Methanolic Extract of *Tapinanthus bangwensis* Exhibits Pro-Fertility and Antioxidant Activities in Female Albino Rats

ABSTRACT

Aims: To determine the effect of methanolic extract of *Tapinanthus bangwensis* on reproductive hormones and antioxidant enzymes in female Wistar rats.

Study Design: Experimental Research.

Place and Duration of Study: Department of Biochemistry, Lagos State University, Lagos Nigeria between November 2019 and February 2020.

Methodology: A total of 40 rats were used and were divided into eight groups of 5 rats. The study was carried out for 21 days, the rats were induced with Postinor-2 for 7 days after which *Tapinanthus bangwensis* extract was administered for 14 days and was compared against the vehicle (oil), Postinor-2, and extract controls. Hormonal level estimation (Progesterone and prolactin) and *in-vivo* antioxidant enzyme activity (catalase, superoxide dismutase (SOD), and alkaline phosphatase activity) were estimated using standard procedures.

Results: The extract administration showed a significant increase ($p<0.01$, $p<0.05$) in the level of prolactin concentration in the PLD group (2.027ng/ml) compared to other groups. Also, there was an increased ($p<0.01$) progesterone concentration in the ILD group (65.35ng/ml) as compared to other groups (CIC). The extract-only (EO) group had the highest level of catalase activity (2.331U/ml), compared with all extract groups and there was no significance in the SOD level across the different groups. Lastly, there was a significant increase in the activity of alkaline phosphatase in the EO and ILD groups ($p<0.05$) compared to the COO group.

Conclusion: The ethanolic extract of *Urtica dioica* roots exhibits pro-fertility and antioxidant activities.

Keywords; *Infertility, Reproductive hormones, Oxidative stress, Tapinanthus bangwensis.*

1.0 INTRODUCTION

"Demographers define infertility as the absence of live birth in a sexually active non-contracepting woman" [1]. "This approach is more in line with the public perception, as well as the dictionary definition, as couples seek live birth rather than conception. Lack of agreement on outcomes representing reproductive 'success' (e.g. pregnancy, ongoing pregnancy, and live birth) has led to considerable variation in the estimated prevalence of infertility" [2]. "There are many causes of infertility, including some that medical intervention can treat" [3]. "Male infertility is responsible for 20–30% of infertility cases, while 20–35% are due to female infertility, and 25–40% are due to combined problems in both parties [4]. In 10–20% of cases, no cause is found" [4]. "The most common cause of female infertility is ovulatory problems, manifesting through sparse or absent menstrual periods" [5].

Several research studies have shown that an imbalance in the hormonal levels leads to infertility in females through several actions like ovulation disruption, prevention of the thickening of the uterine lining, or in most cases preventing the establishment of pregnancy [6]. "A balance between free radicals and antioxidants is necessary for the proper physiological function of the body system. If free radicals are more than the body's ability to regulate them, a condition known as oxidative stress occurs" [7]. Reactive oxygen species have been implicated in the development of premature rupture of the fetal membranes[8] and evidence suggests that oxidative stress may be associated with pre-eclampsia) [9].

Various plant extracts have been analyzed over the years to check their medicinal effect on the enhancement of fertility in male and female populations [10]. Mistletoe, with the common name(s) – bird lime, all-heal, devil's fuge, Iscador, etc. (*Tapinanthus bangwensis*) belongs to the family Loranthaceae [11]. "It is a well-known evergreen parasitic plant, which grows on deciduous trees in a ball-like bush" [12]. It is an excellent medicinal plant. These hemiparasitic plants, mistletoes of the Loranthaceae and Viscaceae, are widely used in various cultures on almost every continent to treat ailments including hypertension, cancer, and diabetes, or used as a diuretic agent [13]. "Traditionally, extracts of mistletoes (*Tapnanthus bangwensis*) have been used against a variety of diseases such as disorders in the female reproductive system, cancer, arthritis, rheumatism, epithelial tumour, hypertension, asthma, nervousness, and epilepsy" [14]. Phytoconstituents of the leaves of *Tapinanthus bangwensis* (methanolic extract) have been said to have anti-cancer, antibacterial, antidiabetic, and hepatoprotective properties [15]. The methanolic extract (MeE) of leaves of *T. bangwensis* has hepatoprotective effects on CCl_4-induced hepatic damage in rats [16]. While the anti-inflammatory activity exhibited by the methanolic extract and fractions of *T. bangwensis* may be attributed to the presence of flavonoids and tannins present in the plant [17]. Despite the local usage of *Tapinanthus bangwensis* in the treatment of female fertility disorders, there is little or no scientific evidence supporting the usage. Hence this study aims to

investigate the profertility effect of the methanolic leaf extract of *Tapinanthus bangwensis* by evaluating the hormone modulatory and antioxidant potentials in female albino rats.

2.0 METHODOLOGY

2.1 COLLECTION OF PLANT SAMPLES

Fresh leaves of *Tapinanthus bangwensis* were collected from a local farm in Ogun state, Nigeria. It was properly identified and authenticated, LUH 8560 at the Herbarium unit of the Botany Department, University of Lagos, Nigeria.

2.2 METHANOLIC EXTRACTION OF *Tapinanthus bangwensis*

The soxhlet extraction method was employed for the extraction of the methanolic extract of *Tapinanthus bangwensis*. The extraction method is based on the solubility of the constituents of the *Tapinanthus* sample in methanol. 200g of dried and blended *Tapinanthus* was placed into the thimble of the soxhlet extraction apparatus chamber using 2 litres of 85% methanol. The thimble was loaded into the main chamber of the soxhlet extractor, and the sample was extracted for 24 hours. After extraction, the solvent was removed using a water bath, yielding 15.22 grams of the extract.

2.3 PHYTOCHEMICAL SCREENING

Qualitative phytochemical screening was carried out on the methanolic extract of the plant sample using standard procedures to identify the phytoconstituents as described by [18].

2.4 COLLECTION AND ACCLIMATIZATION OF ANIMALS

Female albino rats numbering 49, weighing between 60-100g were obtained from the animal house of the Department of Biochemistry, Lagos University Teaching Hospital, Lagos state. The rats were allowed to acclimatize for two weeks. The animals fed with commercial rat feed were kept under hygienic and favorable conditions and maintained under a 12h light/ 12h dark cycle, with rat feeds and water available. After acclimatization for two weeks then the commencement of extract administration began.

2.5 CHRONIC TOXICITY TEST

The toxicity test of the extract was carried out to determine safe dosages for extract administration. A total of nine rats were randomly selected with an average weight of 125g and divided into three groups each containing three rats [19]. The dosages tested for are:

1. 80mg/kg for group 1
2. 200mg/kg for group 2
3. 400mg/kg for group 3

The different extract doses were dissolved in 1ml of carrier oil for administration to the rats. After 24 hours of dosage administration, no death was recorded.

2.6 ANIMAL GROUPING

The animals were divided into eight groups, with an average weight of 122g. Every group contained five animals each and was treated as follows:

Group 1- the control distilled water group (fed 1ml of distilled water): CDW

Group 2- carrier oil only group (fed 1ml of carrier oil): COO

Group 3- the infertile control group (fed 0.14mg/g Postinor-2): CIC

Group 4- pregnant control group (pregnant rats fed 1ml of distilled water): PC

Group 5- extract only group (fed with 12mg/g *T. bangwensis* extract): EO

Group 6- infertile + low-dose extract (fed 0.14mg/g Postinor-2 for 7 days and later administered 12mg/g of extract for 14 days): ILD

Group 7- infertile + high-dose extract (fed 0.14mg/g Postinor-2 for 7 days and later administered 25mg/g of extract for 14 days): IHD

Group 8- pregnant + low-dose extract (pregnant rats fed 12mg/g extract only for 14 days): PLD

All dosage was administered via the oral route.

2.7 COLLECTION OF BLOOD AND ORGANS

The animals were sacrificed after 2 weeks of treatment with the extract. They were anaesthetized with petroleum ether and blood was collected through cardiac puncture. The organs (liver, kidney, brain, and ovaries) were collected and soaked in a physiological saline solution.

2.8 DETERMINATION OF BIOCHEMICAL PARAMETERS

2.8.1 ESTIMATION OF HORMONES

The reproductive hormones progesterone and prolactin were estimated using the ELISA technique based on the principle of a solid-phase enzyme-linked immunosorbent assay. Thawed serum samples have been assayed for prolactin and progesterone using (Bio-inteco, UK) kits. Hormone level is calculated using a standard curve of the absorbance of the standards against their concentrations and the results are expressed as ng/ml [20].

2.8.2 ESTIMATION OF ANTIOXIDANT ACTIVITY

2.8.2.1 CATALASE ACTIVITY

The catalase activity was measured using Aebi's method [21]. A molar extinction coefficient of 0.041(mmol/l) was used to determine the catalase activity which was then expressed as nmol H2O2 decreased/mg protein/min [22].

2.8.2.2 ESTIMATION OF SOD ACTIVITY

The superoxide dismutase activity was measured using the method of Marklund [23]. One unit of activity was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed as units/mg of protein.

2.8.3 DETERMINATION OF ALKALINE PHOSPHATASE ACTIVITY

The substrate p-nitrophenyl phosphate is hydrolyzed by alkaline phosphatase from the sample in the presence of magnesium ions, to form nitrophenol which is yellow and can be read at 405 nm. The intensity of colour produced is proportional to the activity of alkaline phosphatase. The ALP activity was determined using RANDOX kits (USA). Where: 2760 = Extinction coefficient; ΔA 405 nm/min = change in absorbance per minute for the serum sample [24].

2.8.4 ESTIMATION OF BLOOD GLUCOSE

The blood glucose was determined using an Accu-check glucometer and strip on cardiac blood collected in fluoride oxalate blood collection bottles [25].

2.9 STATISTICAL ANALYSIS

Data were analyzed by one-way analysis of variance (ANOVA), to test for significant differences among the groups of rats using Graph-pad prism software version 8.0, and data were expressed as mean ± standard error of the mean. Bars having *, ** or # are significantly different, taking $P < 0.05$ as the significant level.

3.0 RESULTS

3.1 PHYTOCHEMICAL CONSTITUENTS

The results in **Table 1** show the qualitative and quantitative concentration of the phytochemical constituent of *Tapinanthus bangwensis* leaf indicating the presence of phenol, flavonoid, alkaloid, steroid, reducing sugar, tannin, saponin, and terpenoid as the present phytoconstituents. The result table shows the quantitative analysis which indicates the various concentrations of the phytochemicals: phenol (48.72mg/100g), flavonoid (54.29mg/100g), alkaloid (37.51mg/100g), steroid(24.53mg/100g), reducing

sugar (60.15mg/100g), and tannin (35.45mg/100g). Reducing sugar was the major phytoconstituent of the plant sample.

Table 1. Phytochemicals present in the *Tapinanthus bangwensis* leaf extract

Phytoconstituents	Inference	Concentration(mg/100g)
Phenol	Present	48.73
Flavonoid	Present	54.29
Alkaloid	Present	37.51
Steroid	Present	24.53
Reducing sugar	Present	60.15
Tannin	Present	35.45
Phlobatannin	Absent	
Saponin	Present	
Terpenoid	Present	

3.2 EFFECT ON REPRODUCTIVE HORMONES

The progesterone concentration across the different groups are shown in (***Figure 1)*** as follows; CDW: control distilled water progesterone concentration level (53.19ng/ml), COO: control oil only (32.59ng/ml), PC: pregnant control (52.25ng/ml), CIC: infertile control group (31.77ng/ml), EO: extract only (53.61ng/ml), IHD: infertile high dose (48.93ng/ml), ILD: infertile low dose (65.35ng/ml), PLD: pregnant low dose (52.06ng/ml).

The result as expressed as mean ± SEM; n=5 animals in each group, ** $p<0.01$ shows a statistical significance. This result shows a significant ($p<0.01$) decrease in the COO group compared to the ILD group and also in the CIC Group compared to the ILD group.

*a shows the relationship with COO and b shows the relationship with CIC.

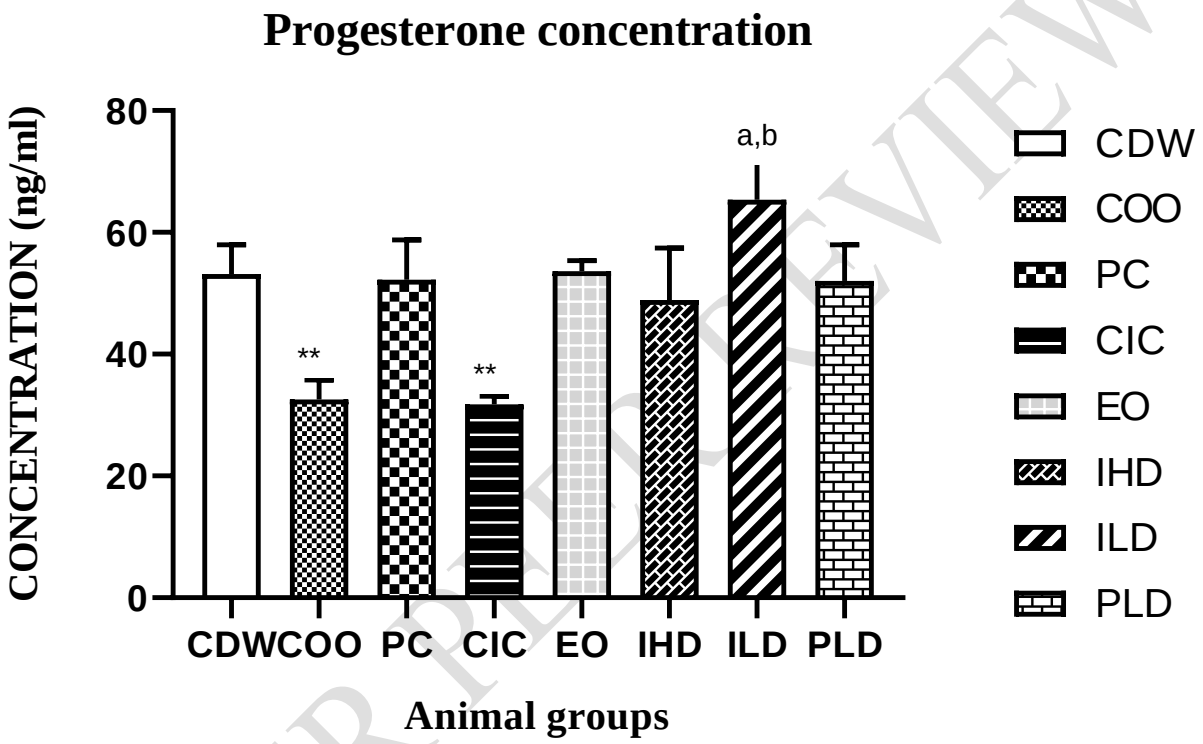

Figure 1. Serum progesterone concentration across different female rat groups.

The result of prolactin concentration across the different groups is shown in (***Figure 2)*** as follows; CDW: control distilled water (1.091ng/ml), COO: control oil only (1.406ng/ml), PC: pregnant control (1.062ng/ml), CIC: infertile control group (1.313ng/ml), EO: extract only (1.137ng/ml), IHD: infertile high dose (1.524ng/ml), ILD: infertile low dose (1.035ng/ml), PLD: pregnant low dose (2.027ng/ml).

The result as expressed as mean ± SEM; n=5 animals in each group, ** $p<0.01$, *$p<0.05$ shows a statistical significance. This result shows a statistical decrease in CDW, PC, EO, and ILD as compared to the PLD group at $p<0.01$ and there is also a significant decrease in the CIC Group as compared to the PLD group at $p<0.05$.

*a shows the relationship with CDW, b shows the relationship with PC, c shows the relationship with CIC, d shows the relationship with EO and e shows the relationship with ILD.

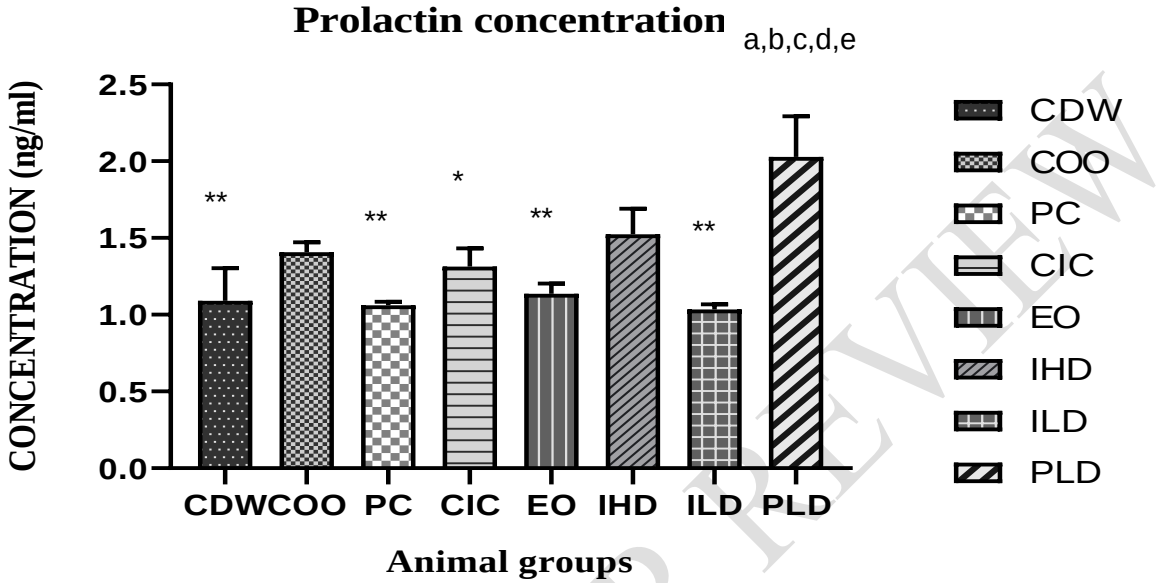

Figure 2. Serum prolactin concentrations across different female rat groups.

3.3 EFFECT ON OVARIAN ANTIOXIDANT ENZYMES

Figure 3 shows the SOD activity in the ovary of the different female rat groups as follows; CDW: control distilled water (70.00U/ml), COO: control oil only (66.67U/ml), PC: pregnant control (60.00U/ml), CIC: infertile control group (63.33U/ml), EO: extract only (66.67U/ml), IHD: infertile high-dose (63.33U/ml), ILD: infertile low dose (60.00U/ml), PLD: pregnant low-dose (46.67U/ml).

The result as expressed as mean ± SEM; n=5 animals in each group, ** $p<0.01$ shows a statistical significance. This result shows no significant difference across the extract groups compared to the controls.

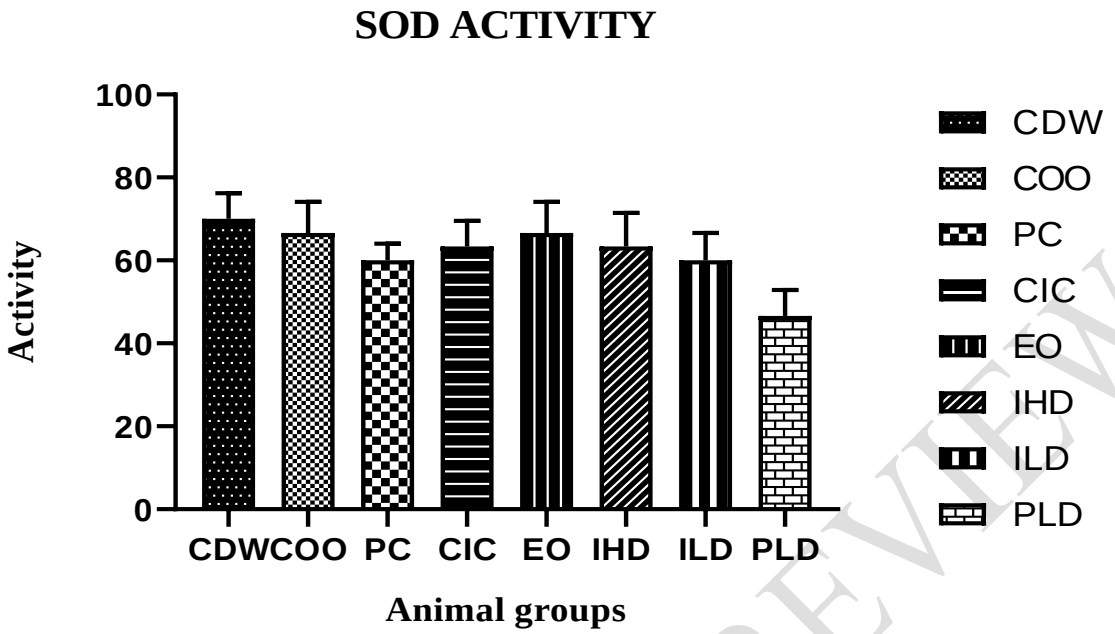

Figure 3. Superoxide dismutase (SOD) activity in the ovary across the different groups.

Figure 4 shows the Catalase activity in the ovary of the different female rat groups as follows; CDW: control distilled water (1.936U/ml), COO: control oil only (1.936U/ml), PC: pregnant control (1.931U/ml), CIC: infertile control group (1.949U/ml), EO: extract only (2.331U/ml), IHD: infertile high dose (2.093U/ml), ILD: infertile low dose (2.046U/ml), PLD: pregnant low dose (2.029U/ml).

The result as expressed as mean ± SEM; n=5 animals in each group, * $p<0.05$ shows a statistical significance. This result shows a statistical decrease in the control groups (CDW, COO, PC, and CIC) as compared to the EO group at $p<0.05$.

*a shows the relationship with CDW, b shows the relationship with COO, c shows the relationship with PC and d shows the relationship with CIC.

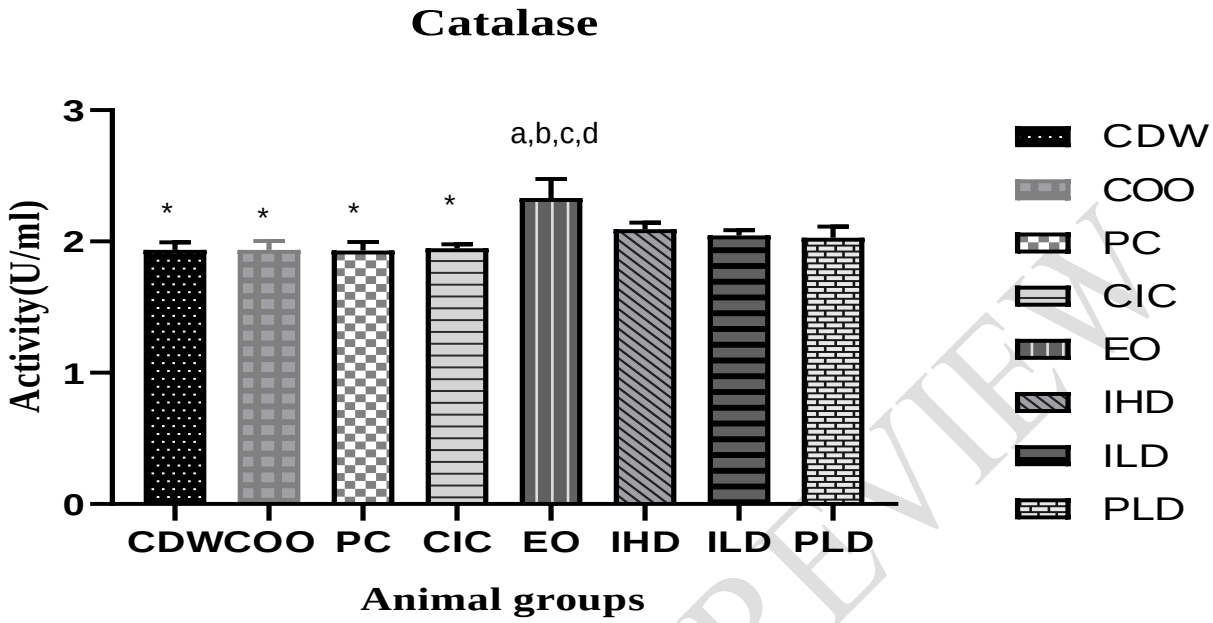

Figure 4. Catalase activity in the ovary across the different groups.

Figure 5 shows the concentration level of alkaline phosphatase in the different groups expressed as mean ± SEM with $p<0.05$ as follows: CDW-control distilled water group (103.6U/l), COO- oil only control (28.98U/l), PC-pregnant control group (71.76U/l), EO- extract only (183.5U/l), IHD- infertile + high dose extract (153.2U/l), ILD- infertile + low dose extract (187.7U/l) and PLD- pregnant + low dose extract (67.62U/l).

This result shows a statistical increase in the EO group as compared to the COO group at $p<0.05$ and also an increase in the ILD group as compared to the COO group at $p<0.05$.

*a shows the relationship with EO and b shows the relationship with ILD.

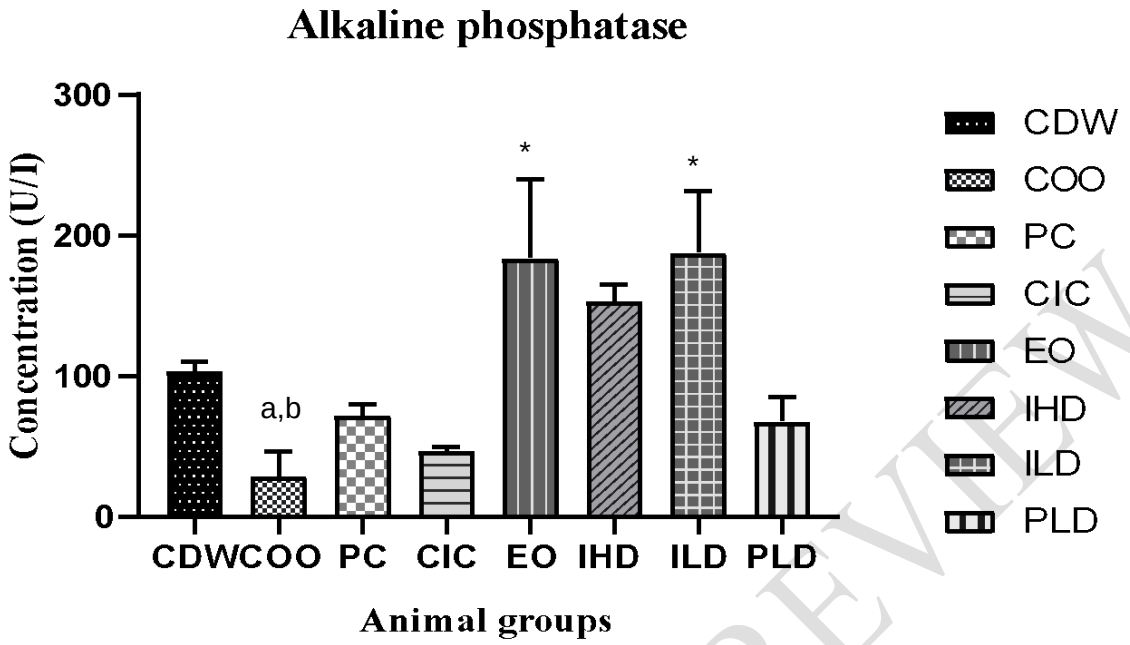

Figure 5. The concentration of alkaline phosphatase across different groups.

3.4 EFFECT ON BLOOD GLUCOSE LEVEL

The blood glucose concentration in the different groups are shown in (***Figure 6)*** as follows; CDW: control distilled water (24.00mg/dl), COO: control oil only (60.00mg/dl), PC: pregnant control (33.33mg/dl), CIC: infertile control group (47.67mg/dl), EO: extract only (31.67mg/dl), IHD: infertile high dose (46.33mg/dl), ILD: infertile low dose (64.00mg/dl), PLD: pregnant low dose (39.00mg/dl).

The result as expressed as mean ± SEM; n=5 animals in each group, ** $p<0.01$, *$p<0.05$ shows a statistical significance. This result shows a statistical decrease in PC and EO groups compared to the ILD group at $p<0.05$ and a statistical decrease in CDW as compared to the ILD group at a value of $p<0.01$.

*a shows the relationship with CDW, b shows the relationship with PC, and c shows the relationship with EO.

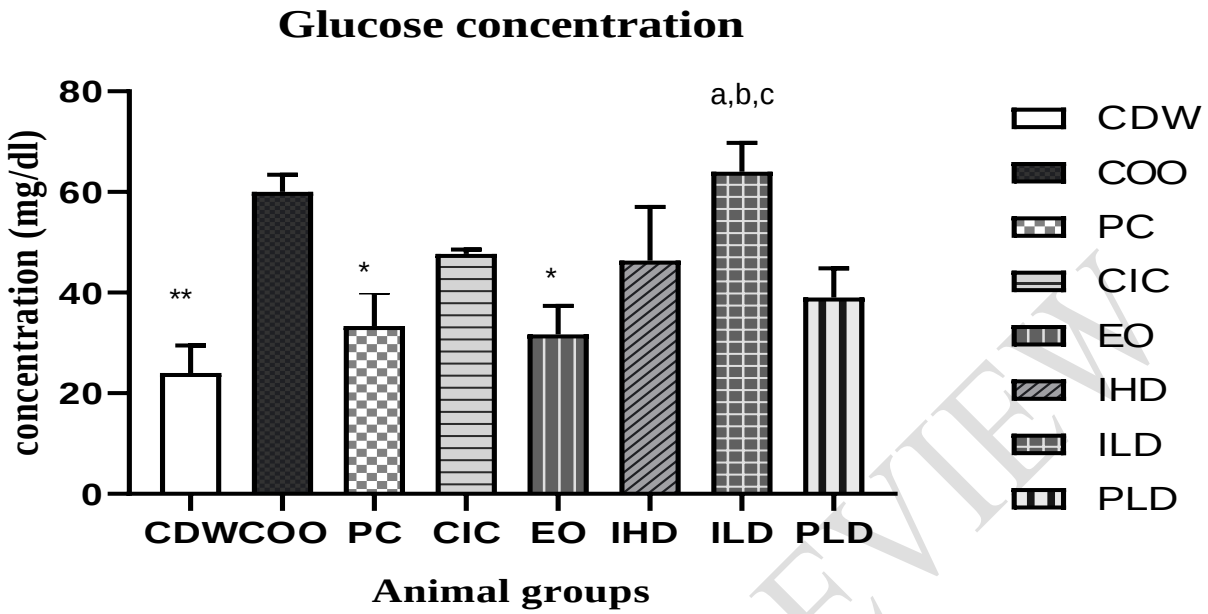

Figure 6. Blood glucose concentrations across different groups.

4.0 DISCUSSION

Infertility is a prominent medical issue that takes its toll on social life in many parts of the world, which require interventions [26]. In this study, the preliminary phytochemical screening of *Tapinanthus bangwensis* leaf extract revealed the presence of reducing sugars and flavonoids in larger amounts with the inclusion of other phytochemicals such as phenols, alkaloids, steroids, tannins, saponins, and terpenoids were also present. This is an indication that the plant possesses some possible antioxidant activities that when properly harnessed could be used in the treatment and management of some diseases.

The methanolic extract of *T. bangwensis* have no significant effect on progesterone concentration. However, continuous administration of the extract to the rats in the group that were induced with infertility showed a significant increase ($p<0.01$) in progesterone concentration compared to the control groups, which is a reversal of the infertility induction. This indicates the progestogenic effect of the extracts, suggesting its profertility potential. Progesterone, also called the pregnancy hormone, is said to be increased in other to help the uterus prepare for and maintain a pregnancy [27].

There is a significant increase in the prolactin concentration ($p<0.05$, $p<0.01$) of the pregnant group fed with the extract (PLD) as compared to the other controls and extract groups, this shows that the extract can be said to possess an enhancing capacity to help increase the level of prolactin due to the increase

of the progesterone levels. According to [28], the upregulation of prolactin gene expression steadily increases due to high progesterone levels during pregnancy. Indeed, progesterone potently stimulates prolactin production. Prolactin is a protein hormone of pituitary origin; however, its synthesis is not limited to the pituitary gland since numerous extra pituitary tissues also express this protein, including the placenta, ovary, testis, mammary gland, skin, adipose tissue, endothelial cells, and immune cells [29]. This widespread prolactin expression might explain its involvement in very different processes such as reproduction, metabolism, immunology, and behaviour [29]. In the immune system, prolactin is thought to act as a locally produced cytokine with relevance for immune regulation and modulation of T- and B-cell function. However, in peripheral blood mononuclear cells (PBMC), [30], prolactin acts by stimulating the secretion of other cytokines and the expression of cytokine receptors, and also as a growth and survival factor. In pathological conditions, increased levels of prolactin could cause deterioration of the subject's condition [31].

The catalase activity of the extract-only group (EO) significantly increased ($p<0.01$, $p<0.05$) compared to all other groups suggesting the antioxidant activity of the methanolic extract, also there were no significant differences in the superoxide dismutase activity (SOD) across the different groups. The antioxidant activity and inhibitory effect of both aqueous and ethanolic extract against lipid peroxidation could be attributed to the phenolic phytochemicals present in the extracts. It could therefore be concluded that both extracts protect the pancreas against oxidative damage, but the aqueous extract exhibited better protection [32]. This recent study also affirms that there is antioxidant activity in the methanolic extract of *Tapinanthus bangwensis.*

The blood glucose concentration increased significantly ($p<0.05$, $p<0.01$) in the infertile low-dose treated group compared to the control and extract groups. In a case-controlled study of diabetic patients, decreased serum prolactin levels contributed to the development and progression of diabetic retinopathy [33]. This suggests that the extract can be used to increase the level of prolactin expression to prevent the occurrence of diabetic retinopathy but should also be regulated to prevent an elevated blood glucose level (hyperglycemia). The alkaline phosphatase concentrations show that the use of the extract does not cause any mild or severe damage to the liver function of the groups fed with the extract.

5.0 CONCLUSION

In conclusion, the methanolic extract of *Tapinanthus bangwensis* exhibited pro-fertility effect on pregnancy by stimulating the increase in the level of prolactin during pregnancy. Likewise, the extract possesses antioxidant and hypoglycemic activities, demonstrating its oxidative stress ameliorative and likely antidiabetic properties. Also, the methanolic extract of *T. bangwensis* does not affect liver function and can be considered safe for consumption. However, there is a need to conduct further studies to

ascertain the specific phytochemicals in *T. bangwensis* leaf extract that was explicitly responsible for the observed activities alongside investigating the underlying mechanisms involved.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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