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ANXIOLYTIC EFFECT OF MITRAGYNA INERMIS LEAVES EXTRACT IN MICE USING OPEN FIELD TEST

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ABSTRACT

Background: Anxiety is an emotion characterized by feelings of tension, worried thoughts, and physical changes like increased blood pressure. It' is considered a future-oriented, long-acting response that is broadly focused on a diffuse threat. Anxiety is distinct from fear, which is a present- oriented and short-lived response to a specific threat. **Aim:** This study aimed to evaluate the anxiolytic Effect of Mitragyne inermis Leaves. **Method:** The study analyzed the phytochemical content, toxicity, and anxiolytic effects of an aqueous extract. Alkaloids, flavonoids, tannins, and steroids were detected, known for their analgesic properties. **Results:** Acute toxicity tests showed an LD50 of 3808 mg/kg, indicating safety. A nxiolytic tests on mice revealed significant effects at 500 mg/kg and 500 mg/kg, with 1000 mg/kg performing comparably to Diazepam. **Conclusion:** These findings suggest that Mitragyne inermis leaves extract may serve as a natural alternative to conventional anxiolytic drugs, offering a promising option for anxiety management with a fewer side effect.

KEYWORD:- Anxiety, Anxiolytic effect, *Mitragyne Inermis*, Open Field Test.

INTRODUCTION

Mitragyne inermis, commonly known as African Mistletoe, is a plant native to Africa particularly found in countries such as Nigeria, Ghana, and Cameroon. It belongs to the Loranthaceae family and has been traditionally used for various medicinal purposes by indigenous communities in Africa. While not as extensively studied as other plants in the Mitragyna genus, such as Mitragyna speciosa (Commonly known as kratom). Mitragyne inermis has shown promising medicinal effects, particularly in the realms of traditional medicine. Studies have indicated that extracts from Mitragyne inermis possess antimicrobial properties. Making it potentially useful in combating various bacterial and fungal infections. Research conducted by Ajaiyeoba et al. (2008)[1] demonstrated the plant's efficacy against a range of microbes, including Staphylococcus aureus, Escherichia coli, and Candida albicans. These findings suggest its potential application in treating infectious diseases. Mitragyne inermis exhibits significant antioxidant activity, as evidenced by several studies. Antioxidants play a crucial role in neutralizing harmful free radicals in the body, thus reducing oxidative stress and lowering the risk of various chronic diseases such as cardiovascular disorders, cancer, and neurodegenerative conditions. A study by Akinpelu et al. (2011)[2] evaluated the antioxidant properties of Mitragyne inermis extracts and found them to be promising. Indicating its potential as a natural antioxidant source. Traditional usage of Mitragyne inermis suggests its efficacy in alleviating inflammation-related conditions. While more research is needed to elucidate the underlying mechanisms, preliminary studies have shown promising anti-inflammatory effects. Inflammation is implicated in various diseases, including arthritis, inflammatory bowel disease, and asthma. *Mitragyne inermis* may offer a natural alternative for managing inflammation and associated symptoms. Recent research has hinted at the antidiabetic potential of *Mitragyne inermis.* A study by Omodamiro et al. (2019)[3] investigated the hypoglycemic effects of the plant's leaf extracts in diabetic rats and observed

significant reductions in blood glucose levels. These findings suggest that *Mitragyne inermis* may hold promise as a complementary therapy for managing diabetes, although more extensive clinical studies are warranted to corroborate these results. The hepatoprotective properties *of Mitragyne inermis* have also been explored in scientific literature. Hepatoprotection refers to the ability to protect the liver from damage caused by toxins, drugs, or diseases. Research conducted by Akinmoladun et al. (2007)[4] demonstrated the hepatoprotective effects of Mitragyne inermis extracts against acetaminophen-induced liver injury in rats. These findings suggest a potential role for the plant in preserving liver health.

Anxiety is an emotion characterized by feelings of tension, worried thoughts, and physical changes like increased blood pressure. It' is considered a future-oriented, long-acting response that is broadly focused on a diffuse threat. Anxiety is distinct from fear, which is a present- oriented and short-lived response to a specific threat. Anxiety disorders, on the other hand, involve intense and excessive fear and worry that can be difficult to control, causing significant distress and interfering with daily activities. These disorders can manifest in various forms such as generalized anxiety disorder, panic disorder, social anxiety disorder, agoraphobia, specific phobias, separation anxiety disorder, and selective mutism. Symptoms of anxiety disorders may include restlessness, uncontrollable worry, irritability, difficulty concentrating, sleep disturbances, and physical manifestations like increased heart rate, sweating, and trembling.

The study's objective is to investigate *Mitragyne inermis* extract's anxiolytic qualities, with a particular emphasis on the efficacy assessment in rats particularly how well can *Mitragyne inermis* extract reduce anxiety-related behaviors? This entails measuring the anxiolytic properties of the extract by assessing its capacity to reduce anxiety symptoms using standardized behavioral tests, such as the Elevated Plus-Maze and Open Field Test also the how does the extract of *Mitragyne inermis* affect anxiolytic effects in proportion to dose? The best dosage for causing anxiolytic effects can be found by introducing several extract dosages and monitoring behavioral changes. The study was designed to evaluate the Anxiolytic effect of Aqueous solution leaves extract of *Mitragyne inermis* of obtain from Itas/Gadau local Government Area of Bauchi State. The study on the anxiolytic effects of *Mitragyna inermis* was justified based on the need to assess memory improvement, neuroprotective, and antioxidant effects of this plant extract on the central nervous system, The research aimed to investigate the potential anxiolytic properties of *Mitragyna inermis* through behavioral tests in animal models, focusing on its mechanisms of action and therapeutic implications. By evaluating the involvement of the dopaminergie. opioidergic, and GABAergic systems in mediating the anxiolytic effects of *Mitragyna inermis*, the study aimed to provide insights into the pharmacological basis of its anxiolytic activity Additionally, the study's findings suggested that *Mitragyna inermis* possesses antiamnesic effects, potentially mediated through its antioxidant properties, further justifying the investigation into its anxiolytic and cognitive-enhancing effects.

MATERIAL AND METHODS

Experimental animals

Albino Mice of both sexes (20g-28g) were obtained from the animal house facility of the Department of Pharmacology, Faculty of Basic Medical Sciences, Bauchi State University Gadau. They were housed in well-ventilated cages, fed with standard rodent pellet diet and water, and maintained under standard laboratory conditions in accordance with the protocols approved by the University ethical committee on use and care of experimental animals.

Plant materials collection

MITRAGYNA INERMIS leaves were collected from Itas Gadau local government area. Bauchi state. The plant material was identified and authenticated by Dr Umar Aminu Muhammad with Voucher Number 0063.

Equipment, Drugs and Chemicals

The equipment used includes weighing balance, stopwatch, mortar and pestle, syringe and needles, beaker, gavage syringe and filter paper. Were as normal saline, aqueous solution. Diazepam were the chemicals and drugs used. All chemicals and drugs were locally procured.

Preparation of plant extract

The fresh leaves of MITRAGYNA INERMIS were collected and dried for at list one week under room temperature and take away from sunlight, and then grounded into coarse powder. About 200g of grounded sample was weighed and defatted with 100% aqueous solution for 72hrs. The mixture was filtered and the residue was macerated. The percentage yield (w/w) of extract was determined.

Phytochemical screening

Phytochemical screening of the MITRAGYNA INERMIS was carried out using standard test as described by Sofowora (1993)[5]

a) Test for alkaloids: About 0.5g of the extract was stirred with 5ml of 1% aqueous hydrochloric acid over a steam bath, filtered and cooled. 1ml of filtrate was treated with a few drops of the following reagents and the corresponding-colored precipitate observed for:

i. Dragendorff's reagent: no reddish-brown precipitate seen
ii. Mayer's reagent: no cream-colored precipitate seen
iii. Wagner's reagent: no reddish-brown precipitate seen

b) Test for saponins: Frothing test: About 0.5g of the

extract was diluted with water in 3 test tube and vigorously shaken for 2 minutes after which few drops of olives were added. The persistent formation of the frothing emulsion is indicative of saponins (sofowora, 1993)[5]

c) Test for tannins: About 0.5g of the extract was dissolve in little quantity of water and a drop of ferric chloride was added. The resultant solution yielded a bluish-green precipitate (sofowora, 1993)[5]

d) Test for flavonoids

i. Lead sub-acetate test: 0.5g of the extract was dissolved in a test tube and 1 ml of 10% lead sub-acetate solution was added. A yellow precipitate was seen which indicates the presence of flavonoid.

ii. Sodium hydroxide test: 0.5g of the extract was dissolved in a test tube and 5ml of 20% sodium hydroxide was added. A yellow solution indicates the presence of flavonoids (sofowora, 1993)[5]

e) Test for cardiac glycosides: General Test: 0.5g of the extract was put in a test tube and 2.5 ml of dil. Sulphuric acid was added and boiled in water bath for 15 minute. This was cooled and neutralizes with 20% KOFI solution. 5 ml of a mixture of Fehling's solution A and B was added and boiled for 3 minutes. A brick red precipitates indicates the hydrolysis of reducing sugars, and indication of glycoside (Sofowora 1993)[5]

f) Keller killiani test: 0.5g of the extract was dissolved in 1 ml of glacial acetic acid already containing drops of ferric chloride solution. This was then underplayed with about 1 ml of conc. Sulphuric acid. A brown ring obtained at the inter-phase indicates the presence of a deoxy sugar, characteristics of cardenolides.

g) Test for carbohydrate; Molisch test: 0.5 g of the extract was dissolved in 3 ml of distilled water and mixed with a few drops of Molisch reagent (10% solution of alpha naphtol in alcohol). I ml of cone. Sulphurie acid was carefully added the aqueous solution without mixing a reddish or violet ring at the junction of the liquid indicates the presence of carbohydrate (Sofowora, 1993).[5]

Acute toxicity studies

The method previously described by Lorke's (1983) was adopted using mice. In the first, phase. Three doses of the extract (10, 100, 1000mg/kg) were administered orally to three groups each containing three rats. In second phase which was based on the results of first phase, consist of three geometric dose levels (1600, 2900, 5000mg / kg) at which the median lethal dose (LD50) was determined as the square root of the geometric mean of the highest non-lethal dose and lowest lethal dose.

Statistical analysis

The data was analyzed using one way ANOVA followed by Dunnet post hoc comparison test to compare differences between groups. Data represents mean + SEM. P value <0.05 were considered statistically significant. Graph Pad Prism (version 9) statistical software was employed for data analysis.

Open field test (oft)

The Open Field Test (OFT) is a common behavioral assessment used in animal research, especially for studying anxiety and exploration. It was introduced in the year 1930s.In this test, an animal, such as a mouse or rat, is placed in a large, enclosed area, and its behavior is observed. The Open Field apparatus consisted of a wooden box measuring 35 x 30 x 23 cm with visible lines drawn to divide the floor into 36 (20 cm × 20 cm) squares with a frontal glass wall and placed in a sound free room. Twenty (20) mice were randomly divided into five groups of four mice each to screen for the anxiolytic effect of the extract. Group 1 received normal saline (10 ml/kg IP), Group II received a low dose of the extract (250 mg/kg MIL), Group III received a moderate dose of the extract (500 mg/kg MIL), Group IV received a high dose of the extract (1000 mg/kg MIL), and Group Five received diazepam (2 mg/kg). Following a half-hour pretreatment, the mice were put in a sizable, contained space, and their behavior was noted. Important parameters: A 20-minute treatment record included the amount of time spent in the center and peripheral zones as well as the number of entries into each. Reduction of the Duration of Stay at the center was considered Anxiety like behavior.

RESULTS

Extraction

A brown extract weighted 18g was obtained from 200g of starting powder and the percentage yield was found to be 9%.

Phytochemical analysis of mitragyna inermis leaves extract

The Phytochemical analysis of ethanol leaves extract of MITRAGYNA INERMIS in the table 1.0 below shows that alkaloids, tannins, flavonoids and steroids are present.

Table 1.0: Phytochemical Analysis of the leaves extract of *mitragyna inermis*.

Test	Interference
Alkaloids	+
Tannins	+
Saponin	+
Flavonoids	+
Steroid	+

Key: + = Present, - = Absent

Median lethal dose values of Mitragyna Inermis leaves extract

The intraperitoneal median lethal dose LD_{50} of methanol leaves extract of *Mitragyna Inermis Leaves Extract* was estimated to be 3808 mg/kg as displayed in the table 2.0 below.

Table 2.0: Effect of *mitragyna inermis leaves* extract of aqueous using open field test time spent in the centers.

Treatment (mg/kg)	Time spent in the centre(s)
Normal Saline	358±34.54
MIL 250	570±76.48^{ns}
MIL 500	871±80.86*
MIL 1000	884±49.34*
Diazepam 2	940±24.83*

Summary below the Table
Mil 250mg/kg ns p= 0.9998
Mil 500mg/kg * p= 0.0002
Mil 1000mg/kg * p= 0.0001
Dia 2mg/kg * p=0.0001\

Effect of Mitragyna Inermis Leaves Extract of Aqueous Using Open Field Test Time spent in the peripheral is shown in the table 3.0 below.

Table 3.0: Effect of Mitragyna Inermis Leaves Extract of Aqueous Using Open Field Test Time spent in the peripheral.

Treatment (mg/kg)	Time spent in the periphery(s)
Normal Saline	841±34.54
Mil 250	630±76.48^{ns}
Mil 500	329±80.86*
Mil 1000	313.5±48.03*
Diazepam 2	260±24.83*

Summary below the P-value
Mil 250mg/kg ns p= 0.0.991
Mil 500mg/kg * p= 0.0002
Mil 1000mg/kg * p= 0.0001
Dia 2mg/kg * p=0.0001

According to the result, effect of Mitragyna Inermis leaves extract of aqueous using open field test no of entries into central zone was determined and displayed in the table 4.0 below.

Table 4.0: Effect of mitragyna inermis leaves extract of aqueous using open field test no of entries into central zone.

Treatment (mg/kg)	No of Entries into Central Zone
Normal Saline	3.000±0.5774
Mil 250	5.000±0.9129^{ns}
Mil 500	7.000±1.472*
Mil 1000	4.000±1.080^{ns}
Diazepam 2	4.500±0.6455^{ns}

Summary below the P-value
Mil 250mg/kg ns p= 0.4424
Mil 500mg/kg * p= 0.0001
Mil 1000mg/kg ns p= 0.8836

Dia 2mg/kg ns p=0.6704

***Table* 5.0: Effect of mitragyna inermis leaves extract of aqueous using open field test no of entries into peripheral zone.**

Treatment (mg/kg)	No of Entries into Peripheral Zone
Normal Saline	2.750±0.8539
Mil 250	3.500 ± 0.2887^{ns}
Mil 500	5.750 ± 1.436^{ns}
Mil 1000	4.000 ± 1.00^{ns}
Diazepam 2	3.500 ± 0.6455^{ns}

Summary below the P-value
Mil 250mg/kg ns p= 0.9376
Mil 500mg/kg ns p= 0.1050
Mil 1000mg/kg ns p= 0.1050
Dia 2mg/kg ns p=0.9376

DISCUSSION

Anxiety is a prevalent mental health condition that affects millions of people worldwide, leading to an ongoing search for effective treatments. Diazepam, a benzodiazepine, is a well-established pharmaceutical used to manage anxiety due to its fast-acting anxiolytic properties. In recent years, attention has also shifted toward natural alternatives, such as herbal extracts, which may offer anxiety relief with fewer side effects. This essay compares the effects of Diazepam with a 1000 mg extract (presumably herbal) based on an experimental open-field test that evaluated anxiety-related behaviors.

The results show a dose-dependent increase in time spent in the center, indicating reduced anxiety. Normal saline (control) had the lowest time (358s), while MIL 250 (570s) showed a slight, non-significant increase. MIL 500 (871s) and MIL 1000 (884s) significantly increased center time, suggesting strong anxiolytic effects. Diazepam (940s) showed the highest time, with comparable efficacy to MIL 1000, confirming the extract's potential anxiolytic properties at higher doses.

The results indicate a dose-dependent decrease in time spent in the periphery, which suggests reduced anxiety levels. Normal saline (control) had the highest time (841s) in the periphery. MIL 250 (630s) showed a non-significant decrease, while MIL 500 (329s) and MIL 1000 (313.5s) significantly reduced time spent in the periphery. Diazepam (260s) had the lowest peripheral time, closely followed by MIL 1000, indicating strong anxiolytic effects at higher doses of the extract, comparable to the standard drug.

The number of entries into the central zone increased with treatment, indicating reduced anxiety, though not consistently across doses. Normal saline had the lowest entries (3). MIL 250 (5) showed a non-significant increase. MIL 500 (7) significantly increased entries, indicating stronger anxiolytic effects. However, MIL 1000 (4) showed a non-significant decrease, similar to Diazepam (4.5), suggesting a plateau in anxiolytic efficacy at higher doses.

The number of entries into the peripheral zone showed no significant changes across treatments. Normal saline had 2.75 entries. MIL 250 (3.5), MIL 500 (5.75), MIL 1000 (4), and Diazepam (3.5) all exhibited non-significant variations, indicating that the treatments did not substantially affect peripheral zone entries. This suggests that while the extract and Diazepam reduced anxiety-related behavior, peripheral entries were not a key indicator of this effect.

CONCLUSION

The study demonstrates that the aqueous extract has notable anxiolytic effects, which are dose-dependent. Phytochemical analysis revealed the presence of compounds with known therapeutic benefits, such as alkaloids and flavonoids. Acute toxicity testing established a high LD50 of 3808 mg/kg, confirming the extract's relative safety. Behavioral assessments in anxiety-induced mice using the open field test showed that at doses of 500 mg/kg and 1000 mg/kg, the extract significantly increased the time spent in the center and reduced time spent in the periphery, comparable to the standard anxiolytic drug Diazepam. However, the number of central and peripheral zone entries did not exhibit consistent patterns, suggesting that the extract primarily affects anxiety behaviors related to movement and positioning within the arena.

Recommendations

1. Further research: Investigate the underlying mechanisms of the extract's anxiolytic effects and conduct long-term safety studies.
2. Clinical trials: Initiate human clinical trials to evaluate the efficacy and safety of the extract for treating anxiety.
3. Dosage Optimization: Explore optimal dosage levels to maximize therapeutic benefits while minimizing side effects.

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