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Evaluation of CNS Stimulant Activity of Ethanolic Extract of Cryptostegia Grandiflora Leaves Using Actophotometer in Experimental Animal Models

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ABSTRACT

The current study used an actophotometer on Wistar rats to evaluate the ethanolic extract from Cryptostegia grandiflora leaves' stimulant effects on the central nervous system (CNS). The cold maceration method with ethanol was used to extract the leaves. Three groups of animals were created: a control group, a standard group, and a test group. The standard stimulant medication was 10 mg/kg of caffeine, while normal saline was used as the control. Two doses of the extract were given: 100 mg/kg and 200 mg/kg. Locomotor activity was measured using an actophotometer, which works by detecting interruptions in infrared light beams. The data were statistically analyzed using one-way ANOVA followed by Dunnett's test. An actophotometer, which detects disruptions in infrared light beams, was used to measure locomotor activity. One-way ANOVA and Dunnett's test were used to statistically analyse the data. When compared to the control group, the extract considerably increased locomotor activity. While caffeine generated a stimulation of 70%, the higher dose (200 mg/kg) demonstrated a larger stimulant effect (62%) than the lower dose (51%). The higher dose had effects akin to those of caffeine and showed superior CNS stimulation compared to the lower dose. Significant results at $p < 0.01$ were confirmed by statistical analysis. The plant's phytoconstituents, including flavonoids and alkaloids, are probably what cause the stimulant effect. According to the results, the ethanolic extract from the leaves of Cryptostegia grandiflora has promising dose-dependent CNS stimulant properties and may help improve mental activity and alertness.

Keywords: Cryptostegia grandiflora, Caffeine, CNS stimulant activity, Wistar rat, Dunnett's test.

INTRODUCTION

Psychological stress, anxiety, and poor sleep quality can cause temporary and permanent disability and are linked to the signs of major mental disorders in the whole world. Insomnia is a common problem all over the world that leads to serious mental and heart diseases. About 20 percent of the population experience major mental problems during their lives. These problems are more prevalent among older people and can result in many negative consequences for their personal, social, and occupational life. Poor mental wellbeing and psychological stress are the most common mental illnesses, just like stroke [1]. The effects of stimulants have an impact on the body in a number of ways. The effects are caused by paths that are related to the nervous system. These include adrenergic, dopaminergic, and serotonergic pathways. Stimulants work by having an effect on the cell membranes of nerve cells. In this regard, the stimulants can hinder the removal of neurotransmitters such as serotonin, norepinephrine, and dopamine. Alternatively, the stimulants can cause the release of these neurotransmitters in the reverse direction. There are some stimulants that affect the receptors that exist before and after the synapses where neurotransmitters are used. Some drugs, similar to cocaine, act by hindering the elimination of norepinephrine, causing serious heart conditions [2]. Cryptostegia grandiflora (Roxb.) R.Br. is a plant which is classified under the family of Apocynaceae and is available in

various regions such as tropical Africa, Madagascar, and some parts of India. The juice that is obtained from above ground parts of this plant forms a substance that looks like caoutchouc after being exposed to sunlight. Additionally, the preparation of this plant is useful in addressing conditions that affect the nervous system. An aqueous solution that is made from the ethanol extract of the aerial parts of the plant has been found to reduce the levels of blood sugar considerably in normal rabbits. The latex of the plant is found to be proteolytic and bacteriolytic, and is also enzymatic against the proteins of some diseases [3].

The actophotometer is one of the frequently employed behavioral approaches to determining the spontaneous motor activity of rodents. This approach is highly effective in exploring the impact that a certain substance might have on the central nervous system in relation to its anxiolytic action. Usually, the decreased activity of an animal under test is viewed as anxiolytic effect [4]. Animals, specifically rodents, are commonly used as experimental animals in preclinical tests to evaluate drug influence on behavior due to their actions' ability to be accurately monitored under controlled conditions. Wistar rats are usually chosen for studying the influence of plant extracts and other substances on the central nervous system. Actophotometer is a device used to measure the degree of movement through changes of light beams while moving. Analysis of the activity level both before and after treatment helps determine the influence of a substance on natural animal movements. Nevertheless, it is important to consider the experimental settings and additional behavior reactions while analyzing the data. Prevailing studies prove that measuring locomotor activity is effective in the investigation of medicinal plants. In particular, the study of Rath et al. evaluated the effect of methanolic leaf extract of Clerodendrum viscosum on Wistar rats. It was revealed that the behavioral response depended both on the doses applied and on the kind of tests done including actophotometer test. Thus, the results of this study prove that the influence of plant extracts on the central nervous system depends on the doses and kind of behavior tested [5]. Cryptostegia grandiflora Roxb.ex R.Br., belonging to the family Apocynaceae, is one of the plants, whose leaves have been investigated for their pharmacological properties. Castro et al. have discovered that ethanolic extract and fractions of the leaves possessed anti-inflammatory and antioxidant activities. Another investigation indicated that methanolic extract of the leaves exhibited analgesic activity in Swiss albino mice. These investigations prove that biological activities have been investigated for the plant, but there is no proof that ethanolic extract of the leaves possesses central nervous system stimulating properties. Moreover, results obtained using different solvents and experimental models cannot be used for the analysis of the CNS activity of ethanolic extract [6].

Thus, the current study was conducted to assess the CNS stimulant-like effect of the ethanolic leaf extract of Cryptostegia grandiflora by means of actophotometer in an experimental animal model with caffeine as the reference drug.

METHODS

Plant Profile

Botanical name: Cryptostegia grandiflora R. Br.

Common names: Indian rubber vine, purple allamanda

Family: Apocynaceae

Synonym: Cryptostegia grandiflora

Preparation of plant extract

Cryptostegia grandiflora leaves were gathered, cleansed to get rid of foreign objects and sticky dirt, dried, and ground into a powder. A hydroalcoholic solvent comprising 525 mL of ethanol and 225 mL of distilled water was used to cold macerate about 75 g of the powdered leaves. To aid in the extraction of phytoconstituents, the powdered plant material was combined with the solvent and allowed to macerate while being stirred periodically. The ethanolic extract of Cryptostegia grandiflora leaves (EECG) was obtained by filtering the mixture after maceration, concentrating, and drying the filtrate. The dried extract was collected and stored in a well-sealed container until it was required. The percentage yield was calculated and recorded.

Animals

Wistar rats of both genders, with a body weight ranging from 150 to 250 grams, were acquired from the institutional animal facility. They were kept in a controlled environment with a temperature between 20°C and 24°C, and a relative humidity between 40% and 60%. The rats were provided with a 12-hour light cycle and 12-hour dark cycle. They had unrestricted access to standard food pellets and water. Permission for the experiments was granted by the Institutional Animal Ethics Committee (IAEC) with the approval number NCP/IAEC/UG/2025-2026/14.

Locomotor activity test

Locomotor activity was measured using an actophotometer to evaluate the central nervous system (CNS) stimulant effects of the ethanolic extract of Cryptostegia grandiflora leaves (EECG). Wistar rats were randomly

divided into four groups, each containing four animals. Group I was the control group and received normal saline (0.9% NaCl). Group II was given caffeine at a dose of 10 mg/kg as the standard drug. Groups III and IV were administered EECG at doses of 100 mg/kg and 200 mg/kg, respectively. Each animal was individually placed in the actophotometer for a period of 10 minutes, and locomotor activity was recorded based on photocell counts generated by the interruption of light beams due to animal movement. Baseline activity was recorded prior to treatment, and activity levels were measured one hour after drug administration. The differences in locomotor activity between the control, standard drug, and EECG-treated groups were analyzed to assess the CNS stimulant activity of EECG.

Instrumentation

The dimensions of the device were 30×30×30 cm. Two photocells were positioned 3 cm above the floor. Each wall of the cage had three equally spaced holes arranged horizontally, located 7 cm above the floor. Light beams were directed through these holes from all sides of the apparatus, and they intersected at the center of the cage. Infra-red filters were used to reduce the influence of light on the animal's behavior. The number of times the light beams were interrupted was automatically recorded by digital counters in the instrument. Locomotor activity can serve as an indicator of wakefulness or alertness, reflecting the level of mental activity. An actophotometer is utilized to measure locomotor activity, with photoelectric cells connected to a counter. When the light beam on a photocell was blocked by the animal, a count was registered. The animals were divided into four groups, each containing four animals. The dose administered was chosen based on results from acute toxicity studies, ensuring a safe dosage.

Statistical Analysis

Statistical analysis Data were expressed as Mean ± SEM and statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS

The locomotor activity of the animals was determined and compared with the normal control and standard. The result revealed that EECG leaves extract in low dose (258±12.4 sec) and high dose (292±17.2 sec), both showed significantly lower activity than standard drug (317±17.8sec). The dose of 200 mg/kg has shown significant increase in locomotor activity ($p<0.01$)

Table 1. CNS stimulant activity of EECG leaves in locomotor activity

Group	Treatment	Dose	Locomotor activity (10 min in secs)		% CNS stimulant activity
			Basal score (B)	Treatment (A)	
I	Normal Saline	0.9 % Nacl	182±10.4	178±12.6	----
II	Standard drug (Caffeine)	10 mg/kg	187±14.2	317±17.8	70
III	EECG leaves	100 mg/kg extract	171±11.6	258±12.4	51
IV	EECG leaves	200 mg/kg extract	180±15.8	292±17.2	62

Values were mean ± SEM, n=4, *P*<0.01 vs control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

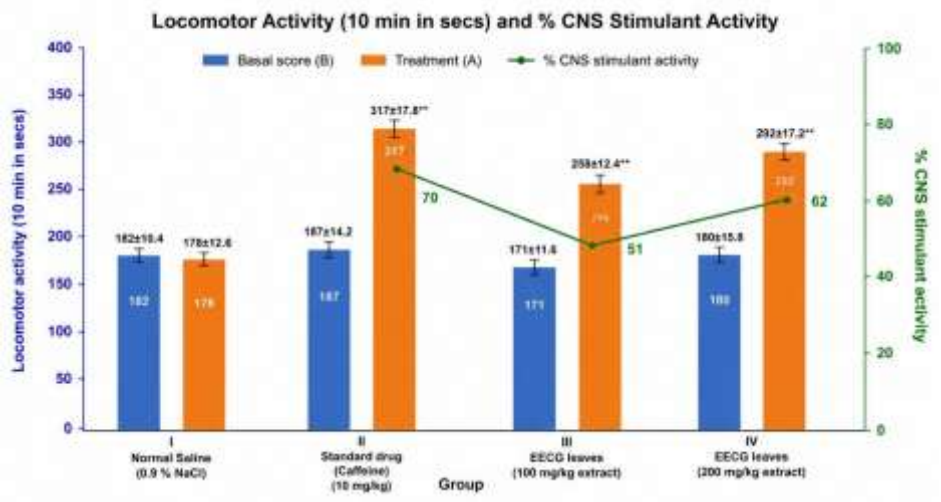


Fig. 1. Locomotor activity (10 min in secs) and % CNS stimulant activity

Values represent mean ± SEM; n= 4; analysis was performed using one-way ANOVA, followed by Dunnett's test. $p<0.01$ was considered as statistically significant

DISCUSSION

The actophotometer method was employed in this study to assess the effect of the EECG leaf extract on locomotor activity in Wistar rats. One common way to measure the effect of the CNS stimulant is the locomotor activity. Compared to the normal control group in this experiment, both doses of EECG extract increased the locomotor activity significantly. The validity of the experimental model was confirmed by the standard drug caffeine (10 mg/kg) with maximum locomotor activity (317±17.8 sec). The EECG extract (100 mg/kg) caused locomotor activity of 258±12.4 (counts/10 min), which signifies the moderate effect of CNS stimulant. A much higher level of locomotor activity was achieved by the 200 mg/kg dose of the EECG extract (292±17.2 sec). The percentage of the CNS stimulant activity was 51% for 100 mg/kg dose and 62% for 200 mg/kg dose. Statistically, both doses were found to be significantly different from the control ($P<0.01$). This indicated the efficacy of the doses. The high dose exhibited activity levels comparable to that of caffeine, indicating that it is more stimulating. The increased locomotor activity can be explained by the fact that the extract contains certain phytoconstituents such as flavonoids, alkaloids or phenolics. This may have an impact on neurotransmitter secretion or activation of the dopaminergic pathways in the brain.

CONCLUSION

From the above analysis, the extract from the EECG leaves is effective as a CNS stimulant compared to other groups. Both the doses used in this experiment caused a rise in locomotor activity in animals. However, the high dose of 200 mg/kg had a stronger stimulating effect compared to the low dose of 100 mg/kg. This means that the stimulating effect is dose-dependent. Moreover, the stimulating effect was comparable to that of the reference substance caffeine. It can be assumed that this stimulating effect of the extract is due to the active phytochemicals contained in the leaves. In conclusion, the present study shows that EECG is pharmacologically important as a CNS stimulant.

Author Contributions

Sivakumar G M was responsible for conceiving and overseeing the study. Perumal P and Shanmugapriya M, who is the corresponding author, played a key role in the planning and management of the research. Ahamed Shameem M, Aravinth K, Karthick S, and Sivakumar M were involved in carrying out the research and preparing the manuscript. All of the authors reviewed the final version of the manuscript and gave their approval.

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Ethical approval

The study was approved by the Institutional Animal Ethics Committee.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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