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EVALUATION OF ANXIOLYTIC ACTIVITY OF AQUEOUS ROOT EXTRACT OF ACACIA NILOTICA IN RATS

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ABSTRACT

Acacia nilotica scientifically known as *Vachellia nilotica* (Fabaceae family, subfamily Mimosoideae), has long been utilised to treat a variety of conditions and shows different activities such as Anti-hypertensive, antipyretics, anti-diabetic property, antioxidant, analgesic etc. This study investigates the anxiolytic effects of *A. nilotica* root extract in albino rat. Two polar solvents were utilised for extracting the plant's root. i.e., ethanol and distilled water. Five groups (n=6) were administered either saline, diazepam or *AN* roots extract administered at 200 & 400 milligram/kg concentrations orally for 28 days. The Light-dark box and Elevated Plus Maze (EPM) were used for determining anxiety levels. The extract significantly reduced anxiety-like behaviors, with treated rats spending more time in the opened arms & light chamber of the plus maze and light box. The results of the higher dose (400 mg/kg) were similar to those of diazepam, indicating that Acacia nilotica roots could potentially hold a role in the natural therapy of anxiety disorders.

Keywords: *Acacia Nilotica*, anti-anxiety, elevation plus maze, light-dark box test.

ANXIETY INTRODUCTION

Anxiety is among the most widespread mental health issues. It affects 1/8th of the world's population and has currently become a highly significant topic of research in psychopharmacology over more than a decade[1]. But still, fewer than 30% of those diagnosed with anxiety symptoms strive for treatment [2]. Although anxiety is a typical human feeling and a natural reaction to threat or danger, it can grow into a mental health issue if it is severe enough, lasts over three weeks, or causes disruption with day-to-day functions [3]. It is now generally acknowledged that, because of their questionable efficacy and potential

for tolerance and drug dependency, anxiolytic medicines should not be taken for prolonged periods of time for the treatment of anxiety disorders [4]. Plants have long been used to treat problems of the CNS [5].

The multipurpose *Acacia nilotica* (Linn.) Willd. Ex Del., also called as the Indian gum Arabic tree, kikar, babool or *Vachellia nilotica* in botanical terms (Fabaceae family, subfamily Mimosoideae), is widely recognised worldwide[6]. Substantial numbers of *Acacia nilotica* are readily present in Saudi Arabia, Sudan, India and Egypt[7]. The *Acacia nilotica* tree has a high content of volatile and essential oils, terpenes, resins, saponins, triterpenoid, flavonoids, alkaloids, phenols, tannins, and oleosins.[8]. In past research, it was determined that the methanol extract of Acacia nilotica root has CNS depressant activity, so it was beneficial in lowering anxiety symptoms by relaxing the central nervous system[9]. Another investigation of the stem of Acacia arabica showed that it contains phytoconstituents that are comparable to those found in the root and offers anti-anxiety properties [10].

MATERIALS & METHODS

Plant Material

The *Acacia nilotica* Linn. root was gathered from the Raja Talab region in Varanasi, Uttar Pradesh, India. The specimen was identified and validated by Mr. Vinay Ranjan (Head of Office and Scientist-E), the BSI Central Regional Centre, Prayagraj 212002.

Drying and size reduction of *Acacia nilotica* root

Acacia nilotica root material was shade-dried for approximately four weeks. After that, the dried root was further ground into a powder, which was thereafter passed via a 40-mesh sieve and stored for subsequent research in an airtight container.

Drugs

Carboxymethylcellulose (CMC) and saline were chosen as the vehicles and also used to prepare the suspension of extract test doses. Standard drug (diazepam) Piramal Healthcare Ltd. was obtained from Utthan Shambhunath Hospital Pharmacy, it received treatment at a doses of (2 milligram/kg). Meta-Chlorophenylpiperazine will be used as anxiety inducing agent. Prior to usage, it was diluted using saline to achieve the desired strength. All of the medications were prepared just before use and given orally.

Preparation of Acacia Nilotica Aqueous Extract

Crude *AN* roots was allowed to dry in the shade air for four weeks. Afterwards, it was crushed utilising a grinder to a fine powder. The powder was stored in a dry, cool environment inside an airtight container. The cold maceration method was used to perform aqueous extraction. 400 grams of powdered *A. nilotica* roots were soaked in one litre of water, and the mixture were stirred occasionally for 48 hours. Following that, muslin cloth and Whatmann filter paper (Number 1) filtered the suspension. Then the extract was then put on a rotary evaporator to separate solvent and concentrate the extract. The filtrate was freeze-dried in a freeze dryer. To prepare the appropriate doses for the experiment, a fractional quantity of the crude extract was weighed and diluted in distilled water[9,11].

Phytochemical Screening

The crude extract was put through phytochemical screening using the techniques outlined by Evans (2005) to identify the secondary metabolites, including glycoside, anthraquinones, alkaloids, phenolics, tannins, saponins, flavonoids and terpenes.

Animals used in the experiment:

Wistar albino rats (weighing around 150-180 grams) of both sexes were acquired from Ms. Chakraborty Enterprises in Kolkata, West Bengal, India, and housed within the Animal house, Department of Pharmacology, Shambhunath Institute of Pharmacy in Prayagraj. They were kept at a regulated room temp. of 26 ± 4 °C with a 12-hrs illuminating and 12 hrs non-illuminating cycle. They also received unrestricted water supply and eatable pellets. The study was conducted from 09:00 o'clock morning to 16:00 o'clock evening. The whole protocol of the experiment was verified by I.A.E.C. (Approval No. SIP/IAEC/003/03/24), dated March 18, 2024, and the animals were cared for in compliance with C.C.S.E.A. guidelines.

Acute toxicity Study (ATS)

ATS of roots of *A. nilotica* in rats was to be assessed in this study. Four groups of twelve healthy, non-pregnant, nulliparous female mice, ages 8 to 12 weeks, were selected. The rats weren't allowed food for the four hours prior to the dosage. Group 1 got 5 milligram/kg of b.w. of *A. nilotica* extract, whereas group 2^{nd} 3^{rd} & 4^{th} received oral doses of 50, 300, & 2000 milligram/kg b.w., respectively. Every day, the mortality rate was recorded, and mice were examined for indications of toxicity. The number of deaths was compared with predefined LD_{50} cut-off values in order to estimate the root extract's LD_{50}[11–14].

Grouping and dosing of animals for screening of anxiety

A sum of thirty wistar albino rats in total were split up into five distinct groups comprising six rats each type:

Group I: Vehicle treated group (0.5 % CMC, 1ml/kg body weight)

Group II: Disease control (mCPP, 0.5 milligram/kg

Group III: standard (Diazepam, 2mg/kg)

Group IV: Test (Aq. Extract of *Acacia nilotica* root 200mg/kg body weight)

Group V: Test (Aq. Extract of *Acacia nilotica* root 400mg/kg body weight)

Model of Elevation + maze

The Elevation + maze apparatus consists of 2 arms opened (50.0 centimetres x 10.0 centimetres) and two arms closed (50.0 centimetres x 10.0 centimetres x 40.0 centimetres), with an opened roof. A central square, measuring 10 x 10 cm, was attached to the arm. In a low light room, the equipment was kept at a ht. of 50-60 cm above the ground by an individual support. The open arms have a 0.25-cm elevated border that gives the animals more hold. The trial took place from 0900 hrs to 1700 hrs. Every rat was positioned individually in the exact middle of the raised model, confronting the open arm. The test continued for the normal 5 minutes, and the maze was completely cleaned in between each subject. At least 45 minutes before the experiment, oral herbal extracts of Acacia nilotica root were given. The elevated plus-maze instruments were being used by each rat in turn due to the dose administration regimen being altered accordingly. Every safety measure was carried out to make sure that the animals wouldn't become anxious from any external stimuli except from the plus-maze's height[15–19].

Light dark box test

Two 20.0 centimetres × 10.0 centimetres × 14.0 centimetres boxes of plastic, transparent and dark, made up the equipment. A door that was left open between the two boxes allowed the mice to travel between them. The only source of light in the chamber was a 100W bulb that was positioned 30 centimetres from the floor of the glass box. The illuminating box with the hole facing was filled with a mouse. Shortly after the mouse entered the dark box, a 5-minute recording of the light box's movements and the mouse's time inside it was made. In between experiments, the equipment was carefully cleaned. The level of duration in each box and alongwith the total no. of incidences in each box will be recorded[5].

Statistics analysis

The data were analysed using GraphPad Prism version 10 and deemed as average ± standard error of mean. Using one-way analysis of variance (A.N.O.V.A.) and the mean differences were examined. P less than 00.05 is accepted as noteworthy.

RESULT

Phytochemical's screening of plant product

The yield of aqueous and ethanolic extract was showed in **Table 1** whereas **Table 2** provides a summary of the phytochemical contents found in the aq. extract of *Acacia nilotica*. The phytochemicals present in the extract include phenolics, alkaloids, tannins, flavonoids, terpenes, and sterols.

Table 01: Extractive yield of various extracts of *A. nilotica* root

Extract	Yield (%w/w)
Water	22 %
Ethanol	16 %

Table 2: Phytoconstituents of root of AEAN.

S.N.	Test Performed	Aqueous Extract	Ethanolic extract
1.	**Alkoloid**	+	+
2.	**Saponin**	+	-
3.	**Tannin & Phenolic compounds**	++	+
4.	**Flavanoids**	+	+
5.	**Glycoside**	+	-
6.	**Protein & Amino acid**	+	-
7.	**Carbohydrate**	+	-
8.	**Terpenoids**	+	+

+ + = moderately present, + = slightly present

Acute-toxicity study

The present research confirmed the non-toxic behaviour of an aqueous root extract that is given orally to animals at a maximum amt. of 2,000 milligrams/kg. Using the OECD Guideline 423 (2001), the oral median-LD_{50} of AEAN in animals was calculated to be 5,000 milligrams/kg.

Anti-anxiety study

Elevation plus maze test: As compared to the saline-treated group, the administration of Diazepam (2 milligram/kg) notably enhanced proportion of indices in open arm and the amount of duration in the same arm ($P < 0.0010$) was mentioned in **Table 3** & **Figure 1**. *A. nilotica* aqueous extract at 200 milligram/kg ($P<00.01$) and 400 milligram/kg ($P < 00.05$) greatly prolonged the duration in the stretched arm also a substantial enlargement in incidence in the open arms at 200 milligram/kg. ($P < 0.050$) and 400 milligram/kg ($P < 0.010$) was seen.

Light & dark box test: In contrast to the saline-treated group, Diazepaam (02 milligram/kg.) visually grow the amt. of time in illuminating compartment ($P < 0.001$) [Table 03]. With administration, there was a noteworthy hike in amt. of time remained in the compartment with light ($P < 0.050$). Anxiolytic effects of *A. nilotica* root aqueous extract at 200 and 400 milligram/kg were compared to a group that received saline treatment and the control groups indicated in **Table 4** & **Figure 2**.

Table 3: Anxiolytic activity of rat in Ele. plus maze test

Group	Treatments	Doses (mg/kg)	Entries in open arm	Duration in open arm (sec)
1	Vehicle (0.5% CMC)	1ml/kg	8.5 ± 1.04	92 ± 4.89
2	mCPP	0.5mg/kg	5.5 ± 1.04	72.5 ± 4.13
3	Diazepam	2mg/kg	15.8 ± 1.60*	158.1 ± 4.26*
4	Aq. Extract of A. Nilotica	200mg/kg	11.8 ± 1.47	137.6 ± 8.09
5	Aq. extract of A. Nilotica	400mg/kg	13.8 ± 1.47*	151.6 ± 5.42*

Values noted above are in avg. ± SD, n=6.
Values of P: <00.010; <00.050 as differentiated to disease vehicle group.

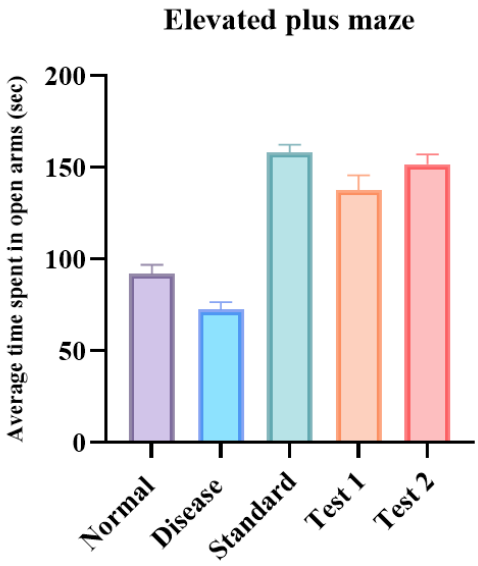

Fig. 1: Results of *A. nilotica* root extract in ele. plus maze. All values are in mean ± SD, n=6.

Table 4: Anti-anxiety activity of rat in Light and dark box test

Group	Treatments	Doses (mg/kg)	Average time in light box (seconds)	No. of transition
1	Vehicle (0.5% CMC)	1ml/kg	111.3 ± 6.18	6.1 ± 1.16
2	mCPP	0.5mg/kg	52.8 ± 6.58	3.5 ± 0.54
3	Diazepam	2mg/kg	174.3 ± 6.15*	10.1 ± 1.16*
4	Aq. Extract of A. Nilotica	200mg/kg	139.8 ± 5.16	7.3 ± 1.21
5	Aq. extract of A. Nilotica	400mg/kg	159.6 ± 4.71*	9.5 ± 1.37*

These values are in mean ± SD, no.= 6. P values: <00.001; <00.05 as compared to disease vehicle group.

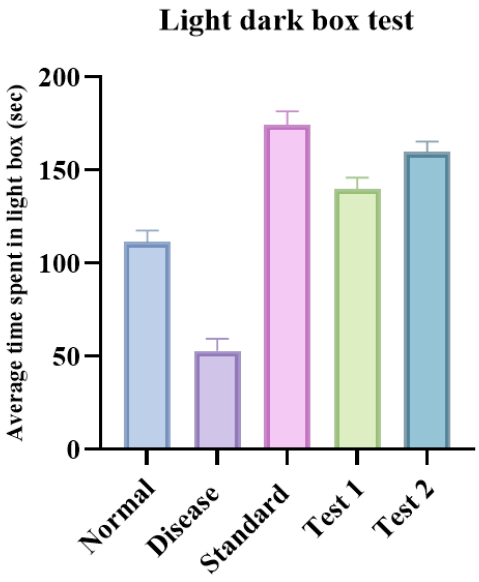

Fig 2: Effect of *A. nilotica* root extract on light & dark box. All values are in mean ± SD, n=6.

DISCUSSION

The discovery of new drugs has benefited greatly from the study of plants. Medicinal plants are the source of most medications used in modern medicine. Since benzodiazepines have been widely utilized over the past 50 years to treat a variety of anxiety disorders, other therapeutic approaches are being considered because of their undesirable side effects. One useful resource for discovering novel treatments for certain conditions is medicinal plants. *A. nilotica* root's anti-anxiety properties were assessed using the EPM & LDB model, which is frequently employed models. These models were selected because it is simple, effective, and requires no time to train. It also doesn't give the animal any distress when being handled. The primary conclusion that forms the basis of the model is that animals who are placed in an Elevated & open Maze experience an approached -avoidance conflict that appears as an exploratory-c-fear drive. Placing animals on the raised plus-maze causes anxiety in them because of their acrophobia, or fear of heights. The signs of dread and anxiety in animals is a reduction in motor activity, which can be assessed by how long the animal stays in the open arms. On the E.P.M. and light-dark box test, the effects of 200 and 400 milligram/kg of *A. nilotica* aq. extract were nearly identical to those of 2 mg/kg of diazepam. The minimum mean duration that animals in the control group spend in the open arms of an EPM indicates that they are completely exhibiting signs of anxiety. The *Acacia nilotica* root, at a amount of 400 milligram/kg., enhanced the arrivals in the light chamber and reduced the durations of entries in the dark chamber. In the same way, diazepam raised the duration and arrivals in the light chamber, indicating that the Light-Dark model exhibited anxiolytic effects.

This indicate that the aqueous extract with the maximum anxiolytic action among the examined extracts was 400 mg/kg. This dose was comparable to that of diazepam, as demonstrated by the statistical equivalency between the extract's effects and the standard drug.

CONCLUSION

The root of *A. nilotica* has an anti-anxiety aqueous extract. The model of controlled studies involving laboratory animals has yielded carefully examined findings. A. nilotica's biologically active compounds can be used to treat anxiety, as demonstrated by the findings' statistical validity, which also explains the plant's clinical significance.

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