiscus sabdriffa is an annual/yearly herbacious drug cultivated and managed for its flowers even though leaves and seeds have also been applied in traditional medicine. The calyces of the herbal plant are applied as a refrigerant in the form of tea to prepare jellies and jams. The plant is found to contain, carbohydrates, flavonoids, acids, proteins, fats minerals and vitamins. The plant has been reported to have Anti- hypertensive, Hepatoprotective and Anti-cancer properties (N. Mahadevan *et al*.).

The leaf of the plant is reported to contain proteins, fat, carbohydrates, fibre, ash, calcium, phosphorous, iron, thiamine, riboflavin, niacin and ascorbic acid. The flower yeilds and yellow dye pigment identified as Daphniphylline. The plant contains flavonoids such as Hibis citrin and Hibiscetin 1 and dried calyces contain flavonoids, gossypetine, Hibiscetine. It also contains alkoloids Anthocyanin, Citric acid, Galactose, Pectin, Quercitine, Straric acid etc., water soluble polysaccharides are isolated from flower buds (Shivali *et al*.).

Researches on Hibiscus species shows the plant pocesses Anti-inflammatory, Anti pyretic, Analgesic, Cough sedative, Anti spasmodic, Anti scrobutic properties. Aldose reductase acitvity has been found in one species. It affects the blood parameters by decreasing fibrinogen content and prolonging the prothrombin time. It also raises blood sugar levels in rats.Hibiscus flowers are safe to use no toxicity are side effects are expected in reasonable doses (Kohlkute *et al*.).

The aerial parts of Hibiscus Sabdariffa are rich in calcium, iron, riboflvin, essential oils, and peculiar saponins. The leaves are used for pungent aroma. It is used traditionally in an infused form taken for fevers, flu, diabetis and constipation. It is also used as diuretic and anti-convulsant treatment (Martinez *et al*.).

Histological study of antiatherosclerotic effect of propolis in induced hypercholestrolemic male albino rabbits. The current findings of experimental rabbits, even though not directly useful to human subjects, advocating that Propolis might be effective as an anti-atherosclerotic agent (Ashok Purohit *et al*.).

Hypotensive Effect of Ethanol Seed Extract of *Hibiscus sabdariffa* Linn (family: *Malvaceae*) on Normotensive Cats. The effects of the ethanol extract were compared to normal basal rhythm and Acetylcholine. The standard drugs used are Ach and different doses of the extracts (three test drug doses 1 mg/ml, 5 mg/ml and 10 mg/ml) were injected by using a cannula inserted in the femoral artery. The extract showed a significant ($P<0.05$) decrease in cat blood pressure (BP). The potent effect of the plant extract appears to be high due to that it has effect at the lowest dose used [14,15]. The 1mg/ml of the extract showed major effective response; Although the standard drug Acetylcholine exhibited a higher potency compared the plant extract. The seed extracts are proved safe by exhibiting less degree of toxicity with LD_{50} more than 5000 mg/kg in rats.

The results of the present study reported that, the ethanolic seed extract Hibiscus subdariffa showed blood pressure lowering effect in normotensive cat with significant statistical difference ($P<0.05$). The blood pressure (systolic and diastolic), pulse pressure and mean arterial pressure in all the three doses (1mg/ml, 5mg/ml and 10mg/ml) of seed extract decreased significantly ($P<0.05$) when compare to normal basal rhythm, except in 5mg/ml of the pulse pressure there was insignificant decrease ($P>0.05$) when compare to normal basal rhythm (Bako *et al*.).

Plant Profile

Origin and Distribution

Hibiscus which is also called as Roselle is indigenous from India to Malaysia, it is usually cultivated, and should have been brought at an earlier date to the Africa[16-19]. The plant has been enormously distributed through the areas of the Tropics and Subtropics of both hemispheres of world, and in various regions of the Central America and West Indies has naturally distributed. Hibiscus or Roselle ... is cultivated in huge amounts in Panama, principally by the West Indians (Figure 1).

In the year of 1971, it was recorded that Roselle calyces, cultivated and harvested and shade dried in Senegal, especially around the areas of Bombay, were usually being exported to Europe (Switzerland, France, Germany and Italy) at the range of 10 to 25 tons per year [20-28].

Figure 1: *Hibiscus Sabdariffa* Linn

Chemical constituents (Table 1)

Sorrel seeds	crude protein	24.0%
	fat	22.3%
	fiber	15.3%
	N-free extract	23.8%
	Ash	7.0%
	Calcium	0.3%
	Phosphorous	0.6%
	Sulphur	0.4%
Component acids of the seed lipids were identified as	Palmitic-acid	35.2%
	Palmitoleic acid	2.0%
	Stearic acid	3.4%
	Oleic acid	34.0%
	Linoleic acid	14.4%
	j3-sitosterol	61.3%
	Cholesterol	5.1%
	Ergosterol	3.2%

Table 1: Chemical constituents in *Hibiscus Sabdariffa* Linn

Medicinal Use

The plant has been used various ailments like cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, resolvent, antiseptic, aphrodisiac, astringent, sedative, stomachic, and tonic. Sorrel is a traditional medicine for dyspepsia, dysuria, fever, hangover, heart ailments, hypertension, abscesses, bilious conditions, cancer, cough, debility, neurosis, scurvy, and strangury. The drink prepared by adding the calyx in water, is reported to be a traditional medicine for cancer.

Materials and Methods

Materials

Plant material: The aqueous extract of powdered flowers of *Hibiscus sabdariffa* Linn.

Collection of plant material: Hibiscus sabdariffa Linn. Flowers were collected from the local gardens of houses and plant nursery and are authenticated.

Methods

Preparation of Hibiscus sabdariff a Linn extract:

- 150gm of dried flowers was macerated with hot water (95 ℃, 6000ml) for two hours.
- The aqueous extract was evaporated under vacuum at 85 ℃.
- The extracted solution was filtered.
- The filtered solution is then lyophilized to obtain 75gms of HSE.
- It is then stored at 4 ℃ before use

Preliminary phytochemical analysis: The crude and successive extracts were tested for the following phytoconstituents, carbohydrate, alkaloids, glycosides, tannins, flavonoids, phytosterols, fats and oils by standard procedures as described by khandelwal and kokate.

The extracts were subjected to the following chemical test for the identification of various active constituents.

Test for alkaloids:

a) Dragondroff's test: Mix 1ml of Dragondroff's reagent with 1ml of the extract produces an orange red precipitate confirms the presence of alkaloids.
b) Mayer's test: Mix 2ml of Mayer's reagent with 1ml of the extract, a cream coloured precipitate indicates the presence of alkaloids.
c) Wagner's test: Mix 2ml of Wagner's reagent with one ml of the extract, the production of reddish brown precipitate confirms the presence of alkaloids.

d) Hager's test: Mix 3ml of Hager's reagent with 1ml of the extract the formation of yellow precipitate indiactes the presence of alkaloids.

Test for carbohydrates:

a) Molisch test: Mix 1ml of α-naphthol solution with 2ml of the extract and then pour concentrated sulphuric acid along the sides of the test tube, purple or reddish violet ring at the junction of the two confirms the presence of carbohydrates.
b) Fehling's test: To 1ml of the extract, add an equal quantity of Fehling's solution A and B and heat. The formation of the brick red precipitate indicates the presence of carbohydrates.
c) Benedict's test: To 5ml of Benedict's reagent add 1ml of extract solution and boil for 2minutes and cool. Formation of a red precipitate shows the presence of carbohydrates.
d) Barfoed's test: To 5ml of Barfoed's reagent, add 1ml of the extract solution and heat to boil, a red precipitate of copper oxide was formed and confirms the presence of carbohydrates in the test extract.

Test for steroids and sterols:

a) LibermannBurchard test: Dissolve the extract in 2ml of chloroform in a dry test tube. Add ten drops of acetic anhydride and two drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green, indicating the presence of steroids.
Salkowaski test: Dissolve the extract in chloroform and add volume of concentrate sulphuric acid. Formation of bluish red to cherry red colour in chloroform layer and whereas the acid layer assumes marked green florescence, represents the steroid and sterol components in the tested extract.

Test for glycosides:

a) Legal test: Dissolve the extract in pyridine and add freshly prepared sodium nitroprusside solution to make it alkaline. The formation of pink to red colour shows the presence of glycoside.
b) Baljet test: To 1ml of the test extract add 1ml sodium picrate solution and the yellow to orange colour reveals the presence of glycoside.
c) Borntrager"s test: Add a few ml of diluted sulphuric acid to 1ml of the extract solution. Boil, filter and the filtrate extract with chloroform. Separate the chloroform layer and treat with 1ml ammonia. The formation of red colour shows the presence
of anthraquinone glycoside.
d) Keller Mililani test: Dissolve the extract in acetic acid containing traces of ferric chloride and transfer to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually becomes blue, confirms the presence of
deoxy sugar attached to the aglycon part of glycoside.

Test for saponins:

a) Foam test: About 1ml of alcoholic extract, dilute separately with 20ml of distilled water and shake in a graduated cylinder for 15 minutes. One centimetre layer of foam confirms the presence of saponins. Add alcoholic vanillin solution o 1ml of the extract; pour a few drops of conc sulphuric acid. A deep violet colour indicates the presence of saponins.

Test for flavonoids:

a) Shinoda test: To 1ml of the extract, add magnesium turnings and 1-2 drops of concentrated hydrochloric acid. Formation of pink or red colour shows the presence of flavonoids. To 1ml of extract, add 1ml of ferric chloride, the formation of brown colour confirms the presence of flavonoids.

Test for triterpenoids: a) Dissolve two or three granules of tin metal in 2ml of thionyl chloride solution. Then add 1ml of the extract into test tube. The formation of a pink colour indicates the presence of triterpenoids.

Detection of phenolics and tannins:

a) Ferric chloride test: The extract was treated with few drops of neutral ferric chloride solution. The formation of bluish black colour indicates the presence of phenolic nucleus.
b) Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. The formation of white

precipitate indicates the presence of tannins.
c) Lead acetate test: The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

Test for protein and amino acid:

a) Biuret test: To 1ml of the extract ad 1ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate solution. Formation of violet colour indicates the presence of protein.
b) Ninhydrine test: Add two drops of freshly prepared 0.2% Ninhydrine reagent to the extract solution and heat. Development of a purple colour reveals the presence of proteins and amino acids.
c) Xanthoprotein test: To 1ml of the extract add 1ml of concentrated nitric acid. The formation of whit precipitate confirms the presence of amino acid.

Test for fixed oils:

a) Spot test: Press a small quantity of extract between two filter paper. Oil stains on paper indicates the presence of fixed oil.

Acute Toxicity Studies

For the pharmacological tests the extracts were suspended in double distilled water containing carboxy methyl cellulose (CMC 1% w/v). The acute toxicity was determined for the aqueous extract of *Hibiscus sabdariffa* Linn. On Wistar albino rats by fixed dose method of OECD (Organisation for co-operation and development) guide line no. 423 (2000) 50-2000mg/kg of the extracts were administered by oral route to the rats. Mortality rate was observed for 5 days. All experiments were performed according to the current guide lines for the care of laboratory animals and ethical guide lines for the investigation of experimental pain in conscious animals. Standard orogastric cannula was used for oral drug administration.

Animal selection: A total of 20 male Wistar rats were obtained from the animal facility Sigma institute of clinical research and admistration and used for the study. All rats were certified with good health at the time of receiving. Age of the animals at the start of the treatment was approximately 8 to 12 weeks.

Acclimatization: Wister rats were allowed to acclimatize to experimental room conditions for a period of 10 days prior to randomization and treatment. During the acclimatization period the rats are observed for the clinical signs.

Environmental conditions: The rats were maintained in the separate polypropylene cages. In the experimental room, temperature of 23±2 °C, controlled humidity (50-55%), 12 hrs of artificial lightening and 12 hrs of darkness cycle were maintained. The experimental room was cleaned and mopped with a disinfectant daily.

Housing conditions: The rats were housed based on the group size per polycarbonate cage. Each cage was fixed with a polypropylene water bottle with stainless steel nozzle. Feed was provided ad libitum throughout the study. The bedding material was changed daily.

For CNS Stimulant activity:

- Weigh the animals and number them.
- Turn on the equipment (check and make sure that all the photo cells are working for accurate recordings) and place individually each rat in the activity case for 10mins.
- Note the basal activity score of all the animals.
- Administer caffeine and plant extracts (1ml/100g) and after 30mins re tests each rat for activity scores for 10mins.
- Note the difference in the activity before and after Caffeine and extracts.
- Calculate % increase in the motor activity.

Results and Discussions

Preliminary pyhtochemical screening studies proves that the extract [*H. Sabdarifa* Linn.] consist the sterols, fixed oils, cardiotonic aglycones, flavonoids, saponins, polyphenols, protiens, dietary fibers.

The aqueous flower extract of *Hibiscus Sabdariffa* Linn shows significant CNS stimulant activity by increasing the pschycomotor activity resp., which exhibits activity equal to that of standards., Thus *Hibiscus Sabdariffa* Linn shows CNS stimulant activity (Table 2-5 and Figure 2).

S. No.	Test	Result
1.	**ALKALOIDAL TEST** a. Dragondroffs test b. Mayer's test c. Wagner's test d. Hager's test	Negative Negative Negative Negative
2.	**CARBOHYDRATES TEST** a. Molish's test b. Fehling's test c. Benedict's test d. Baeford's test	Positive Positive Positive Positive
3.	**STEROIDS TEST** a. LibermannBuchard test b. Salwoski test	Positive Positive
4.	**GLYCOSIDES TEST** a. Legal test b. Baljet test c. Killerkilaini test d. Borntagers test	Positive Positive Positive Positive
5.	**SAPONINS TEST** a. Foam test	Positive
6.	**FLAVONOIDS TEST** a. Shinoda test	Positive
7.	**TRITERPINOIDAL TEST**	Positive
8.	**PHENOLICS & TANNINS TEST** a. Ferric chloride test b. Gelatin test c. Lead acetate test	Positive Positive Positive
9.	**PROTIEN & AMINOACIDS TEST** a. Buret's test b. Ninhydrin test c. Xanthoprotic test	Positive Positive Positive
10.	**FIXED OIL TEST** a. Spot test	Positive
11.	**RESIN TEST** a. Acetic anhydride test	Positive

Table 2: Preliminary Phytochemical Screening of *Hibiscus Sabddarifa* Linn. Flower Extract

Acute Toxicity Studies

Group Number	No. of Animals Per Group	Dose In Mg/Kg	Report
1	3	5 mg/kg	NO DEATH
2	3	50 mg/kg	NO DEATH
3	3	100 mg/kg	NO DEATH
4	3	300 mg/kg	NO DEATH
5	3	500 mg/kg	NO DEATH
6	3	1000 mg/kg	NO DEATH
7	3	2000 mg/kg	NO DEATH

Table 3: Evaluation LD50 Value of the *Hibiscus Sabddarifa* Linn. Extract Based on OECD Guide Line No.423

According to the OECD guide linesno.423 toxicity studies were performed on the mice upto the dose levels of 2000 mg/kg, no death of the mice will be observed. So the LD50was found to be 2000mg/kg. ED50 minimal was 1/10th of LD50 value. So,

ED50 = 2000/40 = 50 mg/kg.
ED50 = 2000/20 = 100 mg/kg.

S. No	Group No	Treatment	Dose in mg/kg	Animal No.	Body Weight in gms	Locomotory Activity For 10 Min [actophotometer]
1	I	Control	NIL	1	150	446
				2	125	550
				3	125	723
				4	150	425
				5	125	530
				1	150	473
2	II	Extract-I *H.Sabddarifa* Linn.	50mg/kg	2	125	560
				3	150	547
				4	125	485
				5	150	550
				1	150	656
3	III	Extract-II *H.Sabddarifa* Linn.	100mg/kg	2	150	745
				3	200	738
				4	125	685
				5	125	725
				1	150	678
4	IV	Standard Caffiene	30mg/kg	2	150	720
				3	150	635
				4	125	625
				5	125	715

Table 4: Evaluation of CNS Stimulant Activity

S. No.	Group No.	Treatment	Dose In mg/kg	Mean±S.D.Of Locomotory Activity
1	I	Control	Nil	525.65±107.81
2	II	Extract-I *H.Sabddarifa* Linn.	50mg/kg	522.5±36.40
3	III	Extract-II *H.Sabddarifa* Linn.	100mg/kg	687.33±37.19
4	IV	Standard Caffiene	30mg/kg	684.66±46.41

Table 5: Mean and S.D. of Locomotory Activity

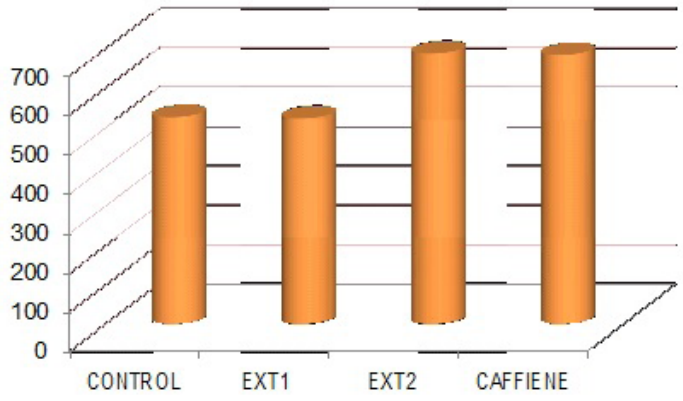

Figure 2: Treatment Vs. Locomotary activity

Conclusions

Phytochemical screening of the extract shows the presence of chemical constituents like carbohydrates, glycosides, flavonoids, saponins, triterpinoids, phenolics and tannins, protiens and aminoacids, fixed oils, resins.

Acute toxicity tests were performed according to the OECD guide line no.423, LD50 value was found to be 200mg/kg.

CNS Stimulant activity was performed by using the locomotary activity by using the actophotometer. The extract will show the increase in the locomotory activity.

From this we can conclude that the extract (***Hibiscus sabdarrifa*** Linn.) shows the CNS Stimulant activity.

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