



CODEN [USA]: IAJPBB

ISSN : 2349-7750

## INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

Available online at: <http://www.iajps.com>

Research Article

### ANTI-ANXIETY SCREENING OF ETHANOLIC EXTRACT'S OF CITRULLUS COLOCYNTHIS LEAVES USING MICE

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**Article Received:** February 2022

**Accepted:** March 2022

**Published:** April 2022

**Abstract:**

*Anxiety disorders are among the most general mental, emotional, and behavioral problems affecting one-eighth of the total population worldwide, and have become a very important area of research interest in psychopharmacology. Anxiety represents a heterogenous group of disorders, probably with no single unifying etiology; various psychodynamic, psychoanalytic, behavioral, cognitive, genetic and biological theories have been proposed to explain the etiology of anxiety disorders. It is reported to have increasing prevalence in recent cohorts in many countries and to have much earlier ages of onset than other commonly occurring chronic conditions<sup>2</sup>. Anxiety disorders affect performance impairments on numerous tasks and are combine with high rates of medically unexplained symptoms, increased utilization of healthcare, strongly and independently combine with chronic medical illnesses, low levels of quality of life and disability. Pharmacologic treatment of anxiety through the ages has included different drugs. The first class of drugs developed (barbiturates) was highly effective, unfortunately, the barbiturates can cause respiratory arrest & have a narrow therapeutic index.<sup>5</sup> The benzodiazepines were developed as a safer alternative to barbiturates, however, their beneficial effects are very shadowed by the emergence of physical & psychological dependence & withdrawal reactions. More drugs used for treatment of anxiety having unfavorable side-effect profiles include buspirone, antidepressants and beta-blockers. The results obtained in the present study conclude that the Citrullus colocynthisethanolic extract has a potent anti-anxiety activity which may be attributed due to the presence of secondary metabolites like antioxidants, alkaloids, phenols, etc. Further investigation are needed to identify and isolate the active constituent for studying mechanism of action and identifying main chemical constituent responsible for anti-anxiety activity.*

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Please cite this article in press Mohd Moiz et al *Anti-Anxiety Screening Of Ethanolic Extract's Of Citrullus Colocynthis Leaves Using Mice., Indo Am. J. P. Sci, 2022; 09(4).*

**INTRODUCTION:**

Anxiety disorders are among the most general mental, emotional, and behavioral problems affecting one-eighth of the total population worldwide, and have become a very important area of research interest in psychopharmacology. Anxiety represents a heterogeneous group of disorders, probably with no single unifying etiology; various psychodynamic, psychoanalytic, behavioral, cognitive, genetic and biological theories have been proposed to explain the etiology of anxiety disorders<sup>1</sup>. It is reported to have increasing prevalence in recent cohorts in many countries and to have much earlier ages of onset than other commonly occurring chronic conditions<sup>2</sup>. Anxiety disorders affect performance impairments on numerous tasks and are combined with high rates of medically unexplained symptoms, increased utilization of healthcare, strongly and independently combined with chronic medical illnesses, low levels of quality of life and disability<sup>3,4</sup>. Pharmacologic treatment of anxiety through the ages has included different drugs. The first class of drugs developed (barbiturates) was highly effective, unfortunately, the barbiturates can cause respiratory arrest & have a narrow therapeutic index.<sup>5</sup> The benzodiazepines were developed as a safer alternative to barbiturates, however, their beneficial effects are very shadowed by the emergence of physical & psychological dependence & withdrawal reactions. More drugs used for treatment of anxiety having unfavorable side-effect profiles include buspirone, antidepressants and beta-blockers. Due to adverse effects combined with the currently available drugs, patients on anxiolytic drugs usually terminate the treatment before full recovery. In addition, one-third of patients in controlled unresponsive to any one of the medications. Thus, there is a critical need for development of newer anxiolytic agents. In the search for latest therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models. Several essential oils taken from plants are employed in order to balance emotions, improve physical and mental wellbeing<sup>6</sup>.

*Citrullus colocynthis* (L.) Schrad, a valuable plant commonly known as Colocynth is member from Cucurbitaceae, reported among all parched, arid zones of world however it is native to Mediterranean region and Asia. Geographically it is distributed in deserts of North Africa, South Europe and whole of Asia, extended up to Egypt. In India, such drought lenient plant species is usually dispersed among all hot arid areas. It is the most generally utilized plant in Indian

traditional medicinal framework and easily propagated by vegetative and generative modes of reproduction in summers. Local people use it to fix disorders like Boils, Pimples, Constipation, Inflammation of joints. To reduce the glucose level, joints aggravations, rheumatism, abdomen enlargement *Citrullus* is being used. Plant species is additionally used to cure Urticaria, constipation, snake poison, stomach ache, Hepatitis, Malaria, Epilepsy and Bowel grievances<sup>7</sup>.

There are several other reports of antidiabetic activity of this herb. Anticancer, anti-fertility effects of the fruit of this plant in rats and human.

In the present study, we investigated the antianxiety and anti-depressant activity in methanolic extract of fruit juice of *Citrullus colocynthis* in animal model.

**MATERIALS AND METHODS****The Collection of plant material and Extraction:**

Porous powder of dried plant leaves of *Citrullus colocynthis* was obtained from the local market and was validated by the Botanical Survey of India, Hyderabad. The porous powder was extracted using methanol by soxhlation process.

**Qualitative Phytochemical Investigation of Extract:**

The ethanolic extract of *Citrullus colocynthis* leaves was subjected to the various chemical tests for the identification of various phytoconstituents.

**Animals:**

Albino swiss albino mice weighting (20-30gm) of both sex were acclimatized for 10 days under laboratory conditions and housed in the animal house for the study. The animals were kept under standard conditions of temperature and light (12 hours light/dark cycles).

The animals were fed with standard pellet diet and water ad libitum. Animals were randomly selected for grouping and male & female mice were kept separately to avoid pregnancy. All experiments were performed in accordance with the CPCSEA guidelines and approved by Animal ethics committee, Shadan Institute of Medical Sciences, Hyderabad-500008.

**Experimental Design:**

The rats were divided five groups (n=6). Drugs/vehicle were administered to the animals 60min prior to study.

**Group I:** Negative control, administer saline 2 ml/kg orally.

**Group II:** Receive standard drug Imipramine (10mg/kg orally).

**Group III:** Group IV: Receive CCE 100 mg/kg orally.

**Group IV:** Receive CCE 200 mg/kg orally.

**Group v:** Receive CCE 400mg/kg orally.

After 30 min of diazepam treatment or 60 min essential oil/vehicle pretreatment, the mice were individually placed in animal models. All the tests were carried out at night with a minimal amount of background noise. After each test, the maze was cleaned with ethyl alcohol to eliminate any olfactory cues to the next animal.<sup>8</sup>

#### Models used to evaluate anti-anxiety activity:

##### Elevated plus maze (EPM)

The test was conducted using apparatus validated by Lister.<sup>9</sup> After each mouse was placed in the center of

the maze facing one of the open arms, the number of entries made into the open and closed arms and the time spent in them was recorded, using a video-camera for the next five min. From these data, the percentage of entries and the percentage of time spent in each arm was calculated.

##### Staircase test (SCT)

SCT was carried out as used by Simiand et al.<sup>10</sup> Each mouse was placed on the floor of the box with its back to the staircase, then the number of steps climbed and the numbers of rears were recorded over 3 min period using a video camera placed over head. A step is considered to be climbed only if the mouse had placed all four paws on the step, the number of steps descended was not considered.

## RESULTS AND DISCUSSION:

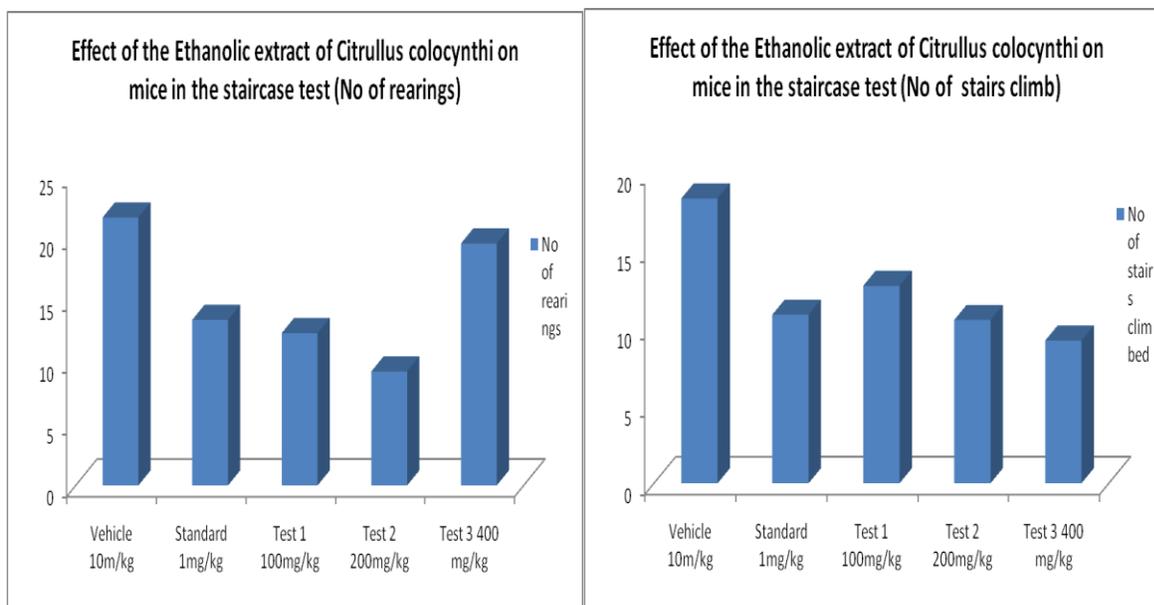
### Anti-Anxiety activity

#### 1. Staircase test (SCT)

##### Effect of the Ethanolic extract of *Citrullus colocynthis* on mice in the staircase test.

| Treatment      | Dose in mg/kg | No of rearings | No of stairs climbed |
|----------------|---------------|----------------|----------------------|
| Vehicle        | 10ml/kg       | 21.67±2.87     | 18.39±1.22           |
| Std (Diazepam) | 1mg/kg        | 13.38 ±4.94**  | 10.88±4.98*          |
| Test2          | 100 mg/kg     | 12.32±5.43*    | 12.75±5.22*          |
| Test3          | 200 mg/kg     | 09.22±3.09*    | 10.54±6.48**         |
| Test1          | 400mg/kg      | 19.56±1.22**   | 9.21±3.02***         |

Values are expressed in Mean ± Standard error mean compared to normal group, n=6, P<0.05 =\*, P<0.01=\*\*, P<0.001=\*\*\* compared to control group. Difference among the groups was analyzed by one-way analysis of variance (ANOVA) followed by tukey's test



## 2. Elevated plus maze model

**Table:1. The behavior of mice treated with the ethanolic extract of *Citrullus colocynthis* in the Elevated plus maze model**

| Treatments     | Dose mg/kg | No of entries (Counts/5 min) |             |              | Time spent (sec/5min) |               |               |
|----------------|------------|------------------------------|-------------|--------------|-----------------------|---------------|---------------|
|                |            | Open arm.                    | Closed arm  | Total        | Open arm              | Closed arm    | Center        |
| Vehicle        | 10ml/kg    | 3.09±2.35                    | 7.79±2.54   | 10.88±3.43   | 43.21±22.12           | 129.81±39.67  | 113.65±44.28  |
| Std (Diazepam) | 0.5 mg/kg  | 11.32±1.87*                  | 6.98±2.30** | 18.30±2.44** | 120.27±10.32*         | 63.43±2*2.54  | 89.23±12.88*  |
| Test1          | 100mg/kg   | 7.04±1.82*                   | 5.74±2.39*  | 12.78±4.45*  | 106.03±39.31*         | 114.27±38.51* | 90.15±38.32*  |
| Test2          | 200 mg/kg  | 10.72±3.98*                  | 7.98±1.22*  | 18.70±3.29*  | 110.32±21.43*         | 96.66±45.65*  | 113.43±11.45* |
| Test3          | 400 mg/kg  | 4.22±2.54**                  | 4.21±1.73*  | 8.43±1.43**  | 88.32±32.54**         | 68.54±18.87*  | 100.09±21.54* |

Values are expressed in Mean ± Standard error mean compared to normal group, n=6, P<0.05 =\*, P<0.01=\*\*, P<0.001=\*\*\* compared to control group. Difference among the groups was analyzed by one-way analysis of variance (ANOVA) followed by tukey's test

The present work has evaluated the antianxiety activity of various doses of the ethanolic extract of leaves of *Citrullus colocynthis* in mice employing two non-conditioned behavioral animal models of anxiety; EPM model, and SCT. These tests are classic &

standard models for screening central nervous system actions providing information about anxiety and psychomotor performance [88]. Further, these models can create an anxiety state in normal rodents in a reproducible paradigm while minimizing some of the confounding factors of other conditioned assays [89].

The EPM test is principally based on the behavior that exposure of animals to an elevated maze alley evokes an approach-avoidance conflict and rodents consistently spend greater time in the closed arms when placed in mazes comprising of open and closed arms [90]. Based on these assertions, EPM tests are reliable means of identifying selective anxiolytic effect of drugs and used as a tool in the investigation of the psychological and neuro-chemical basis of anxiety, for screening anxiety modulating drugs or mouse genotypes [91].

The EPM test have been validated pharmacologically, physiologically and behaviorally, and has become one of the most widely used behavioral tests for anxiety [92]. Anxiety is induced by a fear due to height in

rodents when placed on the EPM. The ultimate manifestation of anxiety is exhibited by preference to

remain at safer places and a decrease in the motor activity. Treatment of mice with the ethanolic extract of *Citrullus colocynthis* (CCE) resulted in significant alterations on the behavioral responses measured in the EPM test. Experimental animals treated with 200 and 400 mg/kg doses of CCE showed significantly increased percentage of number of entries into the open arms. This decreased aversion to open arms compared to control group indicated its anxiolytic activity [93]. The decrease in aversion to open arms by the 200 and 400 mg/kg doses of the oil was also verified by the increased percentage of time spent in the open arms compared with the negative control. Time spent in the central platform of EPM appears to be related to decision making and/or risk assessment. A decreased time spent on the central platform serves as indicator of a reduced decision-making behavior, a parameter accepted as reliable indicators of anxiety and fearfulness [94]. However, neither doses of the CCE nor diazepam altered the parameter significantly compared to control. At a dose of 400 mg/kg, however, the CCE showed a significant decrease on the number of entries in the closed arms. The absence of significant modification in the number of closed arm entries, in the anxiolytic doses of the oil, in the EPM indicated that the anxiolytic activities was observed at doses that did not impair motor activity [95].

The EPM test is one of the most popular tests for search of new benzodiazepine-like anxiolytic agents. In this context, the activity of the ethanolic extract of *Citrullus colocynthis* (100 and 200 mg/kg) in relieving anxiety in this model may suggest a possible positive modulation of the GABA-A/benzodiazepine receptor

complex. that anxiolytic activity was activity is a simple and rapid procedure for preliminary screening of anxiolytic agents. Step climbing reflects exploratory or locomotor activity, while rearing behavior was a manifestation of anxiety state. The present study showed that exposure of the experimental animals to the ethanolic extract of *Citrullus colocynthis* significantly reduced rearing activity at doses (100,200 and 400 mg/kg) that did not suppress climbing, aligning with a behavior of an anxiolytic compound in a staircase model At a dose of 400 mg/kg, the essential oil produced a significant reduction in the number of steps ascended indicating suppression of locomotor activity, which is interpreted as a sedative, rather than anxiolytic effect indifferent studies . Only the GABA receptor complex active agents have been shown to reduce rearing at doses that do not reduce climbing in the SCT. Other non-benzodiazepine compounds induce non-specific suppression of both rearing & climbing behavior, strengthening the suggested possible mechanism of the CCE.

#### CONCLUSION:

The results obtained in the present study conclude that the *Citrullus colocynthis*ethanolic extract has a potent anti-anxiety activity which may be attributed due to the presence of secondary metabolites like antioxidants, alkaloids, phenols, etc. Further investigation are needed to identify and isolate the active constituent for studying mechanism of action and identifying main chemical constituent responsible for anti-anxiety activity.

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