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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *TERMINALIA CATAPPA* LEAF EXTRACTS FOR ITS ANTIANXIETY ACTIVITY

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ABSTRACT

The present study reports physicochemical characterization, anti-microbial activity and antianxiety activity of extracts from *Terminalia catappa* leaves collected from local region of Pusad and Digras, Maharashtra, India. Powdered drug were evaluated for physical parameters like ash values, extractive value, Loss on drying and solubility etc. By using Acetone and methanol as solvents extracts were obtained from Soxhlet method for extraction and subjected for preliminary physicochemical evaluation and antioxidant studies. Analysis of phenolic and flavonoids content were done. Preliminary phyto-chemical analysis confirmed the presence of primary and secondary metabolites like carbohydrate, proteins, alkaloids, phenolic compounds, saponins. Elevated plus maze model was used for evaluating *in vivo* Antianxiety activity of *Terminalia catappa* leaf by using diazepam as standard in rats. Significant to highly significant number of entries with time spent in P zone were shown by both the extract at 200mg/kg conc^{n}. Due to might be presense of flavonoids Phenolic compound, steroid and proteins present in extract. *Terminalia catappa* leaf extracts possess Antianxiety activity from result it was cleared.

KEYWORDS

Terminalia catappa, Acetone and Methanolic extract, Phytochemical screening, Antioxidant effect and Antianxiety activity.

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INTRODUCTION[1]

Latin word “anxietas” meaning intense excessive persistence worry it can be elaborated by responses to the perception of danger in behavioral. Anxiety has capacity to stimulate adaptive response to challenging stressful events. If this condition of anxiety excesses it may destabilizes individuals. If it happens without reason it is considered as pathological. Most commonly neurotic disorders are

related to stress, response to it by individual. Stress as well as coping associated with socio cultural factors. The feeling of fear and anxiety is termed as anxiety. Anxiety=worry of future, fear= reaction to current event. This can cause increased heart rate and shakiness. Anxiety is a normal human emotion. Many anxiety disorders, like generalized anxiety disorder, specific fear disorders, excess introverts, fear of missing out, fear of places and fear of situations, panic disorder and inability to speak due to fear. According to symptoms disorder is characterized and differentiated. An individual may have more than one anxiety disorder. The cause of anxiety disorders is thought to be a combination of genetic and environmental factors. Risk factors are a history of child abuse, family history of mental disorders, and poverty. Other mental disorders, particularly major depressive disorder, personality disorder, and substance use disorder are also causes. Treatment may include lifestyle changes, counseling, and medications this may be included in treatment. Cognitive behavioral therapy is one of the most common counseling techniques used in treatment of anxiety disorders. 2 Medications, such as antidepressants, benzodiazepines, or beta blockers, may improve symptoms. About 12% of people are affected by an anxiety disorder in a given year, and between 5% and 30% are affected over a lifetime. They occur in females about twice as often as in males and generally begin before age 25. The most common are specific phobias, which affect nearly 12%, and social anxiety disorder, which affects 10%. Phobias largly affect people in the age range of 15 and 35, and reduce commonality after range age of 55 United States and Europe have high rates than in other parts of the world.

Reasons of anxiety

Drugs

Anxiety and depression can be caused by alcohol abuse is one of the reason for anxiety.

Medical conditions

Occasionally, an anxiety disorder may be a side-effect of an underlying endocrine disease that causes nervous system hyperactivity, such as pheochromocytoma or hyperthyroidism.

Stress

Anxiety disorders can arise in response to life stresses, such as financial worries, chronic physical illness, social interaction, ethnicity, and body image, particularly among young adults.

Genetics

GAD runs in families and is six times more common in the children of someone with the condition. While anxiety arose as an adaptation, in modern it is almost always thought of negatively in the context of anxiety disorders. Anatomy of brain Responsible for anxiety there are two parts of the brain that are thought to be key players in the production and processing of anxiety – the amygdala and the hippocampus. Amygdala can alert the rest of the brain that a threat is present and trigger a fear or anxiety response. The emotional memories stored in the central part of the amygdala may play a role in anxiety disorders involving very distinct fears, such as fears of dogs, spiders, or flying. The hippocampus is the part of the brain that encodes threatening events into memories". (National Institute of Mental Health). Mechanism of action neurotransmitters.

1) GABA
2) Serotonin
3) Noradrenaline
4) Dopamine GABA Low levels of GABA, a neurotransmitter that reduces activity in the central nervous system, contribute to anxiety. Anxiolytics act by modulating the GABA receptors.
5) Serotonin this neurotransmitter responsible for the mood change, happiness. When the level of serotonin decreases, then anxiety like disorders is produced.

Noradrenaline the level of these neurotransmitter increases the symptoms of anxiety produced, like increase Heart rate

METHODOLOGY

Collection, identification and authentication of plant material[2]

The fresh leaves of *Terminalia Catappa* were collected from the nature of its habitat from pusad, Maharashtra in India. Authentication of plant

Terminalia Catappa was done, specimen No. OWNO./AMP/381/2021 is allotted to the plant. The identify of plant was authenticated from Department of Botany, Ayurvedic Mahavidyalaya, Nashik University.

Extraction and phytochemical evaluation[3]

The authenticated leaves were dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 30 mesh sieve and extracted with ethanol in soxhlet apparatus at 60^{o}C. The solvent was completely evaporated in the hot air oven. The presences of phytochemical constituents in the ethanolic extract of leaves *Terminalia catappa L*. was tested using the standard methods. Therefore, these methods revealed the presence of flavonoids.

Experimental animals[4]

Wistar rats of either sex weighing 150 to 200g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of S.N.I.O. Pharmacy, which is approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) Protocol. Animals were kept under 12 h light/dark cycles and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water ad libitum. At least one week before the commencement of experiment all animals were acclimatized to the laboratory environment. According to the norms of Institutional Animal Ethics Committee the experimental protocol for the study was followed.

Phytochemical analysis[5]

The extracts obtained by successive extraction were subjected to quantitative tests for the identification of various secondary metabolites such as carbohydrates, proteins, tannins, saponins, steroids, flavonoids and glycosides. Phytochemical examinations were carried out for all the extracts as per standard methods

The extracts showed presence of alkaloid, glycosides, tannins, carbohydrates, flavonoids, and saponins. The spots at Rf values of methanolic extract is 0.008, 0.24, 0.39 represents the presence of quercitin, kaemferol, Flavone in the extracts

Detection of alkaloids[6]

In dilute HCl extracts were dissolved individually and filtered.

Dragendorff's test

Add few drops of Dragendorff's reagent to 2-3ml Filtrate, formation of orange brown Ppt. shows the presence of alkaloids.

Hager's test

Hager's reagent to 2-3ml Filtrate yellow precipitate forms indicates the presence of alkaloids.

Tannic acid test

Tannic acid solution treated with test solution gives buff colored precipitate indicates the presence of alkaloids

Detection of proteins and amino acid[6]

Million's test

Mix 3 ml test solution with 5ml Million's reagent. White precipitate warm precipitate turns brick red precipitate dissolves giving red colored solution indicates the presence of proteins.

Ninhydrin test

To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Amino acid's presence confirmed by formation of blue color.

Biuret test

To 3ml test solution adds 4% NaOH and few drops of 1% Copper sulphate solution. Violet color appears.

Detection of carbohydrates[7]

Extracts were dissolved individually in 5 ml of distilled water and filtered. The filtrates were used to test for the presence of carbohydrates

Molish's test

2 drops of alcoholic α - naphthol solution+ filtrates in a test tube. Violet ring at the junction confirms the presence of carbohydrates.

Barfoed's test

Barfoed's reagents and test solution mix in equal volume. Heat for 1-2 min in boiling water bath and cool Red precipitate is observed

Benedict's test

Filtrates treated with Benedict's reagent and heated gently. Orange red precipitate confirms reducing sugars is present.

Fehling's test

Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Red precipitate formation indicates reducing sugars is present.

Detections of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides[8].

Modified borntrager's test

Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of Chapter 5 Materials and Methods Dept. of Pharmacology, S.N.I.O.P Pusad 33 benzene. After separating benzene layer was treated with ammonia solution. Formation of rose pink color in the ammonical layer indicates the presence of anthranolglycosides

Detection of tannins

To 2-3ml of aqueous or alcoholic extract, add few drops[9].

5% Ferric chloride test: Deep blue - black color

Lade acetate sol. Test: White precipitate

Detection of flavonoids[10]

Lead acetate test

Few drops of lead acetate solution was subjected for treatment with extract. If yellow color precipitate formed it indicates flavonoids presence.

Shinoda test

To dry powder or extract add 5ml 95% ethanol few drops conc. HCL and .0.5gm. Magnesium turnings. Orange, pink, red to purple color appears. Add t-butyl alcohol before adding the acid to avoid accidents from a violent reaction and magnesium, only flavones give a deep red to magenta color while flavones and flavones observation is it give weak pink to magnetic color

Detection of phytosterols[11,12]

Salkowski's test

Extracts were allowed for treatment with chloroform and then filtered again filtrates subjected to treatment with few drops of Conc. Sulphuric acid which is shaken and allowed to stand. golden yellow color appearance confirms the presence of triterpenes.

Liebermann burchard test

After chloroform treatment extracts were filtered. Few drops of acetic anhydride allowed to treatment with filtrate then boiled and cooled in this add Conc. Sulphuric acid. If brown ring at the junction forms it shows the presence of phytosterols.

Detection of saponin

Foam test

Drug extract or dry powder vigorously shaken with water. Persistent foam observed indicates the presence of saponin

ACUTE ORAL TOXICITY (AOT)[13,14]

The method followed to perform the AOT was the acute toxic class method, i.e., OECD-423. Three doses were chosen from the Annex-II of OECD 423, i.e., medium, minimum, maximum, i.e, 200mg/kg, 100mg/kg and 2000mg/kg after sighting study. A total of 6 animals were chosen, i.e., 2 animals per group and three group were taken, namely, *Terminalia catappa* l extract- 200mg/kg, 100mg/kg and 2000mg. A single dose was administered, and animals were observed for a period of 14 days for clinical signs and mortality.

Selection of dose[14,15]

The result of such studies showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD50) could be greater than 2000mg/kg body weight in mice. Likewise ≤ 200mg/kg safe experimental dose was calculated and accordingly further screening of extracts were done for

Drugs

Drugs extract

Diazepam

Distilled water

Screening for anti-anxiety activity[16, 17]

Elevated plus maze

Animal grouping

Group A - Control: Distilled Water

Group B- Standard: Diazepam (1mg/kg)

Group C - Test Dose-I: TCAE (100mg/kg)

Group D- Test Dose-II: TCAE (200mg/kg)

Group E- Test Dose I: TCME (100mg/kg)

Group F- Test Dose II: TCME (200mg/kg)

Procedure

The elevated plus maze consist of two open arms, 35X15X15cm and two enclosed arms, 35X15X15cm, that extend from a common central platform; with an open arm roof, arrangement is done in a way that open arms (two in number) are opposite to each other. Above the ground level at height of 50cm the entire maze was elevated. The rats were housed in group of six in cages prior to testing in apparatus. During this time the rats were handled by investigator on alternate days to reduce stress. The animals were divided in six groups. Four groups test drug (p.o.) & standard drug (i.p.) were administered 1 hour before testing. After 1 hr rat was placed in centre of maze, facing one of enclosed arms. During a period of 5 min the following parameters were observed by using video tracking system; Number of entries in open arm and enclosed arms, time spent in open arm, enclosed arm and centre, total number of arm entries

Light-dark method[18-20]

Animal grouping

Group A - Control: DMSO 0.1ml
Group B- Standard: Diazepam (1mg/kg)
Group C - Test Dose-I: TCAE (100 mg/kg)
Group D- Test Dose-II: TCAE (200 mg/kg)
Group E- Test Dose -III: TCME (100mg/kg)
Group F- Test Dose –IV: TCME (200mg/kg)

Procedure

Two compartment chamber (47X27X27cm) testing apparatus comprising of two-third brightly illuminated area and one-third dark area separated by a wall with a round hole (13cm long X 5cm high). A partition containing a opening separate the dark one third from the bright two third of the cage. The animals were treated with test drug (p.o.) and standard drug (i.p.) half hour prior testing. The rats were placed individually in the illuminated part of the cage and the electronic video tracking system was used to automatically count movements through the partitions and clocked the time spent in light and dark compartments

Statistical analysis

All values are expressed as mean ±SD. Statistical significance was determined using one way ANOVA, followed by Dunnet's test[21].

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemical analysis of extracts showed presence of alkaloid, glycosides, tannins, carbohydrates, flavonoids, and saponins.

AOT study

Acute toxicity studies (OECD 423: Acute Oral Toxicity-Class Method) of extracts of *Terminalia catappa* leaf conducted by researchers revealed that the graded doses administration of extracts (up to a dose of 2000mg/kg) did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and in appearance of the animals. No death was recorded up to the dose of 2000mg/kg body weight. The result of such studies showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD50) could be greater than 2000mg/kg body weight in mice. Likewise ≤ 200mg/kg safe experimental dose was calculated and accordingly further screening of extracts were done for

Mortality

The extracts were found to be safe of a dose of 2000mg/kg since mortality mortality was not observed.

Elevated plus maze

Evaluation of Anti-anxiety activity was done by observing the parameters like number of entries in open and enclosed arm, time spent by the rats in open and enclosed arms and comparing these parameters with that of control group. The anxiolytic agents increase the motor activity there by open arm exploratory time.

Light-dark method

The parameters like time spent in light compartment, time spent in dark compartment, number of crossings between these two compartments and transfer latency of rats were evaluated. Anxiolytic agents increase the total loco motor activity.

Discussion

The scientists are sincerely trying to evaluate from the last two decades of the century, many plant drugs which are used in system of medicine followed traditionally. Different parts of this plant have been reported to possess anti-inflammatory, antidiabetic, anti-oxidant, hypolipidaemic, antiobesity and antimicrobial activity. The leaves are cooling, emollient, anti-pyretic, hypoglycemic, diuretic, Laxative, digestible, anthelminthic, urinary concretions, sore throats, pain in the joints, flatulence throat. Traditionally it has been reported that, *Terminalia catappa* leaf may exhibit anti-inflammatory potential so it was selected for evaluation of Anti-anxiety studies. Preliminary phytochemical evaluation of Acetone & methanolic extracts was carried out for the determination of presence of phytoconstituents along with TLC fingerprinting. Both extracts showed presence of alkaloid, glycosides, tannins, carbohydrates, flavonoids, and saponins. The spots at Rf values (Acetone extract) 0.07, 0.24, 0.10, 0.32 and (methanolic extract) 0.008, 0.24, 0.39 represents the presence of quercitin, kaemferol, Flavone in the extracts. Acute toxicity studies (OECD 423: Acute Oral Toxicity-Class Method) of extracts of *Terminalia catappa* leaf conducted by researchers revealed that the graded doses administration of extracts (up to a dose of 2000mg/kg) did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and in appearance of the animals. No death was recorded up to the dose of 2000mg/kg body weight. The result of such studies showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD50) could be greater than 2000mg/kg body weight in mice. Likewise ≤ 200mg/kg safe experimental dose was calculated and accordingly further screening of extracts were done for *In-vivo* memory enhancing activity of *Terminalia catappa* leaf extracts was evaluated by using the Elevated plus maze and light and dark model by using Wistar rats as an animal model. The Anti-anxiety activity of acetone and methanolic extracts of *Terminalia catappa* leaf was evaluated in rats by daily exposing them to the Elevated plus maze and light and dark model with the food pellet in a fix arm of maze. Food pellets were placed in a variable arm for evaluation of working Anti-anxiety. It is characterized by increase in latency to find the food and time spent in selected arm. The results were drawn by evaluating time spent and number of entries in P zone. The results showed that the highest dose (200mg/kg) of both the extracts, showed highly significant Anti-anxiety activity when given orally in daily single dose. The findings suggest effect of two different doses of both the extracts (100mg/kg and 200mg/kg) were probably mediated through ability of the animals to cause a significant decrease in number of errors and increase in latency to find the food and time spent in selected zone as well. At the end of the study it was observed that group no. 6 i.e. aqueous extract treated group at dose of 200mg/kg showed maximum number of entries at P zone (37.4±0.42**#). The same group showed maximum time spent at P zone (190.2±12.4#) as well. Whereas the other groups i.e. group no.5 (acetone at dose of 100mg/kg) group no.4 (methanolic at dose of 200mg/kg) and group no.3 (mehtanolic at dose of 100mg/kg) represents 183±5.74*#, 177.2±9.77**#, 172.4±0.91**# values respectively for time spent in P zone. At P zone the number of entries of these groups were found to be 36.8±0.30**#, 36.8±0.30**#, 35.8 ±0.65**# respectively. Comparison was done with standard drug of all these values i.e. Diazepam at dose of 200mg/kg. From the results it was revealed that both extract i.e. acetone and methanolic showed effective Anti-anxiety activity. Although methanolic extract at 200mg/kg showed more superior and significant to highly significant (from $P < 0.05$ to $P < 0.001$) Anti-anxiety activity by using Elevated plus maze and light and dark model in rats.

Elevated plus maze

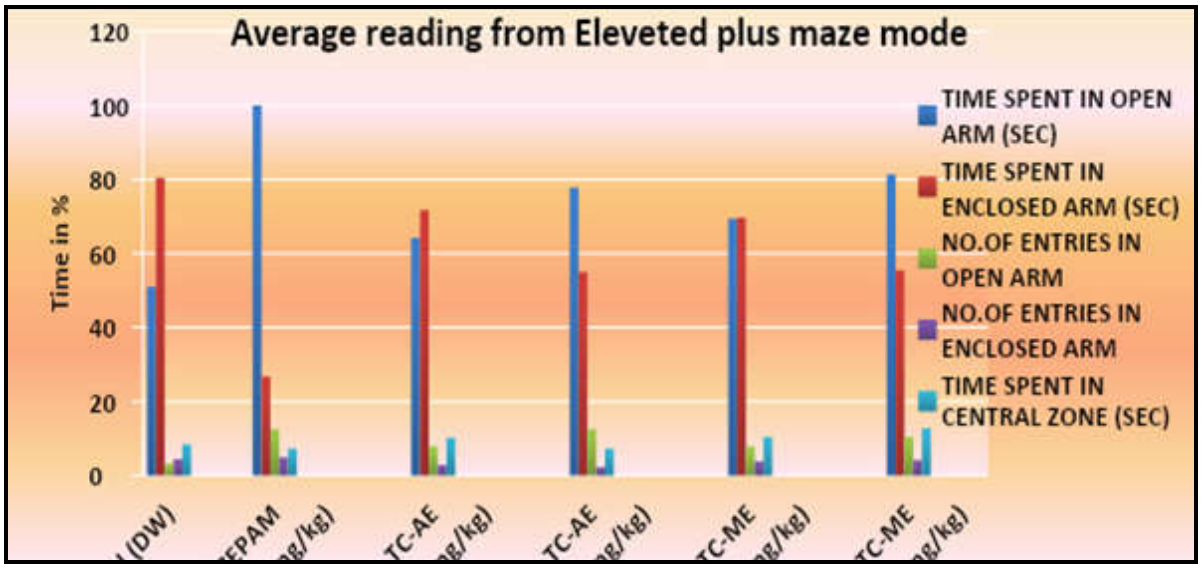
Table No.1

S.No	Treatment	Time spent in open arm (SEC)	Time spent in enclosed arm (SEC)	No.of entries in open ARM	No.of entries in enclosed ARM	Time spent in central zone (SEC)
1	Vehicle (DW)	104.8 ± 3.12	164.1 ± 5.1	6.6 ± 1.6	9 ± 3.1	17.1 ± 1.9
2	TC-AE (100mg/kg)	131 ± 7.8	146.3 ± 6.9	16.1 ± 3.9	5.8 ± 0.7	14.8 ± 1.4
3	TC-AE (200mg/kg)	158.5 ± 4.3	112.1 ± 6.0	25.6 ± 2.6	4.6 ± 1.03	20.8 ± 2.1
4	TC-ME (100mg/kg)	141.5 ± 3.3	142.1 ± 6.0	16 ± 3.6	7.8 ±1.16	14.83 ± 1.6
5	TC-ME (200mg/kg)	166 ± 8.7	113 ± 7.5	21.3 ± 1.8	8.5 ± 1.04	21.16 ± 2.7
6	DIAZEPAM (1mg/kg)	204 ± 4.5	54.6 ± 7.5	25.5 ± 4.6	10 ± 0.89	26 ± 3.6

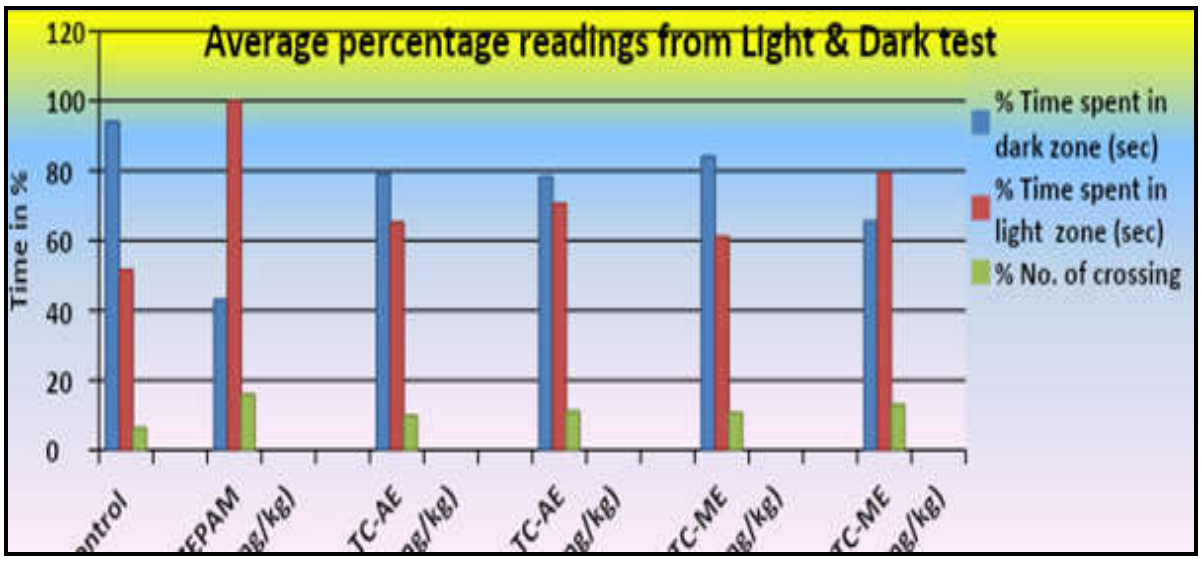
Light and Dark test

Table No.2

S.No	Treatment	No. of crossings	Time spent in dark zone (sec)	Time spent in light zone (sec)	Transfer latency
1	Vehicle (DW)	13 ± 2.2	191.6 ± 4.17	104.8 ± 4.2	22.16 ± 2
2	TC-AE (100mg/kg)	20 ± 1.4	160.3 ± 5.5	132.5 ± 4	22.5 ± 1.8
3	TC-AE (200mg/kg)	22.5 ± 2.1	159.5 ± 4.8	143.16 ± 4	24.16 ± 2.3
4	TC-ME (100mg/kg)	21.8 ± 1.4	170.6 ± 3.3	124 ± 3	20 ± 1.4
5	TC-ME (200mg/kg)	26.5 ± 1.3	133.1 ± 5.5	161.5 ± 3.2	25 ± 1.4
6	DIAZEPAM (1mg/kg)	32.33 ± 2.8	87.5 ± 5.6	203.1 ± 3.6	26.5 ± 1.5



Graph No.1



Graph No.2

CONCLUSION

Terminalia catappa leaf contain several chemical constituents which are pharmacologically important as they have been proved to be beneficial in many specific diseases like cancer, inflammation, infectious, cardiopathy, diabetes, hepatotoxicity and many microbial attacks where its memory enhancing potential is claimed to be useful. The extracts of *Terminalia catappa* leaf tested for antianxiety activity by researchers. No methodical reports on antianxiety activity of *Terminalia catappa* leaf were available. The present study aimed at evaluating the *In-vivo* Antianxiety of *Terminalia catappa* leaf extract in rats. Acetone and methanolic extracts were prepared by the hot extraction process, i.e. by using Soxhlet apparatus. Preliminary phytochemical evaluation of Acetone and methanolic extract was carried out for the determination of presence of phytoconstituents. The result of acute oral toxicity studies of plant extracts as per standard references revealed that in single dose the plant extracts had no adverse effect, indicating that the medium lethal dose (LD50) could be greater than 2000mg/kg body weight in rat. Likewise ≤ 200mg/kg safe experimental dose was calculated and accordingly further screening of extracts were done for In- Vivo study has showed that acetone and methanolic extracts of *Terminalia catappa* does possess significant Antianxiety activity with 100mg/kg and 200mg/kg, but high doses of the acetone extract 200mg/kg being more superior and showed significant to highly significant percentage inhibition (from $P < 0.05$ to $P < 0.001$) when compared with standard Diazepam.

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CONFLICT OF INTEREST

We declare that we don't have any conflict of interest.

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