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Evaluation of Anxiolytic and Antidepressant Activities of the Flavonoid Rich Extract of Leaves of *Cassia obtusifolia* Linn on Mice

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Abstract **Background:** The purpose of this study is to assess how *Cassia obtusifolia Linn* affects the anxiety and depressive-like behaviors, on the other hand, behavioural parameters were assessed without inducing stress. In this study, we use proven models such as brain oxidative indicators, EPM, LDB, anoxic tolerance test for anxiolytic effects and FST & TST for antidepressant effects, antioxidants, and histological observation in conjunction with animal models to evaluate the efficacy of test drugs, i.e., in treating anxiety and depression generated by exposure to stress in mice. **Results:** The test -1 group received a low dose of COE 100 mg/kg, while the test -2 group received an oral median dose of COE 200 mg/kg, and the test- 3 group received an oral higher dose of COE 300 mg/kg. When COE was administered orally, there was a noticeable and dose-dependent increase in both the amount of time spent in the light chamber and the number of entries. Furthermore, COE exhibits a dose-dependent action that significantly increases the time spent in open arm and no. of entries in open arm. However COE exhibits a dose-dependent effect that considerably increases the latency to convulsion. However COE shortens the immobility duration in FST & TST respectively. However, test drugs show a decrease in LPO levels at lower doses. COE, on the other hand, was discovered to boost the repair of GSH at median dose, and CAT levels at higher doses. **Conclusion:** Based on the behavioral study, oxidative parameters, and histological observation data, COE significantly improves anxiety and depression-like behavior in experimental mice.

Keyword Anxiety, Depression, *Cassia obtusifolia*, Diazepam, EPM, LDB, TST, FST

1. Introduction

The body's natural response to stress is anxiety. Another way to think of anxiety is as the body and brain's response to stressors, fear, and anything that makes them feel uneasy about something that is going to happen. Everyone has probably experienced at least one instance when they felt like their hearts were going to burst out of their chests, whether it was while they were waiting for a job interview, asking someone out on a date, or even when they were meeting someone new for the first time. It would be strange if someone had never experienced anxiety because it is a common everyday occurrence [1]. The idea that the current era is "the age of anxiety" accurately depicts the fact that anxiety is a prevalent issue in contemporary life. Numerous risks that come with living in a competitive, technologically advanced postmodern society can cause crippling anxiety. As a result, a vast body of research has been done on the definition, assessment, causes, and treatment of anxiety and anxiety disorders. The fundamental place anxiety holds in personality, counseling, educational, occupational, and clinical psychology—shaping psychology and psychiatry theory and practice—highlights the significance of anxiety as a fundamental construct in both normal and abnormal behaviour. Currently, anxiety is a highly studied and discussed phenomenon in the field of personality and social psychology, with over 100,000 scientific papers covering its various aspects in theory, assessment, research, and clinical practice published in this domain [2]. Hundreds of millions of people worldwide suffer from depression, a potentially fatal mental illness marked by mood swings and a lack of interest in outside events that exacerbate the condition's negative effects on an individual's sense of psychosocial and physical well-being [3]. Currently affecting 350 million people worldwide, depression is seen as a serious public health issue that is predicted to overtake all other causes of mortality by 2020 [4]. The primary causes of depression development are social, genetic, biological, and psychological factors. Of all cases of depression, about 40–50% are inherited, and the remaining 50–60% are brought on by physical illness, emotional stress, and early childhood trauma [5]. Before the age of 40, it affects roughly 7–18% of people at some point in their lives. Approximately two-thirds of patients with depression have suicidal thoughts, and 10-15% of them actually try suicide [6]. Previous research has shown that individuals with persistent pain have a 30% to 54% higher risk of depression than the general population. Generally, women experience depression at a rate that is nearly 50% higher than that of men [7]. Depression can strike people of any age, including young children and adolescents. Depression can also be caused by serious medical illnesses such as heart disease, cancer, and HIV; the use of certain drugs; alcoholism; drug misuse; financial troubles; relationship breakups; and the loss of a loved one [8]. It expresses that depression is achieved by a practical insufficiency of neurotransmitters, especially norepinephrine (NE). Psychotic depression is much more common in older inpatient samples with major depressive illness. Research indicates that in inpatient settings, the prevalence of psychotic depression in adults over 60 ranges from 24% to 53% [9].

Numerous neurotransmitter systems have been proposed as contributing to one or more of the modulatory processes involved. Most common neurotransmitter systems, including serotonergic and noradrenergic, are considered. In general, it is assumed that an underactive serotonergic system and an overactive noradrenergic system are connected. Disregulation is the result of these systems' regulation and regulation by additional routes and neuronal circuits in different parts of the brain of both the emotional response to physiological arousal and its experience [10]. Many believe that it originated from low serotonin system activity and high noradrenergic system activity. Some attention has been paid to the control of corticosteroids and how it relates to anxiety and fear symptoms. Corticosteroids have the ability to change how certain brain circuits function, which can affect not only behaviour under stress but also the functioning of the cognitive ability. Cholecystokinin was widely believed to function as a neurotransmitter that processes fear-inducing stimuli and regulates emotional states. Because these neurotransmitters are so carefully synchronized, modifications to one neurotransmitter system will always cause modifications to another, such as comprehensive feedback systems. Two inhibitory neurotransmitters that reduce the stress response are serotonin and GABA. These neurotransmitters have all emerged as key targets for medicinal substances [11].

Common drugs for anxiety and depression such as SSRI, Benzodiazepines, and Tricyclic antidepressants have become popular in the last few decades. In most instances, these anti anxiety and antidepressant drugs, particularly SSRI, Benzodiazepines, Tricyclic antidepressant drugs. Furthermore, a lot of these medications have unwanted side effects. According to studies, they lead to tolerance, addiction, and physical dependence, whereas antidepressants typically result in gastrointestinal problems [12].

The current investigational protocol aims to investigate anxiolytic and antidepressant effect of Flavonoid Rich Extract of leaves of *Cassia obtusifolia* Linn using the various validated models, antioxidant and histopathological studies. The objective of this investigational study is to overcome serious adverse events of specified categories of drugs and to comprehend the potential mechanism of action of Cassia obtusifolia.

2. Materials and Methods

A) Animals

For the purpose of this investigation, each group consisted of six Swiss albino mice that were in good health and ranged between 25 and 40 grams. The animals were kept in polypropylene cages, which had provision for enough ventilation, and they were kept in full laboratory conditions, which included a temperature of 25± 2 °C, and humidity around 45–55 %. Standard food pellets with unlimited water (Nutrivet Life Science, Pune) were also provided to the animals. A required number of all experimental animals were kept in compliance with the Committee for Control and Prevention of Cruelty to Animals Act, 1960, the Indian government, and the Ministry of Environment and Forests. All required animals for the experimentation were approved by Institutional animal ethical committee of Rajarshi Shahu College of Pharmacy, Malvihir, Buldana, Maharashtra, Pin - 443001, Registration No. 1865/PO/16/CPCSEA and Protocol No. IAEC 1865/23-24/P-18.

B) Drugs: Diazepam, Fluoxetine Hydroxide

C) Collection & Authentication of plant

The plant material was collected in the region of Maharashtra, India, from the native ecosystem. The leaves were collected at the time of collection, and the Botany Department of Jijamata Mahavidyalaya, authenticated them. For subsequent use as a reference, the voucher specimen (number 02/23-3-24) was assigned.

Plant	Family	Parts Used
Cassia obtusifolia Linn.	Caesalpiniaceae	Leaves

D) Extraction process

The leaves of Cassia Obtusifolia Linn were ground into a coarse powder after being shade-dried. The plant's leaves were extracted using the maceration technique. Following the filtering of the solution, alcohol (ethanol) was added to obtain the polysaccharide precipitate. After filtering the solution, the filtrate was evaporated to make up one-fourth of the total volume. Ethyl acetate is used to extract the solution one step at a time after one-fourth of its volume has evaporated. After that, the ethyl acetate extract was evaporated to get the 1% w/w flavonoid-rich fraction of *Cassia obtusifolia Linn*, which had a brownish orange color [13].

E) Groups and Treatment

Treatment and schedule

The control group received the normal saline solution at the dose of 10 ml/kg, the standard group received diazepam at the dose of 2 mg/kg i.p. However the test 1, test 2 and test 3 received the doses of 100 mg/kg, 200 mg/kg and 300 mg/kg (Table 1). After the 7 days treatment (1-7) during this treatment 1hr post behavioural assessment of animal was carried out using LDB and EPM. While in anoxic tolerance test the treatment schedule is 1-14 days after the half an hour behavioural assessment was carried out.

Treatment and schedule

The control group received the normal saline solution at the dose of 10 ml/kg, the standard group received the Fluoxetine Hydroxide at the dose of 20 mg/kg i.p. However the test 1, test 2 and test 3 received the doses of 100 mg/kg, 200 mg/kg and 300 mg/kg (Table 2). After the 7 days treatment (1-7) during this treatment 1hr post behavioural assessment of animal was carried out using TST. While in FST the treatment schedule is 1-14 days after the half an hour behavioural assessment was carried out.

Table 1. Experimental groups and treatments for assessment of anxiolytic effects by using LDBM, EPM and Anoxic Tolerance Test

Sr. No.	Group	Drug	Dose
1)	Control	NaCl Solution	10 ml/kg i.p.
2)	Standard	Diazepam	2 mg/kg i.p.
3)	Test 1	*Cassia Obustifolia* Linn	100 mg/kg
4)	Test 2	*Cassia Obustifolia* Linn	200 mg/kg
5)	Test 3	*Cassia Obustifolia* Linn	300 mg/kg

Table 2. Experimental groups and treatments for assessment of antidepressant effects by using FST and TST.

Sr. No.	Group	Drug	Dose
1)	Control	NaCl Solution	10 ml/kg i.p.
2)	Standard	Fluoxetine Hydroxide	20 mg/kg i.p.
3)	Test 1	*Cassia Obustifolia* Linn	100 mg/kg
4)	Test 2	*Cassia Obustifolia* Linn	200 mg/kg
5)	Test 3	*Cassia Obustifolia* Linn	300 mg/kg

2.1. Experimental Design

2.1.1. Behavioural Parameter

Light-Dark Box Test (LDBT): The Crawley and Goodwin two-compartment exploratory models have been validated in terms of pharmacology, behavior, and physiology. The two-compartment method balances the rat's innate curiosity about a new habitat with the unpleasant characteristics of an open field with intense light. The most accurate metric for determining anxiolytic activity appeared to be time spent in light areas or engaging in exploratory behavior. The light and dark box has two sections: the white-painted light region (27L x 27W x 27H cm) was lit by a 100 W desk lamp, while the black-painted dark area (18L x 27W x 27H cm) was used for lighting. To facilitate movement from one compartment to the other, a 7.5 x 7.5 cm tunnel was used to divide the two sections. The overall number of crossings between the bright and dark compartments, as well as the time spent in the bright and dark compartments, was recorded [14].

Elevated Plus-Maze Test (EPMT): Every test was conducted outside of the colony chamber in a dimly lit space. Rats were kept in the test chamber for an hour before the experimental test. The 40 x 7 open arms, two identically sized closed arms, and 10 cm-high side and end walls comprised the EPM device. The arms were joined by a 7 cm by 7 cm gap in the middle. Animal was placed in the center of the apparatus with its head facing an open arm, and it was then left for three minutes. After that, the animal was removed and placed back in its original cage. The equipment was cleaned with a 10% chlorine bleach solution between each test session. An animal was considered to be in an arm if it had all four paws on the central platform and on the central platform if it had at least two paws. The duration of open arms (OAT: (time in open arm/time in open + closed arm) and the quantity of open arm entries (OAE: (number of open arm entries/number of open + closed arm entries) were used to quantify anxiety. In addition, the total number of arm entries was counted to obtain a measure of spontaneous locomotors activity [15].

Forced Swimming Test (FST): The mice were subjected to FST using Porsolt's test procedure. For 10 minutes at 30 °C, each mouse was made to swim in a glass jar that was 25 × 12 × 25 cm^2 and filled with water up to a height of 15 cm. The immobility time was observed for four out of six minutes. The mouse's time spent floating still in the water and ceasing to struggle was referred to as the immobility phase. It simply moved when necessary to keep its head above the water's surface [16].

Tail Suspension Test (TST): The mice were kept fifty centimeters above the ground during the tail suspension test (TST) by means of adhesive tape that was placed two millimeters from the tips of their tails. As an additional point of interest, the length of immobility was recorded during the last four minutes of the test, which lasted for a total of six minutes [17].

Anoxic Tolerance Test: A 250 mL airtight hermetic vessel was used to hold each animal separately. The animals were then taken out of the vessel immediately after the anoxic tolerance time was noted for recovery. The latency until anoxic convulsions appeared for the first time was known as the anoxic tolerance time [18].

2.1.2. Evaluation of Oxidative Stress Parameters in the Mice Brain

After the behavioral test, oxidative stress parameters were evaluated. In this experiment, independent mice received i.p. injection of ketamine and xylazine (45 mg/kg and 21 mg/kg) for the purpose of anaesthesia. After a cardiac puncture to remove blood, the whole separate portion of the brain was isolated and rapidly placed on an icebag. It was subsequently washed with an isotonic cold saline solution (0.9% NaCl) to filter out any unnecessary surrounding tissue and blood. An isolated individual brain was weighed and noted. Then, using a tissue homogenizer, a 10% w/v brain tissue homogenate was prepared with 50 mM phosphate buffer (pH 7.4; 1% v/v Triton X-100; temperature 4°C). The homogenate was centrifuged at 12,000×g for 15 minutes at 4°C in a high-speed chilled centrifuge. The resulting clear supernatant was considered for analysis as an indicator of oxidative biomarkers study [19].

2.1.3. Estimation of Lipid Peroxidase

For the preparation of the entire 4-ml assay mixture, initially 0.1 ml homogenate was taken and mixed with 1.5 ml glacial acetic acid (20%, pH 3.5), 1.5 ml thiobarbituric acid (TBA) (0.8%), 0.2 ml sodium dodecyl sulphate (8.1%), and 0.7 ml distilled water. After its preparation the resulting mixture was thoroughly mixed using a vortex mixer, then it was heated at 95 °C for an hour in a water bath before cooling down with tap water. Then, using a centrifuge (Remi), a 5 ml combination of n-butanol and pyridine at a 15:1 ratio was added to the resulting assay solution. The mixture was then swiftly centrifuged for 10 minutes at 4000 rpm. The absorbance of the 2 ml organic layer (n-butanol phase) was measured at $\lambda max = 532$ nm with a double beam spectrophotometer (Shimadzu UV-1700) [20].

2.1.4. Estimation of Glutathione Level

Pick up 0.1 mL of the supernatant as a test sample. The supernatant was made less strong by adding 0.9 ml of phosphate (PO4) water. Put in 1 ml of TCA (20%) and let the mix sit for 20 minutes. After that, it was spun at 10,000 rpm for 10 minutes. 0.25 ml of the liquid was taken away. Use 0.75 ml of phosphate water to add. Also, add 2 ml of DTNB, which is 0.0006M in strength. Ten minutes were spent sitting. A spectrophotometer was used to measure absorbance at 412 nm [21].

2.1.5. Estimation of Catalase Level

We measured the catalase (CAT) activity using the method outlined by Claiborne (1985). 0.05 ml of 10%

supernatant makes up the assay mixture. Add one milliliter of H2O2. To a final amount of 3 ml, add 1.95 ml of phosphate buffer (0.05 M, pH 7). Using a UV spectrophotometer, absorbance was measured at 240 nm [22].

2.1.6. Histopathological Examination

Once intracardiac perfusion with phosphate buffer formalin was completed, the brain tissue for histopathology was extracted. An area of a mouse brain was embedded in paraffin wax, sectioned into pieces with a thickness of 2–5 µm, and fixed with 10% (v/v) formalin. Eosin and hematoxylin dye were used to stain the sections for histopathological analysis [23].

2.2. Statistical Analysis

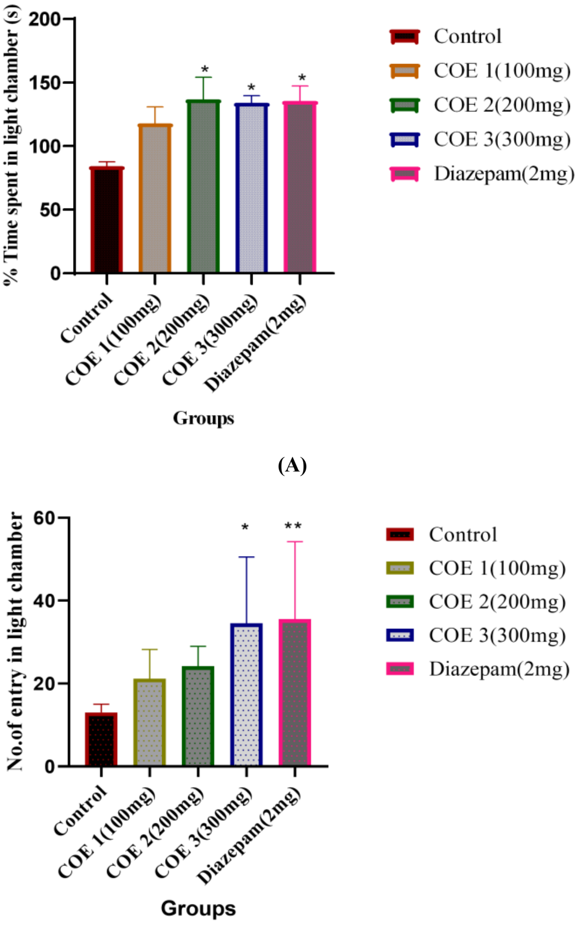
Every result was shown as mean ± SEM. Dunnett's multiple comparison's test was used after one-way analysis of variance (ANOVA) to determine the statistical significance between means. The Graph-Pad Prism software version was used to do the statistical analysis. If $P<0.05$, the values are regarded as statistically significant.

3. Results

3.1. Dose-Dependent Impact of *Cassia obtusifolia* Linn. on Anxiety-Like Behaviours in Light and Dark Box Test

As depicted in **Fig 1 (A)**, it was observed that animals were given dose-dependent treatment of extract *Cassia obtusifolia* Linn and diazepam for 7 days. The saline control decreased the time spent in light chamber in contrast to the treatment group. The different doses of COE 200 mg and 300mg increased the time spent in the light chamber (*P<0.0121, *P<0.0178) in contrast to normal control group. Similarly diazepam treatment animal shows significant improvement in percentage time spent in light chamber (*P<0.0143) as compared to saline treated animals. Whereas a low dose of COE failed to exhibit considerable improvement in light chamber.

As depicted in **Fig 1 (B)**, the saline control decreased the no of entries in light chamber as compared to the treatment group. The dose of COE 300mg/kg increased the no of entries in light chamber (* P<0.0136) more notably as compared to saline treated group. The diazepam treated group considerably increases the no. of entries in light chamber (**P<0.0096) in contrast to saline treated animals. Conversely COE 100 mg /kg and 200 mg/kg failed to increase the no of entries in light chamber found to be non-significant. COE administered orally exhibited a significant and dose-dependent effect on the enhancement of time spent in light chamber, as well as no. of entries in light chamber dose dependently.



(A)

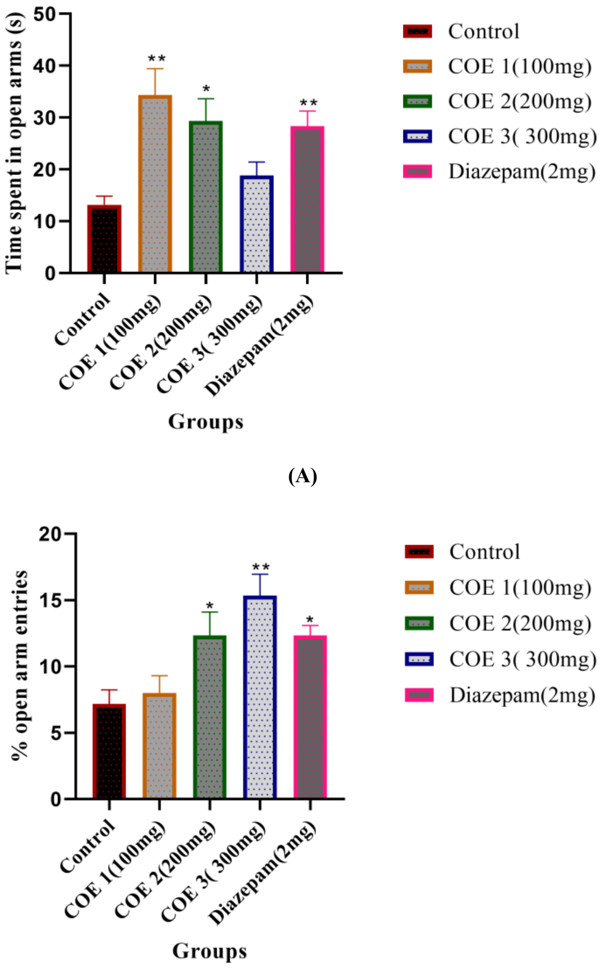
(B)

Figure 1. **(A)** The dose-dependent effect of COE (100, 200 & 300 mg/kg) on time spent in light and dark box. Data were expressed as mean ± SEM (n=6). Statistical comparisons were done by ordinary One way ANOVA followed by Dunnett's multiple comparison's test; *P<0.0121, *P<0.0178 and *P<0.0143 are considered statistically significant Vs Control group. **(B)** The dose-dependent effect of COE (100, 200 & 300 mg/kg) on no of entries in light and dark box. Data were expressed as mean ± SEM (n=6). Statistical comparisons were done by ordinary One way ANOVA followed by Dunnett's multiple comparison's test; *P<0.0136 and **P<0.0096.

3.2. Impact of *Cassia obtusifolia* Linn Treatment on Anxiety-Like Conditions Using Elevated Plus Maze Test

As depicted in **Fig 2 (A)**, the COE 100 mg/kg significantly increased time spent in open arm, (**P<0.0011) in comparison with control group, whereas the COE 200 mg/kg significantly increased the time spent in open arm (*P< 0.0124) in contrast to control group. Similarly the standard group significantly increases the time spent in open arm, (*P<0.0197) as compared to control group. Conversely COE 300 mg /kg failed to increase the time spent in open arm, and the result was found to be non-significant. When COE is taken orally, it can show a notable effect that lengthens the amount of time spent in an open arm position in an elevated plus maze.

As shown in **Fig 2 (B)**, the COE 200 mg/kg significantly increased no. of entry in open arm, (*P < 0.0427) as compared to control group, whereas the COE 300 mg/kg significantly increased the time spent in open arm (**P= 0.0010) in comparison with control group. The standard group significantly increased the no. of entry in open arm, (*P=0.0427) in contrast to control group. Whereas COE 100 mg/kg failed to increase the no of entries in open arm and the result was found to be non-significant. Whenever COE is administered, it can provide some noteworthy and remarkable effects. More precisely, it can selectively enhance the number of entries in the open arm of the elevated plus maze apparatus.



(A)

(B)

Figure 2. **(A)** The effect of COE (100, 200 & 300 mg/kg) on time spent in open arm in EPM test. Data were expressed as mean ± SEM (n=6). Statistical comparisons were done by ordinary One way ANOVA followed by Dunnett's multiple comparison's test; **P<0.0011, *P<0.0124 and **P<0.0197 are considered statistically significant Vs Control group. **(B)** The dose-dependent effect of COE (100, 200 & 300 mg/kg) on no of entries in open arm. Data were expressed as mean ± SEM (n=6). Statistical comparisons were done by ordinary One way ANOVA followed by Dunnett's multiple comparison's test; *P<0.0427, **P<0.0010 and *P<0.0427.

3.3. Dose-Dependent Effect of *Cassia obtusifolia* Linn Treatment on Anoxic Stress Tolerance in Mice

As depicted in **Fig. 3**, it was observed that animals were given dose-dependent treatment of extract of *Cassia obtusifolia* Linn. and diazepam for 14 days. Post hoc treatment of COE 200 mg and 300 mg (*P<0.0109 & **P<0.0050) significantly increased the latency of convulsion (min) in contrast to saline treated animals. The standard group considerably increased the latency of convulsion (*P<0.0230) in contrast to control group. Whereas COE 100 mg/kg failed to increase the latency of convulsion in anoxic tolerance test and it was found to be non-significant. Whenever COE is administered dose-dependently, it can provide some noteworthy and remarkable effects. More precisely, it can selectively enhance the latency to convulsion in anoxic tolerance test.

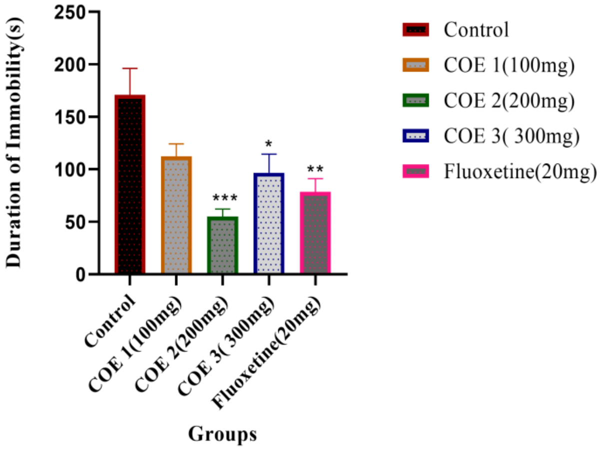


Figure 3. The effect of COE (100, 200 & 300 mg/kg) on latency to convulsion in anoxic tolerance test. Data were expressed as mean ± SEM (n=6). Statistical comparisons were done by ordinary One way ANOVA followed by Dunnett's multiple comparison's test; *P<0.0109, **P<0.0050 and *P<0.0230 are considered statistically significant Vs Control group.

3.4. Impact of *Cassia obtusifolia* Linn Treatment on Depressive Like Behaviors Using Forced Swim Test

As depicted in **Fig 4**, the results of the FST revealed that the COE 200 mg/kg remarkably decreased the immobility time (***P<0.0001) as compared to saline treated animals. Whereas, the COE 300 mg/kg considerably decreased the immobility time (*P<0.0114) in contrast to the saline treated animals. Similarly the standard group treatment significantly reduced the immobility time (**P<0.0016) in comparison with saline treated group. Whereas COE 100 mg/kg failed to reduce the immobility time in FST, it was found to be non-significant. In the force swimming test, COE given orally can show a significant impact that reduces immobility time.

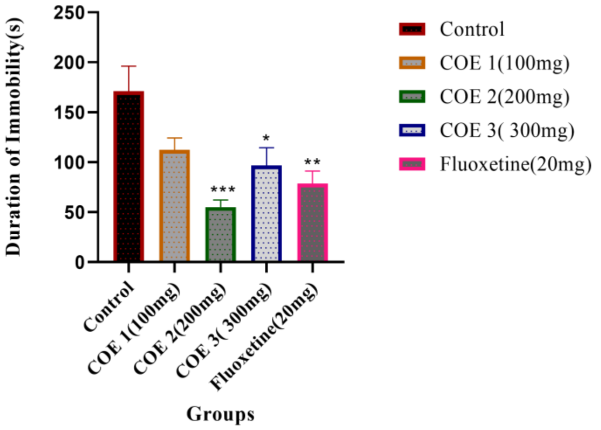


Figure 4. The effect of COE (100, 200 & 300 mg/kg) on duration of immobility in Forced Swim test. Data were expressed as mean ± SEM (n=6). Statistical comparisons were done by ordinary One way ANOVA followed by Dunnett's multiple comparison's test; ***P<0.0001, *P<0.0114 and **P<0.0016 are considered statistically significant Vs Control group.

3.5. Impact of *Cassia obtusifolia* Linn Treatment on Depressive Like Behaviors Using Tail Suspension Test

As depicted in **Fig 5**, the results of the TST revealed that the COE 100 mg/kg remarkably reduced the immobility time (**P<0.0019) as compared to saline treated animals. Whereas, the COE 200 mg/kg considerably decreased the immobility time (*P<0.0102) in contrast to the saline treated animals. Similarly the standard group treatment significantly reduced the immobility time (*P<0.0218) in comparison with saline treated group. Whereas COE 300 mg/kg failed to reduce the immobility time in TST, it was found to be no-significant. In the Tail Suspension test, COE given orally can show a significant impact that reduces immobility time.

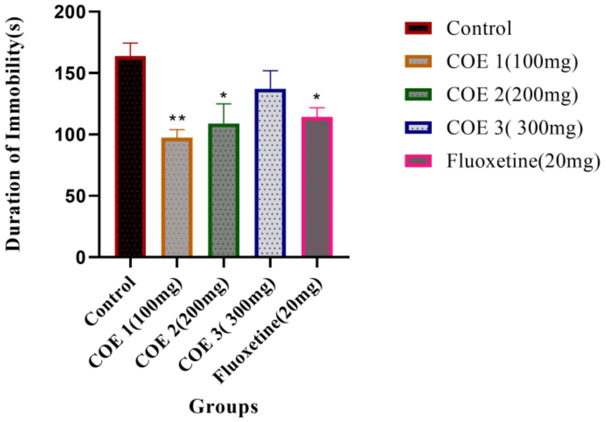


Figure 5. The effect of COE (100, 200 & 300 mg/kg) on duration of immobility in Tail Suspension test. Data were expressed as mean ± SEM (n=6). Statistical comparisons were done by ordinary One way ANOVA followed by Dunnett's multiple comparison's test; **P<0.0019, *P<0.0102 and *P<0.0218 are considered statistically significant Vs Control group.

3.6. Impact of *Cassia obtusifolia* Linn Treatment on LPO Level

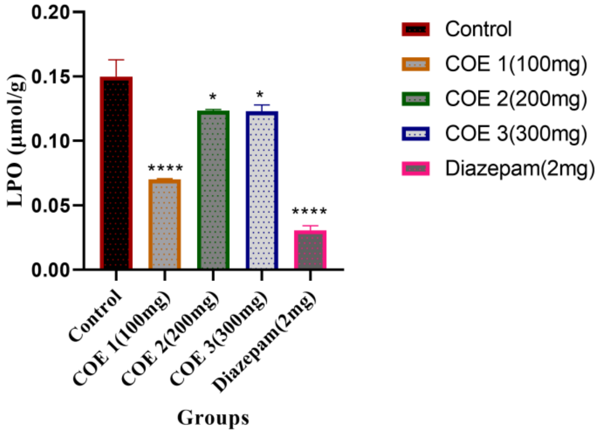
As depicted in **Fig 6 (A)**, the COE 100 mg/kg more significantly decreased the level of LPO (****P<0.0001) as compared to saline treated animals. Similarly the COE 200 mg/kg also considerably decreased the level of LPO (*P<0.0248) in contrast to saline treated animals. Whereas the COE 300 mg/kg remarkably decreased the level of LPO (*P<0.0218) as compared to the control group. Similarly the standard group significantly reduced the level of LPO (****P<0.0001) in comparison with saline treated animals. The LPO level was significantly reduced by the COE when given at doses of 100 mg, 200 mg and 300 mg; in a similar way, the LPO level was most significantly reduced at the 100 mg dose.

3.7. Impact of *Cassia obtusifolia* Linn Treatment on GSH Level

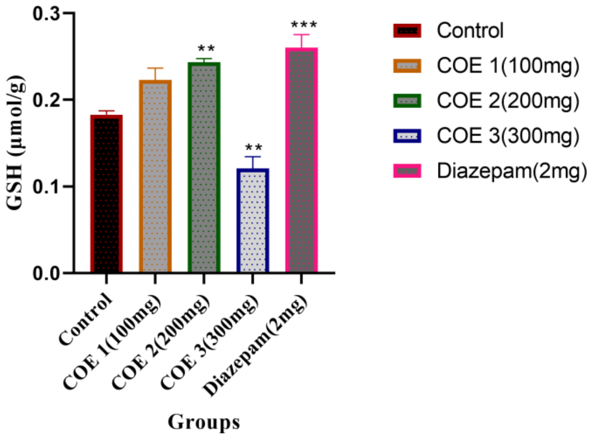
As depicted in **Fig 6 (B)**, the doses of 200 and 300 mg/kg more significantly increased the GSH level (**P<0.0032, **P<0.0024) in contrast to saline treated group. The standard group also more significantly increased the GSH level (***P<0.0002) as compared to saline treated animals. COE Extract can have several remarkable and spectacular impacts whenever it is administered. More specifically, it can boost levels of GSH preferentially. The results were noteworthy since the test group demonstrated a significant increase in GSH when analyzed alongside the standard group.

3.8. Impact of *Cassia obtusifolia* Linn Treatment on CAT Level

As depicted in **Fig 6 (C)**, the doses of 100, 200 and 300 mg/kg more significantly increased the CAT level (*P<0.0291, **P<0.0038 and ***P<0.0008) in contrast to saline treated group. The standard group considerably increased the CAT level (**P<0.0016) in comparison with control group. Oral administration of test drug resulted in a significant and dose-dependent rise in catalase levels.



(A)



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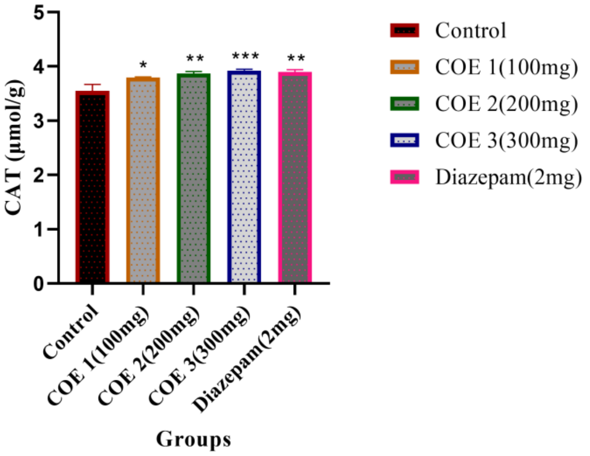
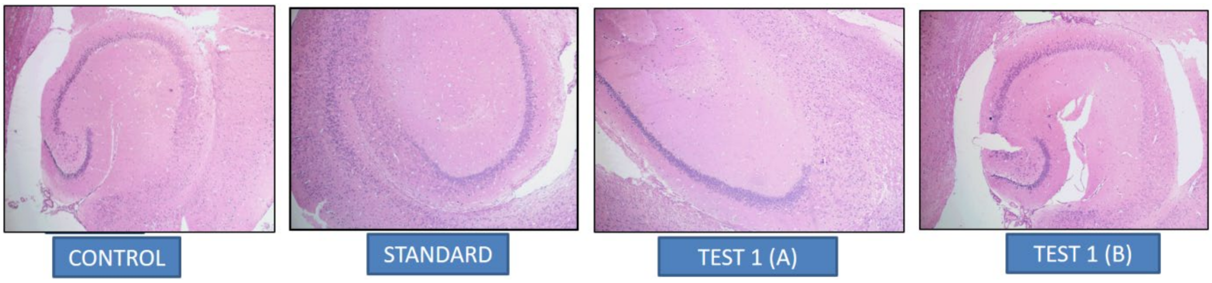


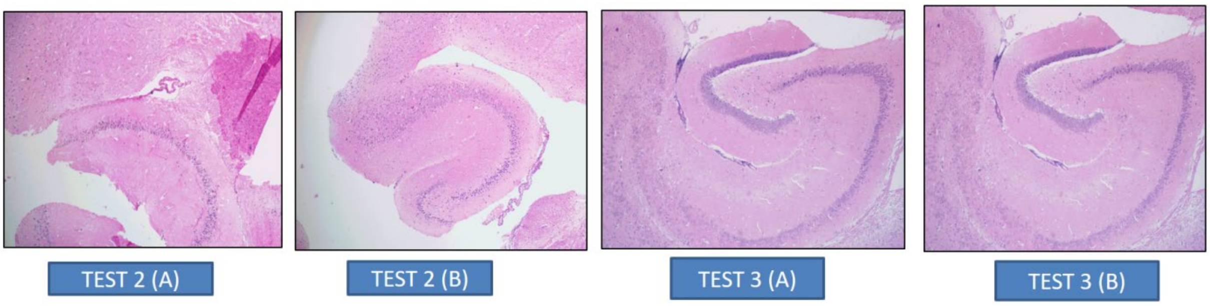
(C)

Figure 6. **(A)** the effects of different doses (100, 200 & 300 mg/kg) of COE extract in LPO level. Each column represents mean ± SEM (n=6) **** P= <0.0001, *P=<0.0248, *P<0.0218, ****P<0.0001 vs control group. (One-way annova Dunnett's multiple comparison's test). **(B)** The effect of COE (100, 200 & 300 mg/kg) on GSH level. Data were expressed as mean ± SEM (n=6). Statistical comparisons were done by ordinary One way ANOVA followed by Dunnett's multiple comparison's test; **P<0.0032, **P<0.0024 and ***P<0.0002 are considered statistically significant Vs Control group. **(C)** The effect of COE (100, 200 & 300 mg/kg) on CAT level. Data were expressed as mean ± SEM (n=6). Statistical comparisons were done by ordinary One way ANOVA followed by Dunnett's multiple comparison's test; *P<0.0291, **P<0.0038, ***P<0.0008, **P<0.0016 are considered statistically significant Vs Control group.

3.9. Histopathological Examination

As depicted in **Fig 7 (a)** Normal group: Microscopic examination of brain tissue shows normal architecture of neuron and normal 5-6 compact layers of small pyramidal cells of CA1 and CA2 regions with most of vesicular nuclei. Molecular layer shows many glial cells among neuronal process. No pyknotic neuron cells. **Fig 7 (b) Standard**: Microscopic examination of brain tissue shows normal architecture of neuron and normal 5-6 compact layers of small pyramidal cells of CA1 and CA2 regions with most of vesicular nuclei. Molecular layer shows many glial cells among neuronal process. No pyknotic neuron cells. **Fig 7 (c) Test I.A:** Microscopic examination of brain tissue shows normal architecture of neuron and normal 5-6 compact layers of small pyramidal cells of CA1 and CA2 regions with most of vesicular nuclei. Molecular layer shows many glial cells among neuronal process. No pyknotic neuron cells. **Test I B:** Microscopic examination of brain tissue shows normal architecture of neuron and normal 5-6 compact layers of small pyramidal cells of CA1 and CA2 regions with most of vesicular nuclei. Molecular layer shows many glial cells among neuronal process. No pyknotic neuron cells. **Fig 7 (d) Test II. A:** Microscopic examination of brain tissue shows degenerative (red arrow) neuron and focal pyknotic (black arrow) neuron cells. **Test II. B:** Microscopic examination of brain tissue shows normal architecture of neuron and normal 5-6 compact layers of small pyramidal cells of CA1 and CA2 regions with most of vesicular nuclei. Molecular layer shows many glial cells among neuronal process. No pyknotic neuron cells. **Fig 7 (e) Test III. A**: Microscopic examination of brain tissue shows normal architecture of neuron and normal 5-6 compact layers of small pyramidal cells of CA1 and CA2 regions with most of vesicular nuclei. Molecular layer shows many glial cells among neuronal process. No pyknotic neuron cells. **Test III. B:** Microscopic examination of brain tissue shows normal architecture of neuron and normal 5-6 compact layers of small pyramidal cells of CA1 and CA2 regions with most of vesicular nuclei. Molecular layer shows many glial cells among neuronal process. No pyknotic neuron cells.





TEST 2 (A) TEST 2 (B) TEST 3 (A) TEST 3 (B)

Figure 7. Histopathological Examination of Brain

4. Discussion

The current investigational protocol was initiated with an aim to assess and contrast the anxiolytic and antidepressant effects of Flavonoid Rich Extract of leaves of *Cassia obtusifolia* Linn using the various validated models, antioxidant and histopathological studies. The main objective of this study is to overcome the serious side effects of specified categories of drugs and to comprehend the potential mechanism of action of Cassia obtusifolia. Anxiety disorders are the most frequent psychiatric disorders among diseases called mood disorders [24]. Stress primarily affects the brain, which can subsequently result in the development of mental diseases associated to stress and cognitive difficulties [25, 26]. Stress exposure has been linked to alterations in the brain's morphology, function, and neurology that are closely linked to stress disorders [27]. Consistent with earlier research, our findings also showed anatomical and functional alterations in a number of brain regions associated with the regulation of behavior and cognition, including the amygdala [28]. Norepinephrine and dopamine are mostly involved in attention, motivation, and regulation of mood and behavior in human and animals but they are usually found to increase in response to stress [29]. Whereas, serotonin level in brain, was found to be decreased during stress, which is primarily involved in the mood and behavior regulation. In addition to this, stress activates specific brain regions, including the amygdala and the prefrontal cortex. Moreover amygdala also plays an important role in regulating the anxiety and phobias in the animals and human [30]. According to the previous studies, the prevalence of anxiety disorders was found to be a total of 34.2% of women who reported having anxiety symptoms after giving birth. The prevalence was, in detail, 34.5% between one and twenty-four weeks after delivery and 30.8% after that point [31]. Based on anxiety prevalence and published data, we analyzed the material and found much research on laboratory animals subjected to anxiety that showed significant behavioral changes related to anxiety disorders. In earlier literature, psychological and physical stress, neonatal isolation stress, circadian rhythm stress, noisy stimulus stress, low-temperature stress, restraint and immobilization stress, social defeat stress, chronic unpredictable stress, and electric foot-shock stress were used to induce anxiety-like behavior in laboratory animals [32]. On the other hand we have the second approach in our study that is the selection of test drug for the treatment of anxiety and depression. The No. of treatment are available in the market but they do not give the satisfactory result with maximum side effect. Authors reported that synthetic drugs have a lot of side effects, by considering this current approach so we had selected the COE. An herbal formulation containing Protein, tannins, phenols, flavonoids, saponins, amino acids, glycosides, and steroids is a phytoconstituent found in COE. The author reported that COE changed a number of antioxidant parameters, showed potent anti-free radical activity, and might have a neuroprotective effect. Cassia obtusifolia L., belonging to Leguminosae family, is used as a diuretic, laxative, tonic, purgative, and natural remedy for headache, dizziness, constipation, tophobia, lacrimation, and eyesight. Its main chemical constituents include anthraquinone derivatives such as emodin, chrysophanol, physcion, obtusifolin, obtusin, au rantio-obtusin, chryso-obtusin, alaternin, questin, aloe-emodin, gluco-aurantio-obtusin, gluco-obtusifolin, naphthopyrone glycosides, and toralactone-9-β-gentiobioside. C. obtusifolia L. can treat Alzheimer's, Parkinson's, and cancer due to its antidiabetic, antibacterial, anti-inflammatory, hepatoprotective, and neuroprotective qualities [33]. As per the earlier research the cassia obtusifolia linn possesses the neuroprotective effect [34].

Based on this current evidence, we designed an experimental design to investigate the effect of COE on anxiety and depression like behavior in experimental animals. The study uses animal models to assess the effects of COE on anxiety and depression like conditions. The aim of the study is to plan several steps, including the selection and purchase of animals and drugs, as well as the assessment of behavioral parameters with various tests such as light and dark box, EPM, TST, FST, and anoxic stress tolerance. Here we observed and evaluated behavioral changes such as time spent in the light chamber, no entry into the light chamber, immobility time, and time spent in the open arm, no entry into the open arm. However a similar study was carried out by Girish C, Raj V, Arya J, Balakrishnan S. [4] and evidenced for the involvement of the monoaminergic system, but not the opioid system in the antidepressant-like activity of ellagic acid in mice. In this investigational protocol female fishes were used and studied out the anxiolytic and antidepressant effect of ellagic acid, and it was concluded that it possesses the anxiogenic activity. In this experiment, Swiss albino mice of either sex or approximately identical weight were employed. The post hoc analysis after 7 days by using the validated models such as LDB and EPM also shows the anxiogenic response.

In our experiment COE was given in a dose-dependent manner (100, 200 and 300 mg/kg) and individual animals were tested after the 30 min by oral administration for the assessment of anxiolytic and antidepressant activities by using validated models like LDB, EPM, FST, TST and Anoxic stress tolerance. Our results show that anxiogenic and antidepressant activity at low to higher doses of COE (100, 200 and 300 mg/kg) in experimental mice. Moreover, the drug senna allata at the dose of 200 mg/kg shows the anxiolytic and antidepressant activity reported by the author [35]. Interestingly COE at the median to higher dose (200 and 300 mg/kg) was attenuated anxiogenic and antidepressant effect by improving the time spent in light chamber and no. of entries in light chamber observed in LDB; however the time spent in open arm and no. of entries in open arm increased in EPM. And the duration of immobility was significantly reduced in TST observed after the post hoc analysis by 7 days. Similarly the duration of immobility was considerably reduced by COE post hoc analysis after 14 days in FST. However in anoxic tolerance test the no. of convulsion dramatically shows increases. More specifically a higher level of anxiolytic and antidepressant effect was achieved by COE that was evaluated by post hoc analysis after 7 days using LDB, EPM and TST or post hoc analysis after 14 days by using FST and anoxic tolerance test. Apart from this test drug COE at median to higher doses in our observation suggested the persistent increase in time spent and no. of entries in light chamber and open arm and reduction in immobility time in FST and TST confirming its more significant anxiolytic and antidepressant effect.

In earlier research Neurada procumbens Linn. was assessed by Khalid AA, et al. [36]. This study tested the dose-dependent effect (50-100 & 200 mg/Kg) of Neurada Procumbens, using models like LDB, EPM, TST and FST. In this experiment 200 mg/Kg of methanolic extract of Neurada Procumbens shows notable anxiolytic and antidepressant activity as compared to control group of animals by LDB, EPM, TST and FST but this study had limitations in which authors did not take the histopath for the confirmation of anxiolytic and antidepressant potential of test drug by considering the standard drug, etc. Similarly, the study performed and reported by H. S. Foyet [37] found Alafia multiflora Stapf (AM) stembarks at the higher dose exhibit anxiolytic and antidepressant effect at 300 mg/kg. Interestingly our study suggested a persistent anxiolytic and antidepressant impact which was achieved in LDB, EPM, TST, FST and anoxic tolerance test. In our experiment the earlier observation of reported study supports our outcomes for anxiogenic and antidepressant effect.

No similar study has been carried out for the evaluation of anxiolytic antidepressant activity of test drug COE by employing LDB, EPM, TST, FST and anoxic tolerance test. This study was done to confirm the test drug's anxiogenic, antidepressant, and antioxidant properties to support our hypothesis and attain another goal. As previously observed, oxidative parameters changed during anxiety and depression in experimental animals. We used the oxidative parameter to confirm anxiolytic and antidepressant efficacy. COE dramatically restored GSH and CAT levels while reversing LPO, a strong oxidant. This shows that the test drug reduced anxiolytic and antidepressant effects in animals and may increase antioxidant levels. Because of its anti-inflammatory qualities, COE has neuroprotective benefits by reducing the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase and increasing the expression of the neurotrophic proteins pCREB and BDNF [33].

More study is to be needed to be required for the confirmation of the precise mechanism of test drug as an anxiolytic and antidepressant potential and factors associated with anxiety and depression.

5. Conclusions

The present study concluded the anxiolytic and antidepressant effect of COE. COE ameliorated behavioral deficits in anxiety and depression like behavior. The result of the present study and main objective of this study were to critically evaluate the effect of test drug i.e. in the treatment of anxiety and depression like behavior in experimental animals. Anxiety and depression in mice were studied by using different parameters in animals by using validated models such as EPM, LDB, TST, FST and brain oxidative markers, antioxidants along with histopathological observation. From the results obtained in behavioral study, oxidative parameters and histopathological analysis, it was concluded that COE (200 & 300 mg/kg p.o.) possesses more significant activity in the experimental models of anxiety and depression. More studies need to be done to find out the mechanism along with factors and neurotransmitters involved for the anxiolytic effect of COE in experimental animals.

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