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Original Article

STUDY TO INVESTIGATE THE ANXIOLYTIC EFFECT OF RUBIA CORDIFOLIA LINN IN MICE

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ABSTRACT

Objective: To evaluate anxiolytic activity of extracts *of Rubia cordifolia* in albino mice by using Actophotometer (locomotor activity), Elevated Zero maze, Mirrored Chamber and biochemical estimation like estimation of plasma corticosterone levels.

Methods: Healthy, Swiss Albino mice weighing 20-35 g and aged 6-8 w were maintained under standard laboratory conditions with controlled temperature (25±2 °C), humidity (40±10 %) and 12 h light and dark cycles each. The animals were fed with standards rodent pellet diet and water *ad libitum*. Rubia cordifolia extract will be administered p. o.. to mice daily in two different doses (300 and 600 mg/kg/p. o.) for 14 d. All the animals will be evaluated for behavioral parameters, i. e. Actophotometer (locomotor activity), Elevated Zero maze, Mirrored Chamber and biochemical estimation like estimation of plasma corticosterone levels will be performed.

Results: In the present study, we have undertaken the anxiolytic activity of extracts of *Rubia cordifolia* in Swiss albino mice, at the dose of (300 and 600 mg/kg/p. o.) with oral administration respectively.

Conclusion: In conclusion extracts of *Rubia cordifolia* have shown significant results as anxiolytic activity using locomotor activity), Elevated Zero maze, Mirrored Chamber.

Keywords: Rubia Cordifolia, Anxiety, Locomotors activity, Plasma corticosterone, Elevated zero maze and mirrored chamber

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INTRODUCTION

Anxiety is a natural response to stress and can be helpful in certain circumstances. The word "anxiety" refers to a common emotion that people have in response to stress, danger, or threats. People who experience anxiety usually feel tense, unpleasant, and upset [1]. Excessive and unreasonable worry over tasks, situations, or even individual things is a hallmark of anxiety disorders. Worry disorders are not a single sickness, but rather a collection of conditions marked by excessive tension, discomfort, and persistently high levels of worry. The substantial prevalence of anxiety disorders in the global general population is demonstrated by recent epidemiological studies [2]. Anxiety disorders affect 13.3% of Americans, with an estimated 15.6 billion Americans experiencing anxiety problems annually [3]. When a person has anxiety to the point where it substantially disrupts their everyday life and prevents them from achieving their goals, they are likely to be diagnosed with an anxiety disorder. One in twenty persons suffers from an anxiety disorder, which is the most prevalent type of mental illness. According to epidemiological data, anxiety is a prevalent and significant issue in later life and has been linked to increased impairment risk in older persons overall [4].

If left untreated, anxiety disorders can cause significant distress and impair a person's life by influencing their thoughts, feelings, and behaviors. anxiety can usually be effectively treated. Women are at least twice as likely as males to experience one of the three anxiety disorders during their lifetime [5, 6]. Anxiety is a normal and necessary part of life. Evolutionarily speaking, it is crucial because people usually feel anxious when confronted with environmental dangers like coming across a lion, which is not a common worry in contemporary society, or when there is a shortage of food or other resources, or when they are accepted by their peers and society as a whole [7]. Anxiety is an adaptive emotion that helps people respond appropriately to stressful situations by changing their behavior and physiology. When faced with a stressful circumstance, everyone feels a certain level of anxiety and trepidation. Anxiety disorders can be defined as situations that fully disrupt a person's daily life. It has been proposed that cognitive biases have a role in the development and may even be the cause of anxiety. Restlessness, exhaustion, mental tension, irritability, sleep difficulties, poor stress management, chest discomfort, abdominal trouble, tachycardia, shortness of breath, shaking, blushing, diarrhea, sweating, and so on are some of the physical signs of anxiety disorders. Anxiety is a mental illness that manifests as excessive worry, motor tension, and exhaustion, along with involuntary symptoms like headache, palpitations, chest tightness, restlessness, and moderate stomach pain that may be triggered by a dangerous stimuli or circumstance [8, 9]. Indian madder, or Rubia cordifolia L. (Rubiaceae), is a significant medicinal plant that is used in Indian traditional medicine to treat a variety of illnesses. The climbing plant Rubia cordifolia grows in the Nilgiris, the North-West Himalayas, and other hilly regions of India at elevations between 1500 and 2500 meters. There are other colloquial names for plant drugs, including Majathi, Assamme Mandar, and Sanskrit Aruna. English Indian Madder in Malayalam, Bengali Manjith, Marathi Manjesta Manjit, Hindi Majit [10]. It is a climbing herb, perennial, or prostate. Sharp, 4-angled, minutely prickly stems; elliptic to ovate-cordate, long petiolate leaves in whorles of 6–8; greenish-yellow flowers in axillary panicles of dichotomous cymes; two-celled, globose, smooth, shining, purplish-black when ripe fruits June through October and Mainly the root and stem are used. Although practically every portion is used, the leaves, root barks, and fruits are crucial for making medications. In the indigenous medical system, Rubia cordifolia is utilized as medication; its fruits and barks are the most effective [11]. According to traditional folklore, it can help with weight control, skin care, diabetes management, cancer prevention, bone health, and inflammation reduction. Additionally, they are used to treat fungal infections, constipation, skin aging, sleep disorders, and stress [12]. The species can tolerate temperatures between 15 and 35 degrees Celsius at high elevations in the Himalayas. The ideal soil for the plant is light, loose, moist, and somewhat shaded. Because the root penetrates deeply into the soil, agriculture benefits from porous, well-aerated soils. In order to keep the beds moist, weekly irrigation is advised. Stem fragments are directly planted for vegetative

propagation [13]. When compared to a group of albino mice that were given Aβ 25-35, the extract from Rubia cordifolia demonstrated a strong protective effect against neurodegeneration and an enhancement in memory retention ability. Due to the GSH and vitamin C content of the plant, Rubia cordifolia exhibits neuroprotective activity by preventing the reduction and increasing GSH levels by inducing GCLC (c-glutamylcysteine ligase) expression, reducing oxidative stress levels by direct scavenging, and decreasing iNOS expression.

MATERIALS AND METHODS

The research protocol of this study has been approved by Institutional Animal Ethics Committee (IAEC) of St. Soldier institute of Pharmacy, Jalandhar, Punjab vide approval no. 2011/PO/Re/S/18/CPCSEA and 1/5/2018 Protocol No. IAEC/SSIP/2020/PR-012.

Animals

Swiss Albino mice weighing 20-35 g and aged 6-8 w were procured from National Institute of Pharmaceutical Education and Research, Mohali, Punjab. The animals were acclimatized for seven days to the housing conditions of Central Animal House Facility of St. Soldier institute of Pharmacy, Jalandhar prior to experiments. Animal were housed and maintained under standard laboratory conditions with controlled temperature (25±2 °C), humidity (40±10 %) and 12 h light and dark cycles each. The animals were fed with standards rodent pellet diet and water *ad libitum*. The experiments were carried out between 09:00 to 17:00 h. The laboratory animals were maintained as per CPCSEA guidelines.

Drugs and chemicals

Rubia cordifolia extract was purchased from Kshipra biotech pvt. Ltd., Indore

Administration of *Rubia cordifolia* extract

Rubia cordifolia extract will be administered p. o. to mice daily in two different doses (300 and 600 mg/kg/p. o.) for 14 d. The feed and water consumption of the treated animals was monitored.

Experimental design

Treatment schedule

Rubia cordifolia extract will be administered p. o. to mice daily in two different doses (300 and 600 mg/kg/p. o.) for 14 d. All the animals will be evaluated for behavioral parameters, i. e. Actophotometer (locomotor activity), Elevated Zero maze, Mirrored Chamber and Biochemical estimation like estimation of plasma corticosterone levels will be performed.

Control group

Mice were handled gently without any stress and after 14 d all the animals will be evaluated for behavioral parameters, i. e. Actophotometer (locomotor activity), Elevated Zero maze, Mirrored Chamber, and Biochemical estimation like estimation of plasma corticosterone levels will be performed.

RCE (300 mg/kg (p. o.))

Rubia cordifolia extract 300 mg/kg (p. o.) were administered for 14 successive days. All the animals will be evaluated for behavioral parameters, i. e. Actophotometer (locomotors activity),. Elevated Zero maze, Mirrored Chamber and Biochemical estimation like estimation of plasma corticosterone levels will be performed.

RCE (600 mg/kg (p. o.))

Rubia cordifolia extract 600 mg/kg (p. o.) were administered for 14 successive days. All the animals will be evaluated for behavioral parameters, i. e. Actophotometer (locomotors activity), Elevated Zero maze, Mirrored Chamber, and Biochemical estimation like estimation of plasma corticosterone levels will be performed.

APZ (0.25 mg/kg *i. p.*)

Alprazolam 0.25 mg/kg *i. p.* was administered for 14 successive days. All the animals will be evaluated for behavioral parameters, i. e. Actophotometer (locomotor activity). Elevated Zero maze, Mirrored Chamber, and Biochemical estimation like estimation of plasma corticosterone levels will be performed.

Table 1: Treatment of different groups

S. No.	Group	Treatment (For 14 d)	Behavioral and biochemical estimations
I	Normal control, normal saline 0.9% NaCl (10 ml/kg, or 1 ml of/100 g body weight p. o.)	Yes	Yes
II	RCE 300 mg/kg (p. o.)	Yes	Yes
III	RCE 600 mg/kg (p. o.)	Yes	Yes
IV	Alprazolam (0.25 mg/kg *i. p)*	Yes	Yes

Behavioral estimations

Locomotors activity

The majority of medications that work on the central nervous system affect how humans and animals move. Alcohol and barbiturates are examples of CNS depressants that decrease motor function. Stated differently, locomotor activity may serve as a gauge of mental activity's alertness or wakefulness. An actophotometer, which uses photoelectric cells that are coupled in circuit with a counter, makes it simple to quantify locomotor activity. A count is made when the animal cuts off the light beam that is hitting the photocell. The animal's movement could be recorded using an actophotometer that is square or circular in shape. This equipment may be tested on both rats and mice [15].

Elevated zero maze

The elevated zero maze (EZM) is a sensitive behavioral test that may be used to identify antineophobic and anxiolytic effects of medications and to disclose an animal's neophobia or nervousness. This labyrinth is 40 cm high, black, and annular, with an inner diameter of 30 cm and an outside circumference of 45 cm. The mouse can explore a runway ring that is 6 cm wide and has four quadrants: two opposing "open" quadrants with no walls and two opposing "closed" quadrants with walls that are 12 cm high. To keep the mouse from falling off, the open quadrants contain ridges of two to three millimeters. The thickness of the walls is 0.75 cm. For six minutes, each animal was put in a closed arm with its back to the open arm, and the following parameters were recorded [16].

Mirrored chamber

A quick, easy, and quantifiable method for evaluating anti-anxiety medications is the mirrored chamber test. The device consists of a square wooden box with a mirror of a cube that is open on one side. The thirty-cm-long mirrored cube is made up of five pieces of mirrored glass, one of which is mirrored and the other is dark brown. The inner cube was facing the floor pane, the top pane, and the three mirrored sides. 40 cm by 40 cm by 30 cm is the size of the container box. In order to create a 5 centimeter corridor that entirely encloses the mirrored chamber, the mirrored cube is positioned in the middle of the wooden container. Additionally, a mirror is positioned on the container sides facing the mirrored chamber's only open side [17].

Biochemical estimation

Collection of blood samples

On 14th d, blood (0.3 ml) was withdrawn from tail vein from all groups of mice. Blood samples were centrifuged at 2500 rpm for 10

min using refrigerated centrifuge (Paramount scientific works, Ambala cantt, India) to separate the plasma, which was used for estimation of corticosterone levels.

(a) Estimation of plasma corticosterone levels

The amount of corticosterone in the blood plasma was quantitatively estimated. After immersing the tubes in cold water for five minutes, 0.50 ml of 0.10 N sodium hydroxide was added to 1.0 ml of the sample in ethanol along with 0.50 ml of a 0.10 percent solution of p-nitroso-N,N-dimethylaniline in ethanol. After being sealed with cotton-wool, the tubes were left in a dark place at 0 °C for five hours. 2.0 ml of pH 9.8 buffer, 5.0 ml of a 0.10 % phenol in ethanol solution, and 0.50 ml of a 1.0 % potassium ferricyanide aqueous solution were added to the aforesaid solution. For ten minutes, the tubes were submerged in a water bath at 20±2 °C. A UV-visible spectrophotometer (UV 3200 UV-VIS Spectrophotometer, Somajiguda, Hyderabad) was used to read the solution at 650 nm.

Statistical analysis

Mean±SEM was used to express all of the data. Graph pad prism in stat (Graph Pad Software Inc., USA) was used to analyze the data for each group using a one-way ANOVA and Turkey's test. A significance level of p<0.05 was applied.

RESULTS

Effect of *Rubia cordifolia* extract on body weight (g) of mice

The mice of *Rubia cordifolia* extract (300 and 600 mg/kg/p. o.) treated group showed significantly (p<0.05) increased in body weight as compared to the control group. Treatment with APZ (0.25 mg/kg i. p) the body weight significantly increased as compared to normal group.

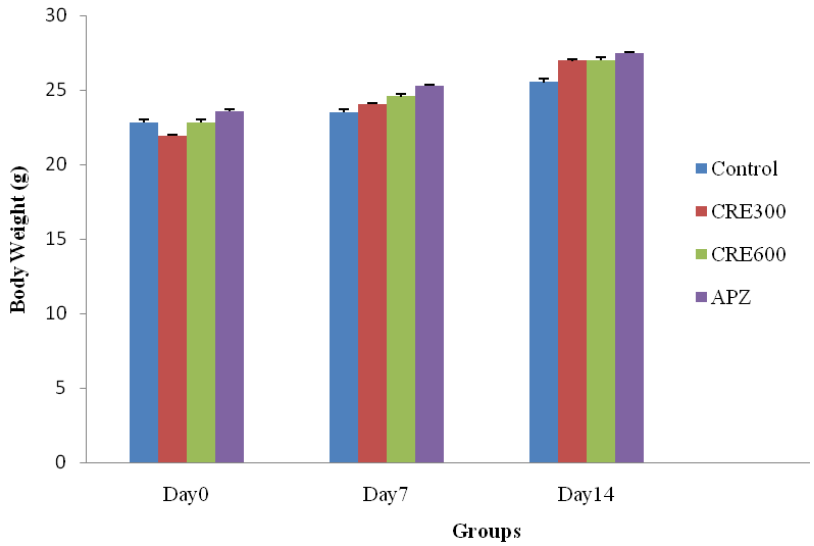

Fig. 1: Effect of *Rubia cordifolia* extract on body weight (g) of mice, values are expressed as mean±SEM [a]denotes p<0.05 as compared with to normal control group and [b]Denotes p<0.05 as compared to APZ (0.25 mg/kg i. p) treated group (One way ANOVA followed by Tukey's test)

Effect of *Rubia cordifolia* extract on feed intake (g) of mice

The mice of *Rubia cordifolia* extract (300 and 600 mg/kg/p. o.) treated group showed significantly (p<0.05) increased in feed intake as compared to the control group. Treatment with APZ (0.25 mg/kg i. p) the feed intake significantly (p<0.05) increased as compared to control group group.

Effect of *Rubia cordifolia* extract on water intake (ml) of mice

The mice of *Rubia cordifolia* extract (300 and600 mg/kg/p. o.) treated group showed significantly (p<0.05) increased in body water intake as compared to the control group. Treatment with APZ (0.25 mg/kg i. p) the water intake significantly (p<0.05) increased as compared to caffeine group.

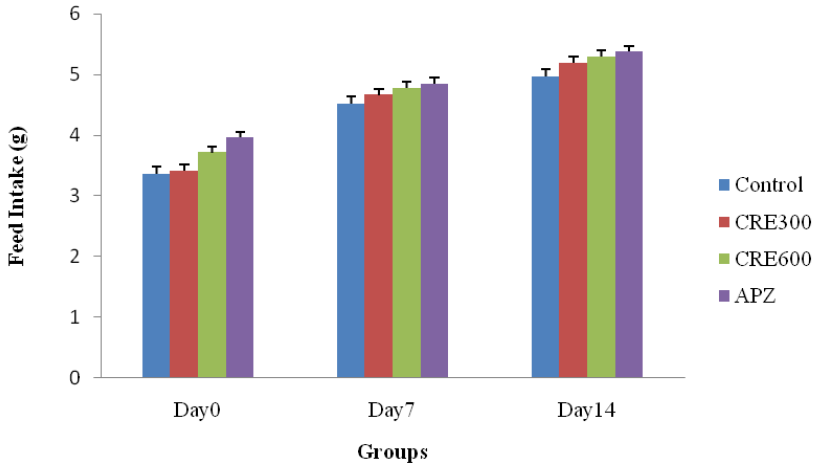

Fig. 2: Effect of *Rubia cordifolia* extract on feed intake (g) of mice, values are expressed as mean±SEM [a]denotes$p$<0.05 as compared with to normal control group and [b]denotes p<0.05 as compared to APZ (0.25 mg/kg i. p) treated group (One way ANOVA followed by Tukey's test)

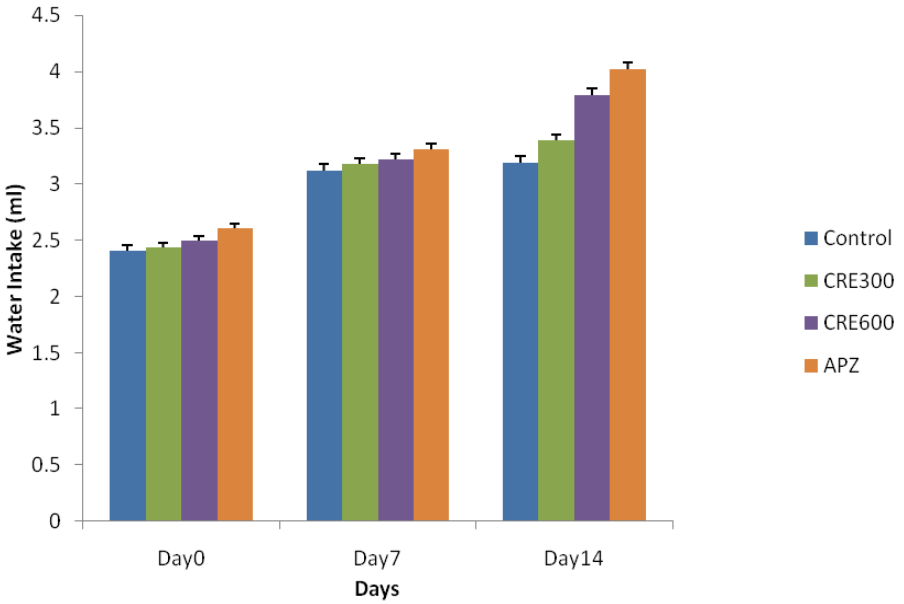

Fig. 3: Effect of *Rubia cordifolia* extract on water intake (ml) of mice, values are expressed as mean±SEM [a]denotes $p<0.05$ as compared with to normal control group and [b]denotes $p<0.05$ as compared to APZ (0.25 mg/kg i. p) treated group (One way ANOVA followed by Tukey's test)

Effect of *Rubia cordifolia* extract on locomotors activity of mice

The mice of *Rubia cordifolia* extract (300 and 600 mg/kg/p. o.) treated group showed no significantly ($p<0.05$) difference in locomotors activity as compared to control group. Treatment with APZ (0.25 mg/kg i. p) also showed no significant change as compared to control group.

Elevated zero maze

Effect of *Rubia cordifolia* extract on latency to enter in open arm (LEO)

An decrease in LEO than control indicates induction of anxiety and vice-versa. The mice of *Rubia cordifolia* extract (300 and 600 mg/kg/p. o.) treated group showed decreased ($p<0.05$) LEO as compared to normal control group. However pretreatment with APZ (0.25 mg/kg i. p) for 14 d also reduced ($p<0.05$) the LEO.

Effect of *Rubia cordifolia* extracts number of entries in open arm (NEO)

NEO indicates how many times the mouse entered in the open arm. Higher the frequency of entry in open arm, lower the anxiety level and vice versa. The mice of *Rubia cordifolia* extract ((300 and 600 mg/kg/p. o.) treated group showed increased ($p<0.05$) NEO as compared to normal control group. However pretreatment with APZ (0.25 mg/kg i. p) 14 d also increased the NEO.

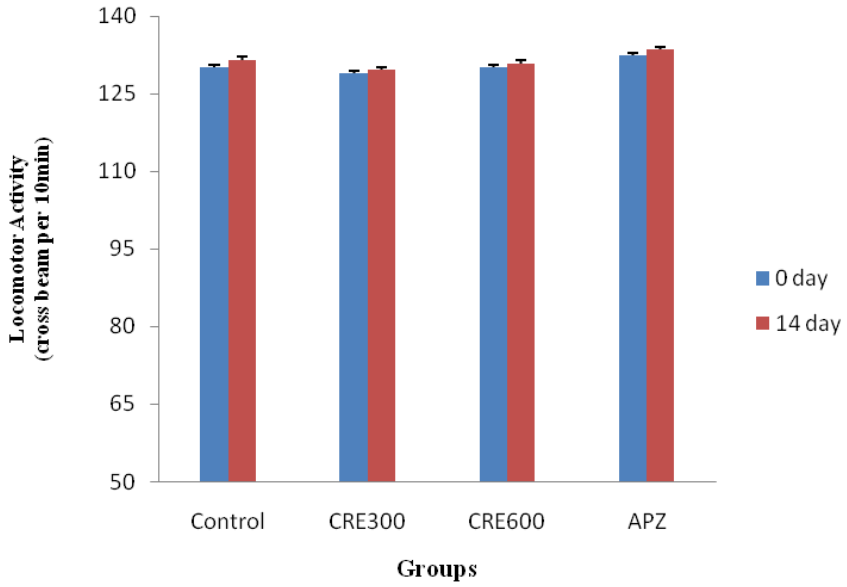

Fig. 4: Effect of *Rubia cordifolia* extract on locomotor activity of mice, values are expressed as mean±SEM (One way ANOVA followed by Tukey's test)

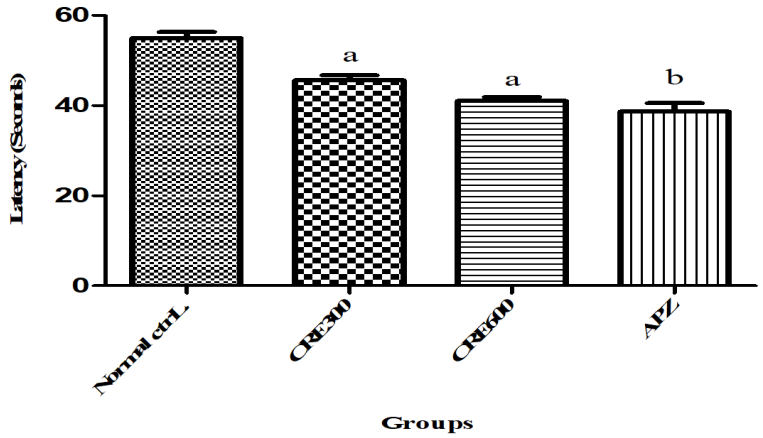

Fig. 5: Effect of *Rubia cordifolia* extract on LEO in elevated zero maze test, values are expressed as mean±SEM [a]denotes*p*<0.05 as compared with to normal control group and [b] denotes *p*<0.05 as compared to APZ (0.25 mg/kg i. p) treated group (One way ANOVA followed by Tukey's test)

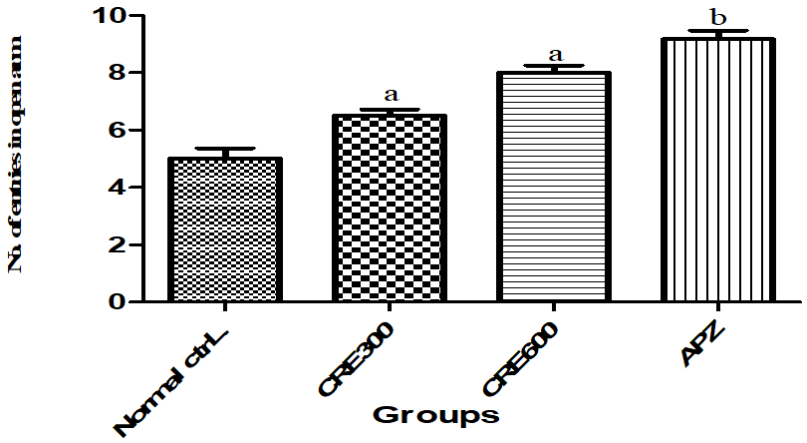

Fig. 6: Effect of *Rubia cordifolia* extract on NEO in elevated zero maze test, values are expressed as mean±SEM [a]denotes *p*<0.05 as compared with to normal control group and [b]denotes *p*<0.05 as compared to APZ (0.25 mg/kg i. p) treated group (One way ANOVA followed by Tukey's test)

Effect of *Rubia cordifolia* extract on time spent open arm (TSO)

TSO indicates the average time spent in open arm by the animal out of total 360 seconds. More the time spent by animal in open arm, lower the anxiety and vice-versa. The mice of *Rubia cordifolia* extract (300 and 600 mg/kg/p. o.) treated group showed increased (*p*<0.05) in TSO as compared to normal control group. However pretreatment with APZ (0.25 mg/kg i. p) 14 d increased the TSO significantly.

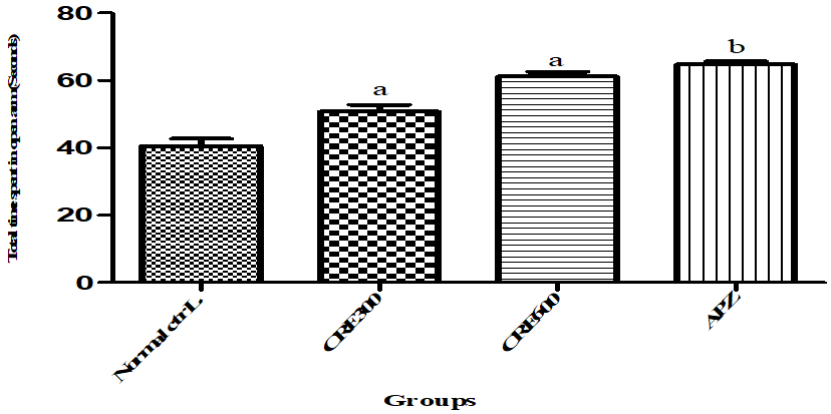

Fig. 7: Effect of *Rubia cordifolia* extract on TSO in elevated zero maze test, values are expressed as mean±SEM [a]denotes *p*<0.05 as compared with to normal control group and [b]denotes *p*<0.05 as compared to APZ (0.25 mg/kg i. p) treated group (One way ANOVA followed by Tukey's test)

Mirrored chamber

Effect of *Rubia cordifolia* extract on latency to enter in mirrored chamber

LEMC is the time taken by animal for first entry into the mirrored chamber. An increase in LEMC than control indicates induction of anxiety and vice-versa. The mice of *Rubia cordifolia* extract (300 and 600 mg/kg/p. o.) treated group showed decreased (p<0.05) LEMC as compared to normal control group. Pretreatment with APZ (0.25 mg/kg i. p) 14 d reduced (p<0.05) the LEMC significantly.

Effect of *Rubia cordifolia* extract on number of entries in mirrored chamber (NEMC)

NEMC indicates how many times the mouse entered in the mirrored chamber. Higher the frequency of entry in mirrored chamber, lower the anxiety level and vice versa. The mice of *Rubia cordifolia* extract (300 and 600 mg/kg/p. o.) treated group showed increased (p<0.05) NEMC as compared to normal control group. Pretreatment with APZ (0.25 mg/kg i. p) 21 d show a increased (p<0.05) the NEMC significantly.

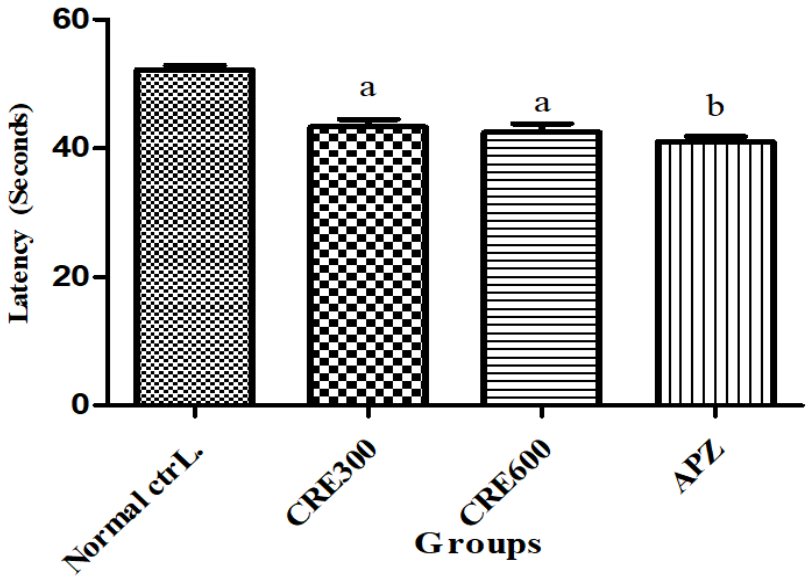

Fig. 8: Effect of *Rubia cordifolia* extract on LEMC, values are expressed as mean±SEM [a]Denotes p<0.05 as compared with to normal control group and [b]Denotes p<0.05 as compared to APZ (0.25 mg/kg i. p) treated group (One way ANOVA followed by Tukey's test)

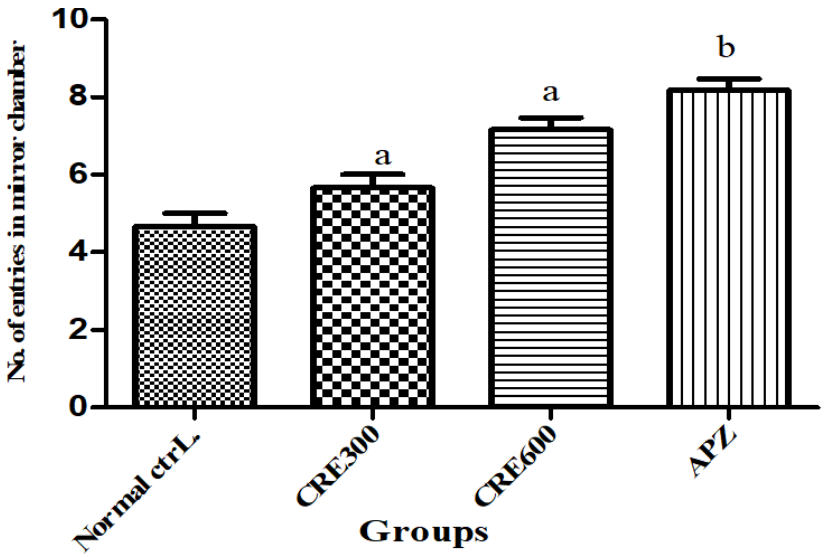

Fig. 9: Effect of *Rubia cordifolia* extract on NEMC, values are expressed as mean±SEM [a]Denotes p<0.05 as compared with to normal control group and [b]Denotes p<0.05 as compared to APZ (0.25 mg/kg i. p) treated group (One way ANOVA followed by Tukey's test)

Effect of *Rubia cordifolia* extract on time spent in mirrored chamber (TSMC)

TSMC indicates average time spent in mirrored chamber by the animal out of total 300 seconds. More the time spent by animal in mirrored chamber, lower the anxiety and vice-versa. The mice of *Rubia cordifolia* extract (300 and 600 mg/kg/p. o.) treated group showed increased (p<0.05) in TSMC as compared to normal control group. Pretreatment with APZ (0.25 mg/kg i. p) 14 d increased (p<0.05) the TSMC significantly.

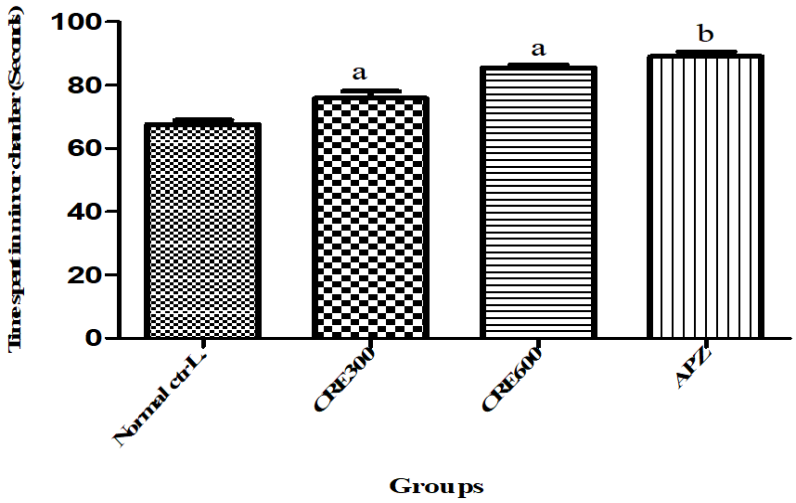

Fig. 10: Effect of *Rubia cordifolia* extract TSMC, values are expressed as mean±SEM [a]Denotes *p*<0.05 as compared with to normal control group and [b]Denotes *p*<0.05 as compared to APZ (0.25 mg/kg i. p) treated group (One way ANOVA followed by Tukey's test)

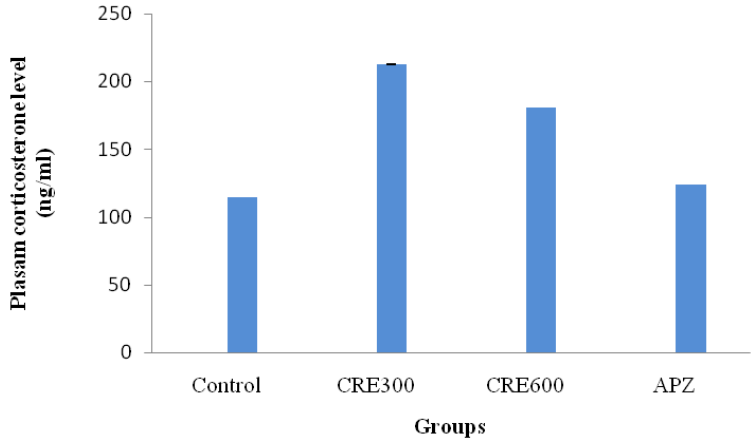

Fig. 11: Effect of *Rubia cordifolia* extract on plasma corticosterone levels, values are expressed as mean±SEM [a]Denotes *p*<0.05 as compared with to normal control group and [b]Denotes *p*<0.05 as compared to APZ (0.25 mg/kg i. p) treated group (One way ANOVA followed by Tukey's test)

Effect of *Rubia cordifolia* extract on plasma corticosterone levels (CORT)

The mice of *Rubia cordifolia* extract (300 and 600 mg/kg/p. o.) treated group showed increased plasma corticosterone level ($p<0.05$) as compared to normal control mice. However pretreatment) APZ (0.25 mg/kg i. p) 14 d showed significant increased ($p<0.05$) in plasma corticosterone significantly.

CONCLUSION

The present "Study to investigate the anxiolytic effect of *Rubia cordifolia* Linn in mice" Male Swiss albino mice of age 6-8 w and weight 20-35 g were used in the present study. *Rubia cordifolia* extract will be administered p. o. to mice daily in three different doses (300 and 600 mg/kg/p. o.) for 14 d regularly. On 15th d, the animal subjected to locomotor activity, elevated zero maze, mirrored chamber test and plasma corticosterone level. Experimental animals as indicated by decrease LEO and increase in NEO as well as TSO in elevated zero maze studies. Also decrease LEMC and increased NEMC as well as TSMC in mirrored chamber studies. Administration of *Rubia cordifolia* extract will be administered p. o. to mice daily in three different doses extract (300and 600 mg/kg/p. o.) for 14 d significantly reduced anxiety. Thus, *Rubia cordifolia* extract may prove to be useful remedy for the management of anxiety owing to its possible neuroprotective and its antioxidant properties.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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