EVALUATION OF PHYTOCHEMICAL AND NEUROPHARMACOLOGICAL EFFECTS OF *CASSIA OBTUSIFOLIA* SEEDS BY USING ALBINO MICE

ABSTRACT:

phytochemical screening revealed the presence of flavonoids, glycosides, phenols, and tannins, all of which have pharmacological potential. The neuropharmacological study was carried out utilizing two classic animal models: Eddy's hot plate method for central analgesic activity and the open field test for locomotor behavior. The extract was given intraperitoneally in two doses: low and high. The results showed that the methanolic seed extract considerably increased the pain response time in the hot plate test and caused significant changes in the time spent in center in the open field test as compared to the control group. The results were statistically significant ($p < 0.05$), suggesting dose-dependent central nervous system action. Finally, the methanolic extract of *Cassia obtusifolia* seeds showed strong neuropharmacological effects, which could be attributed to the phytoconstituents synergistic action. These findings corroborate its traditional use and point to its potential in the development of herbal neurotherapeutics.

Keywords: *Cassia obtusifolia*, Neuropharmacology, Analgesic activity, Anxiolytic activity, Phytochemical screening, Herbal medicine.

1. INTRODUCTION

Neuropharmacology is the study of how medications affect the functioning of cells in the nervous system and the mechanisms by which these effects influence behaviour.

Neurodegenerative disorders (ND) are a significant cause of illness and death worldwide, especially among older adults. With life expectancies rising, the prevalence of these diseases is expected to grow [World Health Organization, Beyond the risk of pathological conditions, individuals over 60 often face natural declines in memory, attention, and multitasking abilities due to normal aging. Importantly, cognitive impairment—whether linked to ND or not—can serve as an early indicator of potential motor decline or the onset of neuropsychiatric symptoms such as apathy and depression (1).

In every nation, mental, neurological, and behavioural diseases are prevalent and lead to great pain. Individuals who suffer from these conditions frequently experience low quality of life, higher mortality, and social isolation. The economic and societal repercussions of these illnesses are enormous (www.brain_dynamics.net/aboutus.php) (2).

Additionally, 80% of people worldwide rely on plant-based medications (WHO, 1996). Both literate and illiterate people in Nigeria and the majority of developing nations mostly use herbal remedies to cure a variety of illnesses. Even though mainstream medicine is available, alternative medical systems like Ayurveda, Sidda, Unnani, and other tribal folklore medicines have made a substantial contribution to India's population's health (3)

This field is divided into two main branches:

- **Molecular Neuropharmacology:**
  This branch explores the chemical interactions within the nervous system at a cellular and molecular level, such as how neurotransmitters and drugs interact with receptors.

- **Behavioural Neuropharmacology:**
  This area examines how these chemical interactions influence behaviour, emotions, and cognitive processes, diverse applications and effects: shedding light on the effects of drugs on mental health and neurological disorders. Drugs that act on the central nervous system (CNS) are indeed integral to the treatment of numerous neurological and psychiatric disorders. These agents function by modifying neurotransmission within the CNS, targeting specific pathways to achieve therapeutic outcomes. (4)

Human-Centric Therapeutic Goals:

1. Neurological Restoration: Developing drugs that not only manage symptoms but also promote neural regeneration and repair.

2. Precision and Selectivity: Enhancing the ability of drugs to target specific receptors, minimizing side effects and improving patient quality of life.

3. Individualized Treatment: Leveraging genetic and biochemical profiles to create personalized medicine strategies for CNS disorders.

CNS-acting drugs have revolutionized medical practice by enabling effective management of previously debilitating conditions. The goal remains to refine these therapies further, ensuring they are not only potent but also "humanize" treatments by prioritizing the well-being and dignity of patients. (5)

PATHOPHYSIOLOGY:(6)

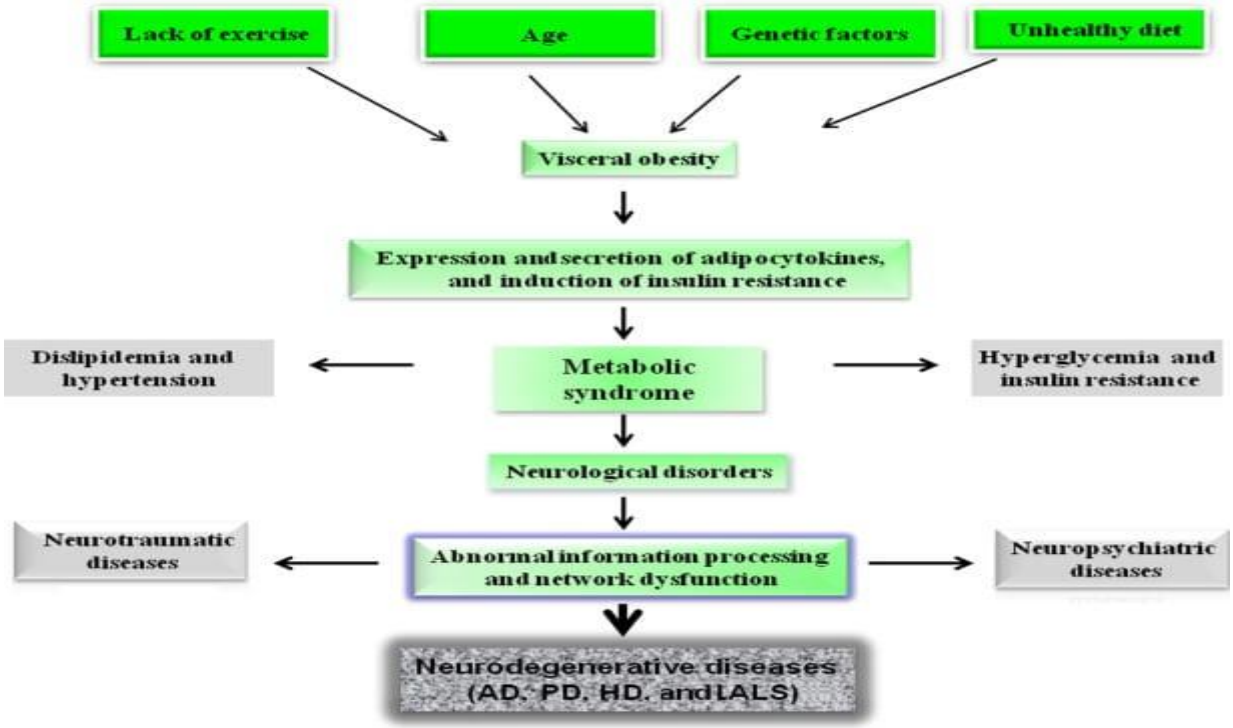


Figure 1. Pathophysiology of neuropharmacological diseases

Causes:

A number of reasons can cause NDs, including aging, which can cause AD and PD, genetic defects that affect the normal functioning of the central nervous system (CNS), which can cause Huntington's illnesses and Amyotrophic Lateral Sclerosis (ALS), and aging itself. Age and specific genetic variations are recognized risk factors for neurological disorders. Gender, low levels of education, endocrine disorders, oxidative stress, inflammation, stroke, high blood pressure, diabetes, smoking, head trauma, depression, infections, cancers, vitamin deficiencies, immunological and metabolic disorders, and chemical exposure are some of the more likely causes of NDs. (7)

2. RESOURCES AND ADVANCES

2.1 THE STUDY AREA:

The Pulla Reddy Institute of Pharmacy’s Department of Pharmacology in Dundigal, Hyderabad, is where the study was carried out.

2.2 PLANT MATERIAL:

Cassia Obtusifolia seeds were collected in December 2024 from online, Telangana, India. After collection, authentication was carried out by the Botanical Survey of India, Room No. 228-238, Sultan Bazar, Koti, Hyderabad-50001.

2.3 PREPARATION OF CRUDE EXTRACT:

Large quantities of fresh *Cassia obtusifolia* seeds were gathered, shade-dried, and grind in a grinder. For additional research, the coarse powder was utilised. The extraction process, known as soxhlation, was carried out using methanol as the solvent. After filtering, the extract was kept for later examination.

2.4 EXTRACTION PROCESS OF CASSIA OBTUSIFOLIA SEEDS BY USING SOXHLET APPARATUS:

REQUIREMENTS AND EQUIPMENT: *Cassia obtusifolia* seeds, Heating mantle, Soxhlet apparatus, round bottom flask, methanol.

PROCEDURE:

- *Cassia obtusifolia* seeds was collected and dried.
- Powder the dried seeds with the help of blender.
- 30 grams of powder was weighed.
- The weighed powder sample was placed in the thimble.
- Add 250 ml of methanol as a solvent in round bottom flask.
- The round bottom flask is placed on the heating mantle
- The solvent get contact with powdered drug which was placed in the thimble.
- After many cycles the desired compound concentrated in the distillation flask.

- The extracted solvent is filtered, heated and cooled.
- Collect the extract.

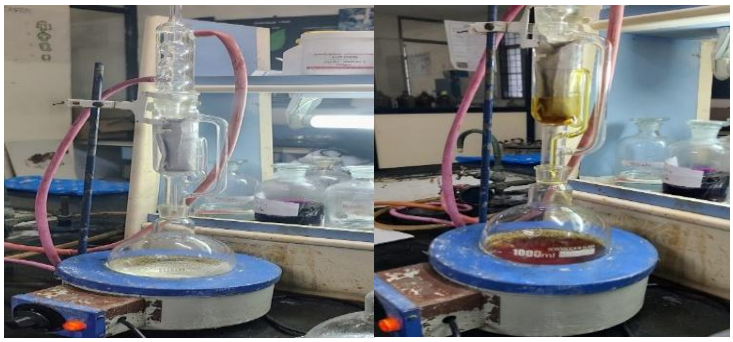


Figure 2: Extraction Process

3. PHYTOCHEMICAL TESTS FOR *CASSIA OBTUSIFOLIA* BY USING METHANOLIC EXTRACT

Phytochemical screening refers to the preliminary analysis of plant materials to identify bioactive compounds such as flavonoids, saponins, tannins, terpenoids, glycosides, and phenolics. These naturally occurring compounds are responsible for a wide range of pharmacological activities, making them important for drug discovery and the development of herbal therapeutics (8).

The qualitative screening of phytochemicals involves classical methods that rely on color change, precipitation, or froth formation using specific chemical reagents. For instance, Dragendorff's, Wagner's, and Mayer's reagents are commonly used for detecting alkaloids; the Shinoda test for flavonoids; and the Salkowski and Liebermann-Burchard tests for steroids and terpenoids (9). These methods help confirm the presence of these bioactive compounds and act as a preliminary step before more advanced chromatographic or spectroscopic analysis (10).

Solvent extraction using polar or non-polar solvents like methanol, ethanol, chloroform, and water plays a crucial role in extracting specific groups of compounds. Methanol, being a polar solvent, is particularly effective in extracting a broad range of phytochemicals such as flavonoids, saponins, and glycosides (11).

The information gathered from phytochemical screening helps in validating the traditional use of medicinal plants and is essential for quality control and standardization of plant-based formulations (12).

3.1 TEST FOR GLYCOSIDES:

- **BRONTRAGERS TEST:**
  2ml of extract was boiled with few drops of sulphuric acid for 15-20 minutes. Cool and filter the extract, add few ml of chloroform to separate the organic layer. Then added ammonia and observed.
  OBSERVATION:
  Glycosides are indicated by colours such as red, pink and orange (13).

3.2 TEST FOR TANNINS:

- **FERRIC CHLORIDE TEST:**
  One ml of extract was mixed with two ml of a 5% neutral ferric chloride solution.
  OBSERVATION:
  The dark blue coloring suggested the presence of tannins and phenolic compounds (14).
- **LEAD ACETATE TEST:**
  Add a few drops of a 10% lead acetate solution to 1 ml of plant extract.
  OBSERVATION:
  Phenolic compounds are indicated by a colour of yellow precipitate (13.1).

3.3 TEST FOR SAPONINS:

- **FOAM TEST:**
  20 ml of distilled water and 5 ml of extract were combined, and the mixture was shaken in a graduated cylinder.
  OBSERVATION:

Stable foam formation was interpreted as a sign that saponins were present (15).

3.4 TEST FOR STEROIDS AND TRITERPENES:

- **SALKOWSKI TEST:**
  0.5 ml of each extract, 2 ml of chloroform was added and then 3 ml of concentrated sulphuric acid was carefully added to form a layer.
  OBSERVATION:
  A reddish-brown colouration at the interface indicates the presence of terpenoids (13.2).

3.5 TEST FOR FLAVONOIDS:

- **SHINNODA TEST:**
  2ml of extract was mixed with few fragments of magnesium turnings and conc. hydrochloric acid was added gradually after few minutes it was observed.
  OBSERVATION:
  The appearance of a pink or orange colour indicates the presence of flavonoids (15.1).

3.6 TEST FOR RESINS:

- 1 ml of various solvent extract was treated with few drops of acetic anhydride solution followed by one ml of conc. Sulphuric acid.
  OBSERVATION:
  The colouration ranging from orange to yellow that indicates the presence of resins (13.3)

3.7 TEST FOR ALKALOIDS:

- **WAGNER TEST:**
  A few drops of Wagner reagent were added to 2 milliliters of extract.
  OBSERVATION:
  The presence of alkaloids is indicated by the production of a reddish-brown precipitate (16).
- **DRAGENDORFF TEST:**

1 ml of the extract was subjected to dragendroff's reagent.

OBSERVATION:

The presence of alkaloids is indicated by the colour of orange-red precipitate.

- **HAGERS TEST:**

  1ml of extract was subjected to hagers reagent

  OBSERVATION:

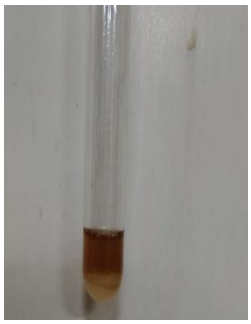
  The presence of alkaloids is indicated by the yellow precipitate.

- **MAYERS TEST:**

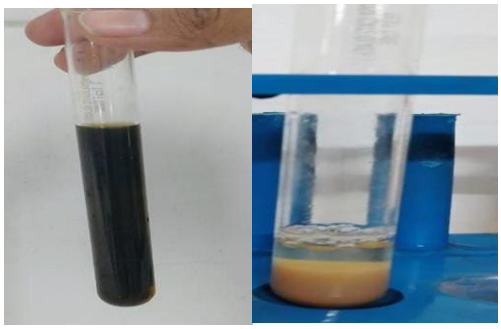
  1 ml of the extract was subjected to Mayers reagent.

  OBSERVATION:

  The presence of alkaloids indicated by yellowish or white precipitate (15.2).

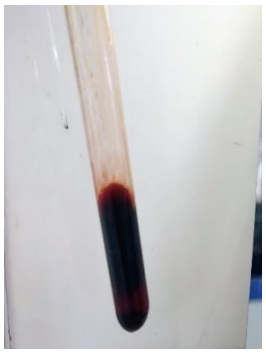


Glycoside Test



Tannins Test

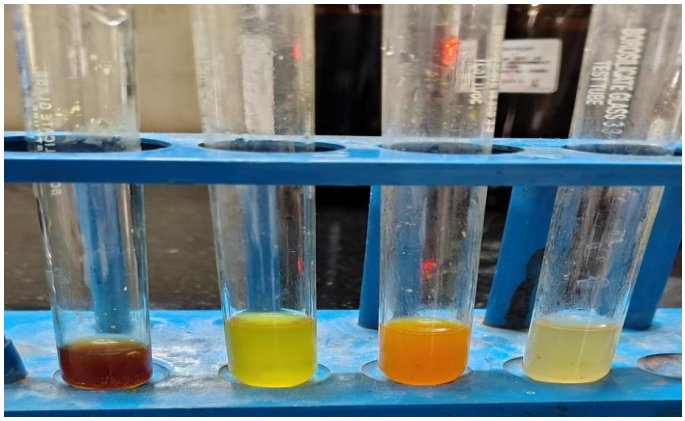
Saponins Test



Steroids & Triterpenes Test

Flavonoids Test

Resins



Alkaloids Test

Figure 3: Phytochemical Tests

INVIVO METHODS:

EDDY'S HOT PLATE:

Procedure:

- Experimental animal's mice were selected and divided into four groups.
- Designated as group1, group2, group 3 and group 4 for control, standard and test respectively.
- The mice were used after 24 hours of fasting and allowing of water only during experiment.
- Each group received a particular treatment i.e.1st group treated with vehicle (gum acacia), The 2nd group was treated with standard drug (diclofenac 10mg/kg intraperitoneally), The 3rd group was treated with low dose of methanolic extract of *cassia obtusifolia* seed 50mg/kg, The 4th group was treated with high dose of methanolic extract of *cassia obtusifolia* seed 200mg/kg.
- All animals were individually weighed and gently placed on a hot plate, with the temperature maintained at a 55 degree celsius.
- The latency period—defined as the time from when the animal was placed on the hot plate until the appearance of a nociceptive response such as paw licking or jumping—was recorded using a stopwatch. To prevent any tissue damage, a cut-off time of 15 seconds was set.

- The reaction times of animals were noted at 0,30,60 and 90 minutes interval after administration of drug and test product. (17)

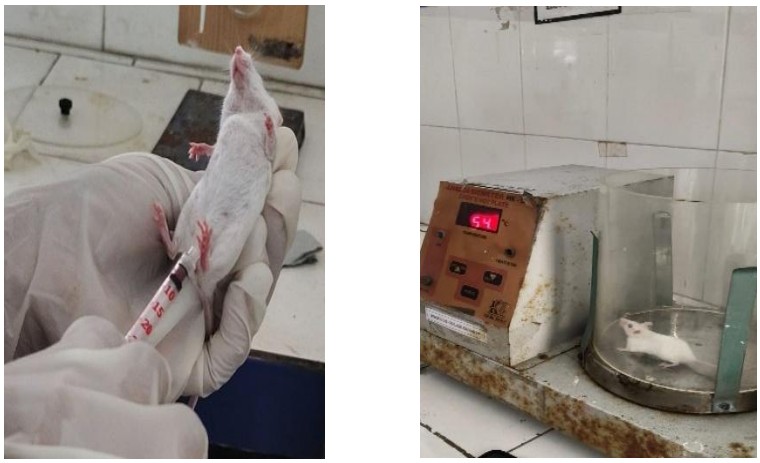


Figure 4: Route Of Administration (I.P.) & Eddy's Hot Plate Apparatus

OPEN FIELD TEST:

Procedure:

The open field test is a widely used method for evaluating general locomotor activity, exploratory behavior, and anxiety-related responses in rodents. In this study, the open field apparatus was constructed from plain wood and measured 45 cm × 45 cm × 20 cm. The floor consisted of a wooden sheet (45 cm × 45 cm), marked with a grid that divided the surface into sixteen equal squares.

- Mice were randomly assigned to four groups were treated. Experimental animal's mice were selected and divided into four groups.
- Designated as group1, group2, group 3 and group 4 for control, standard and test respectively.
- The mice were used after 24 hours of fasting and allowing water only during experiment.

- Each group received a particular treatment i.e.1st group treated with vehicle (gum acacia), The 2nd group was treated with standard drug (Alprazolam 5mg/kg intraperitoneally), The 3rd group was treated with low dose of methanolic extract of *cassia obtusifolia* seed 50mg/kg, The 4th group was treated with high dose of methanolic extract of *cassia obtusifolia* seed 200mg/kg.

Thirty minutes post-treatment, each mouse—whether from the control or treated groups—was gently placed at the center of the open field arena. The behavior of each animal was then observed and recorded over a 5-minute session.

During the observation period, a hand-operated counter and a stopwatch were used to assess the following behavioral parameters:

1. Number of entries and time spent in the central area of the field, which can reflect anxiety-like behavior (less time in the center may indicate increased anxiety).

2. Time spent in the periphery and corners, as animals typically prefer these areas when anxious.

3. Number of crossings (i.e., how many grid squares the mouse entered), used to estimate general locomotor activity or distance traveled.

4. Rearing behavior (standing on hind legs), which reflects exploratory activity.

5. Assisted rearing, when the mouse stood on its hind legs and touched the wall with its forepaws, indicating vertical exploration.

This test setup provides a simple yet effective way to analyze behavioral changes, especially those influenced by anxiolytic or stimulant drugs. (18)

Figure 5: Open Field Test

table 1-

RESULTS FOR PRELIMINARY PHYTOCHEMICAL TEST

S. NO	NAME OF THE CHEMICAL TEST	METHANOLIC EXTRACT
1.	**GLYCOSIDES TEST:**	
	a) Brontragers Test	+
2.	**TANNINS TEST:**	
	a) Ferric Chloride Test	+
	b) Lead Acetate Test	+
3.	**SAPONINS TEST:**	
	a) Foam Test	+
4.	**STEROIDS AND TRITERPENES TEST:**	+
	a) Salkowski Test	
5.	**FLAVANOIDS TEST:**	
	a) Shinoda Test	+
6.	**RESINS TEST:**	+
7.	**ALKALOIDS TEST:**	
	a) Wagner's Test	-
	b) Dragendroff's Test	-
	c) Mayer's Test	-
	d) Hager's test	-

ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *CASSIA OBTUSIFOLIA* SEEDS BY USING EDDY'S HOT PLATE METHOD:

In this evaluation, animals treated with diclofenac (10 mg/kg, I.P) had an evident increase in the latency time 0,30, 60, 90mins. For the groups treated with Methanolic extract (50 mg/kg and 200mg/kg plant extract), less effect was shown in the first 30 min. However, an increase in latency time was found in 60, 90 min. The diclofenac proved to be a potent analgesic, increasing the latency time within the time evolution and paw licking and jumping time was recorded.

TREATMENT	**0 MINS**	**30 MINS**	**60 MINS**	**90 MINS**
CONTROL	8.00±0.365	8.50±0.619	9.10±0.654	10.10±1.013
STANDARD (Diclofenac)	10.16±0.749**	12.80±1.376**	13.16±0.401**	14.16±0.401**
LOWDOSE50mg/kg (*cassia obtusifolia*)	9.40±0.509*	10.83±0.477*	11.66±1.666*	12.83±0.833*
HIGHDOSE200mg/kg (*cassia obtusifolia*)	10.00±0.749*	11.83±0.909*	12.83±0.703*	13.16±1.013*

Table-2 mean and SEM values for eddy's hot plate method (Response Time)

The values are represented as mean ± standard error of mean (SEM), n=6. Statistical significance was analysed by One way ANOVA. Significance was noted as **P < 0.05.**

Analgesic activity of methanolic extract of *cassia obtusifolia* seeds by using eddy's hot plate

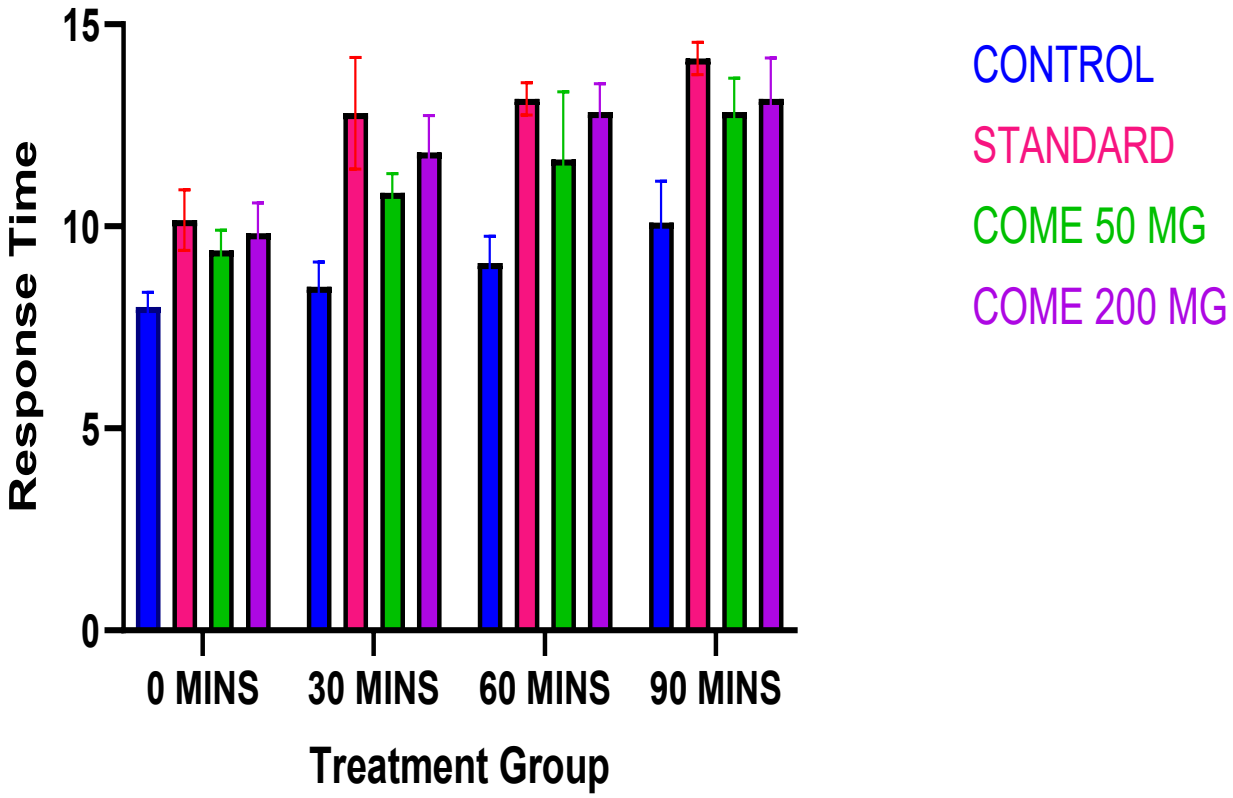


Figure 6: graphical representation of eddy's hot plate

ANXIOLYTIC ACTIVITY OF METHANOLIC EXTRACT OF *CASSIA OBTUSIFOLIA* SEEDS BY USING OPEN FIELD TEST:

In this evaluation, animals treated with Alprazolam (5 mg/kg, i.p.) had an evident anxiolytic activity in time spent in central. For the groups treated with Methanolic extract (50 mg/kg and 200mg/kg plant extract) shows significant ($p<0.05$) when compared to the control.

TREATMENT GROUP	TIME SPENT (MEAN AND SEM VALUE)	NO. OF ANIMALS
CONTROL	13.5±0.764*	6
STANDARD (Alprazolam)	44.5±0.764*	6
LOW DOSE 50mg/kg (*cassia obtusifolia*)	28.5±0.764*	6
HIGH DOSE 200mg/kg (*cassia obtusifolia*)	36.8±0.792*	6

table 3- Evaluation of the Anxiolytic Activity of Cassia obtusifolia in Experimental Animals

The values are represented as mean ± standard error of mean (SEM), n=6. Statistical significance was analysed by One way ANOVA. Significance was noted as *P < 0.05.

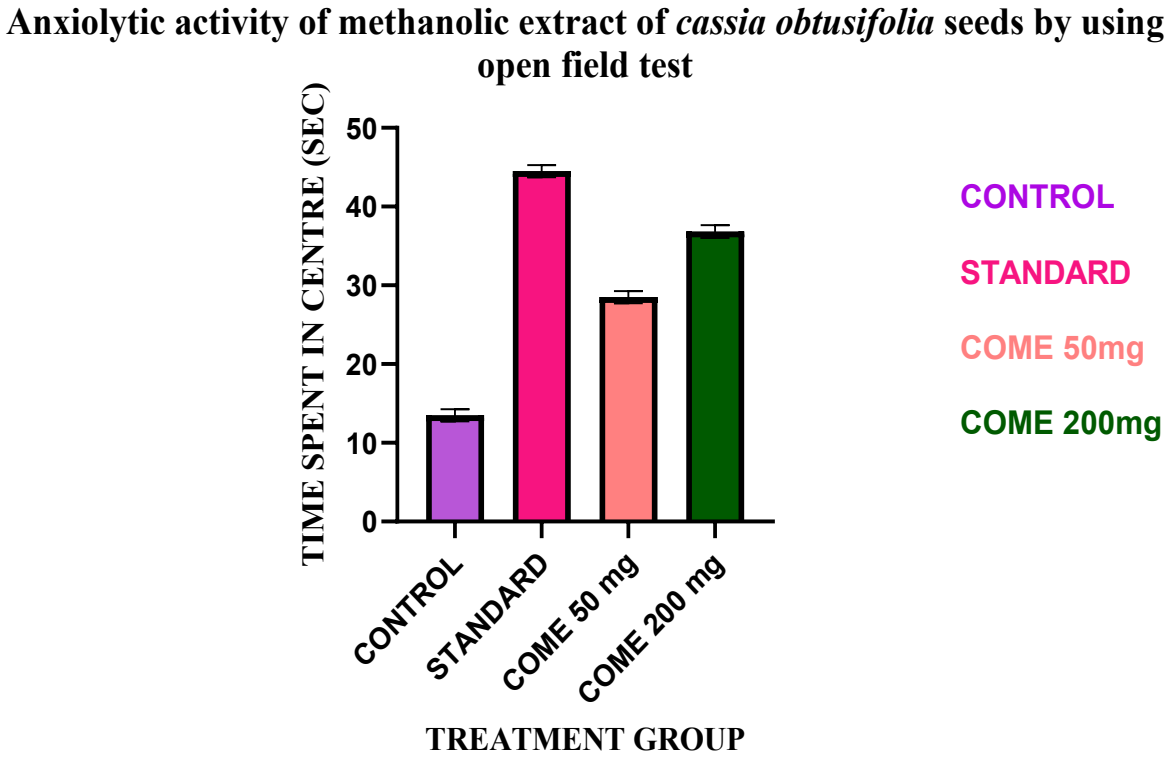


Figure 7: Graphical representation of open field

DISCUSSION

- The present study was aimed at evaluating the phytochemical and neuropharmacological potential of *Cassia obtusifolia* seed extracts using established in vivo models in albino mice. Based on the comprehensive review of literature, *Cassia obtusifolia* has been traditionally reported to possess various medicinal properties, including antioxidant, anti-inflammatory, and central nervous system (CNS)-related effects. However, detailed experimental evaluation of its neuropharmacological properties, especially in seed extracts, remained limited.
- Methanolic extraction via Soxhlet apparatus was employed, as methanol is an efficient solvent for extracting a broad range of phytoconstituents, especially polar compounds. Preliminary phytochemical screening revealed the presence of several active constituents, notably glycosides, flavonoids, and phenolic compounds. These constituents are often associated with neuropharmacological activities such as analgesic and anxiolytic effects, suggesting the potential of the extract for CNS modulation.
- For neuropharmacological evaluation, two validated in vivo models were selected: Eddy's Hot Plate method for assessing central analgesic activity, and the Open Field Test (OFT) to evaluate exploratory behavior and potential anxiolytic effects.
- In the Eddy's Hot Plate test, the extract-treated groups (both low and high doses) showed a statistically significant ($p < 0.05$) increase in latency time for paw licking and jumping when compared to the control group. This confirms the central analgesic effect of the extract. Although the effect was slightly less than the standard drug diclofenac, it was notably consistent and dose-dependent, indicating potential central nervous system involvement.

- In the Open Field Test, mice treated with the extract showed a significant ($p < 0.05$) increase in locomotor activity and frequency of center entries compared to the control group. These behavioral changes suggest a reduction in anxiety-like behavior, indicating possible anxiolytic properties of the extract. Such effects are commonly attributed to the presence of flavonoids and glycosides, which may act through modulation of GABAergic or serotonergic neurotransmission.
- The statistically significant results from both tests support the neuropharmacological potential of *Cassia obtusifolia* seed extract. The findings validate the traditional use of this plant in CNS-related disorders and suggest its potential role in developing new herbal therapeutic agents for pain and anxiety management.
- Further studies involving isolation of individual active compounds, detailed mechanistic pathways, and long-term safety profiling are recommended to substantiate and extend these promising results.

CONCLUSION

The current study successfully evaluated both the phytochemical profile and neuropharmacological activities of *Cassia obtusifolia* seed extract. The preliminary phytochemical screening revealed the presence of various bioactive constituents such as flavonoids, phenols, tannins, saponins, and glycosides, which are known to possess pharmacological effects on the central nervous system (CNS).The in vivo behavioral models provided clear evidence of the extract's CNS activity: In the Eddy’s hot plate test, the extract significantly increased the pain threshold (reaction time) in a dose-dependent manner, indicating central analgesic activity. In the Open Field Test, the extract decreased locomotor activity, rearing, and grooming behaviors, indicating CNS depressant and anxiolytic-like effects. The high-dose group showed more prominent effects compared to the low-dose group and control, though slightly less potent than the standard drug Alprazolam, confirming the extract’s pharmacodynamic potential. These effects may be attributed to the

modulation of neurotransmitter systems, particularly GABAergic and serotonergic pathways, by the active constituents present in the extract. The results thus support the traditional use of *Cassia obtusifolia* in herbal medicine for nervous system-related ailments. In conclusion, the Methanolic extract of *Cassia obtusifolia* seeds exhibits significant neuropharmacological effects, including analgesic and anxiolytic activities, validating its ethnomedicinal use.

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