



***Triclisia Subcordata* Oliv Aqueous Leaf Extract can influence Reproductive Hormones and Haematological Profiles of Mifepristone Threatened Pregnancy in Wistar Rats**

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Abstract

Effect of *Triclisia subcordata* oliv aqueous leaf extract on reproductive hormones and haematological profiles of mifepristone threatened pregnancy in Wistar rats was investigated. Sexually matured rats comprising of 80 male and 80 female rats (150-220g) were purchased and paired up for reproduction after one week acclimatization period in the ratio of 2:2. The rats were sexed, and the pregnant females were housed in different plastic cages of eight groups with ten rats each. Group 1 is the normative control, while Group 2 is the experimental group (mifepristone only). On day 5 of their pregnancies, Groups 2-8 took mifepristone orally. Groups 3-8 were administered orally extract dosages of 100, 200, and 300 mg/kgbw (nonexposed and dew-exposed) respectively daily for 17 days. All analyses were performed using standard procedures. In comparison to the experimental control rats, administration of the extract (nonexposed and dew-exposed) significantly decreased white blood cell, lymphocytes, eosinophil counts but increased oestrogen, progesterone, packed cell volume, haemoglobin, red blood cell, mean cell volume and mean cell haemoglobin levels of treated rats. The 300mg/kgbw extract doses provided better protection there by sustaining the pregnancy as seen in stage 2 especially in those administered with dew-exposed. Histopathological examination of uterus showed histological normal uterus in normal control rats and slight deformation and enlargement of the uterus in the experimental control rats which was restored to normalcy in the treated rats. The result of this study improved suppressed hormones and haemopoietic markers using *Triclisia subcordata* leaf extract against threatened pregnancy in Wistar rats.

Keywords: *Triclisia subcordata* oliv, mifepristone, pregnancy, reproductive hormones, haematology, dew, uterus.

INTRODUCTION

The usage of herbal supplements, natural medications, and traditional regional cures has skyrocketed in the last two decades for both the treatment and prevention of sickness (Enioutina et al., 2017; Barnes et al., 2016; Sammons et al., 2016; World Health Organization Traditional Complementary and Integrative Medicine Definitions, 2013). Dogoua claims that the majority

of customers for these herbal remedies are women, particularly distressed pregnant women (2010). Seven percent to fifty-five percent of pregnant women may take herbal supplements, depending on variables such as location, family history, and cultural norms (Illamola et al., 2020; Dogoua, 2010; Tiran, 2003). Herbal supplements are widely used throughout pregnancy despite concerns about their safety because of the natural bioactive components that lessen and soothe pregnant symptoms including nausea, vomiting, and constipation (Illamola et al., 2020; Frawley et al., 2015). Common pregnancy discomforts include but are not limited to: breast enlargement, heartburn, nausea, vomiting, shortness of breath, candidiasis, bloating, and swollen feet and legs (El Hajj & Holst, 2020). Although there are studies supporting its use during pregnancy and the related pharmacological advantages, the risk linked with long-term use remains unclear. Fetal loss, heavy bleeding, and hormone disruption are all possibilities (Bernstein et al., 2020; Smeriglio, Tomaino, & Rombetta, 2014). Herbal treatments have a long and storied history of use as abortifacients and abortion inducers throughout cultural contexts (Bernstein et al., 2020). Due to the absence of regulation surrounding their use, these plant-based drugs and mixtures are routinely purchased without a prescription and without instructions on dosage or administration (Illamola et al., 2020). Pre-packaged paperwork seldom give user-friendly instructions and requirements in the event of a medical referral (Bernstein et al., 2020). Due to their effectiveness in treating a variety of ailments, herbal treatments were gaining popularity in certain regions. For example, compared to American women, those in India and Ghana are more prone to take herbal medicines to protect their pregnancy and the health of their unborn kids (Adusi-Poku et al., 2015; Bhatt, 2016). Preventing infant hyperbilirubinemia (Tabatabaee, 2011) and treating fetal growth retardation (Maputle et al., 2015; Rahman et al., 2008; Adusi-Poku et al., 2015; Mugomeri et al., 2015; Bhatt et al., 2016; Dika et al., 2017) were its most prevalent clinical uses (Maputle et al., 2015).

Triclisia subcordata Oliv, a plant of the Menispermaceae family, has long been used in West African folk medicine. The Menispermaceae are a family of mostly tropical flowering vines, although they also include certain herbs, shrubs, and even trees. The 70 genera that make up this family include more than 450 different species; one of them is *Triclisia*. The leaves are alternating and unlobed most of the time, although they may sometimes be lobed and have palmate veins (Ezimah et al., 2013).

The Umuoji call *T. subcordata* Oliv. "ike mbekwu," which translates to "shaped leaf," while the Nsukka call it "ogwu-aju," which translates to "antidizziness" and "Alugboran" in Yoruba (Dalziel, 1937). From the stalk, a thick rope called a "tietie" is used as a rough fibre (Irvine, 1961). You could come across this plant in the West Tropical African countries of Senegal, Togo, Sierra Leone, Ghana, Nigeria, and the Ivory Coast (Trease and Evans, 1993). The antioxidant properties of *Triclisia subcordata* have been studied for their potential to cure a wide range of diseases and disorders, including diabetes, cancer, infections, and histamine sensitivity (Asuzu and Anaga 1995; Abo et al., 2011; Ayoola et al., 2017; Li, 2016). Bisbenzylisoquinoline, isochondodendrine, and 2'-norcocculine are the three active alkaloids found in *Triclisia subcordata*. Uche et al. (2016) investigated their cytotoxicity and impact on the survival of ovarian and cancer cells (2017). Muscle relaxation and ulcer protection were found in rats by Gbadamosi and Erinoso (2016). In Nigeria, people utilize root extract to cure a wide variety of medical conditions, including high blood pressure, ulcers, snakebites, diarrhea, malaria, typhoid, pyorrhea, and even joint pain, limb swelling, and anemia (Popoola et al., 2021).



Plate 1: photograph showing *Triclisia subcordata* Oliv leaf

Therefore, this research analyzed the effects of an aqueous leaf extract (nonexposed and dew-exposed) of *Triclisia subcordata* on pregnancy hormones and haematology markers in female Wistar rats over the duration of their pregnancies (first and third trimesters). These findings are important because they will raise public knowledge about the benefits of this plant for expecting mothers.

MATERIALS AND METHODS

Procurement of Plant Material

Triclisia subcordata Oliv leaves were collected fresh in October 2021 at the University of Port Harcourt in Choba, Rivers State, Nigeria. At the university's herbarium, they were catalogued

by a taxonomist (Dr. Suleiman M.) from the college of pharmaceutical sciences' department of pharmacognosy and phytotherapy and given the voucher number UPHMO472.

Procurement of Animals

Jos, Plateau state's National Veterinary Research Institute Vom provided the 160 Wistar rats used in the research (80 male and 80 female rats weighing 150-220g). After a week of acclimation, the test subjects had free access to the standard rat diet and water dispensers. The rats were divided into eight groups of ten each and placed in a chamber heated to 37 degrees Celsius.

Preparation of Plant Extract

Triclisia subcordata is a subspecies of *triclisia*. The goal was to guarantee that only the cleanest dried olive leaves were utilized. In order to extract the liquid, the leaves were ground into a pulp in a mortar. For later usage, the filtrate was collected and stored in the freezer (in the traditional way).

Collection of Dew

A fresh, large-bottomed plastic dish is set outdoors anytime between midnight and daybreak. The dew was moved into an uncontaminated container and taken for analysis.

Experimental Design

Eighty female Wistar rats ranging in size from 150g to 220g were randomly split into eight groups of 10. Rats were separated into the following groups, with the experimental design being influenced by Ayoola et al. (2017):

Groups	Treatment
I (Normal control)	Received normal feed and water daily
II (Experimental control)	Received feed + water + mifepristone on day 5
III (Treatment 1)	Received feed + water + nonexposed extract (100 mg/kg bw) + mifepristone on day 5

IV (Treatment 2)	Received feed + water + nonexposed extract (200 mg/kg bw) + mifepristone on day 5
V (Treatment 3)	Received feed + water + nonexposed extract (300 mg/kg bw) + mifepristone on day 5
VI (Treatment 4)	Received feed + water + dew-exposed extract (100 mg/kg bw) + mifepristone on day 5
VII (Treatment 5)	Received feed + water + dew-exposed extract (200 mg/kg bw) + mifepristone on day 5
VIII (Treatment 6)	Received feed + water + dew-exposed extract (300 mg/kg bw) + mifepristone on day 5.

The rats had the extract (nonexposed and dew-exposed) cannulated into their mouth for 17 days. Telleria et al., 1996's oral cannula administration of mifepristone dose was modified thus: The procedure has two phases.

Eighty male and eighty female rats were coupled in a 2:2 ratios after a week of acclimatization on a conventional rat diet for a 24-hour mating session at stage one (1 day). That was the first detectable pregnancy symptoms. Pregnancy was indicated by the presence of a vaginal plug, vaginal semen, and an open or enlarged vagina. Wistar pregnant rats were split up into eight groups of ten, and given the therapy as described above.

Specimen collection:

Five rats from each group were given an oral dose of mifepristone on day 5, and then they were humanely euthanized the next day. (within few hours after taking mifepristone, rather than waiting the whole 24). Chloroform was used to put the rats to sleep before they were measured. They went without food for eight hours before being put under anesthesia and having their throats cut open to access the jugular vein so that blood samples could be taken. Blood taken after treatment with heparin bottles was utilized for hormonal analysis, whereas blood collected

after treatment with ethylenediaminetetraacetic acid (EDTA) bottles was used for hematology. After sterilizing the rats, their uteri were removed and stored in sample carriers with 10 percent formalin for further histological investigation.

At the second stage, only Groups I and II received feed and water until Day 17. Groups III through VIII received nonexposed/dew-exposed extract (before the start of labour). They were found to have put on a lot of weight. Each group's surviving rats were slaughtered on day 18 of pregnancy, and their blood was collected into heparin and EDTA bottles for the aforementioned tests as described in Nwaichi et al. (2013), while their uteruses were preserved in sample holders with 10 percent formalin for histology.

Estimation of Follicle Stimulating Hormone (FSH) Concentration:

AccuBind Enzyme-Linked Immunosorbent Assay (ELISA) Microwells test kits were used to determine the concentrations of FSH in the samples obtained by Mboso et al (2013). (Monobind Incorporated of the USA). One set of serum samples was used as a standard, while the other two sets were used as controls. The wells were prefilled with 50 μ l of serum reference, control, and specimen before 100 μ l of FSH-Enzyme reagent solution was pipetted in. After 60 minutes of room temperature incubation, the microplate was decanted by gently spinning it for 30 seconds. The decanter was washed in a wash buffer that held 350 microliters in three distinct cycles. Each well had 100 μ l of substrate solution added to it, and then the plates were incubated for 15 minutes at room temperature. After 20 seconds of moderate stirring, 50 microliters of stop solution were poured into each well. In order to determine the absorbance at 450 nm for each well, we used a microplate reader. The optimal absorbance versus FSH concentration curve was determined by fitting the data from each paired serum standard. When the point of intersection between the curves was located, the unknown FSH concentration was calculated using the average absorbance of the duplicates against the vertical axis.

Estimation of Progesterone Concentration:

According to Mbosso et al., the AccuBind Enzyme-Linked Immunosorbent (ELISA) Microwells test kits were used to analyze the samples for progesterone concentrations (2013). (United States-based Monobind Incorporation). Tri-duplicate microplate wells were prepared for the analysis of the serum samples used for the controls, references, and tests. Combine 25 μl of the designated serum reference, control, and specimen with 50 μl of the Progesterone Enzyme reagent solution in each of the designated wells. After incubation for 60 minutes at room temperature and centrifugation for 20 seconds at a moderate speed, the contents of the microplate were decanted. The decanter was cleaned with a wash buffer totaling 350 μl three times. After incubating the substrate solution for 20 minutes at room temperature, 50 μl of the stop solution was added and the mixture was agitated for 20 seconds. Each well's absorbance at 450 nm was measured using a microplate reader. For each pair of serum standards, a curve of absorbance against progesterone concentration and a best-fit curve spanning the points shown were calculated. By reading the progesterone concentration from the left side of the graph and the average absorbance of the duplicate from the right, we were able to locate the spot where the two lines intersected.

Estimation of Estrogen Concentration:

According to Mbosso et al (2013) description, the estrogen concentration in the samples was determined using AccuBind's ELISA Microwells test kits Monobind, Inc., a firm established in the USA. Three serum samples (one each for the reference, control, and test) were placed in separate wells of a microplate. Fifty microliters of the estradiol (estrogen) biotin reagent and 25 microliters of the serum reference, control, and specimen were added to each well. After 90 minutes of room temperature incubation and a moderate spin for 30 seconds, the microplate's contents were drained. Once the decant had been rinsed three times with 350 microliters of wash buffer, we added 100 microliters of substrate solution and 50 microliters of stop solution

to each well and gently mixed for 20 seconds. Both the substrate and the stop solutions were left to incubate for 20 minutes at room temperature before being introduced to the decanter. The absorbance at 450 nm of each well was determined using a microplate reader. Every set of serum standards had its own absorbance versus estrogen curve, and then a line of best fit was drawn between the data points. The unknown estrogen concentration was obtained by plotting the average absorbance of the duplicate on the vertical axis, locating the point where the curve crosses, and reading the estrogen concentration off the horizontal axis.

Haematological Indices

The centrifuge tube with a whole blood sample was inserted into the Model-Mindray BC-10 Auto haematology analyzer, and the findings were printed out.

Histological Examination:

The Awvioro (2002) method of histological analysis was used. Following the rats' deaths, their ovaries were removed and placed in containers containing normal saline at a 10% concentration. Tissue slices were cleaned by immersing them in xylene after water was removed from the material using a calibrated percentage of alcohol in an ascending sequence from the lowest concentration to the absolute. Thin, even slices were cut using a rotary microtome for histological analysis. A slide that had been cleaned of all oil was heated in a dish of pure water. After removing the bulk of the oil from the slide's surface, a piece was put on its surface by gradually expanding the surrounding wax with the connecting needles. As the seas became warmer, the terrain flattened out. After removing the slide from the heating plate, it was labeled and allowed to air dry. Hematoxylin and eosin were used to stain the slides. To prepare the slices for microscopic analysis, they were dyed and then mounted in the appropriate medium under a glass cover slip with a mountant. A microscope was used to look at the slides.

Dew Analysis

Determination of Cations (Metals) by Atomic Absorption Spectrophotometry

Three hours were spent burning the sample to ash in a muffle furnace set at 630 degrees Celsius. A sample of ash was dissolved in 10 mL of strong hydrochloric acid by first heating it on a hotplate using an electro-thermal heater. The ash solution was prepared by mixing 50 mL of distilled water with the ash. The metal content of the sample was determined with the use of an atomic absorption spectrometer (iron, magnesium, calcium, potassium, manganese, sodium, and zinc).

Determination of Anions

Nitrate (NO₃⁻) was detected in the water using the EPA-approved brucine method, whereas chloride (mohr), organic carbon (rapid oxidation) and sulfate were measured using alternative techniques (the turbidimetric method).

Analytical Statistics

Statistics were analyzed using SPSS, a software created by SPSS Inc., version 23.0. One-way analysis of variance (ANOVA) required the determination of means and standard deviations (M and SD, respectively) for use in making multiple comparisons. The level of significance set at 0.05 was deemed appropriate.

RESULTS AND DISCUSSION

RESULTS

Table 1: Chemical composition of dew

Properties	Quantity
pH	6.74±0.02
Cations	
Ca ²⁺ (Mg/L)	7.86±2.11
Mg ²⁺ (Mg/L)	3.28±0.53
K ⁺ (Mg/L)	3.06±0.12

Na (Mg/L)	9.04±2.21
Mn ²⁺ (Mg/L)	0.03±0.01
Zn ²⁺ (Mg/L)	0.23±0.06
Fe ²⁺ (Mg/L)	0.03±0.01
Anions	
Cl ⁻	30.62±5.18
NO ₃ ⁻	2.68±0.41
SO ₄ ²⁻	16.11±3.89
TOC (Mg/L)	0.86±0.10

Values are Mean ± SD of triplicate determinations. n=3

W.H.O. (2017)

Levels of hormonal profile parameters in mifepristone-administered Wistar rats.

Figure 1 shows the effect of *T. subcordata* leaf extract (nonexposed/dew-exposed) on follicular stimulating hormone (FSH) levels of mifepristone-administered Wistar rats

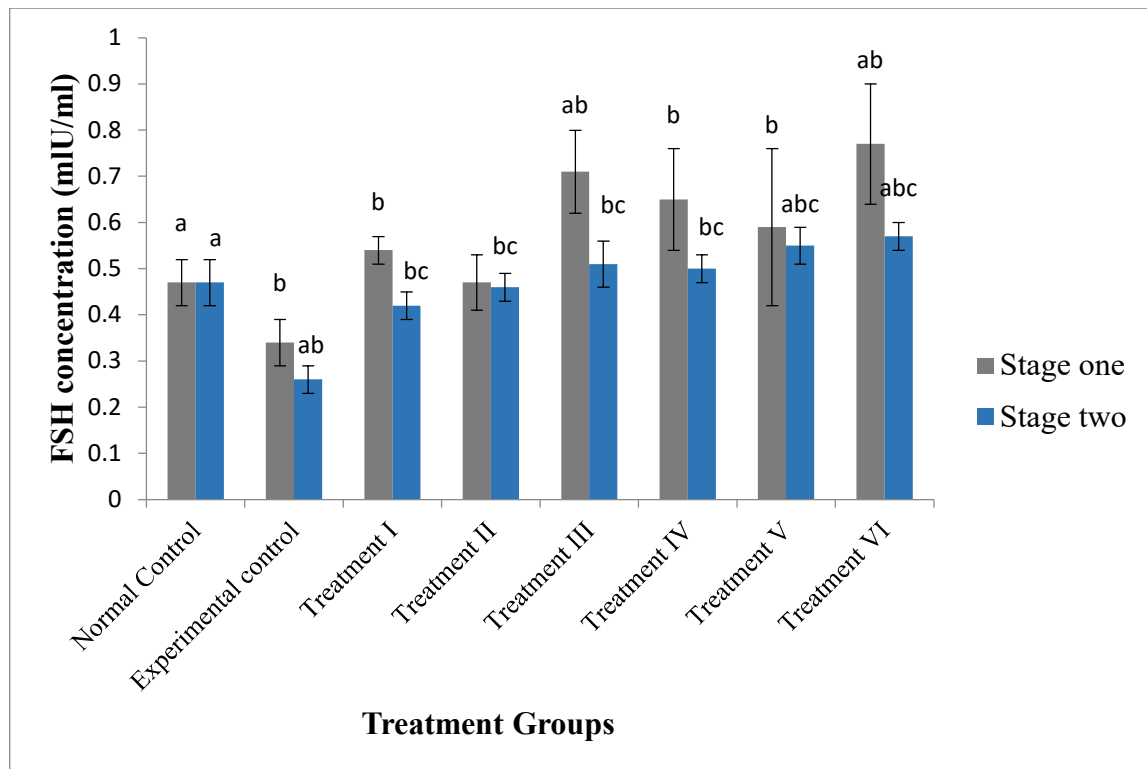


Figure 1: The effect of *Triclisia subcordata* leaf extract on follicle stimulating hormone levels in Wistar rats given mifepristone.

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c,.) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

Treatments IV, V, and VI (100, 200, and 300mg/kg b.w dew-exposed extract) were significantly different from Treatments I, II, and III (100, 200, and 300mg/kg b.w nonexposed extract) at the 0.05 level (superscript C (c)).

body mass index

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

Figure 2 shows the protective effect of *T. subcordata* leaf extract (nonexposed/dew-exposed) on oestrogen (E2) levels of mifepristone-administered Wistar rats.

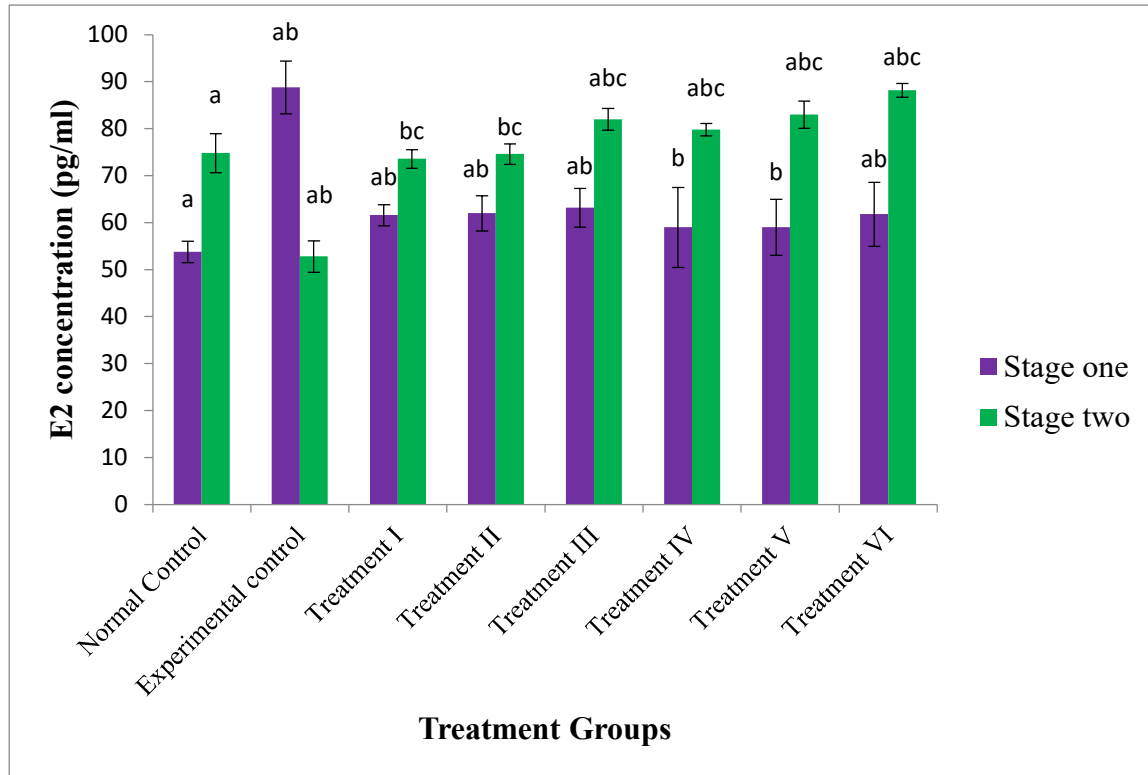


Figure 2: The effect of *Triclisia subcordata* leaf extract on oestrogen levels in mifepristone-treated Wistar rats.

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c..) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

Treatments IV, V, and VI (100, 200, and 300mg/kg b.w dew-exposed extract) were significantly different from Treatments I, II, and III (100, 200, and 300mg/kg b.w nonexposed extract) at the 0.05 level (superscript C (c)).

body mass index

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

Figure 3 shows the protective effect of *T. subcordata* leaf extract (nonexposed/dew-exposed) on progesterone (PG) levels of mifepristone-administered Wistar rats.

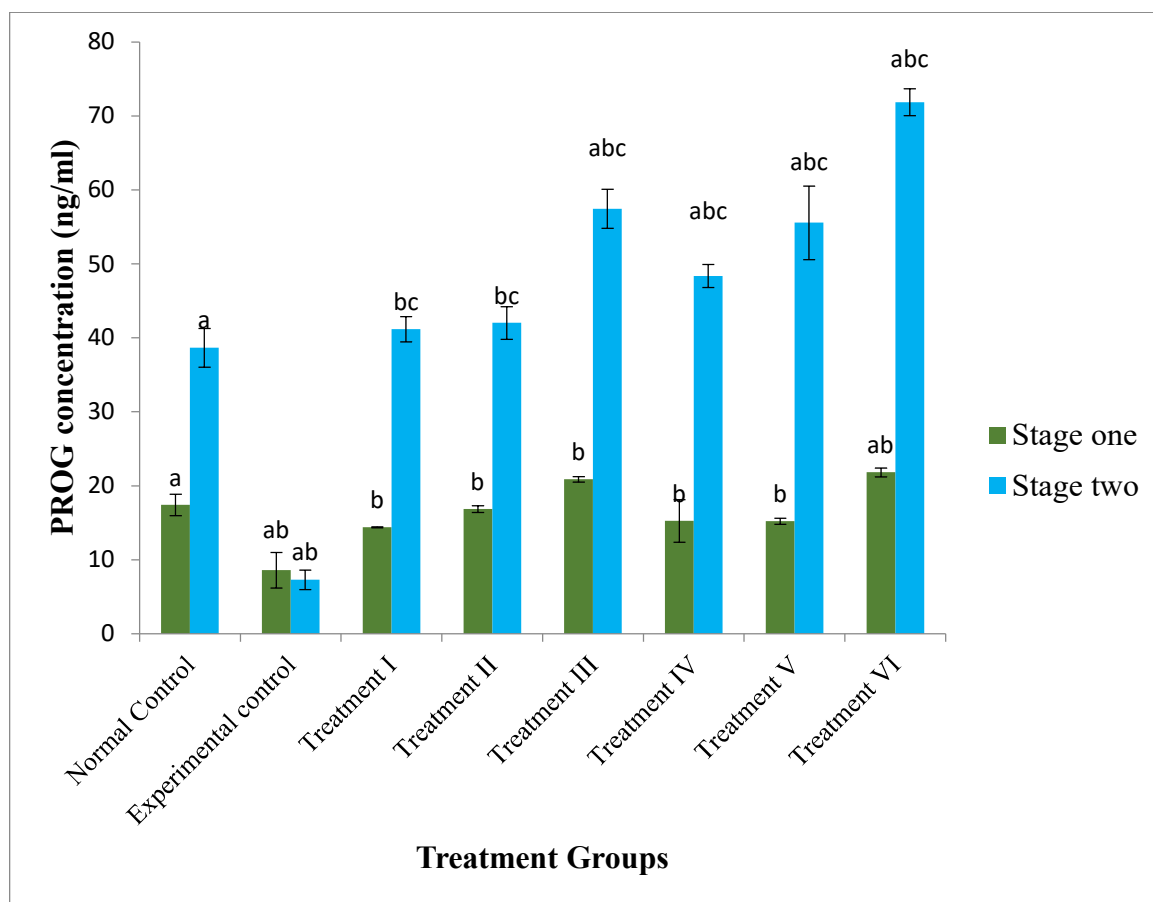


Figure 3: The effect of *Triclisia subcordata* leaf extract on PROG levels in Wistar rats given mifepristone.

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c...) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

Treatments IV, V, and VI (100, 200, and 300mg/kg b.w dew-exposed extract) were significantly different from Treatments I, II, and III (100, 200, and 300mg/kg b.w nonexposed extract) at the 0.05 level (superscript C (c)).

Progesterone (PROG) and body mass index (b.w.)

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

Levels of haematological parameters in mifepristone-administered Wistar rats.

Figure 4 shows the protective effect of leaf extract of *T. subcordata* leaf extract (nonexposed/dew-exposed) on packed cell volume levels of mifepristone-administered Wistar rats.

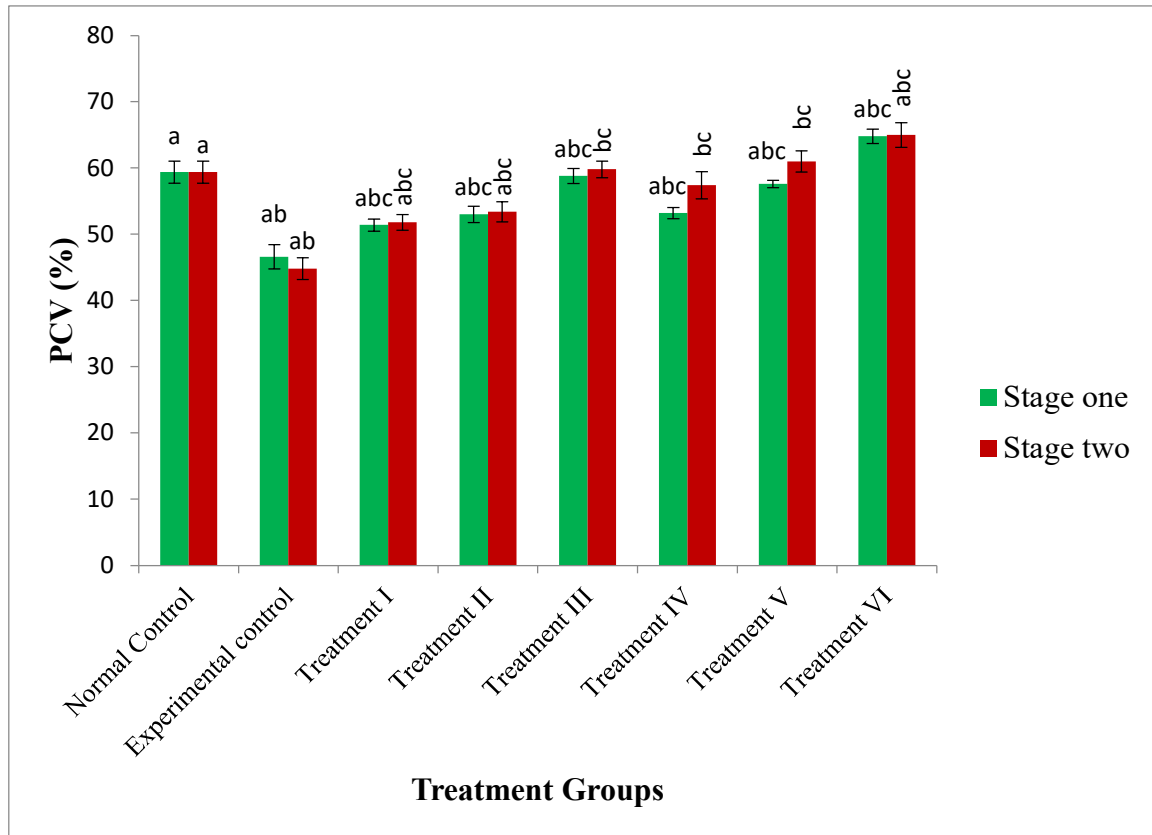


Figure 4: Protective effect of leaf extract of *T. subcordata* leaf extract/extract with dew on packed cell volume levels of mifepristone-administered Wistar rats.

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c...) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

Treatments IV, V, and VI vary significantly from Treatments I, II, and III at the p0.05 level, denoted by the superscript C (c).

Packing cube volume (PCV) and body mass (b.w.)

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

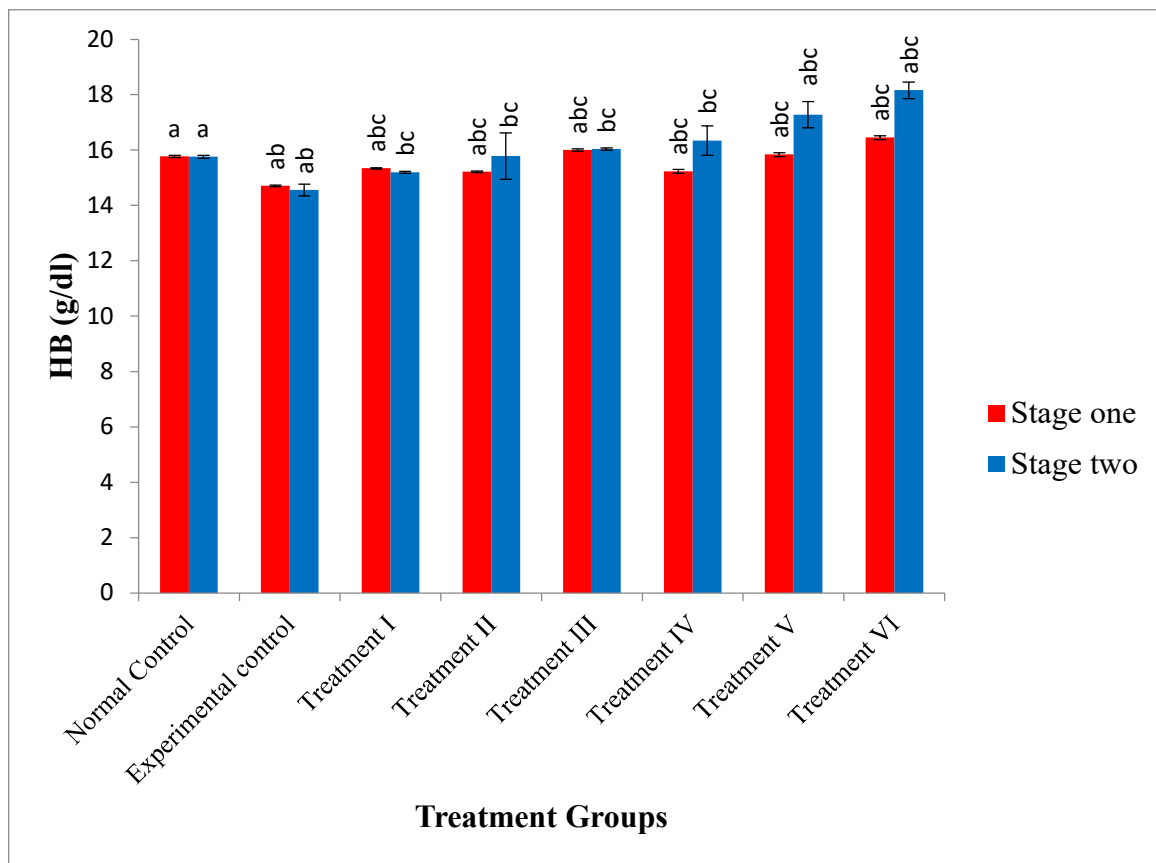


Figure 5 shows the protective effect of *T. subcordata* leaf extract (nonexposed/dew-exposed) on haemoglobin levels of mifepristone-administered Wistar rats.

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c...) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

Treatments IV, V, and VI vary significantly from Treatments I, II, and III at the p0.05 level, denoted by the superscript C (c).

Weight (b.w.) = body mass index

(Hb) = hemoglobin

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

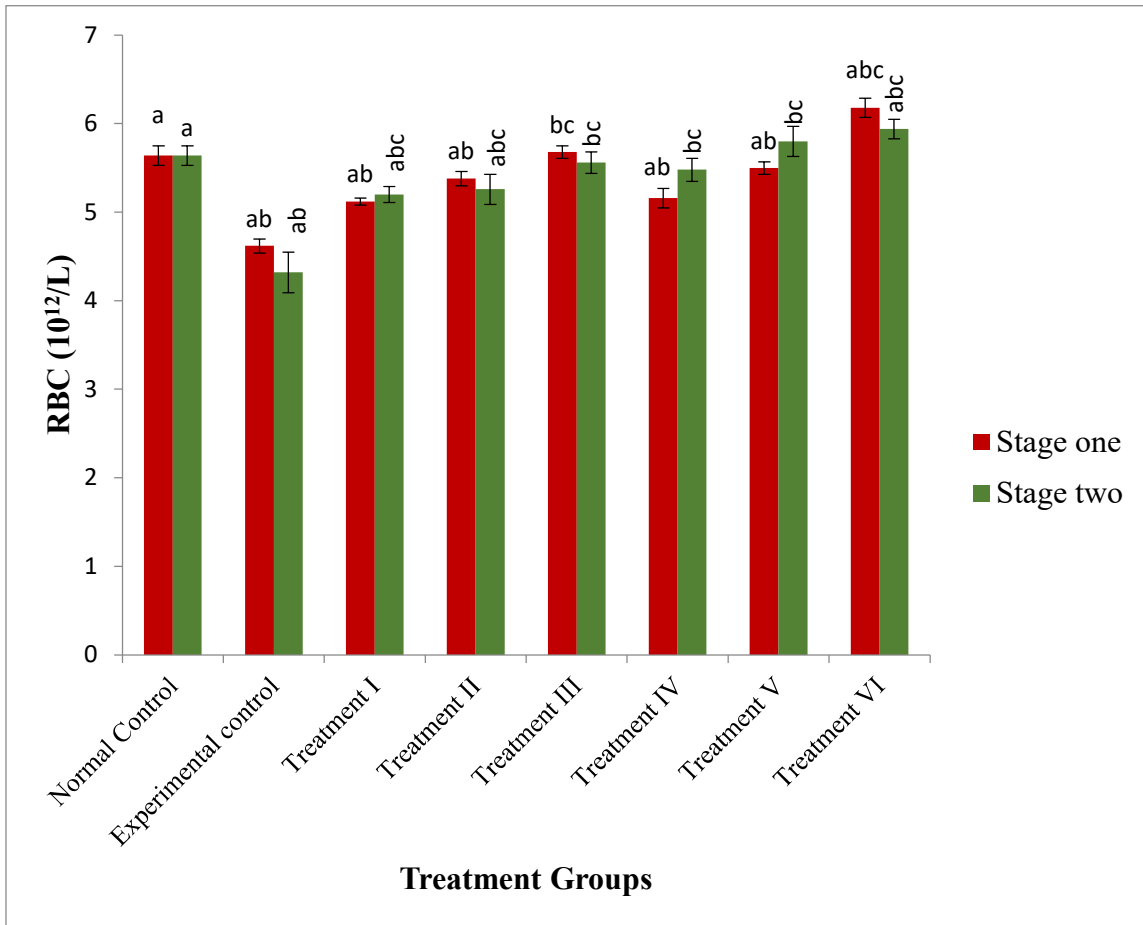


Figure 6: *T. subcordata* leaf extract (nonexposed/dew-exposed) protects the red blood cell count of mifepristone-treated Wistar rats.

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c...) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

Treatments IV, V, and VI vary significantly from Treatments I, II, and III at the p0.05 level, denoted by the superscript C (c).

RBC = red blood cell; b.w. = body mass index

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

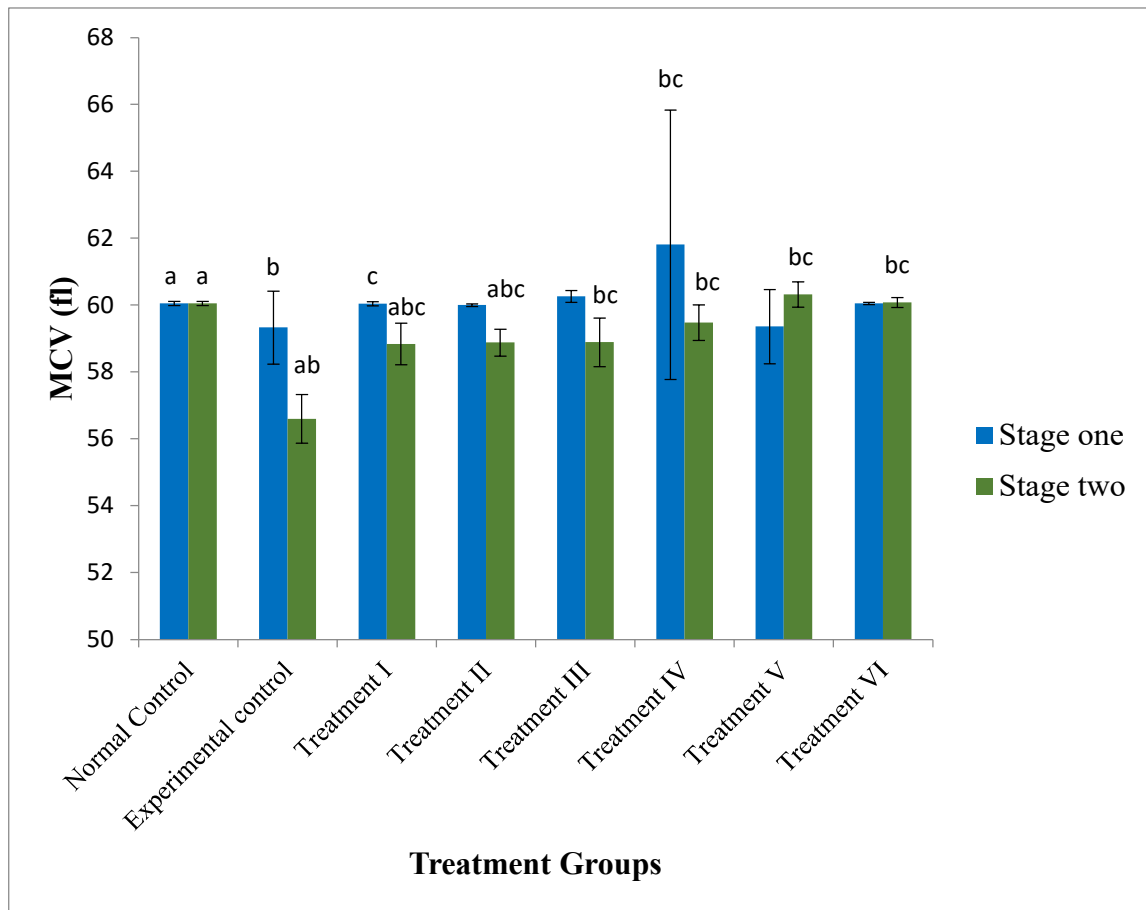


Figure 7: T. subcordata leaf extract (nonexposed/dew-exposed) protects the mean cell volume of mifepristone-treated Wistar rats.

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c...) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

Treatments IV, V, and VI vary significantly from Treatments I, II, and III at the p0.05 level, denoted by the superscript C (c).

Mean cell volume (MCV) and body weight (b.w.) are abbreviations.

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

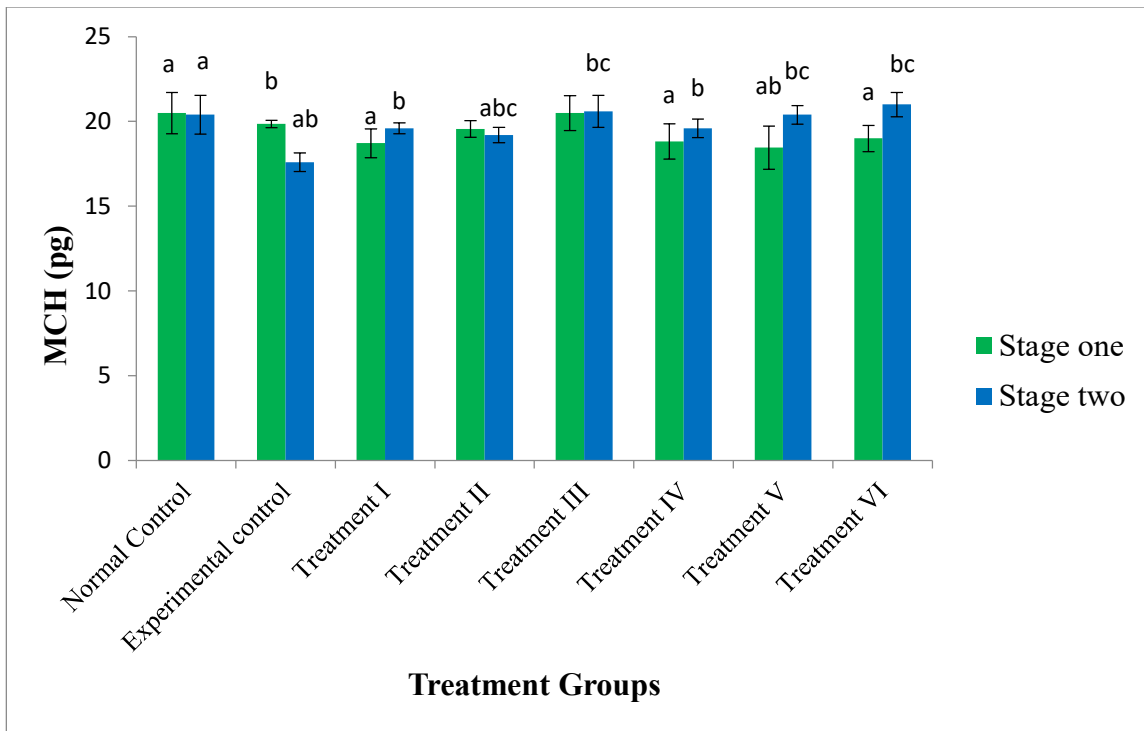


Figure 8: shows the protective effect of *T. subcordata leaf extract* (nonexposed/dew-exposed) on mean cell haemoglobin of mifepristone-administered Wistar rats

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c...) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

Treatments IV, V, and VI vary significantly from Treatments I, II, and III at the p0.05 level, denoted by the superscript C (c).

Mean cell haemoglobin, or MCH, for short.

body mass index

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

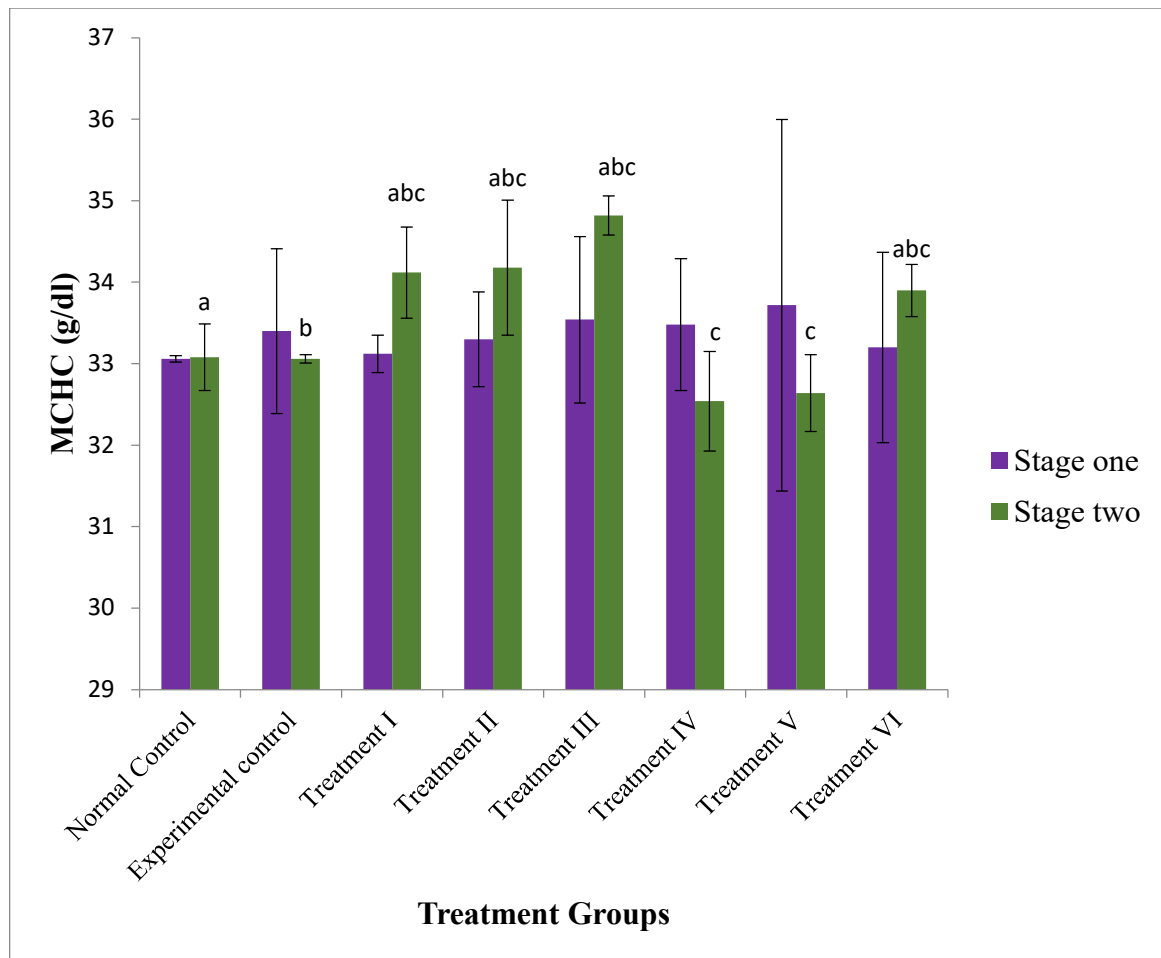


Figure 9: shows the protective effect of *T. subcordata leaf extract* (nonexposed/dew-exposed) on mean cell haemoglobin concentration of mifepristone-administered Wistar rats

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c..) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

Treatments IV, V, and VI vary significantly from Treatments I, II, and III at the p0.05 level, denoted by the superscript C (c).

Mean cell haemoglobin concentration (MCHC)

b.w. (body weight)

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

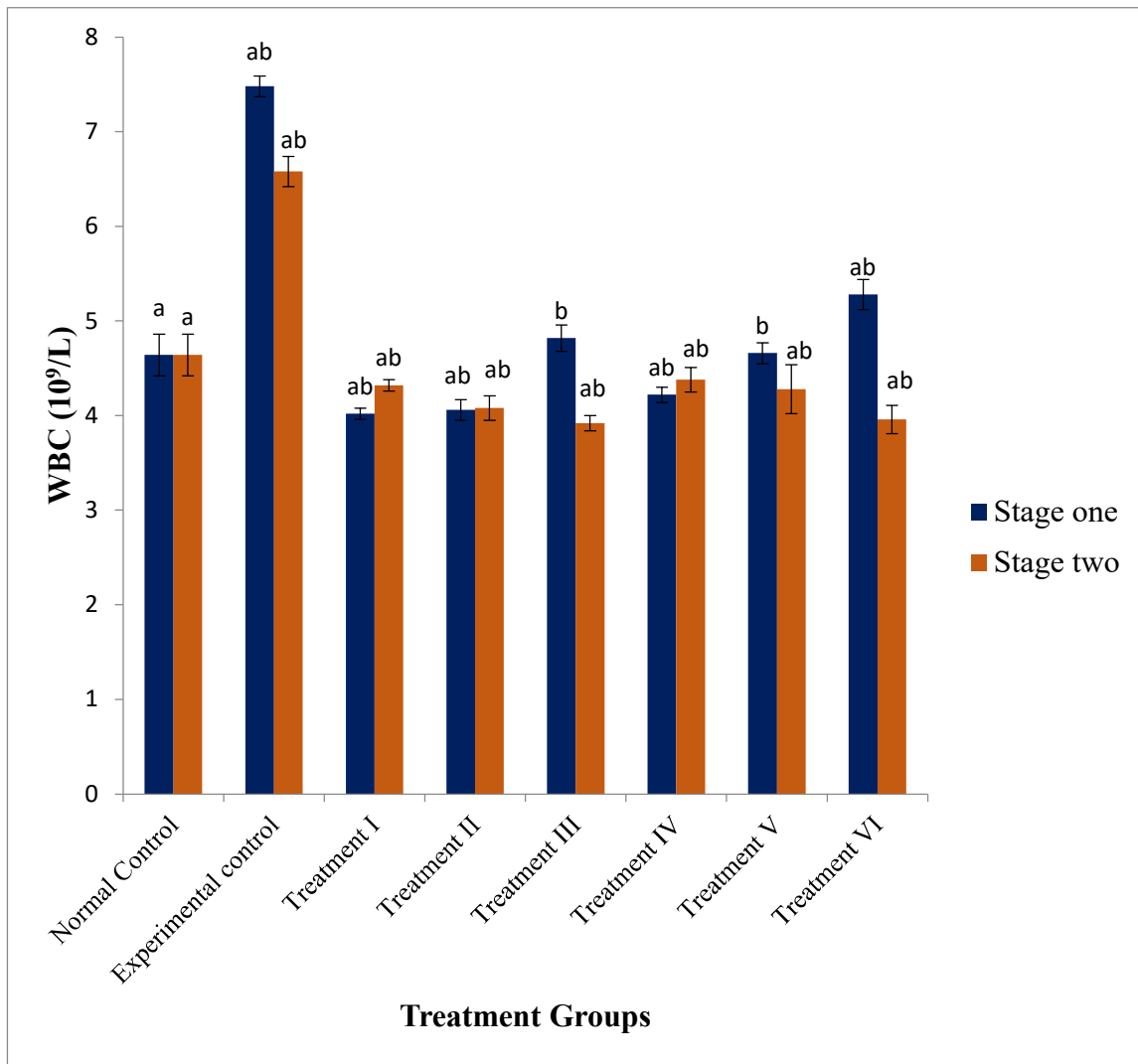


Figure 10: shows the protective effect of *T. subcordata* leaf extract (nonexposed/dew-exposed) on white blood cell count of mifepristone-administered Wistar rats.

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c...) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

White blood cell (WBC); b.w. body weight (b.w.)

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

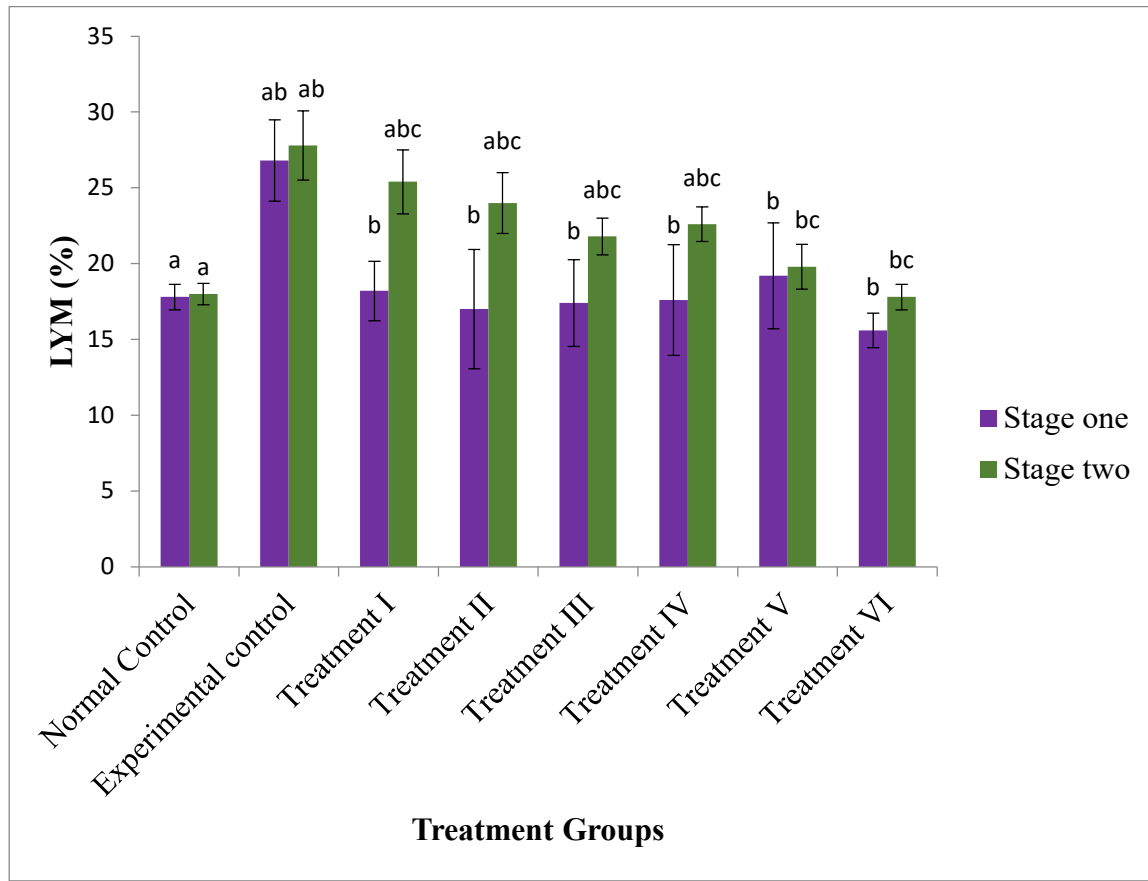


Figure 11: shows the protective effect of *T. subcordata* leaf extract (nonexposed/dew-exposed) on lymphocytes percentage of mifepristone-administered Wistar rats

Data shown is the mean (S.D.) from five separate analyses (n=5). Superscript letters (a, b, c, ..) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

Treatments IV, V, and VI vary significantly from Treatments I, II, and III at the p0.05 level, denoted by the superscript C (c).

Lymphocytes (LYM) and body mass index (b.w.)

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

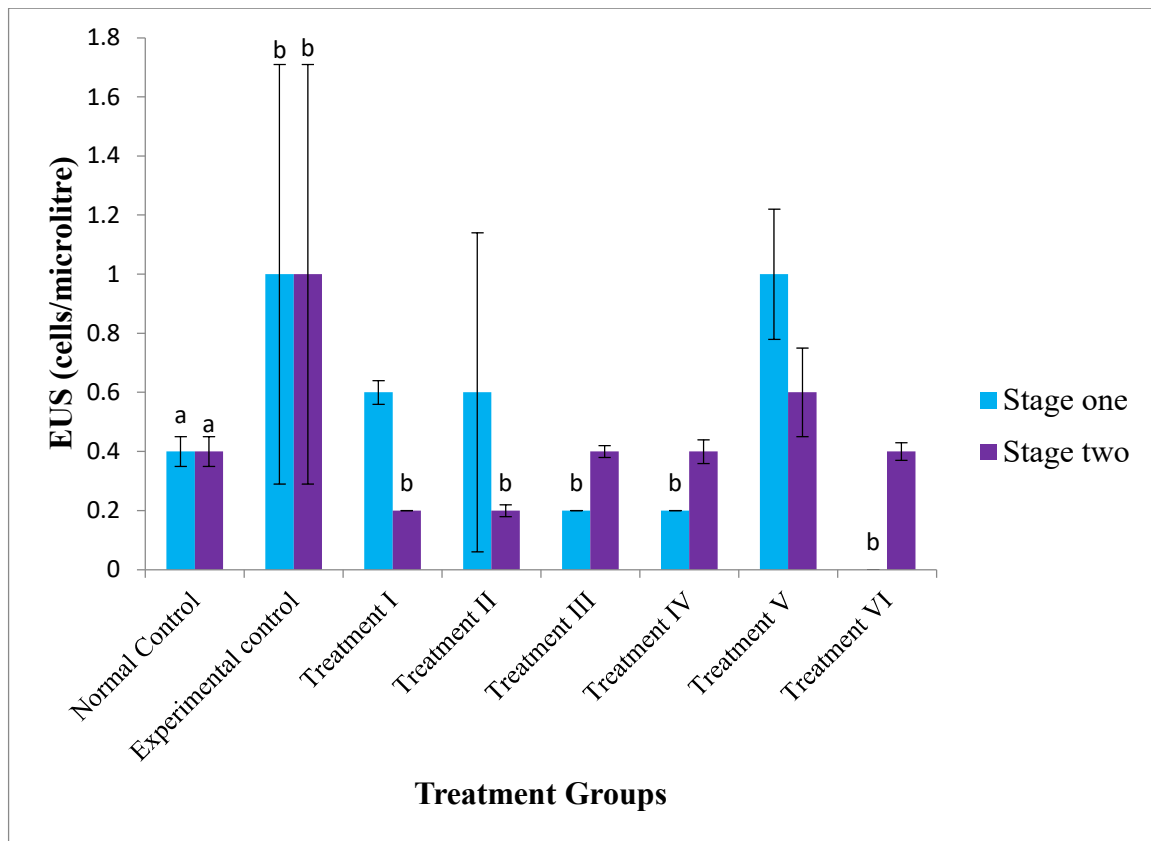


Figure 12: shows the protective effect of *T. subcordata leaf extract* (nonexposed/dew-exposed) on eosinophils count of mifepristone-administered Wistar rats

Mean standard deviation (n=5) of the results. Superscript letters (a, b, c..) on bars indicate statistical significance (p0.05).

Differences indicated by a superscript indicate statistical significance when compared to the baseline control group I (I = p0.05).

When comparing Group II (the experimental control), a value of b indicates statistical significance at the 0.05 level.

EUS = eosinophils

b.w. = body weight

First-stage rats received therapy for five days.

Rats that were treated for a total of seventeen days constitute Stage 2.

Effect of Aqueous Leaf Extract of (Nonexposed/Dew-Exposed) *T. subcordata* Oliv on the Uterus of the Female Wistar Rats.

The impact of *Triclisia subcordata* leaf extract (nonexposed/dew-exposed) on mifepristone-treated albino rats was investigated using histopathology.

H&E staining; magnification X400

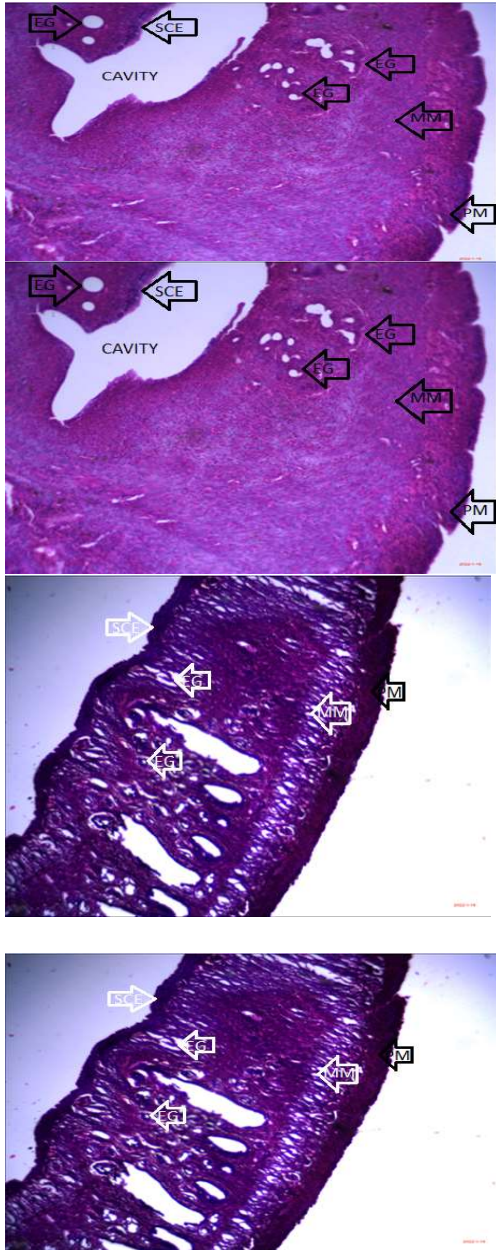


Plate 1: Photomicrograph of uterus section of a **stage one** normal control rat shows histologically normal uterus with empty cavity line by simple columnar epithelia cells (SCE), the endometrium contains endometrial glands (EG), myometrium (mm) contains smooth muscles, perimetrium (pm) covers the outer part of the uterus.

Plate 2: Photomicrograph of uterus section of a **stage two** normal control rat shows histologically normal uterus with empty cavity line by simple columnar epithelia cells (SCE), the endometrium contains endometrial glands (EG), myometrium (mm) contains smooth muscles, perimetrium (pm) covers the outer part of the uterus.

Plate 3: Photomicrograph of uterus section of a **stage one** experimental control rat showing slight deformation and enlargement of uterus section, endometrium contain empty gland (EG), luminal borders lined with simple columnar epithelium (SCE), myometrium (mm) contain vacuoles but an intact perimetrium (pm).

Plate 4: Photomicrograph of uterus section of a **stage two** experimental control rat showing slight deformation and enlargement of uterus section, endometrium contain empty gland (EG), luminal borders lined with simple columnar epithelium (SCE), myometrium (mm) contain vacuoles but an intact perimetrium (pm).

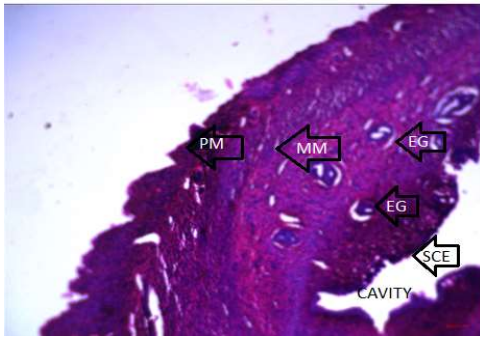


Plate 5: Photomicrograph of uterus section of a stage one rat that received mifepristone plus 100 mg/kg *Triclisia subcordata* leaf extract (nonexposed) showing endometrium lined with simple columnar epithelium (SCE), endometrial glands (EG) are small, intact myometrium (mm), intact perimetrium (pm) and empty cavity. This implies histological normal uterus section.

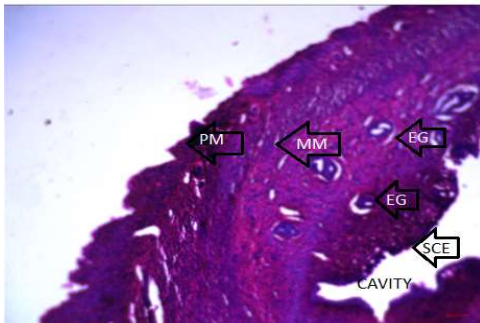


Plate 6: Photomicrograph of uterus section of a stage one rat that received mifepristone plus 100 mg/kg *Triclisia subcordata* leaf extract (dew-exposed) showing endometrium lined with simple columnar epithelium (SCE), endometrial glands (EG) are small, intact myometrium (mm), intact perimetrium (pm) and empty cavity. This implies histological normal uterus section.

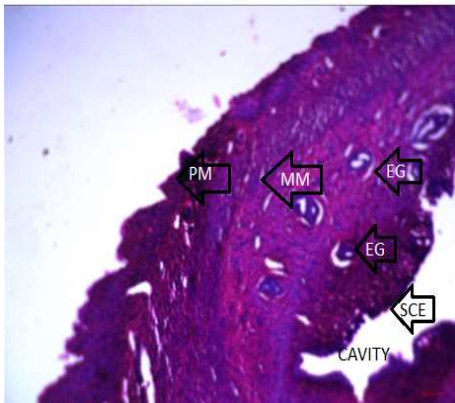


Plate 7: Photomicrograph of uterus section of a stage one rat that received mifepristone plus 200 mg/kg *Triclisia subcordata* leaf extract (nonexposed) showing endometrium lined with simple columnar epithelium (SCE), endometrial glands (EG) are small, intact myometrium (mm), intact perimetrium (pm) and empty cavity. This implies histological normal uterus section.

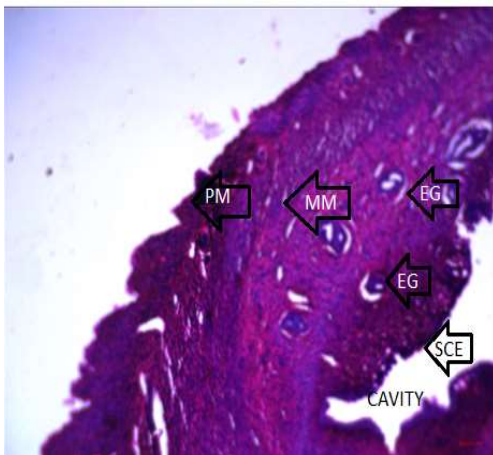


Plate 8: Photomicrograph of uterus section of a stage one rat that received mifepristone plus 200 mg/kg *Triclisia subcordata* leaf extract (dew-exposed) showing empty uterine cavity, endometrium containing numerous glands that are devoid of secretions, luminal border covered with simple columnar epithelium (SCE), intact myometrium (mm) and intact perimetrium (pm). This implies histological normal uterus section.

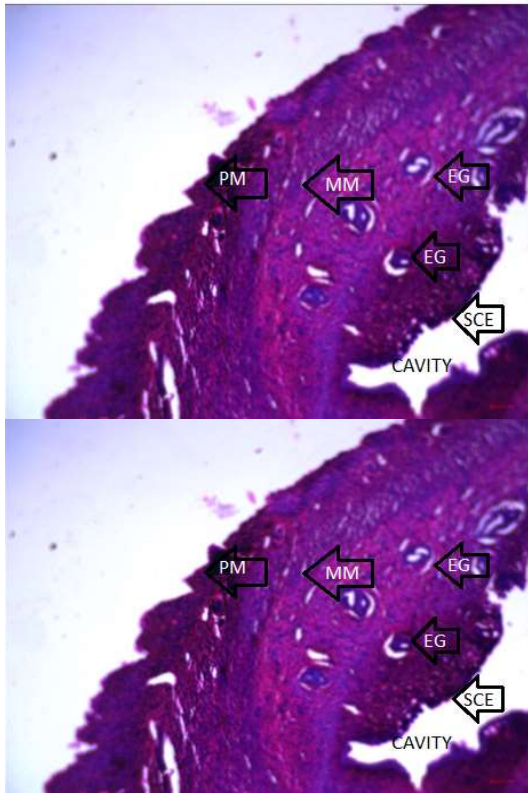


Plate 9: Photomicrograph of uterus section of a stage one rat that received mifepristone plus 300 mg/kg *Triclisia subcordata* leaf extract (nonexposed) showing endometrium lined with simple columnar epithelium (SCE), endometrial glands (EG) are small, intact myometrium (mm), intact perimetrium (pm) and empty cavity. This implies histological normal uterus section.

Plate 10: Photomicrograph of uterus section of a stage one albino rat that received mifepristone plus 300 mg/kg *Triclisia subcordata* leaf extract (dew-exposed) showing empty uterine cavity, endometrium containing numerous glands that are devoid of secretions, luminal border covered with simple columnar epithelium (SCE), intact myometrium (mm) and intact perimetrium (pm). This implies histological normal uterus section.

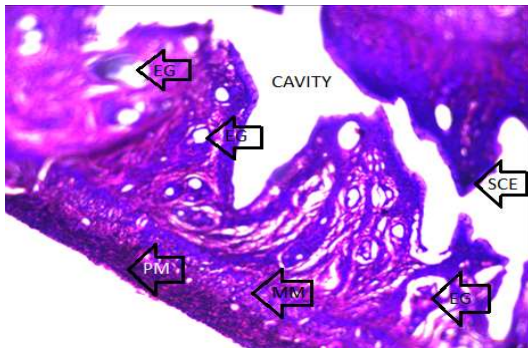


Plate 11: Photomicrograph of uterus section of a stage two rat that received mifepristone plus 100 mg/kg *Triclisia subcordata* leaf extract (nonexposed) showing empty cavity, endometrium lined with simple columnar epithelium (SCE) and contains empty gland (EG), intact myometrium (mm) and intact perimetrium (pm). This implies histological normal uterus section.

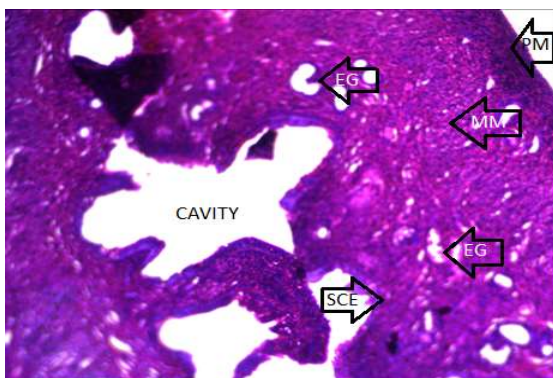


Plate 12: Photomicrograph of uterus section of a stage two rat that received mifepristone plus 100 mg/kg *Triclisia subcordata* leaf extract (dew-exposed) showing empty cavity, endometrium lined with simple columnar epithelium (SCE) and contains empty gland (EG), intact myometrium (mm) and intact perimetrium (pm). This implies histological normal uterus section.

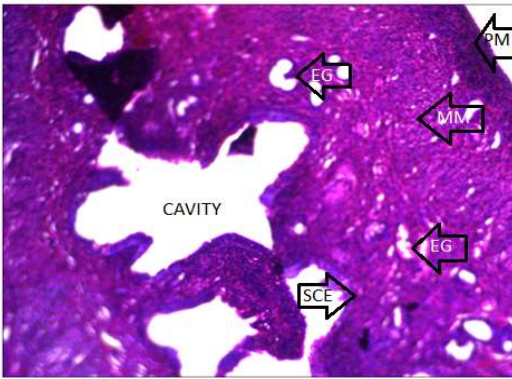


Plate 13: Photomicrograph of uterus section of a stage two rat that received mifepristone plus 200 mg/kg *Triclisia subcordata* leaf extract (nonexposed) showing empty cavity, endometrium lined with simple columnar epithelium (SCE) and contains empty gland (EG), intact myometrium (mm) and intact perimetrium (pm). This implies histological normal uterus section.

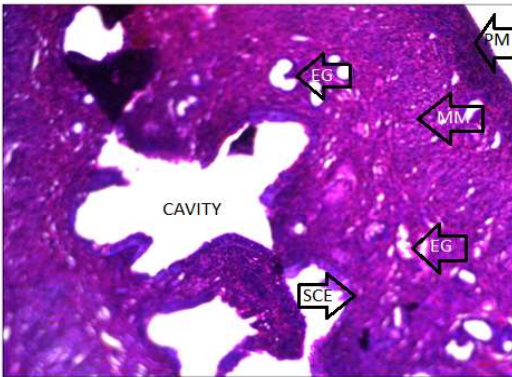


Plate 14: Photomicrograph of uterus section of a stage one rat that received mifepristone plus 200 mg/kg *Triclisia subcordata* leaf extract (dew-exposed) showing empty cavity, endometrium lined with simple columnar epithelium (SCE) and contains empty gland (EG), intact myometrium (mm) and intact perimetrium (pm). This implies histological normal uterus section.

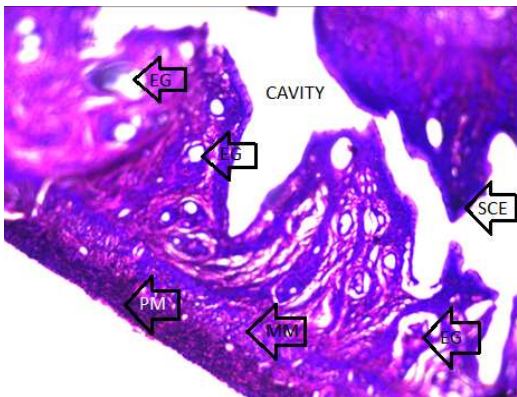


Plate 15: Photomicrograph of uterus section of a stage two rat that received mifepristone plus 300 mg/kg *Triclisia subcordata* leaf extract (nonexposed) showing empty uterine cavity, endometrium containing numerous glands that are devoid of secretions, luminal border covered with simple columnar epithelium (SCE), intact myometrium (mm) and intact perimetrium (pm).

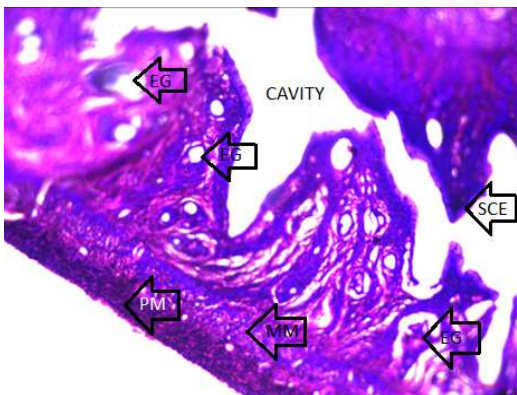


Plate 16: Photomicrograph of uterus section of a stage two rat that received mifepristone plus 300 mg/kg *Triclisia subcordata* leaf extract (dew-exposed) showing empty uterine cavity, endometrium containing numerous glands that are devoid of secretions, luminal border covered with simple columnar epithelium (SCE), intact myometrium (mm) and intact perimetrium (pm). This implies histological normal uterus section.

DISCUSSION

As can be seen in Table 1, dew includes a broad range of elements, including cations such as calcium (Ca), magnesium (Mg), sodium (Na), potassium (k), iron (Fe), zinc (Zn), and manganese (Mn), as well as anions such as sulphate (SO₄), chloride (Cl), nitrates (NO₃), and organic carbon (C) in appreciable amounts. Cations and anions can be seen as macrominerals and microminerals. Out of the seven macro minerals needed for foetal growth and development, the dew content contains six namely Ca, Mg, Na, K, Sulphate and Chloride. Fe, Zn and Mn are needed in small quantity for proper growth. Inadequate intake of these minerals during pregnancy/gestation has been associated with low foetal growth, IUGR (intrauterine growth restriction), preeclampsia, preterm delivery, uterine hyperexcitability, insulin resistance, adverse neurodevelopmental outcomes and metabolic syndrome later in life (Fanni *et al.*, 2021; Khayat *et al.*, 2017; Dawson *et al.*, 2015; Kumar and Kaur, 2017). The amount of these macro and microminerals present in the dew in conjunction with the leaf extract may be effective in protecting against the aforementioned threats.

Aqueous leaf extract (nonexpose/dew-exposed) of *T. subcordata* was studied for its impact on hormonal and haematopoietic parameters in mifepristone-treated rats. We paid particular attention to *T. subcordata* oliv leaves because they could include natural plant components that are helpful as preventative measures in preserving a pregnancy. Figures 1-3 show the changes in reproductive hormone levels caused by *T. subcordata* leaf nonexposed extract and *T. subcordata* leaf dew-exposed extract in rats that had been treated with mifepristone. Pregnancy termination medication mifepristone acts as an antiprogestosterone by preventing hormones from reaching the uterus and maintaining the uterus's natural structure. Yakubu *et al.* (2010) found that the levels of FSH, oestrogen, and progesterone in mifepristone-administered rats were significantly ($p < 0.05$) lower than in the normal control group, and these findings are supported by the present investigation. Consistent with the findings of prior studies, mifepristone is considered an abortifacient (Odoh *et al.*; 2020) and Telleria and Deis (1996). *T. subcordata* leaf

nonexposed extract/dew-exposed extract on the other hand, had a protective effect on the experimental rats, as shown by increases in follicle stimulating hormone, oestrogen, and progesterone concentrations among treated groups in a dose-dependent manner, with 300mg/kg bw providing the best protection, particularly in stage 2. The extract from the leaves was helpful on its own, but it was more effective when exposed to dew.

When compared to the experimental group, both the nonexposed extract and the dew-exposed extract boosted FSH levels dose-dependently ($P < 0.05$) in stage one, however FSH levels fell when the extract was administered continuously (stage two). These findings are consistent with those of (Stilley and Segaloff, 2018), who reported that FSH is no longer needed as such for pregnancy to continue because of the high production of oestrogen and progesterone. This occurs because both oestrogen and progesterone have a suppressive effect on the hypothalamus and pituitary gonadotropes. Given the importance of the pituitary-gonadal axis to reproductive health, its disruption may have negative consequences (Amah et al., 2012; Koneri et al., 2006; Yama et al., 2011).

In stage two, the oestrogen (E_2) levels in the treatment group were significantly higher than the experimental control groups ($P < 0.05$). Since the rise was dose-dependent, we found that 300 mg/kg body weight was optimal.

Progesterone level was significantly increased ($P < 0.05$) dose dependently both at stage one and two when compared to the experimental control. This increment especially in stage two was massive indicating that the extract (nonexposed and dew-exposed) is capable of protecting the rats from mifepristone toxicity thereby sustaining the pregnancy till birth. The action of the leaf extract might be due to its antioxidant properties (Ayoola *et al*; 2017, Akinwunmi *et al*; 2020) and the presence of phytochemicals. Phytochemical screening, as reported by Benie et al. (2003) and Yakubu et al. (2005), has shown several bioactive and detrimental components of

plant extract that may affect ovulation regulation, fertility, and reproduction. Flavonoids raise progesterone and estrogen levels in the blood plasma (Lecomte et al., 2017; Greco et al., 2021). This suggests that the observed changes in circulating hormone levels may be explained by the presence of this phytochemical in *T. subcordata* leaves (Okpara et al., 2021).

Increases in both oestrogen and progesterone were noticeable in dew-exposed extract compared to results from using simply the leaf extract (nonexposed) ($P < 0.05$). Perhaps this is because dew contains minerals (such as calcium, magnesium, potassium, sodium, zinc, iron, manganese, chlorine, and sulfur) that are essential for a developing fetus's health during gestation.

The effects of *T. subcordata* leaf extract (nonexposed/dew-exposed) on the haematological parameters of mifepristone-administered rats were evaluated (Figures 4–12). The extract affected the haemopoietic systems of the test animals in a dose-dependent manner. Experimental control rats treated with mifepristone had significantly lower packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) count, mean cell volume (MCV), and mean cell hemoglobin (MCH) than normal rats, whereas rats given the extract (nonexposed/dew-exposed) had much higher values when compared with experimental control for these measures. In particular, at stage 2, the mean cell hemoglobin concentration (MCHC) of extract-treated rats (groups 3, 4, 5 and group 8 of dew-exposed extract) was substantially greater than that of experimental control rats at the $P < 0.05$ level.

Reduced red blood cell mass (RBC) is often related with low PCV. As expected, this was reflected in a decrease in RBC and Hb levels, but although this was also the case in the mifepristone group in this study. Treated rats exhibited significant improvement ($P < 0.05$), demonstrating the plant's safety for red blood cells. These results agree with those found by Ikewuchi, hence his work is credible (2012). An increase in packed cell volume, hemoglobin, red blood cell count, mean cell volume, and mean cell hemoglobin are all positive results that

have been seen with the use of this plant in the treatment of anemia. As red blood cells and hemoglobin (Hb) are required for transferring breathing gases, *T. subcordata* leaves or leaf extract (nonexposed/dew-exposed) may increase erythropoietin synthesis from the kidneys, the blood's oxygen-carrying capacity, and the amount of oxygen delivered to the tissues (Airaodion et al., 2019). These properties may be attributable to the appreciable amount of protein contained by the leaves (Okpara *et al.*, 2021) and the presence of iron seen in the dew making the rats administered with dew-exposed extract more potent. Its protective effect on erythrocyte parameters against mifepristone toxicity might also be due to their phytochemicals and antioxidants. These cells and haemoglobin (Hb), which are crucial for transferring breathing gases, may restrict the release of erythropoietin from the kidneys, the humoral regulator of RBC synthesis, which may explain the reduction in erythrocyte parameters reported in the blood of mifepristone-administered rats in this study. Mifepristone may have undiscovered side effects on tissue oxygenation and blood oxygen transport (Polenakovic and Sikole, 1996; Oyedeji et al., 2013).

Counts of white blood cells, lymphocytes, and eosinophils were significantly ($P < 0.05$) higher in experimental control rats due to mifepristone toxicity compared to normal control rats; however, they were significantly ($P < 0.05$) lower in rats treated with extract (nonexposed) and dew-exposed extract, demonstrating that their immune systems were not compromised. This significant changes are observed mainly in stage 2 (at continuous administration of the leaf extract (nonexposed/dew-exposed)). A high peripheral white blood cell count is indicative of coronary artery disease and inflammation, and white blood cells have been linked to the destabilization of coronary artery plaques at the onset of acute coronary syndrome (Morreno et al., 1994; van Der Wal et al., 1994; Libby, 2001; Takeda et al., 2003). (Takeda et al., 2003; Chillaci et al., 2007; Kho et al., 2007). Accordingly, the decreased incidence of coronary artery disease and the lower white blood cell (WBC) counts reported in rats fed with *T. subcordata*

leaf extract (nonexposed and dew-exposed) at all doses show that this plant's extract has the ability to protect against the increase in WBC generated by mifepristone. White blood cell (WBC) composition, and lymphocytes in particular, have been linked to enhanced performance in very stressful situations (Farombi, 2003). An increase in the proportion of white blood cells and lymphocytes suggests that the bioactive compounds present in the extracts stimulated a stress response.

Photomicrograph upon sectioning (Plate 1-16) of the uteri of all groups except group 2 (experimental control) showed normal uterine architecture. Group 2 showed slight deformation and enlargement of uterus section which was corrected by the extract (nonexposed/dew-exposed) at different doses (groups 3-8). This implies that the extract (nonexposed/dew-exposed) greatly attenuate toxicity caused by mifepristone in pregnant rats, as shown by these results.

Conclusion

Even though mifepristone is toxic, *T. subcordata* is still able to stimulate haemopoiesis in Wistar rats. Oestrogen and progesterone levels are raised while the follicle stimulating hormone level remains stable, guaranteeing a safe pregnancy up to the time of delivery. Increased blood levels in pregnant rats suggest that *T. subcordata* might be marketed as a fertility-boosting vegetable and herbal medication that mitigates the negative effects of mifepristone on blood counts.

Pregnancy preservation is enhanced by the exposure of *T. subcordata* leaf extract to dew.

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