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Ethanolic Root Extract of *Urtica dioica* Exhibits Pro-fertility and Antioxidant Activities in Female Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author SOO designed the study and wrote the protocol. Authors JOI and OKA performed the statistical analysis. Authors ASS and AKA wrote the first draft of the manuscript. Authors ASS, JOI and AKA managed the analyses of the study. Authors JOI, OKA and ZOA managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To determine the effect of ethanolic extract of *Urtica dioica* roots 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 used, were divided into eight groups of 5 rats. The study was carried out for a period of 21 days, the rats were induced with levonorgestrel for 7 days after which *Urtica dioica* extract was administered for 14 days and were compared against vehicle, levonorgestrel and extract controls. Hormonal level estimation (Progesterone, estradiol, prolactin and testosterone) and *in-vivo* antioxidant enzyme activity (catalase and alkaline phosphatase activity) were estimated using standard procedures.
Results: The administration of the extract showed no statistically significant difference in estradiol levels across all groups. Progesterone levels decreased significantly ($p<0.05$) compared to the controls while prolactin and testosterone levels also decreased, although not significant. The extract

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increased catalase and alkaline phosphatase activities significantly in IHD group compared to the control.
Conclusion: The ethanolic extract of *Urtica dioica* roots exhibits pro-fertility and antioxidant activities.

Keywords: Infertility; reproductive hormones; oxidative stress; Urtica dioica.

1. INTRODUCTION

According to WHO, infertility is a disease of the reproductive systems defined by the failure to achieve a clinical pregnancy after 12 months of regular unprotected sexual intercourse [1]. Infertility is a big health problem affecting an estimated 80 million couples worldwide [2]. As at 2011, it was estimated that female infertility occurs in about 37% of all infertile couples and ovulatory disorders account for more than half of these [3]. In Nigeria, infertility is the commonest presenting complaint in gynaecological clinics and prevalence of 14.8% to 38.8% of outpatient gynaecological consultation has been reported [4, 5].

Hormones and inflammatory mechanisms are implicated in the major events of female reproduction function, including ovulation, menstruation, embryo implantation and pregnancy. Increasing evidence shows that hormonal imbalance may lead to pregnancy complications [6]. Likewise, oxidative stress, characterized by an imbalance between reactive oxygen species and antioxidant defences has been identified to play a key role in pathogenesis of subfertility in both males and females. This imbalance between reactive oxygen species and antioxidants can lead to a number of reproductive diseases such as endometriosis, hydro salpinges, polycystic ovary syndrome (PCOS), and unexplained subfertility [7].

Medicinal plants' extracts and their bioactive metabolites have played important roles in the treatment and prevention of various diseases and with proven efficacy [8]. *Urtica dioica*, stinging nettle, is a valuable medicinal plant that belongs to the Urticaceae family. Nettle is an herbaceous perennial flowering herb [9], which has been known for a long time as a plant of therapeutic relevance in folk medicine [10]. *U. dioica* contains various beneficial compounds such as flavonoids, fatty acids, polysaccharides, sterols, lignans, lecithin [11], minerals (iron, manganese, magnesium, potassium, and calcium), vitamins (A, C and D), proteins, antioxidants and carotenoids [12]. Among the long-time utilization of the aqueous and alcoholic extracts of *U. dioica* is for the treatment of anaemia [13], urinary, bladder and kidney dysfunctions [14]. Additional reported beneficial properties of this plant include anti-inflammatory [15], anticancer [16], anti-osteoporotic [17], antihypertensive, hypoglycaemic, hepatoprotective [18], antioxidant [19], testicular protective effects, as well as increasing the quality of spermatozoa and sperm parameters [8, 20]. There is paucity of information on the effect of *Urtica dioica* extract on female reproductive parameters. Hence this study aims to evaluate the fertility regulatory effect of the ethanolic root extract of *U. dioica* using female albino rats, where some biochemical and hormonal parameters were investigated.

2. METHODOLOGY

2.1 Collection of Test Samples

Urtica dioica plants, including roots, were collected from the Lagos state university environment. The plant was assigned the ID LUH8510 when it was presented for proper identification and authentication at the University of Lagos herbarium.

2.2 Preparation of Ethanolic Extract of Nettle Roots

Nettle roots were collected and washed with distilled water. The roots were dried in the oven at 40^0C for 2 days. The dried roots were blended to powder using a kitchen blender. 100 g of the powdered roots was soaked in 1 L of 95% ethanol for 72 hours with intermittent shaking as a cold maceration extraction. The root extract was concentrated using a rotary evaporator and further using a water-bath.

2.3 Collection and Acclimatization of Animals

A total of 49 female Wistar albino rats weighing between 60-100 g were purchased from the animal house Lagos University Teaching Hospital (LUTH), Lagos State. The animals were

fed with commercial rat feed. They were kept under hygienic and favourable conditions and maintained under a 12h light/ 12h dark cycle with free access to rat feed and water. The animals were allowed to acclimatize at the animal house, department of biochemistry, for two weeks before commencement of treatment.

2.4 Chronic Toxicity Testing

The toxicity test of the extract was carried out to determine safe dosages for extract administration following a reported procedure [21]. A total of nine rats were randomly selected with average weight of 125 g and divided into three groups with each group containing three rats. The dosages tested for are:

1. 100 mg/kg for group 1
2. 200 mg/kg for group 2, and
3. 400 mg/kg for group 3.

To dose the extract, the different doses were dissolved in 1ml of carrier oil. No death was recorded after 24 hours of extract administration.

2.5 Animal Grouping

The animals were divided into eight groups, with the average weight of 122g. Each group contained five animals and were treated as follows:

Group 1- control group (fed 1ml of distilled water): CDW
Group 2- carrier oil group (fed 1ml of carrier oil): COO
Group 3- infertile group (fed 0.14 mg/g levonorgestrel): CIC
Group 4- pregnant control group (pregnant rats fed 1ml of distilled water): PC
Group 5- extract only group (fed with 100 mg/kg *U. dioica* extract): EO
Group 6- pregnant + low dose extract (pregnant rats fed 100 mg/kg extract only for 14 days): PLD
Group 7- infertile + low dose extract (fed 0.14 mg/g of levonorgestrel for 7 days and later received 100 mg/kg of extract for 14 days): ILD
Group 8- infertile + high dose extract (fed 0.14 mg/g of levonorgestrel for 7 days and later received 400 mg/kg of extract for 14 days): IHD

All administration was through oral route.

2.6 Phytochemical Screening

Qualitative phytochemical screening to determine the phytochemicals present in the ethanolic extract of the test sample was carried out according to Usman, Abdulrahman [22] as follows;

- Test for phenols: 1 ml of extract + 2 ml of $FeCl_3$ solution
- Test for flavonoids: 2 g of dried sample + 5 ml of distilled water + few drops of NaOH
- Test for saponins: 2 g of dried sample + 2 ml of distilled water + vigorous shaking
- Test for terpenoids:1 ml of extract + 2 ml of chloroform + few drops of conc. H_2SO_4
- Test for steroids: 0.2 g of extract+ 2 ml of acetic acid + conc. H_2SO_4
- Test for tannins: 0.5 g of sample + 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution + 2 ml of the filtrate

2.7 Collection of Blood and Organs

The animals were sacrificed after 2 weeks of treatment with 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 physiological saline solution.

2.8 Determination of Biochemical Parameters

2.8.1 Estimation of hormones

The reproductive hormones progesterone, prolactin, estradiol and testosterone were estimated using ELISA technique based on the principle of a solid-phase enzyme-linked immunosorbent assay. Thawed serum samples have been assayed for prolactin, progesterone, estradiol and testosterone using (Bio-inteco, UK) kits. A series of standards and serum samples were added to specific wells and then 100 µL of enzyme conjugate was added to all wells and incubated for 60/90 minutes, as specified. The wells were then washed 4 times with the wash buffer and 100 µL of substrate was added and incubated for 20 minutes. 100 µL of stop solution was then added and a yellow colour formed and read at 450nm on a microplate reader. Hormone level is calculated using a standard curve of the absorbance of the standards against their concentrations and the results expressed as ng/ml [23].

2.8.2 Estimation of catalase activity

The catalase activity was measured using Aebi method [24]. The mitochondria pellet was

dissolved in 1.0ml of 0.1mol/L potassium phosphate buffer (pH 7.4). 10 µL of the mitochondria homogenate was then added to a cuvette containing 2.89ml of a 50mmol/L phosphate buffer (pH 7.0). the reaction was initiated by adding 0.1ml of freshly prepared 30mmol/L H_2O_2 to make a final volume of 3.0ml at 25^0C. The decomposition rate of H_2O_2 was measured at 240nm for 5 min on a spectrophotometer. A molar extinction coefficient of $0.041(mmol/L)^{-1}cm^{-1}$ was used to determine the catalase activity which was then expressed as nmol H_2O_2 decreased/mg protein/min [25].

2.8.3 Determination of alkaline phosphatase activity

The substrate p-nitrophenyl phosphate is hydrolysed 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). In a cuvette, 10 µl of sample was mixed with 500 µl of the reagent. The initial absorbance was read at 405 nm, and subsequently over 3 minutes. The mean absorbance per minute was used in the calculation: ALP activity (IU/l) = 2760 × ΔA 405 nm/min. Where: 2760 = Extinction coefficient; ΔA 405 nm/min = change in absorbance per minute for the serum sample [26].

2.9 Statistical Analysis

Data were analysed 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 mean.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Constituents

The use of medicinal plants for therapeutic purposes has gained much acceptability traditionally for health care in local areas worldwide, either due to low or no cost, and poverty or scarcity or lack of access to modern drugs [27]. Edirne et al. (2020) suggested that the different phytochemical content present in the leaves and root of *Urtica dioica* (Nettle) extract could be employed to treat infertility [28]. In this study, the phytochemical analysis of the ethanolic root extract of *Urtica dioica* shows that phenols, flavonoids, alkaloids, steroids, reducing sugar and tannins are the active phytoconstituents present (Table 1). Meanwhile, quantitative analysis reveals that reducing sugar is the major constituent at 53.20 mg/100 g, followed by alkaloid, phenol, flavonoid and tannin at 49.43, 48.77, 46.16 and 35.48 mg/100g respectively. Steroid concentration is the lowest at 19.95 mg/100 g (Table 1). Our result is in agreement with a previous report that *Urtica dioica* is rich in several phytoconstituents such as, phytosterols, terpenoids, phenols, fatty acids, saponins, flavonoids, tannins, proteins and amino acid [29], which may be responsible for its pro-fertility potential.

Table 1. Phytochemicals present in the *Urtica dioica* root extract

Phytoconstituent	Inference	Concentration (mg/100 g)
Phenol	Present	48.77
Flavonoid	Present	46.16
Alkaloid	Present	49.43
Steroid	Present	19.95
Reducing Sugar	Present	53.20
Tannin	Present	35.48
Phlobatannins	Absent	-
Saponins	Absent	-

3.2 Effect on Reproductive Hormones

Urtica dioica is known as a natural aromatase inhibitor and a valuable medicine that serves a therapeutic purpose in the treatment of oestrogen dependent disorders by decreasing plasma concentration of oestrogen [30]. Several studies have confirmed that *Urtica dioica* products inhibit aromatase and interfere with the conversion of testosterone into oestrogens [31]. Hence, administration of aromatase inhibitor may be a promising means of normalising oestrogen levels in female that can result in fertility improvement. This study found no significance difference (*P= .05*) in estradiol concentration across the treated groups (Fig. 1). The administration of the extract normalised estradiol concentration. However, Kargar Jahromi and Karimi Jashni [32] in their study reported a significant increase in the levels of serum oestrogen concentrations using ethanol root extract of *Urtica dioica* at dosage of 300 mg/kg in extract treated groups.

Progesterone is an important part of infertility treatment since it supports implantation. It is essential in establishing and maintaining early

pregnancy and female reproduction by acting as a regulator all along the female reproductive axis [33]. Progesterone concentrations across the different groups are presented in (Fig. 2). The serum progesterone concentrations in the infertile + extract groups show increased concentrations compared to the infertile control group, however, all extract treated groups showed reduced progesterone concentrations compared to the control groups. This is in contrast with results from Kargar Jahromi and Karimi Jashni [32] who reported significant increase in progesterone levels compared to the control group at a dosage of 300 mg/kg. The PLD group also showed a reduced progesterone concentration compared to the positive control group, although not statistically significant.

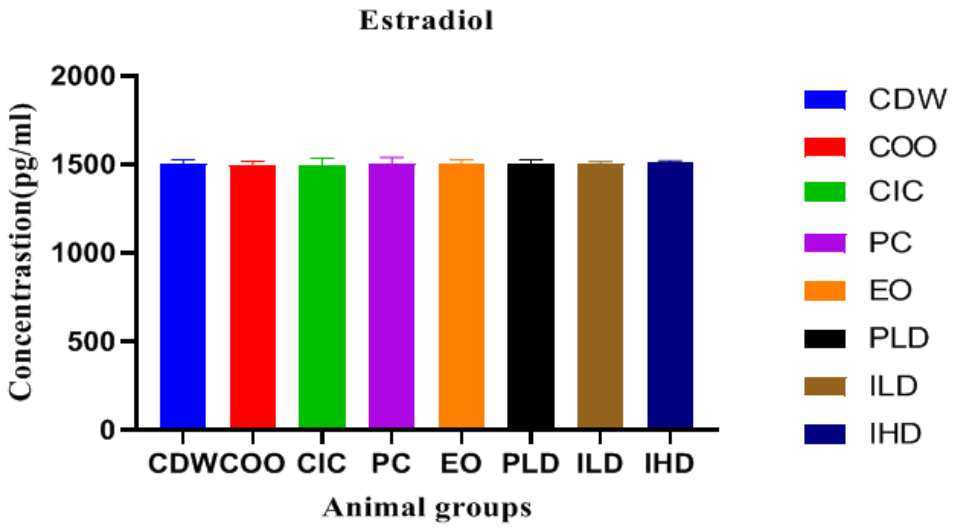

Fig. 1. Serum estradiol concentrations across the different groups
The results are expressed as Mean ± SEM

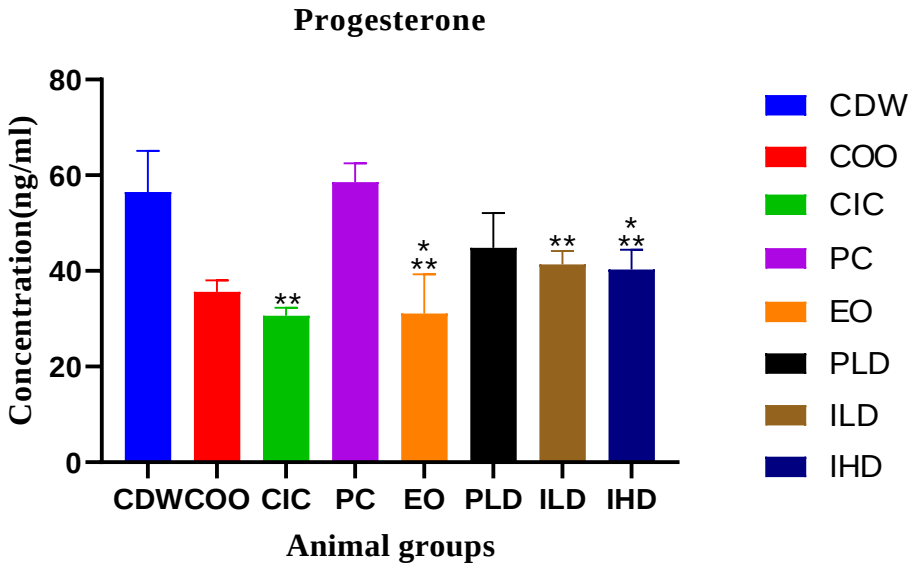

Fig. 2. Serum progesterone concentration across the different groups
*Significant from normal control, *P < 0.05: **P < 0.01*
The results are expressed as Mean ± SEM

Prolactin is a pregnancy maintenance hormone. It is a known polypeptide hormone synthesized in but not limited to lactotrophs of anterior pituitary gland; it plays other important biological roles in mammalian reproduction other than lactating effect which includes, gonadotropin (follicle stimulating hormone, FSH and luteinizing hormone, LH) synthesis and secretion suppression [34]. It is also an important component of the reproductive system but its hypersecretion inhibits gonadotropin-releasing hormone (GnRH) secretion and decreases GnRH receptor response to GnRH in both animals and humans, as well as a decrease in luteinizing hormone (LH) pulse frequency and amplitude [35], consequently causing decreased libido, sexual dysfunction, irregular ovulation and the loss of menstrual periods, which will hinder conception. This study shows that *Urtica dioica* has no statistically significant effect (*P*= .05) on serum prolactin concentration across the groups (Fig. 3). However, it is noteworthy that the PLD group showed a higher concentration of prolactin compared to the PC group. The EO group showed decreased prolactin concentration compared to the CDW group. The IHD and ILD groups showed reduced concentrations of prolactin compared to the controls and CIC group. This indicates that *Urtica dioica* root ethanol extract may play a role in keeping prolactin concentrations at favourable levels in both infertile and pregnant groups when treated with the extract, thus exhibiting profertility properties. Slightly elevated prolactin levels have been associated with the consumption of serotoninergic agents, including fluoxetine, escitalopram, and venlafaxine, and *Urtica dioica* have also been reported to contain serotonin [12,36].

Testosterone is a steroid hormone involved in many bodily processes, including reproductive physiology (e.g., spermatogenesis), morphology (e.g., development of secondary sexual characteristics), psychology (e.g., sexual desire), and behaviour (e.g., aggression) — each of which plays an important role in survival and reproduction. In this study, despite observing a decreased testosterone concentration in all the *Urtica dioica* root extract treated groups when compared with the control, the difference was not to a significance extent (*P*= .05), as shown in (Fig. 4). However, Jalili et al. (2014) reported a dose-dependent increase in testosterone level of rats when treated with *U. dioica* extract [20].

3.3 Effect on Ovarian Antioxidant Enzymes

Antioxidant enzymes are biomarkers that constitute the first line of cell defence in living system against free radicals [37]. These biomarkers are useful in the determination of the extent and effect of oxidative stress. The disordered physiological processes in human sex cells have been associated with oxidative stress [38], which is seen as the consequence of an imbalance reactive oxygen species (ROS) production and degradation.

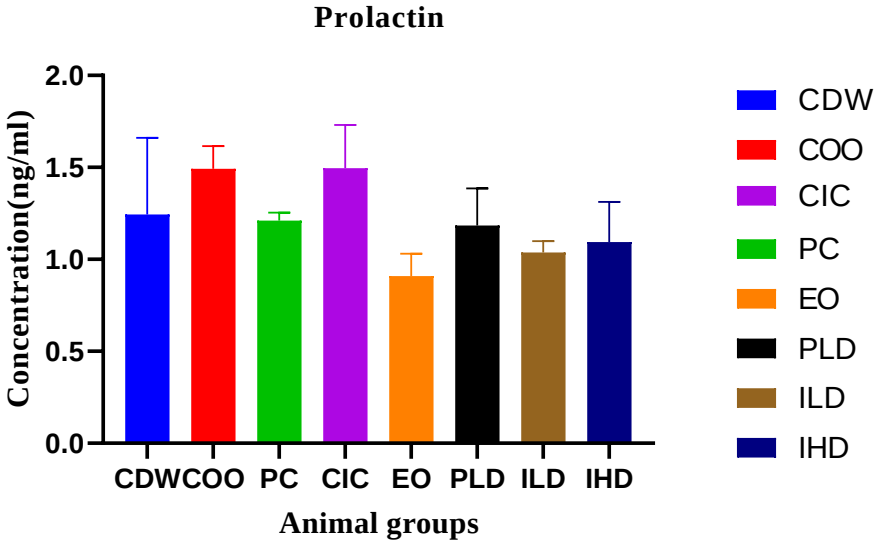

Fig. 3. Serum prolactin concentrations across the different groups
The results are expressed as Mean ± SEM

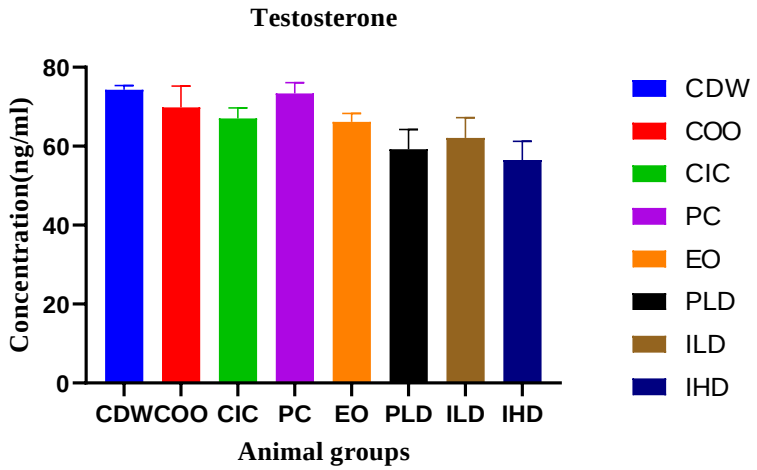

Fig. 4. Serum testosterone concentrations across the different groups
The results are expressed as Mean ± SEM

Catalase (CAT), is known to be a vital *in-vivo* antioxidant biomarker that catalyses H_2O_2 (hydrogen peroxide) into water (H_2O) and oxygen (O_2) in an energy-efficient manner in the cells exposed to environmental stress [39]. The knowledge of CAT activity in seminal plasma and other biological fluids has been long used as a tool to improve the diagnosis and prevention of female infertility, and bioactive constituents might have a unique effect on its activity by playing a preventive role in clinical condition caused by oxidative stress-derived female infertility [40]. (Fig. 5) shows the CAT activity in the ovary of the different treated rat groups in this study. Here, the extract-only treated group showed an increase in catalase activity, although not significant compared to the control groups. The pregnant + extract group did not show appreciable increase in catalase activity compared to the pregnant control group. The infertile + extract treated groups also showed an increase in catalase activity compared to the infertile control group, although not significant. The treatment of infertility with the extract (low dose), that is, the ILD group increased catalase activity significantly (P = .05) when compared to CDW (Fig. 5). Hence, high CAT activities suggest that *U. dioica* may offer protection to the cells against oxidants. This agrees with the findings of Kataki et al., who reported the hepatoprotective activity of nettle extract as a result of increase in catalase activity and other hepatic enzymes [41].

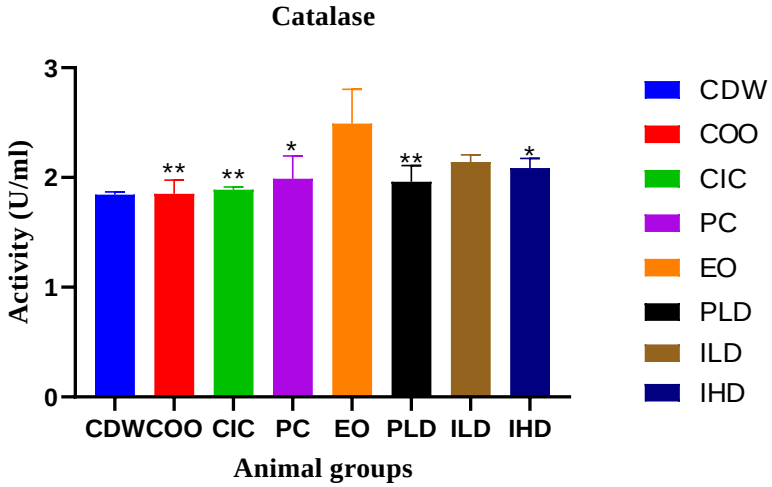

Fig. 5. Catalase activity in the ovary across the different groups
*Significant from normal control, *P < 0.05: **P < 0.01*
The results are expressed as Mean ± SEM

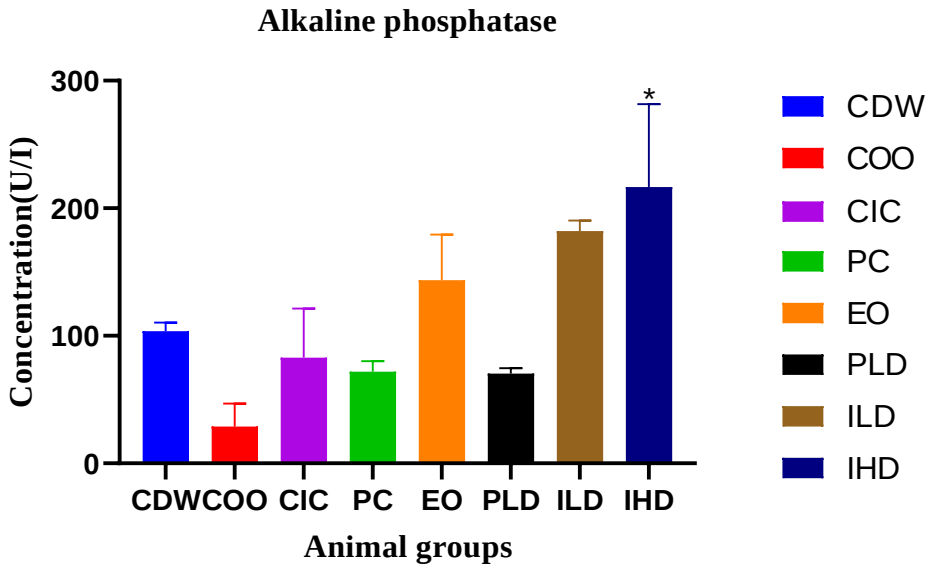

Fig. 6. Serum alkaline phosphatase concentrations across the different groups
*Significant from CDW, *P < 0.05*
The results are expressed as Mean ± SEM

Alkaline phosphatase (ALP) is a marker of pathological alteration in biliary flow, and its activity have been reported to significantly increase with the administration of nettle extracts [42]. Alkaline phosphatase concentration in the serum of the different rat groups is shown in (Fig. 6). Treatment with the extract showed an increased ALP activity in the EO and IHD groups compared to CDW and PC control groups. However, the ILD treated group showed a statistically significant increase (*P* = .05) in ALP activity compared to CDW (Fig. 6). Also, this possibly suggests the ovary protective role of the ethanolic root extract of *Urtica dioica*.

4. CONCLUSION

In conclusion, ethanol root extract of *U. dioica* possesses profertility and antioxidant activities, possibly due to its phytoconstituents, maintenance of favourable prolactin levels and ovary protective activities. However, more studies need to be conducted in order to ascertain which among the various phytochemicals present in *U. dioica* root extract was specifically responsible for the observed activities as well as 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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