

**Evaluation of anxiolytic potential of *Cichorium Intybus* methanolic root extracts**

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Article Info: Received 25 November 2020; Accepted 18 December. 2020**DOI:** <https://doi.org/10.32553/jbpr.v9i6.817>**Corresponding author:** S.Tripathy**Conflict of interest statement:** No conflict of interest**Abstract:**

Cichorium Intybus L. is a medicinally important plant that belongs to the family Asteraceae. The plant root is used as anti-hepatotoxic, antiulcerogenic, antiinflammatory, appetizer, digestive, stomachic, liver tonic, cholagogue, cardiogenic, depurative, diuretic, emmenagogue, febrifuge, alexeteric and also as tonic. The present study was carried to screen the anxiolytic potential of *Cichorium Intybus* L methanolic root extracts by using different animal models like actophotometer, rotarod, staircase, holeboard, elevated plus maze tests . In the experiment the extracts found to decrease the anticipatory anxiety of the animal without significant alteration in motor coordination. Efficacy may be attributed to the presence of different phytochemicals like coumarins, flavonoids in the extracts.

Introduction:

Anxiety and related disorders, most common mental illnesses worldwide, represent a prominent healthcare problem throughout in current scenarios. Reports from the World Health Organization (WHO) suggest that anxiety and related disorders will become the second leading cause of disability in both developed and developing countries. Pharmacotherapeutic approaches for the management of anxiety disorders include psychotropic drugs, but these agents are limited by their side-effect profile, the need for dietary precautions, and drug interactions (Baldessarini R2001). Due to the side effects of anxiolytic treatment, there has been increasing interest in the use of alternative therapies including aromatherapy and herbal therapy (Bradley BF,2007). Therefore, the search for new therapeutic agents continues. Interest in alternative medicine and plant-derived functional food to conquer stress and promote relaxation has increased recently.

Cichorium Intybus L. is a medicinally important plant that belongs to the family Asteraceae. The tuberous root of this plant contains number of medicinally important compounds such as inulin, bitter sesquiterpene lactones, coumarins, flavonoids and vitamins (Varotto, S2000.). The plant root is used as anti-hepatotoxic, antiulcerogenic, antiinflammatory, appetizer, digestive, stomachic, liver tonic, cholagogue, cardiogenic, depurative, diuretic, emmenagogue, febrifuge, alexeteric and also as tonic. It is useful in vitiated conditions of kapha and pitta, cephalalgia, hepatomegaly, inflammations, anorexia, dyspepsia, flatulence, colic, gout, burning sensation, allergic conditions of skin, jaundice, splenomegaly, hyperdipsia, skin diseases, leprosy, strangury, amenorrhoea, chronic and bilious fevers, ophthalmia, pharyngitis, vomiting, arthralgia, lumbago, asthma and general debility (Nadkarni, A.K., 1976). This plant is also used to treat AIDS, cancer,

diabetes, dysmenorrhoea, impotence, insomnia, splenitis and tachycardia (Duke, J.A., 1983.). Inulin is used to replace fat or sugar and reduce the calories of food. It is suitable for consumption by diabetics (Niness, K.R., 1999.)and is also used in inulin clearance test to measure glomerular filtration rate-GFR (Vasudevan, D.M. 1995). Recent pharmacological investigation of the root extract of this plant revealed immunomodulator, antitumor and anticancer properties (Angelina etal 1999. Hazra, B., R. et al 2002.,). The sesquiterpene lactones such as lactucin and lactucopicrin were isolated from chicory and reported for its antibacterial and antimalarial activity (Bischoff,2004). Therefore, we undertook the study to evaluate the anxiolytic potential of *Cichorium Intybus* root extractsby using different animal models and studying the effect of the plant on their exploratory behavior.

Materials and methods:**Collection of plant material:**

The dried roots of *Cichorium Intybus* are collected from the Chicory Factory, located in Ananthapur District. The plant material was authenticated a specimen was kept in the department for future referances. The roots were cleaned, shade dried, coarse powdered, and subjected to extraction with methanol by using Soxhlet's apparatus.

Preparation of ethanolic extract:

The plant material was cut into small pieces and powdered coarsely and then placed in the extraction thimble. The 100grams of chicory root powder was placed in an extraction chamber which was suspended above the flask containing the solvent methanol and below a condenser. The flask was heated and the methanol evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The

extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the methanol extract was removed and methanol was evaporated by heating mantle.

Drugs: Diazepam inj. i.p, (Ranbaxy laboratories limited, Boiser, Thane, India) was used as a reference drug. It was diluted using saline before use. Different doses of *Cichorium Intybus* flowers extract were prepared by dissolving with 1% of gum acacia in distilled water. All solutions were prepared freshly on the test days and administered orally.

Treatments: The animals were divided into 3 groups with 8 animals each. All the treatments were given orally 30 minutes prior to the experiment. Group 1 is vehicle control, Group 2 is Standard drug: Diazepam(2mg/kg), Group 3 is Test: *Cichorium Intybus* extract (500mg/kg),

Animals: Healthy adult Swiss albino mice (20-25 g) were used for the study of anxiolytic activity. Experimental animals were purchased from Mahaveer Enterprises Ltd, Hyd. The animals were housed in a group of six per cage and maintained under standard environmental conditions: $25 \pm 2^\circ\text{C}$ temperature, 12:12 h light and dark cycle, and 45-55% relative humidity, with free access to food and water *ad libitum*. Mice were treated with Diazepam (2 mg/kg, i.p.), *Cichorium Intybus* extract for 15 days. 30 min after treatment with diazepam and 1 h after treatment with control and/ or extracts, the following tests were performed.

Pharmacological screening:

General behavioural profile

Evaluation of general behavioural profile was performed by the method of Irwin S. The animals were under observation for behavioral changes if any, at 30 minutes interval in the first hour and at one hour intervals for next 6 h.

Elevated plus maze: The plus maze apparatus consisted of two open arms ($35 \times 5 \text{ cm}^2$) crossed with two closed arms ($35 \times 5 \times 20 \text{ cm}^3$) with a central square of $5 \times 5 \text{ cm}^2$. The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Each treated mouse was individually placed on the center of the elevated plus maze with its head facing the open arm. The time spent and the numbers of entries in both the open and the closed arms were recorded for 5 min. [Adeyemi OO].

Locomotor activity monitoring: The locomotor activity was measured using a digital actophotometer(INCA, Ambala, India) which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. Mice were placed individually in the actophotometer and all the mice were tested for activity score for 5 min[Reddy DS].

Rotarod test: The apparatus consisted of a horizontal metallic rod of 2.5 cm diameter and 45 cm in length and

was divided into 4 sections by 3 plexiglass partitions, thereby allowing simultaneous testing of 4 mice. It was attached to a motor with the variable adjusted speed of 20 rpm. The rod was positioned at a height of 20 cm above the table top. The mice were placed on the rotating rod and observed for the time required falling from the rod in a 5min test session. [R.R. Somani].

Staircase test: Staircase was made up of wooden ply material and composed of 5 identical steps of 3 cm height, 15 cm width and 3 cm depth. The internal height of the walls was constant along the whole length of the staircase. The mice were placed on the floor of the box with their back to the staircase. The number of steps climbed and the number of rears were counted over a period of 5 min.. The average number of steps and rearings were recorded[Yukio Ago].

Hole board: The hole board apparatus consisted of a wooden chamber ($40 \times 40 \times 25 \text{ cm}^3$) with 16 holes evenly distributed on the floor. The apparatus was elevated to a height of 25 cm from the ground so that the mice could peep through the holes. Each mouse kept in the apparatus. The number of head pokings were recorded during the 5 min observation period[Sonovane GS].

Results:

Table 1: Effect of *Cichorium.intybus* methanolic root extract on general behavioral profiles in mice (n=10).

Evaluation Parameters	Scores(average of 10 animals)	
	Control (0.5% Sodium CMC)	Chicory extract (500mg/kg body wt.)
Alertness	0	
Grooming	0.1	0.4
Vocalization	0	0
Restlessness	0.1	0.2
Irritability	0	0.8
Reactivity	0.1	0.8
Spontaneous action	4	2.6
Touch response	0	0.2
Pinna response	0.1	0.2
Corneal reflex	0	0
Gait	4	3.2
Righting reflex	0	0

Cichorium intybus methanolic extract at a dose of 500mg/kg affected spontaneous activity, irritability gait and touch responses. They also produce moderate or slight depression relating to awareness and alertness animals. However, the righting reflex corneal reflex and vocalization remain normal throughout the study process. Above studies indicates that there is a mild sedation action of the extract on the animals.

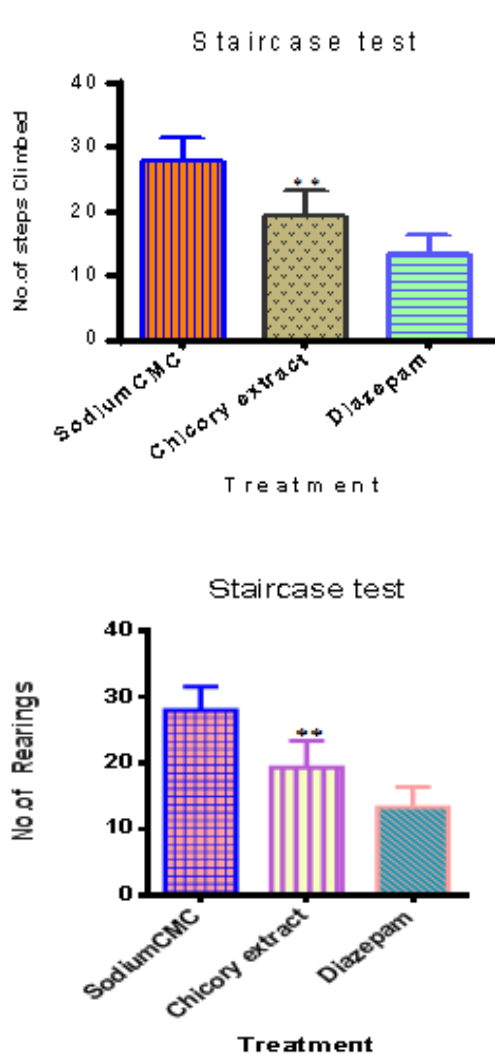


Figure 1: Effect of Chicory extract in staircase test on mice.

All values are Mean \pm SEM of n= 6, One way ANOVA followed by Brown-Forsythe test, Bartlett's test, ** p <0.05. Ns- non significant when compared to control(0.5% sodium CMC) treated animal.

In the current study methanolic extract of chicory root had shown significant (p<0.05) decrease in no.of rearings when compared to control which indicates anxiolytic potential of extract. And also shown significant (p < 0.05) increase in no. of steps ascended by mice is seen with administration of methanolic extract treated animals as compared to standard.

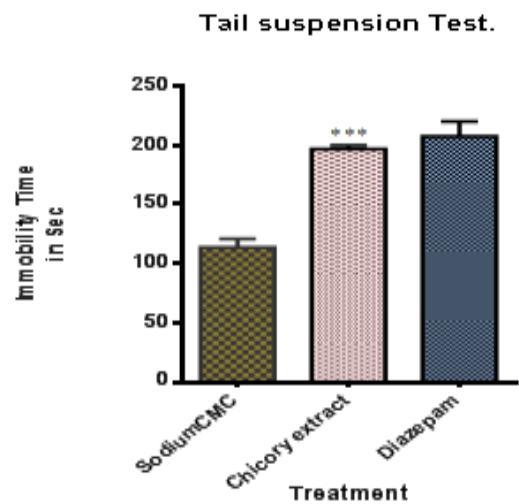


Figure 2: Effect of Chicory extract in Tail Suspension test on mice.

All values are Mean \pm SEM of n= 6, One way ANOVA followed by Brown-Forsythe test, Bartlett's test, ** p <0.05. Ns- non significant when compared to control(0.5% sodium CMC) treated animal.

The methanolic chicory root extract has shown significant (p<0.05) increase in immobility time when compared to control group of animals. But less significant (p<0.05) when compared to diazepam.

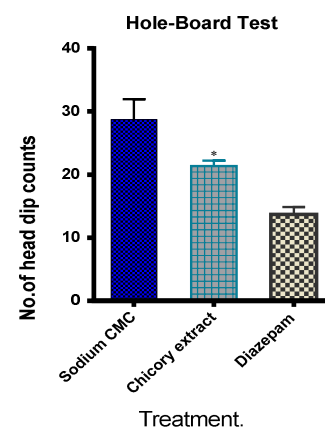


Figure 3: Effect of Chicory extract in Hole Board test on mice.

All values are Mean \pm SEM of n= 6, One way ANOVA followed by Brown-Forsythe test, Bartlett's test, * p <0.05, ** p<0.01, Ns- non significant when compared to control(0.5% sodium CMC) treated animal.

In the present study, methanolic extract of chicory root at dose 500 mg/kg significantly (p<0.05) decreased head - dip counts as compare to the control.

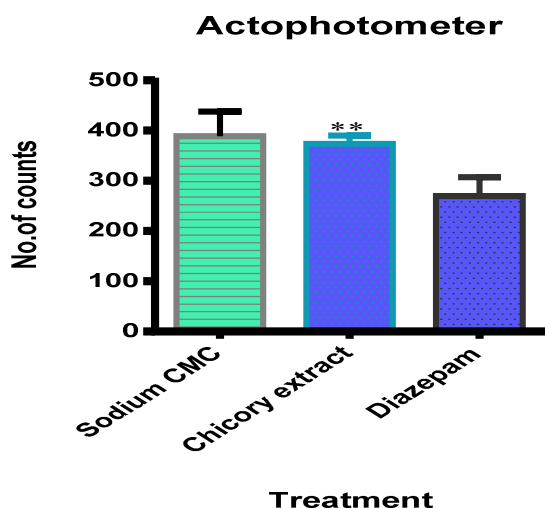


Figure 4: Effect of Chicory extract in locomotor activity test on mice.

All values are Mean± SEM of n= 6, One way ANOVA followed by Brown-Forsythe test, Bartlett's test , * p <0.05, ** p<0.01, Ns- non significant when compared to control(0.5% sodium CMC) treated animal.

The mice treated with methanolic chicory root extract has shown significant(p< 0.05) decrease in locomotor activity scores when compared to control. But scores are less significant as compared to standard.

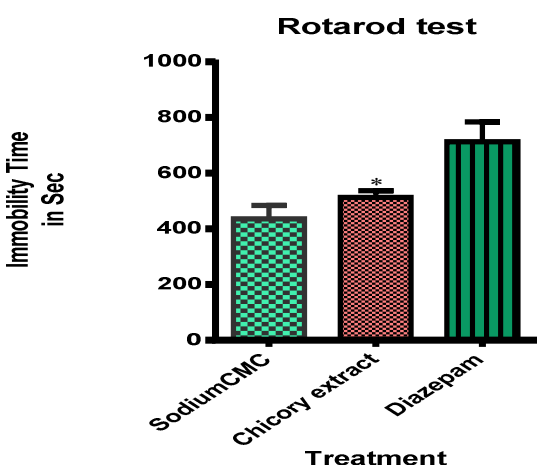


Figure 5: Effect of Chicory extract in Rotarod test on mice.

All values are Mean± SEM of n= 6, One way ANOVA followed by Brown-Forsythe test, Bartlett's test , * p <0.05, ** p<0.01, Ns- non significant when compared to control(0.5% sodium CMC) treated animal.

In case of rotarod test mice treated with methanolic chicory root extract has shown significant (p< 0.05) increase in immobility time is recorded when compared with control. This effect is less significant (p<0.05) when compared to standard.

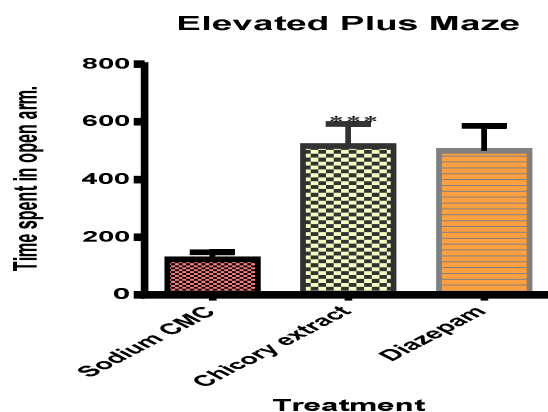
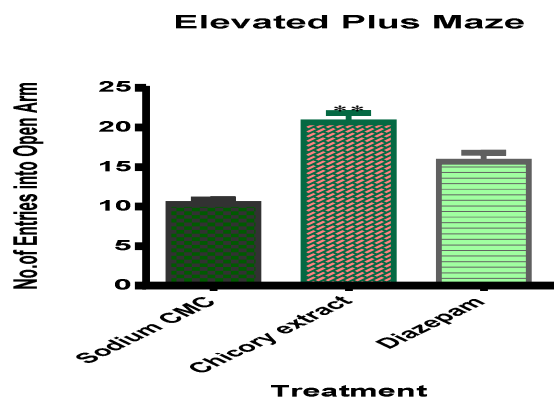


Figure 6: Effect of Chicory extract in Elevated Plus Maze on mice.

All values are Mean± SEM of n= 6, One way ANOVA followed by Brown-Forsythe test, Bartlett's test , * p <0.05, ** p<0.01, Ns- non significant when compared to control(0.5% sodium CMC) treated animal.

In current study, administration of methanolic extract of chicory root prolonged the time spent in the open arms, and also shown significant (p<0.05) the entries into open arms. The results showed that chicory at 500mg/kg could increase the time in the light area and the number of entries into the open arms.

Discussions

The loss of irritability, reactivity and grooming indicates anxiolytic effect of the extracts on the animal. Irritability is an extension of restlessness and in higher degree it is demonstrated by aggressiveness. The test extract suppress the normal irritability when exposed to stressful external stimuli. The gait of the animal indicates the control disturbance or neuromuscular blocker upon administration of the test drug some animals show a bit staggering gait. Above studies indicate that there is a mild sedation action of the extract on the animals. The loss of irritability, reactivity and grooming indicates an anxiolytic effect of the extracts on the animal.

The elevated plus-maze is a well-established animal model for testing anxiolytic drugs (Dawson 1995). It

represents a forced exploration, i.e., no non-stressed situation, since all parts of the situation are novel (Belzung and Le Pape, 1994). Exploratory behaviours include open arm activity and head dipping. A greater number of these measures indicate a greater level of exploration. In this test, the percentages of entries into open arms and of time spent in open arms have generally been used as indices of anxiety. Generally, an anxiolytic agent can increase the frequency of entries into open arms and increased the time spent in open arms of the elevated plus maze. In the present study, single administration of methanolic extract of chicory root prolonged the time spent in the open arms, and also shown significant ($p < 0.05$) the entries into open arms. The results showed that chicory at 500mg/kg could increase the time in the light area and the number of entries into the open arms, suggesting again that chicory possesses anxiolytic properties. It at the same time, the entries of closed arms was not affected by the administered chicory.

The evaluation of certain components of behaviour of mice such as curiosity or exploration has been attempted by Boissier et al. (1964) and Boissier and Simon (1964). The "hole-board" test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess emotionality, anxiety and/or responses to stress in animals. In the present study, methanolic extract of chicory root at dose 500 mg/kg significantly ($p < 0.05$) decreased head - dip counts as compare to the control. These results indicate the significant anxiolytic potential of chicory extracts in this paradigm. They do not alter spontaneous behaviour of the animals at the chosen dosage regimen.

The stair case test, one of the exteroceptive model chosen for the current study was originally described by Thiebot *et al.*, and subsequently used by several workers conforming the utility of this test in the successful and rapid assessment of anxiolytic activity. When (test animal) introduced into novel environment, rodents experience a conflict between anxiety and exploratory behaviour manifesting as increased vigilance and behavioural activity. Steps ascended is believed to reflect exploratory or locomotor activity, while rearing recorded as number of rearing in observation duration (5minutes) is an index of anxiety. In the present study methanolic extract of chicory root had shown significant ($p < 0.05$) decrease in no.of rearing and increase in no. of steps ascended by mice when compared to control which indicates anxiolytic potential of extract.

The locomotor activity can be an index of wakefulness (alertness) of mental activity (Arora 2011). Most of the CNS drugs influence the locomotor activities in animals. The locomotor activity (horizontal activity) can be easily measured using actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. In locomotor activity test (Actophotometer) the mice treated with methanolic chicory root extract has shown

significant ($p < 0.05$) decrease in locomotor activity scores when compared to control. But scores are less significant as compared to standard. The results indicates that the extract possess anxiolytic potential.

Rotarod test is used to evaluate the activity of drugs interfering with motor coordination. In 1956, Dunham and Miya suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. (Deacon 2013) An anxiolytic drug usually shows skeletal muscle relaxation property together with taming or calming effect which reduces anxiety and tension. The loss of muscle-grip strength is an indication of muscle relaxation. In case of rotarod test mice treated with methanolic chicory root extract has shown significant ($p < 0.05$) increase in immobility time is recorded when compared with control. It indicates that extract shows anxiolytic effect.

Plants containing tannins, sterols, flavonoids etc. are reported to have anxiolytic activity and preliminary phyto - chemical screening of chicory root revealed the presence of sterols, tannins, flavonoids and carbohydrates in the chicory roots. Therefore, the anxiolytic activity of root chicory is may be due to the presence of tannins, sterols, flavonoids etc. However, further investigations are required to isolate the phytoconstituents responsible for anxiolytic activity and to find their mechanism of action. As described by several authors and accepted universally, a novel environment created anxiety in these test animals, which reflected in its behaviour typical of a state of anxiety and conflict. It was demonstrated that the administration of the methanolic extract of chicory root in mice was able to induce anxiolytic effects, without modifying significantly the spontaneous motor activity.

Conclusion:

From the above study, it was concluded that *Cichorium Intybus* root extract possess significant anxiolytic effect. These findings give a scientific rationale for the future work of *Cichorium Intybus*

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