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Research Article

# Pharmacological evaluation of anxiolytic activity of ethanolic extract of *Hemidesmus indicus* in rodent model

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Abstract

The current study uses rodent models to explore the anxiolytic properties of Hemidesmus indicus' ethanolic extract (EEHI). Hemidesmus indicus, an evergreen plant well-known for itstherapeutic characteristics, has been widely used in Ayurvedic medicine to treat a variety of conditions, including anxiety. This study seeks to give scientific validation for its anxiolytic properties. The ethanolic extract was made using normal extraction processes and given to rodents in varied doses (100,200 and 400 mg/kg). The anxiolytic activity was assessed using avariety of widely recognized behavioural assays, including the Elevated Plus Maze (EPM), Open Field Test (OFT), and Light-Dark Box (LDB) test. Diazepam (2 mg/kg) served as the standard reference drug. The results revealed that EEHI significantly reduced anxiety-like behaviours in all three tests when compared to the control group. In the EPM test, EEHI-treated rodents spent more time and entered the open arms. In the OFT, ambulation, rearing and assisted rearing increased, indicating lower levels of anxiety. The LDB test supported these findings, with a significant increase in time spent in the light compartment. The study suggeststhat EEHI has promising anxiolytic activity and may offer a natural treatment alternative for anxiety disorders. Further research is needed to isolate specific active molecules and better understand the underlying mechanisms of action.

**Keywords:** *Hemidesmus indicus*, Anxiolytic activity, Rodent model, Elevated plus maze, Open field test, Light-dark box test.

## INTRODUCTION

The main class of drugs that are frequently administered to alleviate anxiety are benzodiazepines. However, using them is linked to negative effects including addiction risk, potentiation of other central depressant medications, and psychomotor impairment. Numerousplants are being researched for use as supplementary and alternative anxiety medications[1,2]. Studies have been carried out to find a different, more targeted, and affordable treatment. *Hemidesmus indicus* also known as Indian sarsaparilla in English, Anantmul in Hindi, and Ananta in Sanskrit language. It is a member of the Apocynaceae family that can be found throughout India, particularly in various territories of West Bengal. It is a thin, laticiferous,semi-erect and fragile bush, specifically known for its therapeutic properties[3]. It is reported that Indian traditional healers utilize it for nephritic symptoms, syphilis, and paediatric sore mouths. The decoction of roots is beneficial for skin conditions, syphilis, elephantiasis, anorexia, blood purification, and renal and urinary illnesses. It is commonly used in folk medicine and Ayurvedic and Unani preparations for treating biliousness, blood disorders, diarrhoea, skin illnesses, respiratory conditions, fever, asthma, eye diseases, burning sensation, arthritic conditions and gastric problems [4-6]. Despite many reports in scientific publications onthe uses of HI, relatively little research has been done on CNS applications. Because of this, the present study was taken to evaluate the anti-anxiety activity of the ethanolic extract from the roots of *Hemidesmus indicus.*

## MATERIAL AND METHOD

### Collection and authentication of plant material

Root of *Hemidesmus indicus* R.Br. (locally called Anantmul) were obtained from Doctor Hari Singh Gaur Vishwavidyalaya botanical garden and authenticated from Departmentof Botany, Dr. H.S. Gour University, Sagar (M.P). Herbarium number-: BOT/H/04/104/12

### Preparation of ethanolic extract of *Hemidesmus indicus*:

Dried *Hemidesmus indicus* powder 500 grams were thoroughly extracted using 95% ethanolin a Soxhlet system for a whole day. The solvent evaporated, leaving behind a dark brown residue. It was discovered that the yield of *Hemidesmus indicus* root extract was 20.7% w/w. In preparation for their use in phytochemical and toxicological analyses, the extracts were dehydrated at normal temperature & then refrigerated at 4°C.

### Animals

Adult Swiss mice (20–30 gm) of either sex were used for the study. The animals were acquired from the College of veterinary Science and Animal Husbandry NDVSU, Mhow, Madhya Pradesh. They were housed in polypropylene cages in the air-conditioned room with the temperature maintained at 25 ± 3°C, and 12 h alternating light and dark cycles. the mice wereprovided with a nutritionally adequate diet (Hindustan Lever Limited, India) and drinking water ad libitum throughout the study. Approval by the Animal Ethics Committee for the experimental procedures obtained.

### Acute oral toxicity study

Studies on acute toxicity were carried out in accordance with OECD 423 criteria (OECD, 2001). After 24 h of fasting, different groups of mice were administered single oral dose (500,1000 or 2000 mg/kg) of ethanolic extract of H. indicus. Three mice were used for each doselevel in the study. Immediately after dosing, animals were observed for signs of toxicity during the first 0.5, 1, 2, 4, 8 and 12 h, and at every 24 h for 14 days. Behavioural parameters,tremors, lethargy, death, amount of water and feed taken were observed [7].

### Drugs

Benzodiazepines are recognized to be anxiolytics at low dosages and induce drowsiness and muscular relaxation at greater levels[8]. We used diazepam (2 mg/kg) as a standard to evaluate anxiolytic effects. Diazepam (Calmpose 5 mg, Ranbaxy Laboratories Limited, Navi-Mumbai) was suspended in 0.5% of carboxymethyl cellulose in distilled water. Accurately weighed quantity of ethanolic extract of Hemidesmus indicus was separately suspended in distilled water using 1% (w/v) of gum acacia as suspending agent and administered toexperimental animals.

### Treatment Schedule

The anxiolytic activity was examined by using the elevated plus maze, open field test and lightand dark model. A total of 30 mice of either sex were randomly divided into five groups of 6 each and treated as follows: Group 1 received vehicle (normal saline); group 2 received diazepam (2 mg/kg, p.o.); groups 3,4 and 5 received ethanolic extract of *Hemidesmus indicus* roots (100, 200 and 400 mg/kg, p.o., respectively).

### Elevated Plus-Maze Test

The plus-maze instrument, made up of two open arms (16×5 cm) and two closed arms (16×5×12cm) with an open roof, was used to examine anxiolytic behaviour in animals. The plus-maze was elevated (25 cm) from the floor. After 1 hour of oral administration of vehicle, diazepam, and an ethanolic extract of H. indicus, each mouse was put on the central platform,facing the open arm. The proportion of time spent (duration) in open arms and the frequency of open arm entries were recorded for 5 minutes. All precautions were made to ensure that noexternal stimuli, other than the height of the plus maze, would cause anxiety in the animals. Arm entry was defined as all four paws crossing the line separating an arm and the core area. The proportion of time spent in open arms and number of open arm entries were computed using the following formulas: [100 × open/(open + enclosed)] and (100 × open/total entries), respectively [9-12].

### Open Field

This assay was used to detect both angiogenic and anxiolytic action under identical conditions. Mice were tested using a variety of open field devices. The apparatus was a wooden box measuring 60 x 60 x 60 cm. The open field area was divided into 16 squares (15 × 15 cm), withfour in the centre and 12 along the walls. The experimental chamber was dark and soundproof.The open field arena was lighted with a 40-W bulb that focused on the field from a height of 75-100 cm. After 60 min of oral administration with vehicle, diazepam, and an ethanolic extract of H. indicus extract, mice were placed individually in one of the corner squares, and the ambulation (no. of square crossed),number of rearing (the number of times an animal stands on its hind legs, often with its forepaws raised off the ground.) and number of assisted rearing(Instances where the animal rears with the support of the arena walls , using them to balance or explore.) were recorded for 5 minutes [13-15].

### Light/dark test

The device was made up of two 20 cm x 10 cm x 14 cm plastic boxes. One compartment was painted white and had bright lighting, while the other compartment was painted dark black and had red dimly illuminated light. The mice were allowed to move between boxes via an open door. The illumination in the black compartment was 50 lux, but in the white region it was raised to 1000 lux using an additional light source. After 60min of oral treatment with vehicle,diazepam and ethanolic extract of *H. indicus* mice was placed in the light box facing the hole.The transition between the light and dark boxes, as well as the duration spent in the light box,were recorded for 5 minutes [16].

## RESULTS

### Result of OECD toxicity study

The OECD 423 standards were followed in conducting the acute oral toxicity research. No adverse changes and mortality were observed in animals up to the dose of 2000 mg/kg, whichorally received ethanolic extract of *Hemidesmus indicus.* This indicates 2000mg/kg is maximum safe dose. Hence, the three doses were selected as 100mg/kg, 200mg/kg and 400mg/kg for studying In vivo antianxiety activity.

### Evaluation of anti-anxiety activity

### Elevated plus maze

In EPM, (Table No.1,2) all the mice were treated with three measurements of HIEE (100, 200 and 400 mg/kg) which indicated increase in open arm entries as well as time spent in open armsof EPM model which showed significantly reduce in anxiety and also, animals treated with diazepam (2 mg/kg), obviously, indicated increase in number of entries in open arms as well as time spent at open arm. Furthermost animals treated with each of the three dosages indicated reduction in number of passages in closed arms of EPM model when compared with control group. Which demonstrated decline in time spent at closed arms of EPM which was huge in vehicle treated control group. Additionally, animals treated with diazepam obviously, indicated maximum reduction in number of passages at closed arm.

**Table 1: Anxiolytic activity profile on Number of entries (Mean ± SEM) in EPM apparatus of *Hemidesmus indicus* ethanolic extract.**

| GROUP NO. | TREATMENT GROUP | DOSE (mg/kg) | Number of entries (Mean ± SEM) | | % OAE (percentage open arm entries) |
|---|---|---|---|---|---|
| | | | Open arm | Closed arm | |
| 1 | CONTROL | saline | 7.33 ± 0.21 | 11.1 ± 0.47 | 39.77 ± 1.59 |
| 2 | DIAZEPAM | 2 | 13.8 ± 0.34*** | 6.5 ± 0.22*** | 67.98 ± 2.16*** |
| 3 | HIEE | 100 | 6.5 ± 0.22 | 9 ± 0.36 | 41.94 ± 1.82 |
| 4 | HIEE | 200 | 8.16 ± 0.30* | 8.33 ± 0.36* | 50.51 ± 2.61* |
| 5 | HIEE | 400 | 10.83 ± 0.41*** | 7.5 ± 0.22** | 59.09 ± 2.69*** |

Results are shown as MEAN ± SEM (n=6); *P<0.05, **P<0.01, ***P<0.001: when compared to control group by one way ANOVA followed by Dunnett multiple comparison test

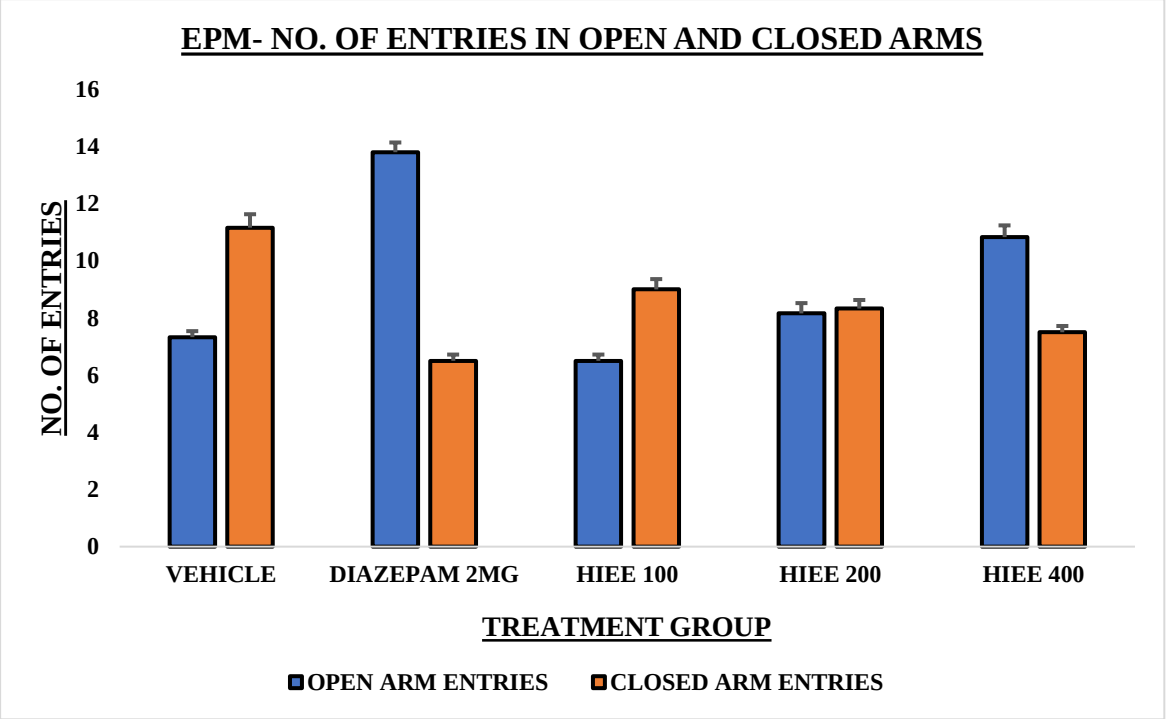


**Figure 1: Graphical representation for no. of entries in open and closed arms in EPM**

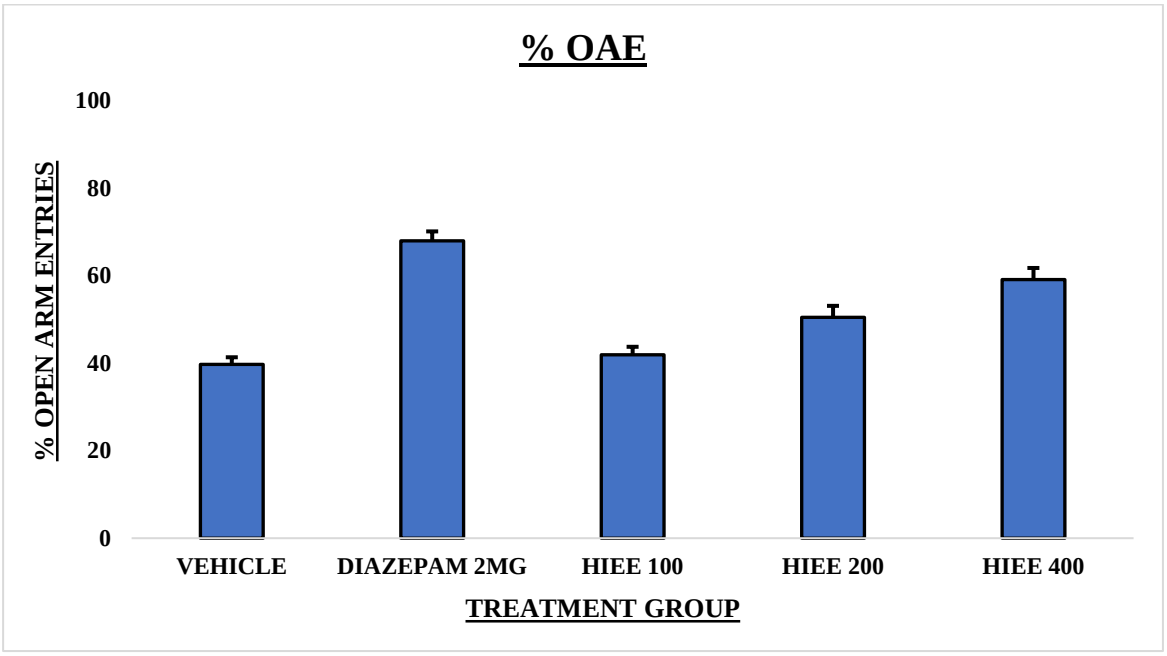


**Figure: 2 – Graphical representation for percentage open arm entries.**

**Table 2: Anxiolytic activity profile on total time spent in open and closed arms in sec (Mean ± SEM) of *Hemidesmus indicus* ethanolic extract in EPM.**

| GROUP NO. | TREATMENT GROUP | DOSE (mg/kg) | Time spent in sec (Mean ± SEM) | | %TSOA (Percentage time spent in open arms) |
|---|---|---|---|---|---|
| | | | Open arm | Closed arm | |
| 1 | CONTROL | saline | 53.33 ± 1.64 | 238.16 ± 2.48 | 18.27 ± 0.59 |
| 2 | DIAZEPAM | 2 | 161.66 ± 3.37*** | 130 ± 2.12*** | 55.42 ± 1.47*** |
| 3 | HIEE | 100 | 92.5 ± 1.74* | 194.16 ± 2.08* | 32.28 ± 0.70* |
| 4 | HIEE | 200 | 136.16 ± 3.87** | 155.33 ± 1.89* | 46.71 ± 1.44** |
| 5 | HIEE | 400 | 148.67 ± 2.92*** | 146.83 ± 2.65** | 50.31 ± 1.34*** |

Results are shown as MEAN ± SEM (n=6); *P<0.05, **P<0.01, ***P<0.001: when compared to control group by one way ANOVA followed by Dunnett multiple comparison test

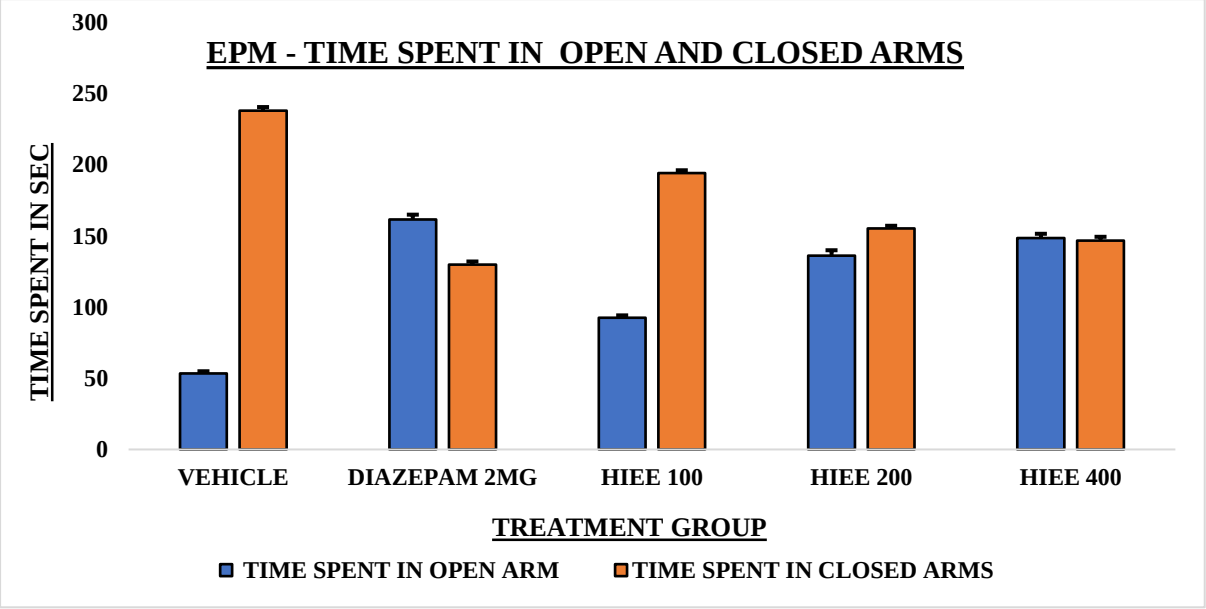


**Figure 3: Graphical representation for time spent in open and closed arms in EPM**

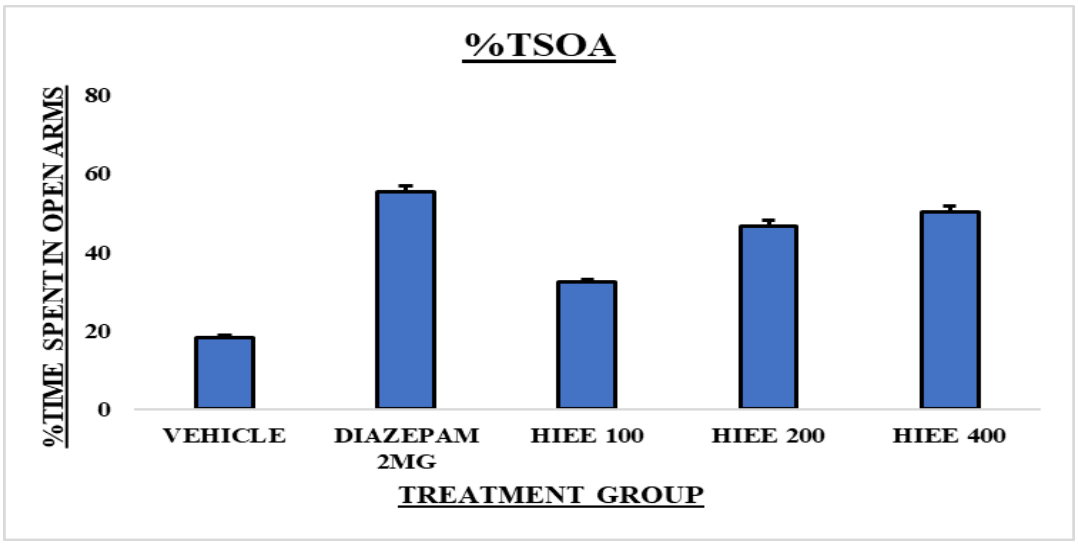


**Figure 4: Graphical representation for %TSOA (Percentage time spent in open arms) in EPM**

**Light and dark model**

In LDT, (Table No. 3) all the mice were treated with three measurements of HIEE (100, 200 and 400 mg/kg) which indicated lessened time spent in dark box with corresponding increase in time spent in light chamber when compared with controls. Essentially, animals treated withdiazepam (1mg/kg) obviously demonstrated decreased time spent in dark box with corresponding increase in time spent in light chamber. Animals treated with moderate and high dosage (200 and 400mg/kg) indicates more critical results when compared with the low dosage (100 mg/kg). In the HIEE groups, a dose-dependent increase in light entrances and time spent in light was seen. Higher doses (200 mg/kg and 400 mg/kg) produced results similar to diazepam, implying that HIEE might exhibit anxiolytic properties.

**Table 3: Anxiolytic activity profile on light and dark apparatus of *Hemidesmus indicus* ethanolic extract.**

| GROUP NO. | TREATMENT GROUP | DOSE (mg/kg) | TIME SPENT IN SEC (Mean ± SEM) | | %TSLB (Percentage time spent in light box) (Mean ± SEM) |
|---|---|---|---|---|---|
| | | | LIGHT | DARK | |
| 1 | CONTROL | saline | 44.66 ± 1.83 | 254.66 ± 2.65 | 14.92 ± 3.10 |
| 2 | DIAZEPAM | 2 | 153.33± 2.73** | 146.16 ± 2.104** | 51.19 ± 1.78*** |
| 3 | HIEE | 100 | 78 ± 2.54* | 221.33 ± 1.42* | 26.05 ± 3.26** |
| 4 | HIEE | 200 | 126.83 ± 2.84** | 172.66 ± 2.77** | 42.34 ± 2.24** |
| 5 | HIEE | 400 | 138.87 ± 2.31*** | 160.16 ± 2.49*** | 46.44 ± 1.66*** |

Results are shown as MEAN ± SEM (n=6); a=P<0.05, b=P<0.01, c=P<0.001: when compared to control group by one way ANOVA followed by Dunnett multiple comparison test

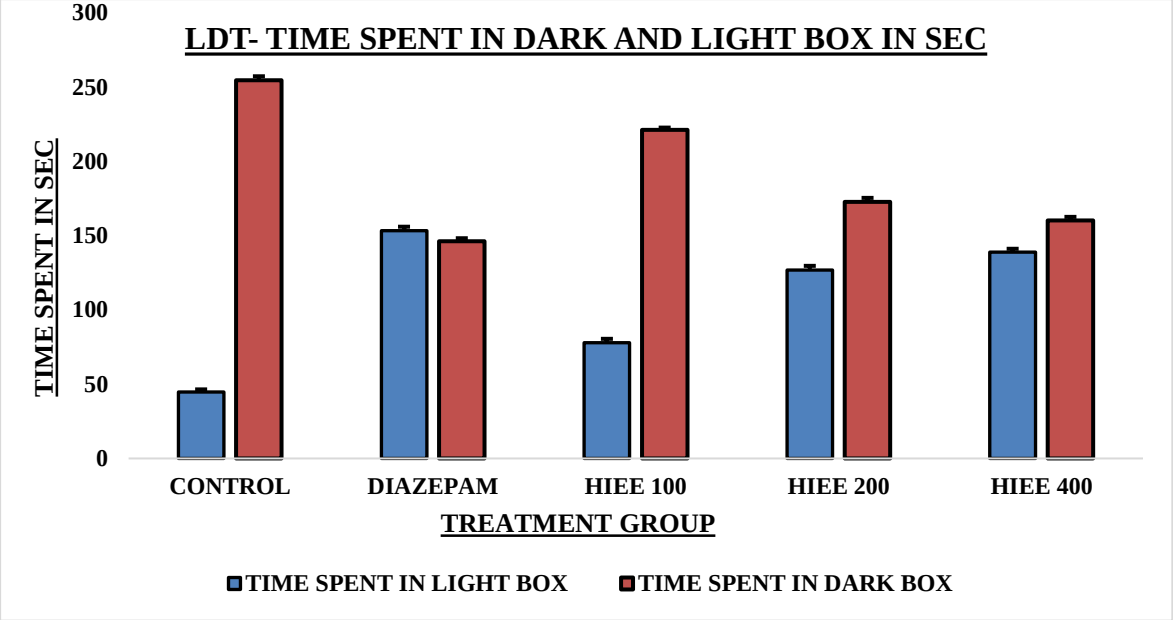


**Figure 5: Graphical representation for time spent in light and dark box in LDT**

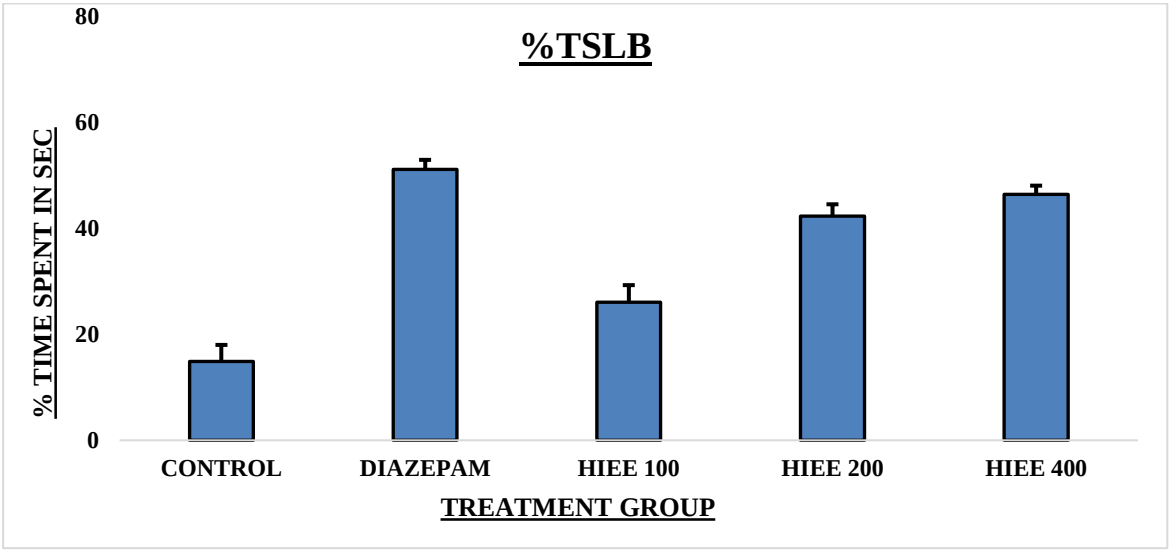


**Figure 6: Graphical representation for %TSLB percentage time spent in light and dark box in LDT**

**Open field test**

In OFT, (Table No. 4) all the mice were treated with three measurements of HIEE (100, 200 and 400 mg/kg) which indicted increase in every single exploratory parameter. The experiment assessed the effects of various doses of drugs on ambulation, rearing, and assisted rearing. The control group displayed moderate ambulation and rearing, with minimal assistance rearing. When compared to the control group, the diazepam group had considerably higher ambulation,rearing, and assisted rearing rates. Doses of 100, 200, and 400 mg/kg among the HIEE groups resulted in varying degrees of ambulation, rearing, and assisted rearing; the highest dose led to the highest level of activity. Overall, diazepam markedly enhanced locomotor activity, while doses of HIEE indicated potential therapeutic use by showing dose-dependent effects on motor behaviour.

**Table 4: Anxiolytic activity profile on open field apparatus of *Hemidesmus indicus* ethanolic extract.**

| GROUP NO. | TREATMENT GROUP | DOSE (mg/kg) | AMBULATION | NO. OF REARING | NO. OF ASSISTED REARING |
|---|---|---|---|---|---|
| 1 | CONTROL | saline | 112.5 ± 1.33 | 8.33 ± 0.49 | 18.16 ± 0.47 |
| 2 | DIAZEPAM | 2 | 192.16 ± 1.57*** | 13.83 ± 0.307*** | 36.33 ± 0.61*** |
| 3 | HIEE | 100 | 139.66 ± 1.99** | 9.16 ± 0.477 | 22.66 ± 0.91* |
| 4 | HIEE | 200 | 171 ± 1.36*** | 11.41 ± 0.33** | 32 ± 0.57*** |
| 5 | HIEE | 400 | 182.21 ± 1.61*** | 11.84 ± 0.47** | 34.5 ± 0.68*** |

Results are shown as MEAN ± SEM (n=6); *P<0.05, **P<0.01, ***P<0.001: when compared to control group by one way ANOVA followed by Dunnett multiple comparison test

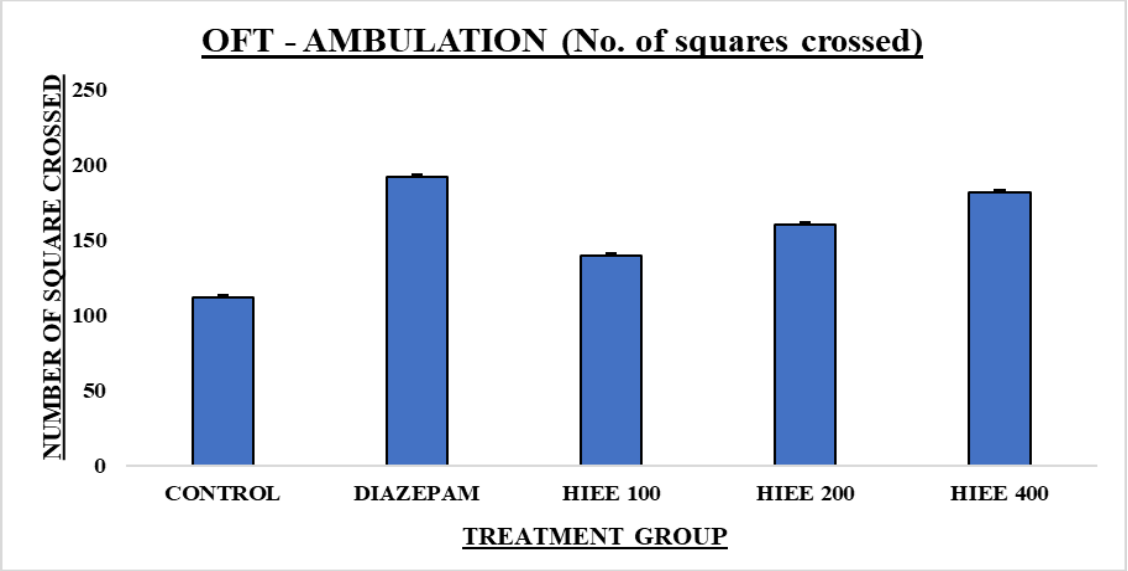


**Figure 7: Graphical representation for AMBULATION (no. of square crossed) in OFT.**

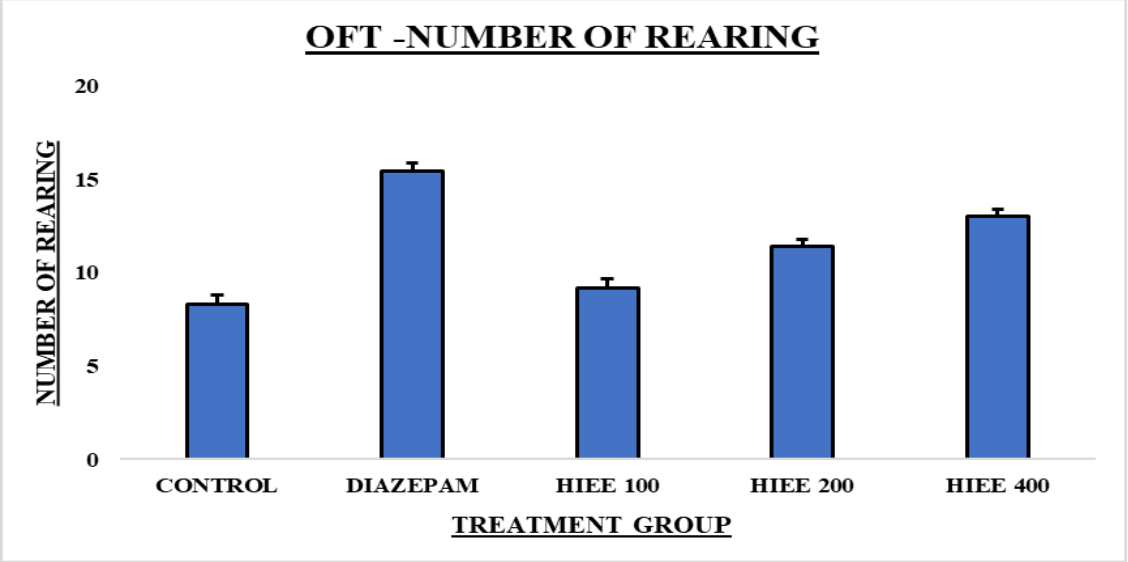


**Figure 8: Graphical representation for no. of REARING in OFT.**

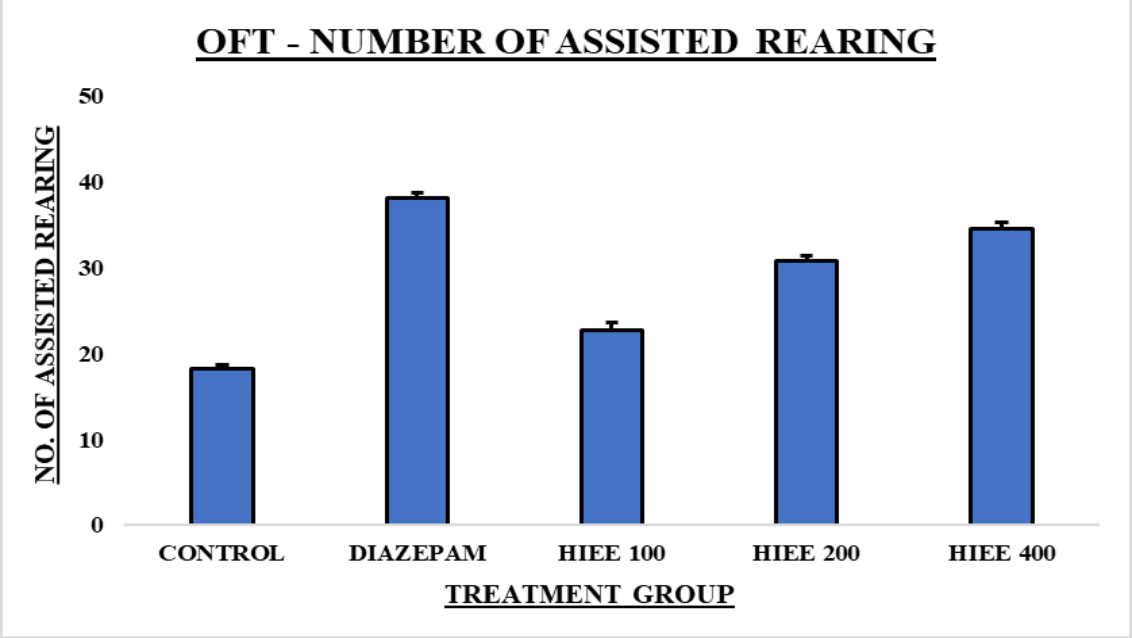


**Figure 9: Graphical representation for no. of assisted rearing in OFT**

## DISCUSSION

Anxiety disorders are among the most common mental disorders. Anxiety is a typical reaction that includes dread, tension, high blood pressure, perspiration, and trembling. However, whenit gets excessive, it has a negative impact on everyday life [17]. The plant includes a diverse range of bioactive chemicals, including saponins, tannins, flavonoid ds, steroids, and alkaloids, many of which have pharmacological properties relevant tothe central nervous system and may contribute to anxiolytic (anti-anxiety) effects[18]. The elevated plus maze (EPM) is a popular behavioural assay for measuring anxiety in rodents. In our study, mice administered with the ethanolic extract of Hemidesmus indicus (HIEE) spentmore time in the open arms and entered them more frequently than the control group. These behaviours are indicative of decreased anxiety, as rodents naturally avoid open and elevated regions due to their sensitivity to potentially dangerous places. The EPM data imply that HIEEhas a relaxing impact on rodents at dose of 200mg/kg and 400mg/kg, lowering their anxiety and increasing their willingness to explore potentially dangerous locations. This experiment gave strong data supporting the anxiolytic effects of HIEE [19,20]. In light-dark box test (LDBT) is a popular behavioural assay for detecting anxiety-likebehaviours in rodents, taking use of their natural sensitivity to highly lighted environments andpreference for dark, confined places[21]. In our study, mice given the ethanolic extract of Hemidesmus indicus (HEEI) 200mg/kg and 400mg/kg spent much more time in the light compartment than the control, indicating a decrease in anxiety levels. Furthermore, the frequency of transitions between the light and dark compartments was greater in the HIEE- treated group, indicating more exploratory activity and less fearfulness. The results of the LDBT support the anxiolytic effects of HIEE found in other behavioural tests, such as the elevated plus maze (EPM) and the open field test (OFT) [22, 23]. The open field test (OFT) results revealed significant evidence for the anxiolytic effects of Hemidesmus indicus' ethanolic extract (HIEE). Treated mice had a much higher number of squares crossed, indicating increased locomotor activity and decreased anxiety. Furthermore, the frequency of rearing, a measure of exploratory behaviour, was significantly higher in the EEHI group than in controls, indicating increased curiosity and decreased fearfulness. The risein assisted raising, in which rodents explore while leaning against walls, demonstrated a balanced reduction in anxiety while remaining cautious. These behaviours underscore EEHI'sanxiolytic effect, complementing the findings of the elevated plus maze (EPM) and light-darkbox test (LDBT), and imply that Hemidesmus indicus could be a promising natural anxiety treatment [24]. Thus, in this study, both the higher doses of HIEE (200 and 400 mg/kg) and diazepam significantly reduced the anxiety effect in mice and were proven to be effective.

## CONCLUSION

The study shows that the ethanolic extract of Hemidesmus indicus (EEHI) has strong anxiolyticactivity in mice models and has a good safety profile. These data indicate that EEHI may be apromising natural alternative or complementary therapy for anxiety disorders. However, morestudy is needed to investigate different extracts, understand mechanisms, optimize dose, and prove efficacy and safety through long-term and clinical investigations. If these procedures arecompleted successfully, Hemidesmus indicus may be recognized as a viable choice in the pharmaceutical management of anxiety disorders, providing a natural and effective treatment alternative.

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