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EVALUATION OF ANTIDEPRESSANT ACTIVITY AND SUBCHRONIC TOXICITY STUDIES OF METHANOL LEAF EXTRACT OF *URTICA DIOICA L.* IN MICE AND RATS

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INTRODUCTION

DEPRESSION

Depression is a multifactorial, chronic, and life-threatening disease characterised by unusual feelings of sadness, reduced energy, social withdrawal, lack of motivation, sexual dysfunction, sleep disorders (in 75% of the patients), nightmares, depressed mood, anhedonia, worthlessness, feelings of guilt, hopelessness, and suicidal thoughts are the main symptoms of depression.[1] All of these symptoms hurt physical and social functioning. About 80% of all people facing depression have some form of impairment in their daily functioning. Globally, over 700 thousand people with depression die by suicide every year.[2]

Depression may affect everyone regardless of age, gender or race. The pathophysiology of depression is still poorly understood. Several hypotheses have been advanced to explain its mechanism.[3] The biogenic amine (monoamine) hypothesis was the first. According to this hypothesis, the typical symptoms of depression are the results of a changed concentration of monoamines or incorrect monoaminergic transmission. This is described by the Basic Emotion Theory (BET), put forward for the first time in the 1950s. Environmental stressors are another set of factors associated with the development of depressive disorder including job loss, death of a spouse, divorce, unwanted pregnancy, social isolation, rape, war, etc. About 280 million people are documented as suffering from depression.[4]

ANTIDEPRESSANTS

MAOIs were the first class of antidepressant drugs available for pharmacological therapy and they were used very often in the past to treat different forms of depression. Currently, available tricyclic antidepressants include amitriptyline, clomipramine, desipramine, imipramine, nortriptyline, amoxapine, doxepin, protriptyline and trimipramine. Specific serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed group of antidepressant drugs. Available SSRIs include Fluoxetine, fluvoxamine, paroxetine, sertraline, citalopram, and escitalopram. Noradrenaline–dopamine reuptake inhibitors (NDRIs) inhibit the re-uptake of Noradrenaline NA and Dopamine. They are newer than first-generation antidepressants and include amineptine or bupropion.

Among the greatest unmet needs in depression is a lack of effective pharmacotherapies for patients who do not respond to first- and second-line antidepressant medications.[2] Treatment-resistant depression (TRD) is a growing area of interest among researchers and mental health professionals.[3] The unfavourable side effects of many modern antidepressants contribute to low patient compliance.[5] Finding new, safer substances with antidepressant efficacy is imperative. This study evaluated the antidepressant activities of *Urtica dioica* methanol leaf extract and determined its toxicological profile. The objectives were to determine the phytochemical constituents of the methanol leaf extract of Urtica dioica, the median lethal dose (LD_{50}) of the methanol leaf extract of Urtica dioica, the safety profile of the methanol leaf extract of Urtica dioica in a 28-day administration in Rats, and evaluate the effect of methanol leaf extract of Urtica dioica in neurobehavioral models of depression in mice.

MATERIALS AND METHODS

Materials

Test Drug-*Urtica Dioica L.*

- **Standard Drug-** Standard (Imipramine) drug --- dissolved in distilled water before orally administered to the Mice.
- **Animal Used-**Rats and Mice
- **Number of Rats-**42 (male and female)
- **Strain of Rats-**Albino Wister Rats
- **Weight of Rats**-120-180mg
- **Number of Mice-** 12
- **Strain-** Swiss Albino Mice
- **Weight of Mice-** 20-30g

Source: Animal House, Department of Pharmacology and Toxicology, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria.

Housing Conditions
I separate cages 12-12hour light and dark cycle
Relative humidity-40-60%
Temperature-25 (-/+2^{0}C) **Diet**-Standard Food pellets consumed by Rats

Instruments
The following materials were used in this study; ruler (80cm long, 3cm wide), metal support (30cm), wooden blocks, wire mesh cages, electronic weighing balance, test tubes, Gavage needle, Automated haematology analyzer (Pentra-XL 80, Horiba ABX, USA) Syringes Beaker, Conical Flask and Spatula, pestle and mortar, Automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany).

Preliminary work (Selection of Plant)
Gathering sufficient information from various articles and journals it was concluded that there is scope to explore some more pharmacological activities in the plant *Urtica Dioica L.*, hence it was selected for further studies.

Collection and Authentication of Plant material
- Collection – Wild cultivar, in Dange Shuni Local Government Area of Sokoto, wild cultivar, Sokoto State Northwest Nigeria in October 2023.
- Authentication of Plant- Identified and authenticated by a taxonomist (Malam Halilu) in the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences Usmanu Danfodiyo University Sokoto, Nigeria. Plant material was manually washed with distilled water and deposited in the herbarium Voucher number PCG/UDU/DG/0017.
- Drying- Dried under the shade.
- Size reduction- Test materials were pulverised to powder using a wooden pestle and mortar

Preparation of Urtica Dioica L. leaves extract- 500g of the dried powder was weighed and subjected to cold maceration for 72hrs with 2L of 95% methanol as the solvent of extraction. The mixture was allowed to stand for 5 hours and was then shaken periodically.
- Filteration- Whatman filter paper was used for filtration of the mixture and the extract was concentrated in a water bath at 40^{0}C.
- Packaging- To maintain uniformity, the dried extract was packed into an airtight container and stored in a cool and dry place. This material was used for further study.

Phytochemical Analysis of Crude Extracts (Qualitative)
These tests were conducted at the Department of Pharmacognosy and Ethnopharmacy Laboratory, Faculty of Pharmaceutical Sciences Usmanu Danfodiyo University Sokoto, Nigeria. Analytical methods described by Trease and Evans (2012) were employed.[6]

- **Test for flavonoids**

a) Sodium hydroxide test: 1 ml of 10% sodium hydroxide solution was added to the extract. A yellow colour indicates the presence of flavonoids.
b) Ferric chloride test: The extract was boiled with water and filtered. Two drops of ferric chloride solution were added to 2mls of the filtrate. A blue-green or violet color indicates the presence of a phenolic nucleus.
c) Shinoda test: Small quantity of extract dissolved in water. Concentrated hydrochloric was added and magnesium chips. Cherry red precipitate indicates the presence of flavonoids.

- **Test for alkaloids**

About 0.5g of the extract was stirred with 5ml of 1% aqueous hydrochloric acid in a water bath and filtered. 3ml of the filtrate was divided into three test tubes;
a) Dragendroffs test: To the first test tube, a few drops of freshly prepared Dragendroff's reagent were added and observed for the formation of an orange-to-brownish precipitate.
b) Mayer's test: To the second test tube, a few drops of Mayer's reagent were added and observed in the formation of a white to yellowish or cream precipitate.
c) Wagner's test: To the third test tube, one drop of Wagner's reagent was added and observed for brown-reddish or brown precipitate.

- **Test for Saponins**

a. Frothing Test: Small amount of extract was put in a test tube and about 10 mls of water was added to it. It was thoroughly shaken for 30 seconds. The test is positive if honeycomb froth persists in the test tube for 10-15 minutes.

- **Tests for Carbohydrates**

a. Molisch's Test: a little amount of the extract was put in a test tube and a little amount of Molisch's reagent was added. Few drops of concentrated sulphuric acid were added. Appearance of a reddish colored ring indicates the presence of carbohydrates.

b. Fehling's Test: a little amount of extract was put in a test tube and a few drops of Fehling's reagents A and B were added. It was then heated. Presence of brick red precipitate indicates the presence of carbohydrates.

- **Test for Tannins**

a. Lead Sub acetate Test: a little amount of extract was put in separate test tubes. They were dissolved with chloroform. Then a few drops of lead sub acetate were added. Presence of a heavy precipitate indicates that tannins are present in the plant extract.
b. Ferric Chloride Test: a little amount of extract was put in separate test tubes. A small amount of water was added. Then a few drops of ferric chloride were added. Presence of blue black color indicates that tannins are present.

- **Test for Cardiac Glycosides**

a. Kella – Kelliani Test: a small amount of extract of was dissolved in glacial acetic acid. Few drops of ferric chloride were added, shaken andfew drops of concentrated sulphuric acid were added. Appearance of a purple-brown ring at the interface indicates the presence of cardiac glycosides.

- **Tests for Steroids**

a. Liebermann-Burchard's Test: a small amount of extract was dissolved in chloroform and equal volume of acetic anhydride and then a few drops of concentrated sulphuric acid were added. The extract is positive for steroids if the upper layer is blue-green in colour while the lower layer is red.
b. Salkowski's Test: A small amount of extract was taken and dissolved in some chloroform. A few drops of concentrated sulphuric acid was added. The appearance of a brown ring indicates the presence of steroids.

- **Experimental Work**

Animals

- Adult Wistar rats of 120-180 g and Mice of 20-30g were used for the study. The rats and mice were obtained from the Animal House of the Department of Pharmacology and Toxicology for experimental purposes. The animals were maintained under controlled conditions, temperature (23 ± 2°C), humidity (50 ±5%) and 12 h light-dark cycles. All the animals were acclimatised for seven days before the study. The animals were randomised into experimental and control groups and housed individually in sanitised polypropylene cages containing sterile husks as bedding. They had free access to standard pellets such as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 hours before the experimental protocol to minimise non-specific stress. All the studies were conducted according to the prescribed International and institutional guidelines of the Committee for Control and Supervision of animal studies.
- Preparation of dose

The test extract Urtica Dioica L. was dissolved in distilled water to make the appropriate concentration for various experimental doses before being orally administered to the Rats and Mice.

- **Toxicity Studies**

Acute toxicity test

The acute oral toxicity test of the methanol leaf powder extract of Urtica Dioica L. was determined before the efficacy study following Lorke's method (Lork, 1983). The study was conducted in two phases using a total of twelve rats. In the first phase, nine rats were divided into 3 groups of 3 rats each. Groups 1, 2 and 3 animals were given 10, 100 and 1000 mg/kg body weight (b.w.) of the extract, respectively. In the second phase, further specific doses of 1600, 2900 and 5000 mg/kg body weight (b.w.) of the extract were administered to three rats (one rat per dose) to determine the correct LD50 value further.

Subchronic toxicity test

The subchronic toxicity test of methanolic leaf powder extract was also determined before the efficacy study as per the Organization for Economic and Community Development (OECD Test Guideline 408) method. Twenty-four rats of either sex were randomly divided into four groups of six rats each (3 male and 3 female). The first group received 10ml/kg distilled water (vehicle) orally. The second, third and fourth received 100, 200 and 400mg/kg of Urtica dioica respectively, daily for 28 days orally. The Rats had access to food and water ad libitum throughout the experiment (28 days). Observation was done daily for general symptoms of toxicity and mortality. The weights of the rats were taken weekly. On the 29^{th} day, under light chloroform anaesthesia, the animals were sacrificed. Just before death, a cardiac puncture was carried out to collect blood samples for haematological and biochemical tests. The blood samples for analysis for all the rats were collected using 6mls bottles. One bottle was EDTA coated (3mls) and the second bottle non EDTA coated (3mls). Blood samples for EDTA bottle was analysed for WBC, RBC, PCR, PCV, MCV, HRT and platelets. The blood in the non EDTA bottles centrifuged and the plasma was decanted to test for LFT and EUCR. The tissue samples of various organs (brain, kidney, liver and heart) were collected from one rat from each group and were subjected to histopathological examinations to assess any cellular changes or abnormalities at the histopathology laboratory of the university teaching hospital. The tissues were collected in 10% formalin containers.

Experimental Protocol for Efficacy study

Animals were divided into five groups with six animals in each group. The experimental design was as follows;
Group 1: This group was administered 10ml/kg of distilled water each (negative control)
Group 2: This group was administered 100mg/kg of the extract each.
Group 3: This group was administered 200mg/kg of the extract each.

Group 4: This group was administered 400mg/kg of the extract each.
Group 5: This group was administered 10 mg/kg of imipramine each (positive control).

Behavioral Models for Depression
These procedures were carried out at the animal house of the Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Health Sciences, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria. Four animal models for depression were conducted to evaluate the antidepressant activity of the methanol leaf extract of Urtica dioica.

All the behavioural models for the efficacy study, Force Swim Test (FST), Tail Suspension Test (TST), Sucrose Preference Test (SPT) and Swim Induced Grooming (SIG) models were conducted using the same experimental protocol.

FST
In 1977, Porsolt described this model which is the frequently adopted model used for screening antidepressant-like activity in mice (Porsolt et al. 1977).

Video recording and a live observer were used to measure the immobility time. Increased immobility time in the forced swim test is considered indicative of depressive-like behaviour. A decrease in immobility time following treatment with the potential antidepressant (extract) suggests an antidepressant effect.

TST
The total duration of immobility induced by the tail suspension test was measured according to the method described by Steru et al. (1985). Increased immobility time is considered indicative of depressive-like behaviour. A decrease in immobility time following treatment with the potential antidepressant (extract) suggests an antidepressant effect.

SPT
The Sucrose preference test is one of the most widespread and commonly used animal models of depression. A decrease in the sucrose consumption ratio of an experimental animal is indicative of a successful depression model with anhedonia.

SIGT
The method previously described by Chesher and Jackson (1984) as modified by Magaji et al. was adopted (Magaji et al. 2014). Mice were treated orally with either a graded dose of the extract and distilled water (10 ml/kg) for fourteen days. On the 15th day, each mouse was placed individually in a transparent beaker (1000 ml) containing water (at 25 °C) up to 6 cm and allowed to swim for 3 min. Mice were dried with a hand towel for 20s and placed immediately in a transparent glass cage and the total grooming duration was recorded over 5 minutes.

STATISTICAL ANALYSIS
Results were expressed as Mean ± Standard Error of Mean (SEM) and percentages. Data analysis was performed using SPSS statistical software (version 25). Comparison between groups was made using analysis of variance (ANOVA). A post hoc Dunnet test was performed for multiple comparisons when statistical difference was obtained, values of $p< 0.05$ were considered significant.

RESULTS
Phytochemical Constituents of Methanol leaf Extract of Urtica dioica Phytochemical analysis of methanol leaf extract of Urtica dioica revealed tannins, flavonoids, steroids, carbohydrates, Anthraquinones, proteins, alkaloids and cardiac glycosides (Table 1).

Table 1: Phytochemical Constituents of Methanol Leaf Extract of Urtica dioica.

TEST	RESULT
Saponins	+
Carbohydrates	+
Phenols	+
Flavonoids	+
Tannins	+
Cardiac glycosides	+
Steroids	+
Alkaloids	+
Anthraquinones	+
Proteins	+

Key: + = Present

Acute Toxicity Study
No mortalities were observed in the first and second phases of the study (Table 2)

Table 2: Acute toxicity studies of methanol Leaf extract of Urtica dioica.

Dose (mg/kg) PO	Number of rats dead/used	% Mortality
Phase I 10	0/3	0
100	0/3	0
1000	0/3	0
Phase II 1600	0/1	0
2900	0/1	0
5000	0/1	0

Subchronic Toxicity Study
Effect of Methanol Leaf Extract of Urtical Dioica on Haematological Indices Following 28 Days Oral Treatment in Albino Rats
After twenty-eight days of oral administration of methanol leaf extracts Urtica dioica, there was no significant ($p<0.05$) alteration in the haematological indices in all the extract-treated groups (100, 200 and 400mg/kg) compared with the control group treated with distilled water (Table 3).

Table 3: Effect of Urtical udioica Methanol Leaf Extract on Haematological indices.

Treatment/ HGB Dose (%)	WBC ($x10^3$µL)	RBC ($x10^3$µL)	(g/dl)	HCT ($x10^3$µL)	PLT (fl)	MCV
Distilled water10 ml/kg	13.6 ±1.21	10.9±1.30	136.1±1.54	38.1 ±2.12	597.5 ±2.41	53.3 ±1.18
Extract 100 mg/kg	13.3 ±1.32	10.9±2.31	176.5±3.14	43.2 ±1.08	593.5 ±2.54	59.1 ±1.74
Extract 200 mg/kg	14.6 ±2.10	10.2±0.98	141.8±1.65	35.9 ±3.12	531.3 ±5.61	57.5 ±2.14
Extract 400 mg/kg	13.5 ±.097±1.32	11.0	137.8±4.32	38.2 ±1.03	662.1±5.37	57.0 ±0.57

Data expressed as Mean± SEM, WBC = White Blood Cells, RBC = Red Blood Cells, HCT = Hematocrit, HGB =Hemoglobin, PCV = Packed Cell Volume, PLT = Platelet, MCV = Mean Cells Volume. Dunnet post-hoc test.

Effect of Methanol Leaf Extract of Urtica dioica on Renal Function Indices Following 28 Days Administration of the Extract

The result indicates a slight increase in urea in certain groups, but the increase is not statistically significant (Table 4).

Table 4: Effect of Methanol Leaf Extract of Urtica dioica on Renal Function Markers.

Treatment/Dose (mg/kg)	Urea (mmol)	Creatinine (mmol)	Na^+ (mmol)	K^+ (mmol)	Cl^- (mmol)	HCO_3 (mmol)
Distilled water	4.25±0.15	2.06±0.17	133.1±1.22	2.55±0.23	112.6±2.10	19.80±1.50
Extract 100	5.43±0.24	2.05±0.07	136.8 ±2.43	3.10±0.15	113.8±2.11	19.33±1.25
Extract 200	4.65±0.12	2.11±0.08	136.83±2.11	3.03±0.15	116.3±2.13	19.80±1.25
Extract 400	4.53±0.21	2.45±0.14	134.66±1.50	3.16±0.27	113.1±4.10	20.10±1.23

SEM = Standard Error of Mean n= 6, Na^+ = Sodium ion, K^+ = Potassium ion, Cl^- = Chloride ion, HCO_3 = Bicarbonate, Dunnet Post-hoc test.

Effect of Methanol Leaf Extract of Urtica dioica On Liver Function Indices Following 28 Days Oral Treatment in Rats

There was no significant alteration in the liver function markers following 28 days' oral administration of (100, 200 and 400 mg/kg) methanol leaf extract of Urtica dioica (Table 5).

Table 5: Effect of Methanol Leaf Extract of Urtica dioica On Liver Function Indices.

Treatment/Dose (mg/kg)	T.B (mg %)	D.B (mg %)	ALK (m/l)	AST (m/l)	ALT (m/l)	T.P (g/l)	Albumin (g/l)
Distilled water	0.91±0.04	0.46±0.03	80.66±2.11	6.33±2.12	6.60±2.15	62.10±2.42	36.15±1.01
Extract 100	0.90±0.02	0.45±0.03	75.33±2.21	6.50±1.44	7.50±2.30	64.16±3.15	39.33±2.27
Extract 200	0.93±0.10	0.53±0.06	75.83±2.40	6.83±2.42	8.00±1.24	63.50±1.10	34.15±1.21
Extract 400	0.90±0.06	0.45±0.02	63.83±6.34	6.80±2.17	7.86±2.68	65.61±3.21	40.01±0.19

Data expressed as Mean±SEM, SEM = Standard Error of Mean n= 6, T.B = Total Bilirubin, D.B = Direct Biluribin, ALK = Alkaline Phosphatase, AST = Aspartase Transaminase, ALT= Alanine Transaminase, T.P = Total Protein. Dunnet Post-hoc test.

Histology of Rat's Kidney Treated with Urtica dioica leaf extract for 28 days Toxicity Studies

The histological examinations of the kidney organ revealed no tissue change upon administration of Urtica dioica extract for twenty-eight days when compared with the distilled water-treated group for the same period of intervention (Plate I).

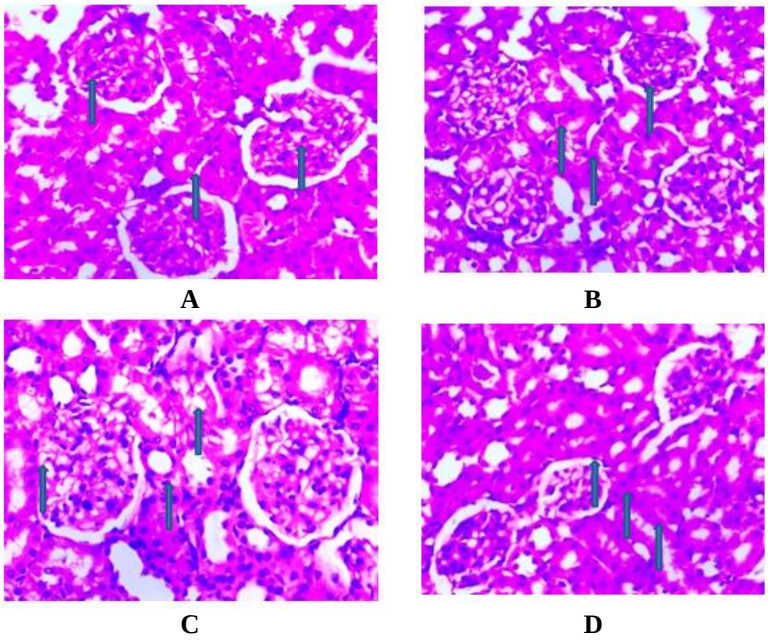

Plate I: Photomicrograph of a section of rat kidney treated with methanol leaf extract of Urtica dioica following 28 days oral administration (H&EX 100). (A) Distilled water treated showing normal glomeruli, tubules and interstitium. B, C, and D are 100, 200 and 400mg/kg extract treated groups respectively.

Histology of Rat's Liver Treated with Urtica dioica leaf extract for 28 days Toxicity Studies

The histological examinations of the liver organ revealed no tissue damage upon administration of Urtica dioica extract for twenty-eight days when compared with the distilled water treated group for the same period of intervention (Plate II).

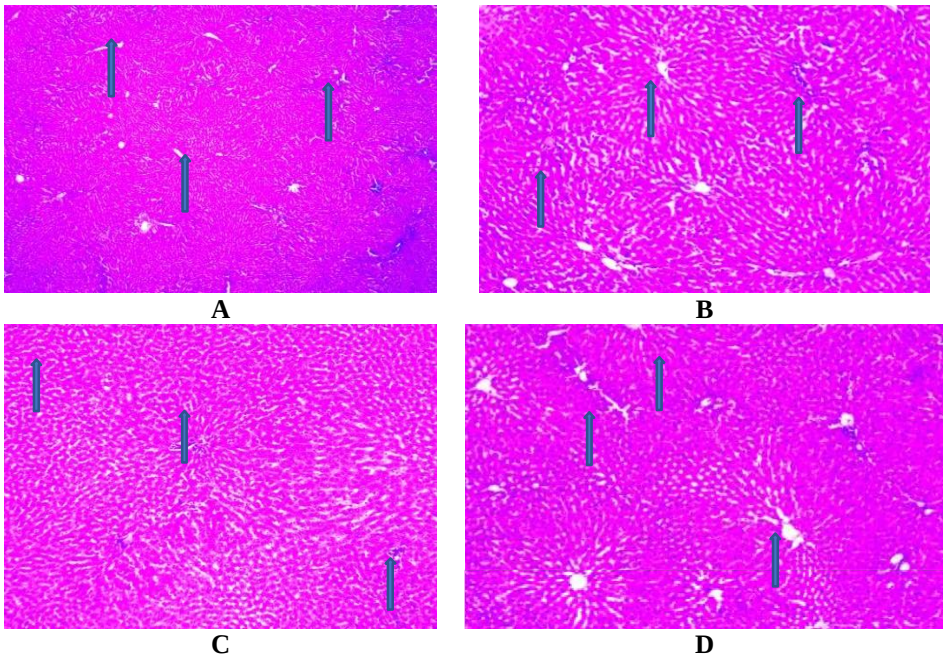

Plate II: Photomicrograph of a section of rat liver treated with methanol leaf extract of Urtica dioica following 28 days of oral administration (H&EX 100). (A) Distilled water treated showing normal portal triad, central vein and hepatocytes. B, C, and D are 100, 200 and 400mg/kg extract-treated groups respectively.

Histology of Rat's Spleen Treated with Urtica dioica leaf extract for 28 days Toxicity Studies

The histological examinations of the spleen organ revealed no tissue damage upon administration of Urtica dioica extract for twenty-eight days when compared with the distilled water treated group for the same period of intervention (Plate III).

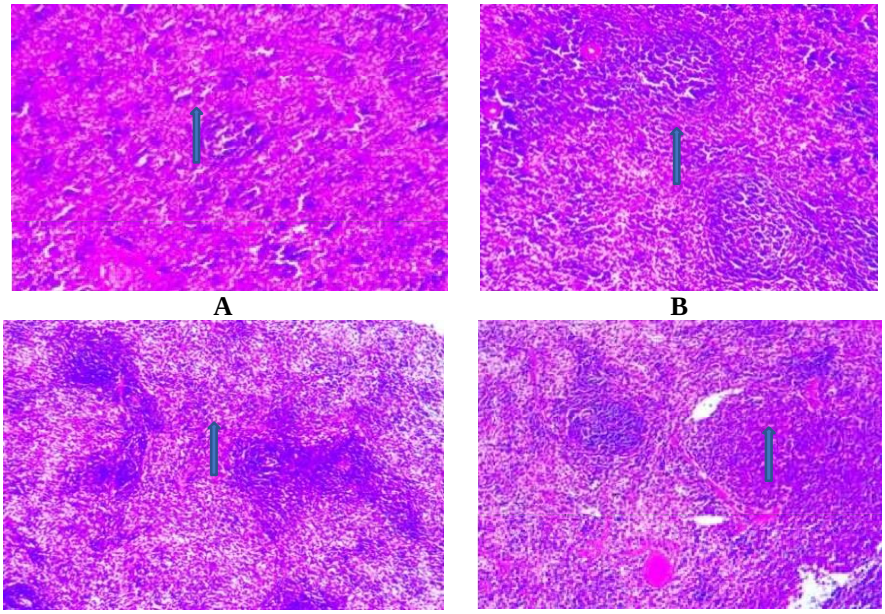

Plate III: Photomicrograph of a section of rat spleen treated with methanol leaf extract of Urtica dioica following 28 days of oral administration (H&EX 100). (A) Distilled water treated showing white and red pulp. B, C, and D are 100, 200 and 400mg/kg extract-treated groups respectively.

Histology of Rat's Heart Treated with Urtica dioica leaf extract for 28 days Toxicity Studies

The histological examinations of the heart organ revealed no tissue damage upon administration of Urtica dioica extract for twenty-eight days when compared with the distilled water treated group for the same period of intervention Plate IV).

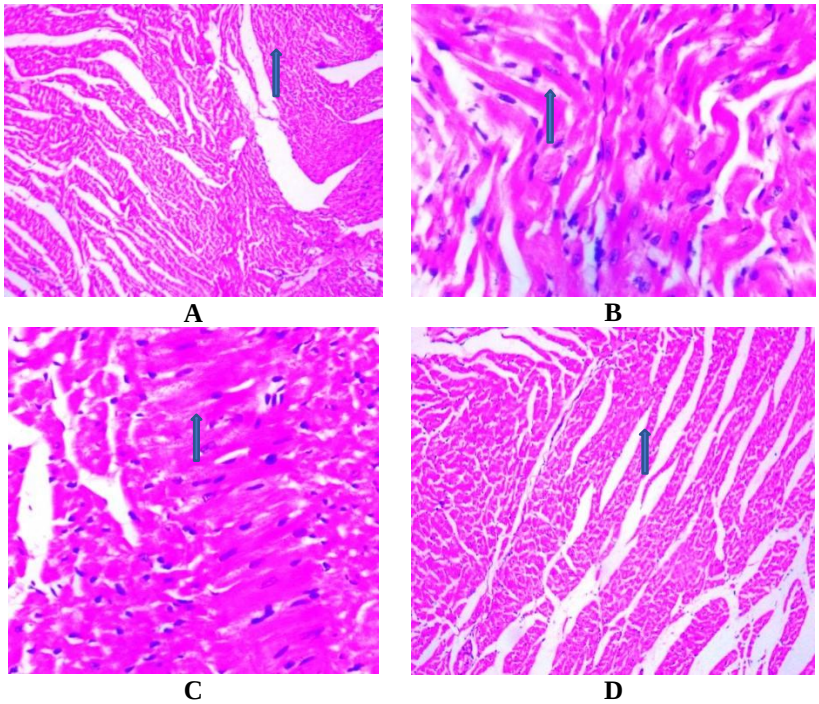

Plate IV: Photomicrograph of a section of rat heart treated with methanol leaf extract of Urtica dioica following 28 days of oral administration (H&EX 100). (A) Distilled water treated showing cardiac myocytes. B, C, and D are 100, 200 and 400mg/kg extract-treated groups respectively.

Histology of Rat's Brain Treated with Urtica dioica leaf extract for 28 days Toxicity Studies

The histological examinations of brain organs revealed no tissue damage upon administration of Urtica dioica extract for twenty-eight days when compared with the distilled water treated group for the same period of intervention (Plate v).

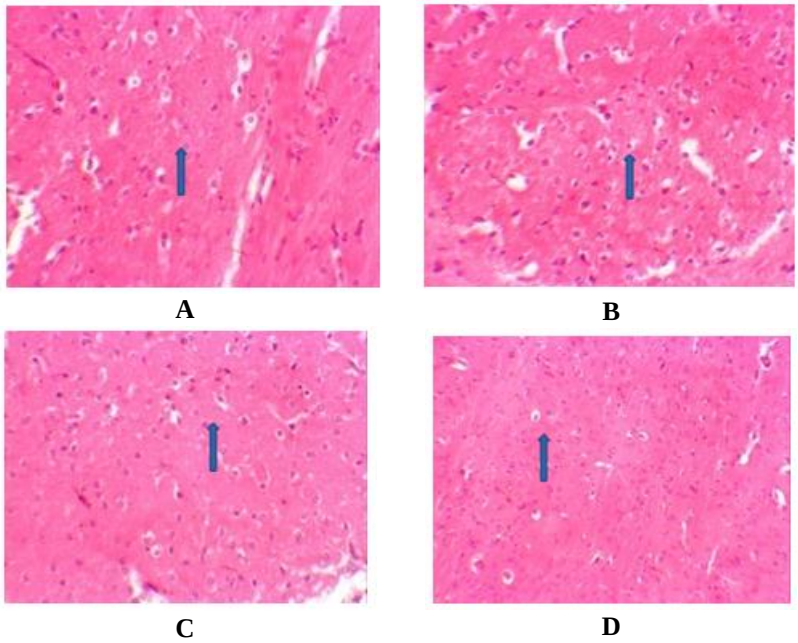

Plate V: Photomicrograph of a section of rat brain treated with methanol leaf extract of Urtica dioica following 28 days oral administration (H&EX 100). (A) Distilled water treated showing pyramidal neuronal cells neutrophils. B, C, and D are 100, 200 and 400mg/kg extract treated groups respectively, showing also pyramidal neuronal cells.

Effect of the Methanol Leaf Extract of Urtica dioica on Neurobiological Models of Depression (Efficacy Study)

Effect of Urtica dioica Leaf Extract on Mice Immobility in Forced Swim Test

The extract at the dose of 200 and 400 mg/kg significantly ($p < 0.05$) decreases the duration of immobility induced by the forced swim test as reported in Figure 1 below.

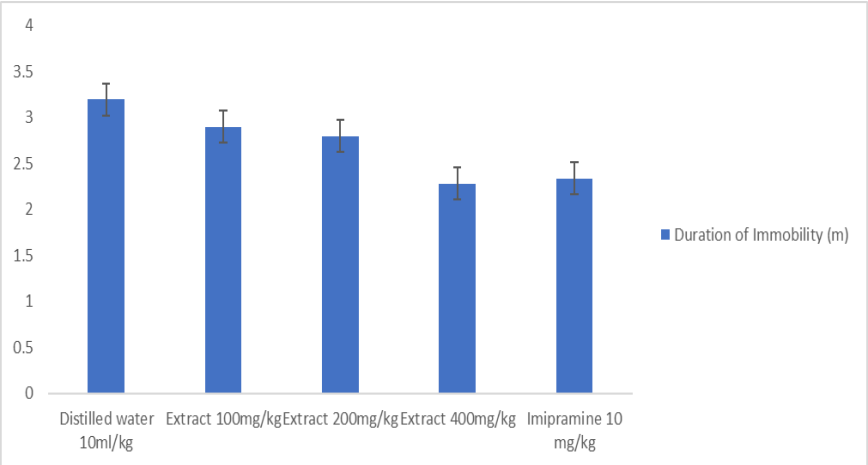

Figure 1: Effect of Methanol Leaf Extract of Urtica dioica on Duration of Immobility in Forced Swim Test. Data was presented as mean ± SEM, 200mg/kg and 400mg/kg dose of the extract were significant at $p < 0.05$ as compared distilled water treated groups with other groups by one-way ANOVA followed by Dunnet post-hoc.

Effect of Urtica dioica Leaf Extract on Mice Immobility in Tail Suspension Test

The extract at the dose of 400 mg/kg significantly ($p < 0.05$) decreases the duration of immobility induced by tail suspension posture as reported in Figure 2 below

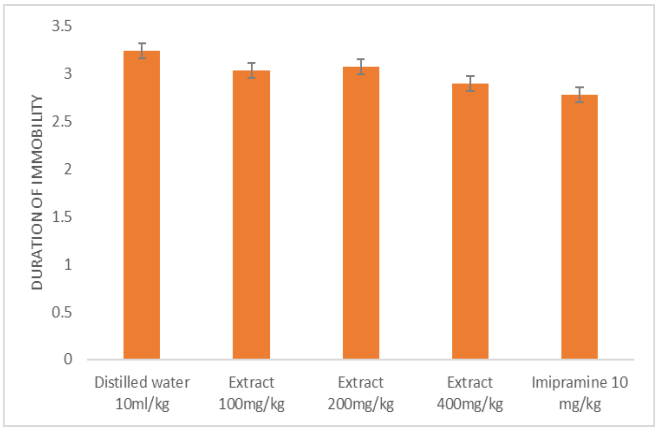

Figure 2: Effect of Methanol Leaf Extract of Urtica dioica on Duration of Immobility in Tail Suspension Test. Data was presented as mean ± SEM, 400 mg/kg of the extract was significant at p< 0.05 as compared distilled water treated groups with other groups by one-way ANOVA followed by Dunnet post-hoc.

Effect of Urtica dioica Leaf Extract on Mice in Sucrose Preference Test

The extract at the dose of 400 mg/kg significantly (p< 0.05) increases the percentage of sucrose preference in the sucrose preference test as displayed in Figure 3 below

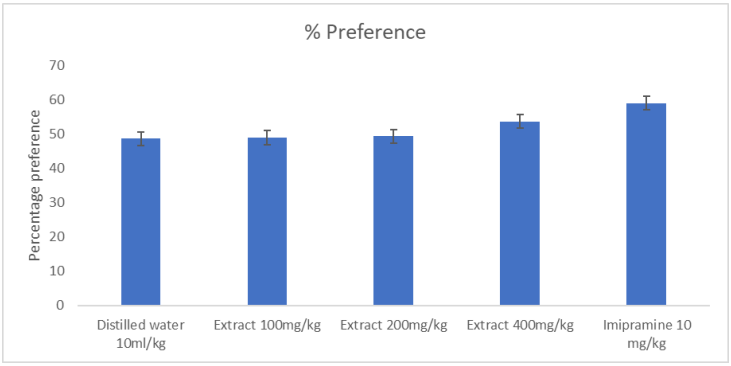

Figure 3: Effect of Urtica dioica Leaf Extract on Mice in Sucrose Preference Test. Data was presented as mean ± SEM, 400 mg/kg of the extract was significant at p< 0.05 as compared distilled water treated groups with other groups by one-way ANOVA followed by Dunnet post-hoc.

Effect of Urtica dioica Leaf Extract on Mice Grooming in Post-Swimming Test

The extract at the dose of 400 mg/kg significantly (p< 0.05) increases the duration of grooming in post-swimming behavioural observations as reported in Figure 4 below.

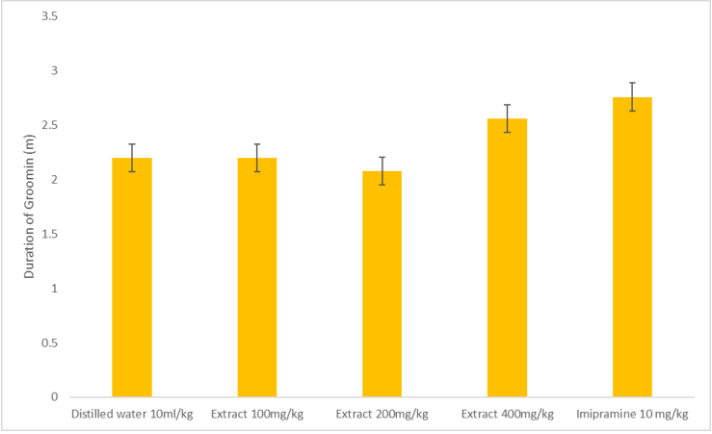

Figure 4: Effect of Methanol Leaf Extract of Urtica dioica on Duration of Grooming in Post Swimming Test. Data was presented as mean ± SEM, 400mg/kg of the extract was significant at p< 0.05 as compared distilled water treated groups with other groups by one-way ANOVA followed by Dunnet post-hoc.

DISCUSSION

The preliminary phytochemical screening of the methanol leaf extract of Urtica dioica revealed the presence of tannins, saponins, flavonoids, steroids, alkaloids, carbohydrates and reducing sugar. Significant numbers of plants containing a wide variety of phytochemicals as their bioactive constituents have been reported to alter central nervous system activities.[7]

In the oral acute toxicity study of the plant extract, the LD_{50} was determined to be greater than 5000mg/kg as no mortality was observed in both phase I and II of the Lork's method. According to Dietrich Lorke, 1mg/kg is considered highly toxic, 10mg/kg is considered toxic, 100mg/kg is moderately toxic, 1000mg/kg is slightly toxic and 5000mg/kg is considered not toxic.[8] The results indicated that large doses of the extract can be toxic and low doses are relatively safe. According to the American Society for Testing Safety of Materials, any chemical substance with LD_{50} less than 2000mg/kg/oral route but greater than 1000mg/kg/oral could be considered to be slightly toxic.

Generally, conventional drugs are used to treat depression (especially MAOIs and SSRI-like drugs) but adverse drug reaction (ADR) is very common with conventional agents, these effects are dependent on about 8% of people are hospitalized due to their ADRs their consumption in the United States.[9] Medicinal plants have an important role in primary health care having better compliance and good efficacy in the prevention and treatment of depression.[10] Urtica dioica is one of the most common types of medicinal plants used to treat different kinds of illnesses. In this study, we use the methanol leaf extract of Urtica dioica as an antidepressant in Swiss mice. There are various models available to screen the antidepressant activity in Swiss mice including, the sucrose preference test and swim-induced grooming for anhedonic behaviour, forced swim test and tail suspension test for despair behaviour. The forced swim and tail suspension test is the most widely used model for antidepressant screening.

Urtica dioica methanol leaf extract significantly decreased the duration of immobility in forced swim and tail suspension tests indicating a possible antidepressant effect, the phytochemicals contents of the plant indicated the presence of alkaloids which have been reported to possess central nervous system effects, including antidepressant activity.[11] The immobility displayed by rats and mice when subjected to unavoidable stress such as the force swim test and tail suspension test respectively are thought to reflect state of despair or low mood, which is thought to reflect depressive disorders in human.[12] The results obtained in this study showed the reduction in the duration of immobility observed after administering the extract doses to the animals in both models, indicates possible antidepressant effect through enhancing the level of extracellular monoamines neurotransmitters. Urtica dioica methanol leaf extract may contain some phytochemicals that enhances the extracellular concentrations of the monoamines such as norepinephrine, epinephrine, dopamine and serotonin by either inhibiting their up-take mechanism or inhibiting their metabolism.

The methanol leaf extract of Urtica dioica also significantly increases the percentage sucrose preference when compared with the distilled water group. Lost in motivation toward pleasurable and reward earning activity is termed anhedonia, which remained one of the cardinal symptoms of depressive disorder, the ability of this extract to reverse stress-induced anhedonia toward sucrose water is an indicator that the plant may resolve this depressive symptom. These findings portrayed decreased levels of dopamine transmitters within certain regions of the brain, specifically in the limbic system regions such as the ventral tegmental area, nuclear accumbens, prefrontal cortex, hippocampus and amygdala can precipitate anhedonic depressive symptoms. Our Urtica dioica extract significantly prolongs the duration of grooming behaviour and this is suggestive that, our extract elicits its effect via enhancing the level of dopamine in this region, an activity similar to that produced by the monoamine oxidase B inhibitors.[13] The primary aim of evaluating the safety profile of medicinal plant extract is to identify the pattern and extent of lethality of that extract to internal organs and systems on exposure to a specified time.[14] In the oral acute toxicity study of the plant extract, the median lethal dose was found to be > 5000 mg/kg in rats. According to Dietrich Lorke, 1 mg/kg is considered highly toxic, 10 mg/kg is considered toxic, 100 mg/kg is moderately toxic, 1000 mg/kg is slightly toxic, and 5000 mg/kg is considered not toxic. The results of the acute toxicity study indicate that the extract of Urtica dioica administered through an oral route up to 5000 mg/kg dose do not produce mortality in all the phases. Furthermore, according to OECD criteria under its Globally Harmonized Classification System, for xenobiotics and mixtures, substances with LD50 > 2000 mg/kg are categorized as category 5 (relatively safe).[15] Our result indicates that on acute administration, the extract of Urtica dioica is relatively safe. Sub-chronic toxicity testing is useful in assessing the safety of target organs and the haematological or biochemical effects of extracts since these effects are often invisible upon acute low-dose administration of the test substance. Haematological parameters are used as markers in ascertaining the extent of toxicity of xenobiotics and other biologically active substances, plant extracts inclusive.[14] The haematopoietic system has a great sensitivity to toxic substances and hence its parameters serve as great markers for systemic toxicity.[16] Blood cells are derived from pluripotent stem cells, immature cells with the tendency of differentiating to become an erythrocyte (RBC), a leukocyte (WBC), or a thrombocyte (platelet).[17] In this study, administration of Urtica dioica to rats for 28 days produced no significant change in red blood cells, white blood cells,

haemoglobin and packed cell volume and other haematological parameters indicating possible safety of this plant on haematopoietic tissues**.**

CONCLUSION

It may be concluded that the methanol leaf extract of Urtica dioica contains bioactive phytochemicals with antidepressant effects. The toxicity studies of the methanol leaf extract of Urtica dioica indicate relative safety. Mechanistic studies should be employed to determine the exact mechanism through which this extract elicited its antidepressant effect, and activities guided fraction should employed to isolate the phytochemical(s) responsible for the observed activity.

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