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THERAPEUTIC POTENTIAL OF *Zingiber officinale* and *Syzygium aromaticum* (ZOSA) CO-THERAPY: INSIGHTS FROM BIOCHEMICAL, HISTOPATHOLOGICAL, AND IMMUNOHISTOCHEMICAL STUDIES

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ABSTRACT

In recent times, the combined therapy of *Zingiber officinale* and *Syzygium aromaticum*, known as ZOSA, has gained popularity among women, sparking curiosity regarding its safety and physiological effects. This research aimed to explore the influence of ZOSA extract on sex hormone profiles, oxidative stress markers, as well as histopathological and immunohistochemical parameters. Twenty female Wistar rats were segregated into four groups and administered varying doses of ZOSA extract (100, 200, and 300 mg/kg) for a duration of 30 days. Diverse biochemical, histopathological, and immunohistochemical analyses were carried out. The findings revealed that ZOSA exhibited substantial antioxidative characteristics, notably reflected in the reduced levels of malondialdehyde (MDA). Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone were significantly increased ($p<0.05$) compared to estrogen levels. Histopathological assessments unveiled active follicular development and maturation, as evidenced by the abundant presence of corpus luteum. Similarly, immunohistochemistry investigations demonstrated a robust expression of progesterone receptors ($p<0.05–0.001$) in the ZOSA-treated groups in contrast to estrogen receptors. Overall, our data elucidate that ZOSA extract exerts remarkable effects on female fertility. Nevertheless, the modifications observed in the biochemical assays suggest a potential for organ toxicity at higher doses.

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INTRODUCTION

Herbal remedies are becoming increasingly popular among younger generations, especially with the rise of health trends on social media. In recent decades, do-it-yourself (DIY) herbal remedies have gained wider acceptance due to several factors: (i) the common misconception that natural products have fewer side effects, (ii) the belief that they are more effective, (iii) their cost-effectiveness, (iv) easier accessibility compared to conventional drugs, and a general preference for self-medication [1]. Among these trends is the co-therapy of *Zingiber officinale* and *Syzygium aromaticum* (ZOSA) for enhancing female reproductive health and increasing conception rates.

Zingiber officinale, commonly known as ginger, has been used for centuries for its medicinal properties. Research has

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reported that the rhizome of *Z. officinale* possesses anti-inflammatory [2], antioxidative [2], and anti-cancer effects [3]. Its bioactive components, such as gingerol and shogaol, are known for their antimicrobial [4], anti-inflammatory [2], and analgesic effects [5]. On the other hand, *Syzygium aromaticum*, or cloves, has a well-documented history in traditional medicines, including Ayurveda and various folkloric remedies from China and Nigeria. The phytoconstituents of *S. aromaticum* buds, such as eugenol, acetyleugenol, gallic acid, hydroxybenzoic acids, hydroxycinnamic acids, quercetin, and kaempferol, are reported to have similar pharmacological activities [6] to those of *Z. officinale*.

Furthermore, the antioxidant properties of *S. aromaticum* buds have been compared to synthetic antioxidants like BHA (Butylated Hydroxyanisole) and pyrogallol [7]. These overlapping pharmacological activities have stimulated interest in evaluating the potential of ZOSA as a co-therapy in animal models.

MATERIALS AND METHODS

Chemicals

All chemicals and drugs used in this study were of high grade and sourced from reputable pharmaceutical companies. The specific chemicals and solvents utilized included distilled water, chloroform (SAFC, Germany), 98% methanol (Florida, USA), and dimethyl sulfoxide (Thistle Scientific, UK).

Plant Collection and Extraction

Zingiber officinale rhizomes and *Syzygium aromaticum* buds (ZOSA) were purchased from a local market, identified, and allocated herbarium voucher numbers (Bot/Herb/UCC/013, Bot/Herb/UCC/269). The rhizomes of *Z. officinale* underwent a process of shade-drying spanning 21 days, after which both botanical specimens were pulverized into fine powder. A total quantity of 890 g of *S. aromaticum* was subjected to maceration in 1500 ml of 98% methanol, while 1000 g of *Z. officinale* underwent a similar maceration process in 2500 ml of methanol for 48 hours (ambient temperature: 28-31 ºC). After maceration, filtration was carried out utilizing No. 1 Whatman filter paper, followed by a concentration process employing a rotary evaporator set at 40 ºC and 110 rpm. The calculated percentage yields for the extracts obtained from *Z. officinale* rhizomes and *S. aromaticum* buds were 35.9% and 3.64%, respectively.

Experimental Animals

Twenty female Wistar rats, aged 4 weeks (weighing 55-60 g), were obtained from the Department of Pharmacology's animal house and acclimatized for two weeks. They were randomized into four groups, each containing five rats. All animals were housed in plastic cages under controlled environmental conditions (temperature: 25-30 ºC, photoperiod: 12 hours light/12 hours night, relative humidity: 50-55%). They were fed standard chows and had access to water ad libitum. Animal handling was conducted following the OECD guidelines, and ethical approval (270APHA1124) was obtained from the Faculty Animal Research Ethics Committee (FAREC-FBMS) at the University of Calabar.

Acute Toxicity Determination

The acute toxicity test was conducted following OECD guidelines No. 423. The study was carried out in two phases, following previously described methods with slight modifications [8-9]. Post-administration, the animals were observed, and no deaths or signs of toxicity were recorded.

Study Design

The duration of the study was 30 days, with the following treatment administrations (p.o: 1 ml):

Group 1 (Sham): 2% DMSO solution (Vehicle)
Group 2 (100 mg/kg ZOSA): 100 mg/kg ZOSA (w/w: 1:1) in 2% DMSO solution
Group 3 (200 mg/kg ZOSA): 200 mg/kg ZOSA (w/w: 1:1) in 2% DMSO solution
Group 4 (300 mg/kg ZOSA): 300 mg/kg ZOSA (w/w: 1:1) in 2% DMSO solution

Post-treatment, the rats were fasted overnight and weighed before being anesthetized and sacrificed. Blood samples were collected via cardiac puncture and stored for further analysis.

Determination of Sex Hormonal Profile

Hormonal assays were performed to measure serum levels of estradiol, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) concentrations using commercial ELISA kits designed for rats. Absorbance readings were obtained using a spectrophotometer (Thermo Fisher Scientific, USA), and the varying hormone concentrations were calculated based on these readings.

Determination of Oxidative Stress Indices

Serum levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA) were measured using methods described in previous studies [10-12], utilizing commercial rat kits (CUSABIO, USA) for quantification via spectrophotometry.

Determination of Some Biochemical Assays

Liver function tests (ALT, AST, ALP) and kidney function tests (Na^+, K^+, Cl^-, HCO^{3-}, creatinine, urea) were evaluated using Randox kits (Randox Laboratories Limited, UK), while lipid profiling was conducted with Cusabio kits (Cosmo Bio, USA).

Histomorphometry of Ovary and Uterus

Tissues were fixed in 10% buffered formalin to preserve their structure for further analysis. After fixation, the tissues underwent a series of processing stages to prepare them for microscopic examination. One important step involved in the processing was the graded dehydration in ethanol. This process helps to remove water from the tissues gradually,

ensuring that they are adequately dehydrated before proceeding to the next stage. Thereafter, the tissues were cleared in xylene and embedded in paraffin wax at 60 ºC. Once embedded, the tissues were sliced into thin sections and stained using hematoxylin and eosin (H&E). The stained tissue sections were then examined under a light microscope (Leica, Germany), while their histomorphometric studies were conducted using the ImageJ Analyze plugin software.

Immunohistochemistry of the Ovary and *Uterus*

Immunohistochemical staining of ovarian and uterine tissues (2–5 µm thick) was performed on silane-coated slides. The samples were deparaffinized and rehydrated before sequential application of primary and secondary antibodies, following previously established protocols [13]. Staining was carried out using the Avidin-Biotin (LAB) method in citrate buffer (pH 6.0). Visualization occurred with a light microscope, and quantification was performed using the ImageJ Analyze Plugin.

Data Analysis

Data were presented as Mean ± Standard Deviation (SD) (n=5). One-way ANOVA with Tukey's post-hoc test was employed using GraphPad Prism software (version 8.1), with statistical significance set at $p < 0.05$.

RESULTS

ZOSA Effects on Female Sex Hormones

ZOSA significantly increased ($p<0.05$) serum levels of FSH, LH, and progesterone, while decreasing ($p<0.05$) estrogen levels in a dose-dependent manner when compared to the Sham group (Figure 1a-d).

ZOSA Demonstrates Significant Anti-Oxidative Stress Activities

As shown in Figure 2 (a-c), the levels of SOD, CAT, and GST were significantly elevated ($p<0.05$) with increasing doses of ZOSA co-therapy compared to the Sham group. In contrast, the MDA level was highest in the Sham group (127.0 ± 2.83 pmol/ml) and decreased ($p<0.05$) with increasing doses of ZOSA (43.0 – 37.33 pmol/ml), as illustrated in Figure 2 (d).

ZOSA Effects on Biochemical Indices

The effects of ZOSA co-therapy on various biochemical indices are detailed in Table 1:

Liver: Increases in serum levels of AST, ALT, and ALP were noted in the ZOSA groups in comparison to the Sham group, albeit these alterations did not reach statistical significance ($p>0.05$) for AST and ALT.

Lipid: Elevations in serum concentrations of Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL), Very Low-Density Lipoprotein (VLDL), and Low-Density Lipoprotein (LDL) were detected in the ZOSA groups relative to the Sham group, exhibiting pronounced effects at the 300 mg/kg ZOSA dose for Total Cholesterol (3.62 mmol/l) and Very Low-Density Lipoprotein (0.94 mmol/l).

Kidney: Marked elevations in renal function tests were observed, particularly at the 300 mg/kg ZOSA dosage, resulting in significant increases ($p>0.05$) in urea and creatinine levels. Conversely, there was a reduction in the HCO_3^- concentration.

ZOSA Effects on Ovarian and Uterine Histology

Ovary: As illustrated in Figure 3 (a-d), ovarian sections exhibited proliferating follicles at varying stages of maturation, encompassing primordial, primary, and fully developed Graafian follicles housing viable oocytes. A greater abundance of corpora lutea and luteinized cells was evident in the ZOSA groups in comparison to the Sham group (Figure 3a-d, Table 2).

Uterus: In Fig. 4 (a-d), sections of the uterus displayed an intact superficial layer of the endometrium, abundant cellular endometrial stroma, and a thickened endometrium with numerous tubular glands and an inflammatory cellular infiltrate (predominantly eosinophils and neutrophils) in the ZOSA groups in comparison to the Sham group (Fig. 4a- d, Table 2).

ZOSA Effects on Progesterone and Estrogen Receptor Expressions

Progesterone: Stromal cells in all uterine sections stained positively for progesterone receptors at varying intensities (Fig. 5a-d). Epithelial cells in the ZOSA groups also stained positively for progesterone receptors, with significant effects observed at the 300 mg/kg ZOSA dose (Fig. 5d). Cell counts were remarkably elevated ($p<0.05 – 0.01$) (Fig. 5e), while the elevation in total area (%) was statistically insignificant (Fig. 5f) compared to the Sham group.

Estrogen: The uterine sections shown in Fig. 6 (a-d) exhibited an intact superficial epithelial lining, along with populations of endometrial gland and stromal cells. These epithelial and stromal cells were weakly stained for estrogen receptors. As illustrated in Fig. 6 (e-f), cell counts for estrogen receptors were significantly diminished ($p<0.05 – 0.001$) in the ZOSA groups compared to the Sham group.

DISCUSSION

The emergence of do-it-yourself (DIY) remedies has underscored the necessity to assess their effectiveness, toxicity, and safety, prompting our exploration of the co-therapy trend involving *Zingiber officinale* and *Syzygium aromaticum*. Our study revealed that the combined extracts of *Z. officinale* and *S. aromaticum* significantly increased ($p<0.05$) the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone in the serum, while concurrently decreasing ($p<0.05$) estrogen levels. This impact was further substantiated by the expression patterns of progesterone and estrogen receptors following ZOSA administrations, as

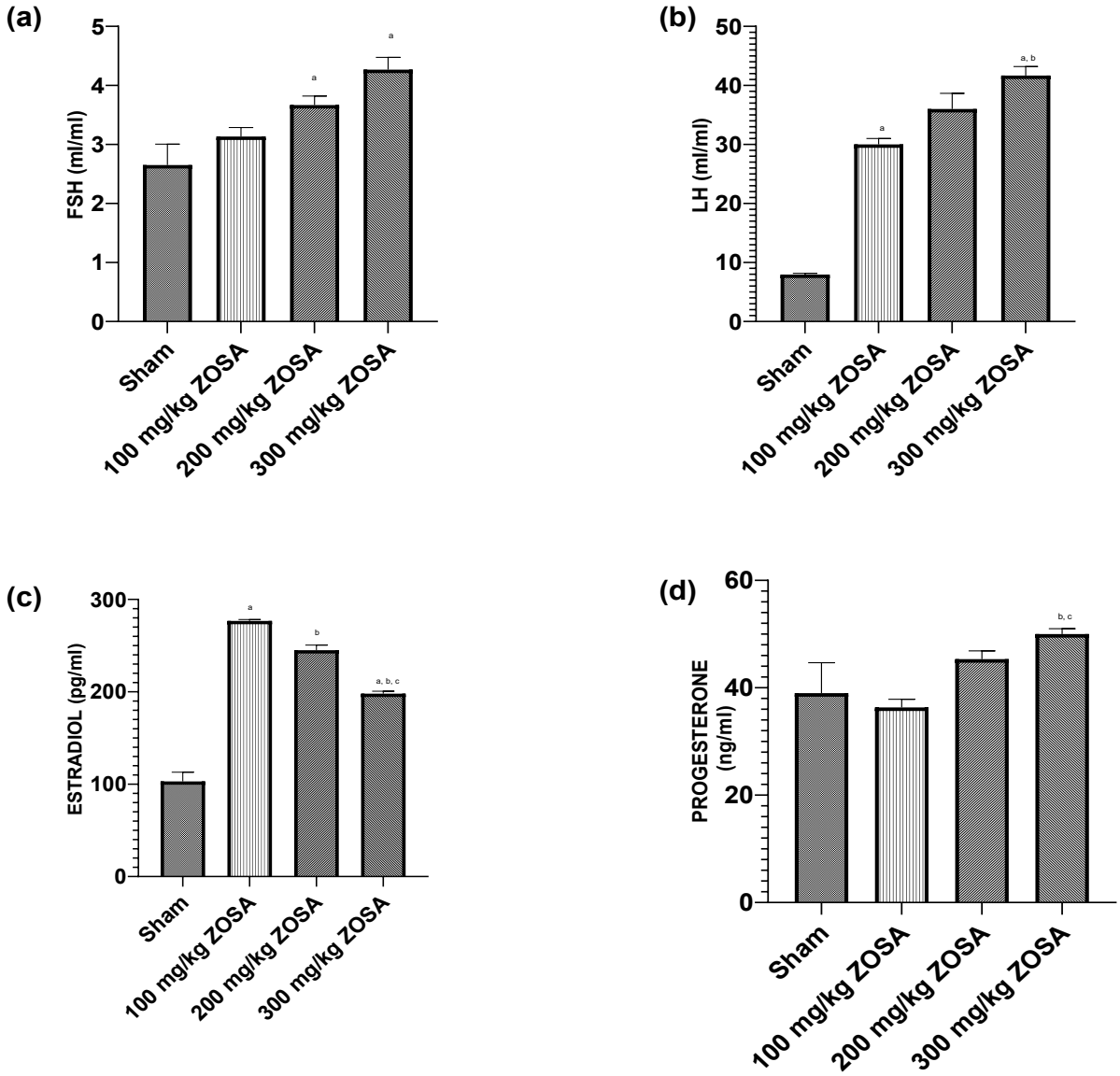

Figure 1. Effect of ZOSA co-therapy on the following hormones was analyzed: (a) FSH, (b) LH, (c) estradiol, and (d) progesterone. The results are presented as Mean ± SD, with a sample size of n = 5. Significant differences were observed with the following statistical outcomes: a $p < 0.05$ compared to the Sham group, b $p < 0.05$ compared to the 100 mg/kg ZOSA group, and c $p < 0.05$ compared to the 200 mg/kg ZOSA group. Data were analyzed using one-way ANOVA followed by Tukey's HSD test. Abbreviations: ZOSA: *Zingiber officinale Syzygium aromaticum,* FSH: follicle-stimulating hormone, LH: luteinizing hormone.

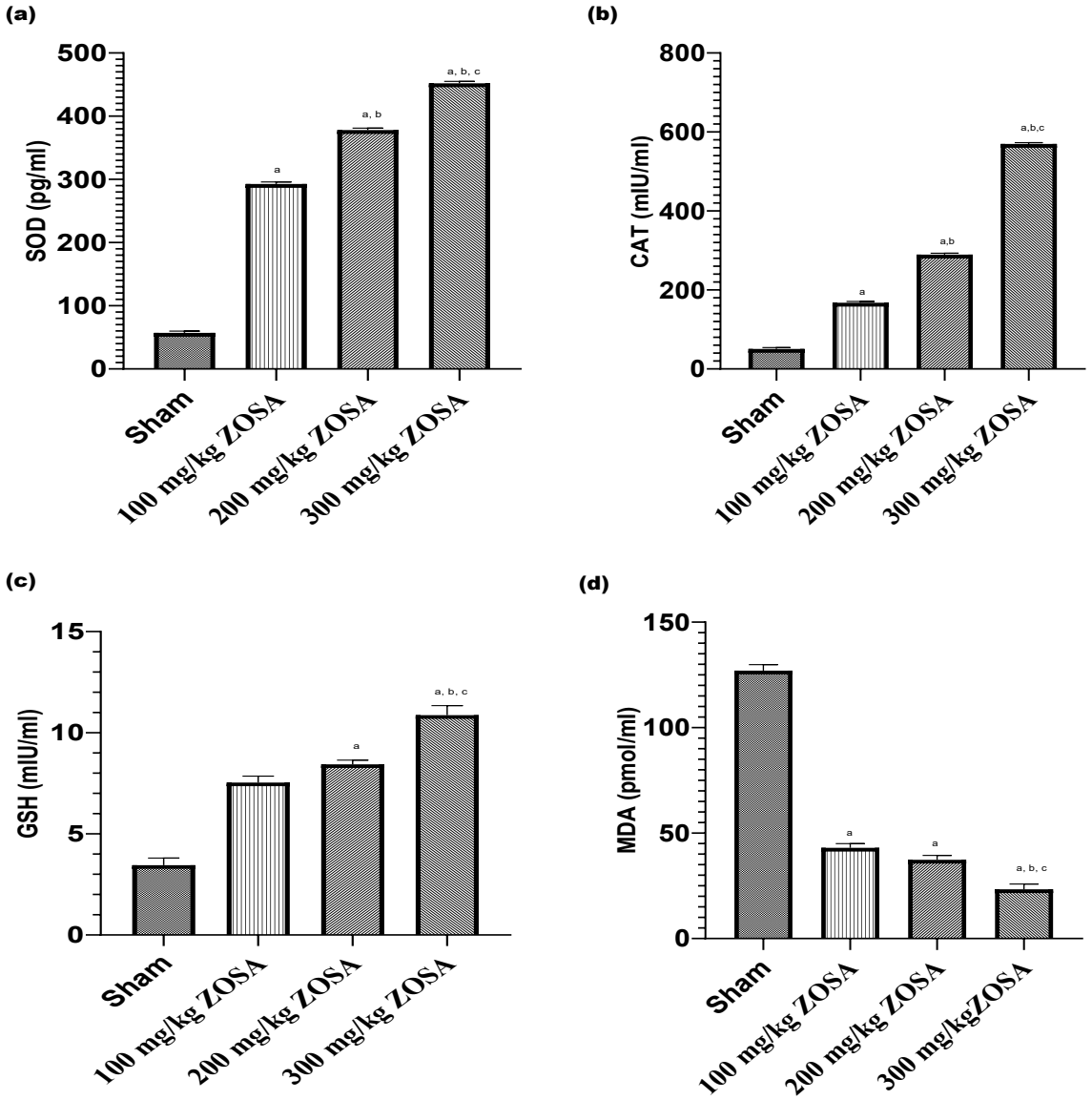

Figure 2: Effect of ZOSA co-therapy on the following parameters: (a) SOD, (b) CAT, (c) GSH, and (d) MDA. The values are expressed as Mean ± SD, with n = 5. Statistical significance was determined with: [a] $p < 0.05$ compared to the Sham group, [b] $p < 0.05$ compared to the 100 mg/kg ZOSA group, and [c] $p < 0.05$ compared to the 200 mg/kg ZOSA group. Data were analyzed using one-way ANOVA followed by Tukey's HSD test. Abbreviations: ZOSA: Zingiber *officinale Syzygium aromaticum,* SOD: Superoxide dismutase, CAT: catalase, GSH: glutathione, MDA: malonaldehyde.

Table 1. *ZOSA* effects on certain biochemical indicators

Parameters	Sham	100 mg/kg ZOSA	200 mg/kg ZOSA	300 mg/kg ZOSA
Liver function				
AST (IU/L)	32.50 ± 1.50	40.33 ± 7.59	39.67 ± 6.34	57.75 ± 8.04
ALT (IU/L)	16.50 ± 1.45	26.67 ± 7.41	30.01 ± 7.78	43.25 ± 4.11
ALP (IU/L)	146.01 ± 2.00	147 ± 19.20	151.33 ± 9.46	172.25 ± 10.68 [c]
Lipid profile				
TC (mmol/l)	2.23 ± 0.03	2.02 ± 0.05 [c]	2.16 ± 0.02 [b, c]	3.62 ± 0.03 [a, b, c]
TG (mmol/l)	1.24 ± 0.01	1.82 ± 0.03	1.89 ± 0.03	2.06 ± 0.03
HDL (mmol/l)	0.56 ± 0.01	0.51 ± 0.01 [c]	0.54 ± 0.01 [c]	0.91 ± 0.01 [b, c]
VLDL (mmol/l)	0.56 ± 0.01	0.83 ± 0.01 [a, b, c]	0.86 ± 0.01 [a, b, c]	0.94 ± 0.01 [a, b, c]
LDL (mmol/l)	1.11 ± 0.02	0.69 ± 0.04 [b, c]	0.76 ± 0.01 [b, c]	1.77 ± 0.04 [b]
Kidney function				
Na^+ (mmol/l)	135 ± 0.89	137.40 ± 0.49	137.40 ± 0.49	141.80 ± 0.40
K^+ (mmol/l)	4.0 ± 0.09	4.74 ± 0.12	5.28 ± 0.18	6.72 ± 0.18
Cl^- (mmol/l)	106 ± 0.89	110.80 ± 0.75	112.40 ± 0.8	118.20 ± 0.75
HCO^3 (mmol/l)	19.40 ± 0.49	16.40 ± 0.49 [a]	15.80 ± 0.4 [a]	13.20 ± 0.40 [a, b, c]
Urea (mmol/l)	3.42 ± 0.31	6.11 ± 0.11 [a]	7.48 ± 0.09 [a, b]	6.92 ± 0.08 [a, b, c]
Cr (mmol/l)	50.36 ± 1.21	116.10 ± 1.11 [a]	133.92 ± 1.04 [a, b]	124.30 ± 1.39 [a, b, c]

Values are expressed as Mean ± SD, n=5. [a] $p<0.05$ vs Sham, [b] $p<0.05$ vs 100 mg/kg ZOSA, [c] $p<0.05$ vs 200 mg/kg ZOSA (Analyzed using one-way ANOVA; followed by Tukey's HSD). Abbreviations: ZOSA: *Zingiber officinale Syzygium aromaticum,* AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, TC: total cholesterol, TG: triglyceride, HDL: high-density lipoprotein, VLDL: very low-density lipoprotein, LDL: low-density lipoprotein, Na+: sodium, K+: potassium, Cl-: chloride, HCO3-: bicarbonate, Cr: creatinine.

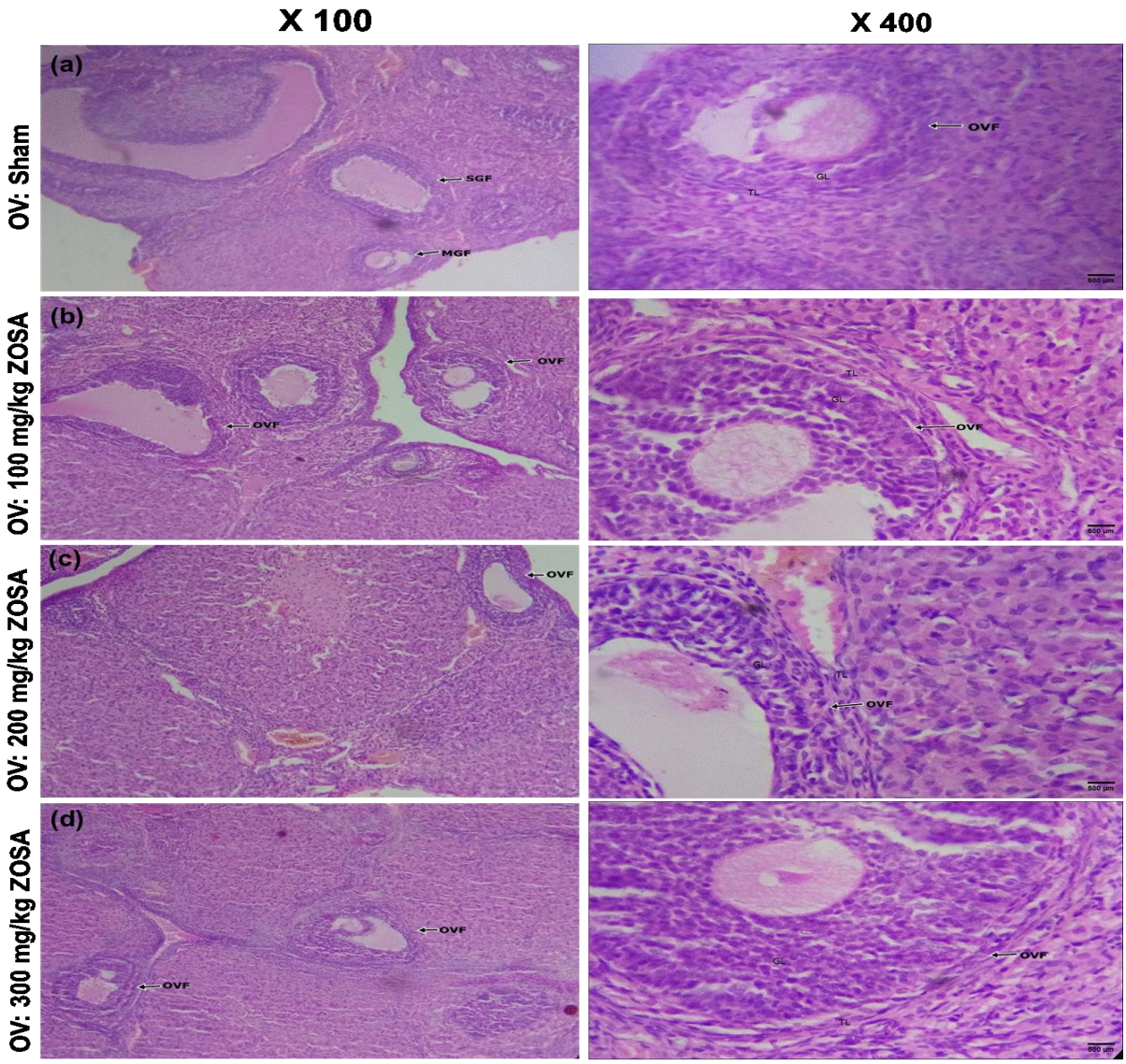

Figure 3. Representative photomicrographs of hematoxylin & eosin-stained sections of ovarian sections of (a) Sham, (b) 100 mg/kg ZOSA, (c) 200 mg/kg ZOSA, (d) 300 mg/kg ZOSA. Magnifications x100 and x400 (scale bar: 500 µm). Abbreviations: ZOSA: *Zingiber officinale Syzygium aromaticum,* OVF: ovarian follicle, CL: corpus luteum, E: empty ovarian follicle.

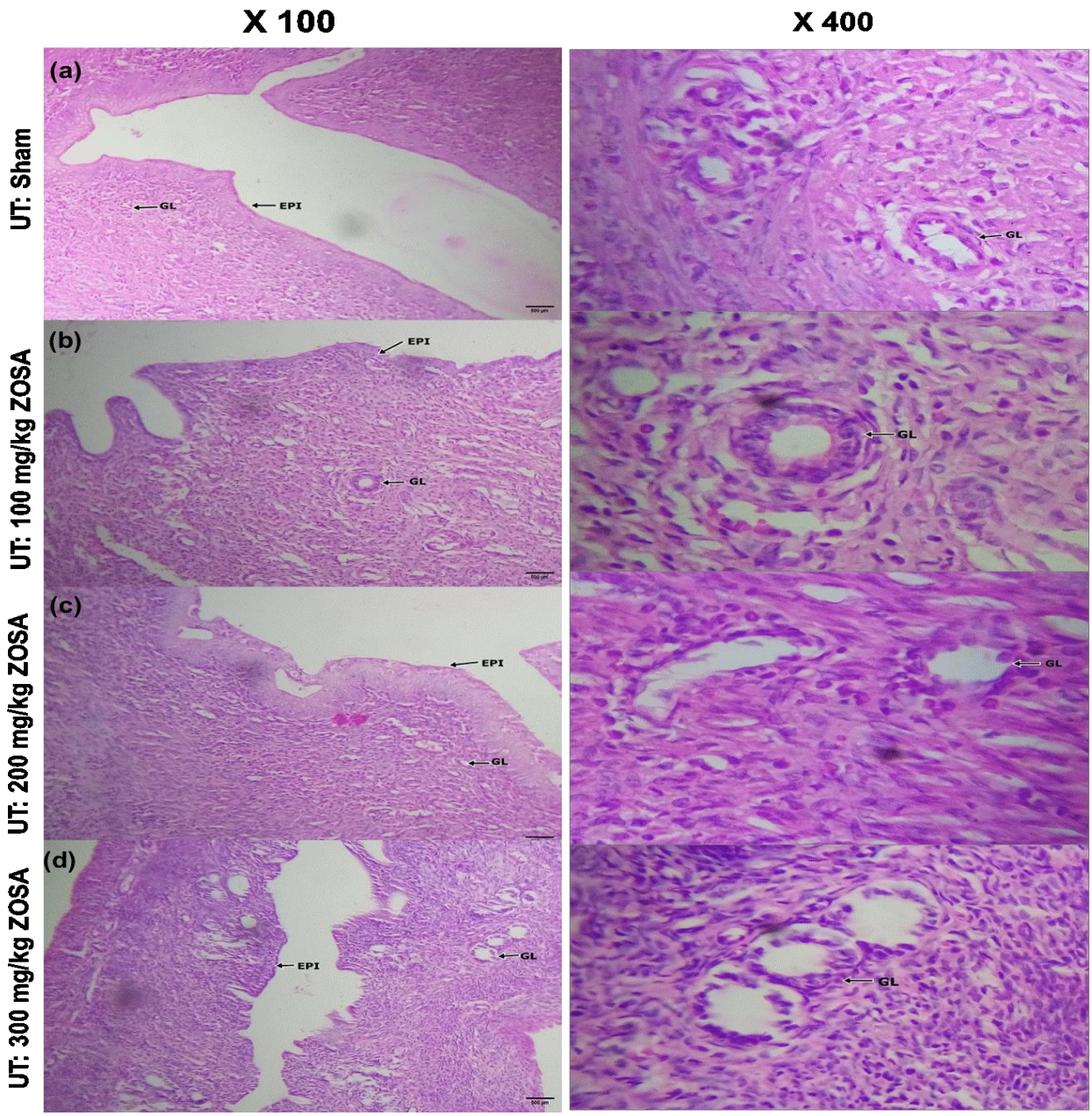

Figure 4: Representative photomicrographs of hematoxylin & eosin-stained sections of uterine sections of (a) Sham, (b) 100 mg/kg ZOSA, (c) 200 mg/kg ZOSA, (d) 300 mg/kg ZOSA. Magnifications x100 and x400 (scale bar: 500 µm). Abbreviations: ZOSA: *Zingiber officinale Syzygium aromaticum,* SGF: secondary Graafian follicle, MGF: mature Graafian follicle, GL: gland, EPI: epithelium.

Table 2: ZOSA Effects on the Reproductive Characteristics

Parameters	Sham	100 mg/kg ZOSA	200 mg/kg ZOSA	300 mg/kg ZOSA
Ovarian histomorphometry				
Ovary area (mm^2)	17.79 ± 17.35	11.69 ± 21.18	22.05 ± 17.69 [a, b]	28.06 ± 16.66 [a, b]
Corpus luteal number	2.40 ± 0.49	2.20 ± 0.40	4.40 ± 0.80 [a, b]	4.20 ± 0.75 [a, b]
GC thickness (µm)	918.10 ± 13.86	1621.20 ± 23.53 [a]	1400 ± 23.15 [a, b]	2333.09 ± 17.47 [a, b, c]
TL thickness (µm)	330.15 ± 11.46	556.46 ± 20.87 [a]	670 ± 15.76 [a, b]	1146.30 ± 13.68 [a, b, c]
Uterine histomorphometry				
Endometrial thickness(µm)	223.61 ± 11.48	280.13 ± 10.38	898.89 ± 15.34 [a]	1149.78 ± 15.09 [a, b]
Glandular thickness(µm)	544.06 ± 9.88	996.39 ± 9.09 [a]	1291. 20 ± 23.97 [a, b]	891.96 ± 21.08 [a, c]

Values are expressed as Mean ± SD, n=5. [a] $p<0.05$ vs Sham, [b] $p<0.05$ vs 100 mg/kg ZOSA, [c] $p<0.05$ vs 200 mg/kg ZOSA (Analyzed using one-way ANOVA; followed by Tukey's HSD). Abbreviations: ZOSA: *Zingiber officinale Syzygium aromaticum*, GC: granulosa cells

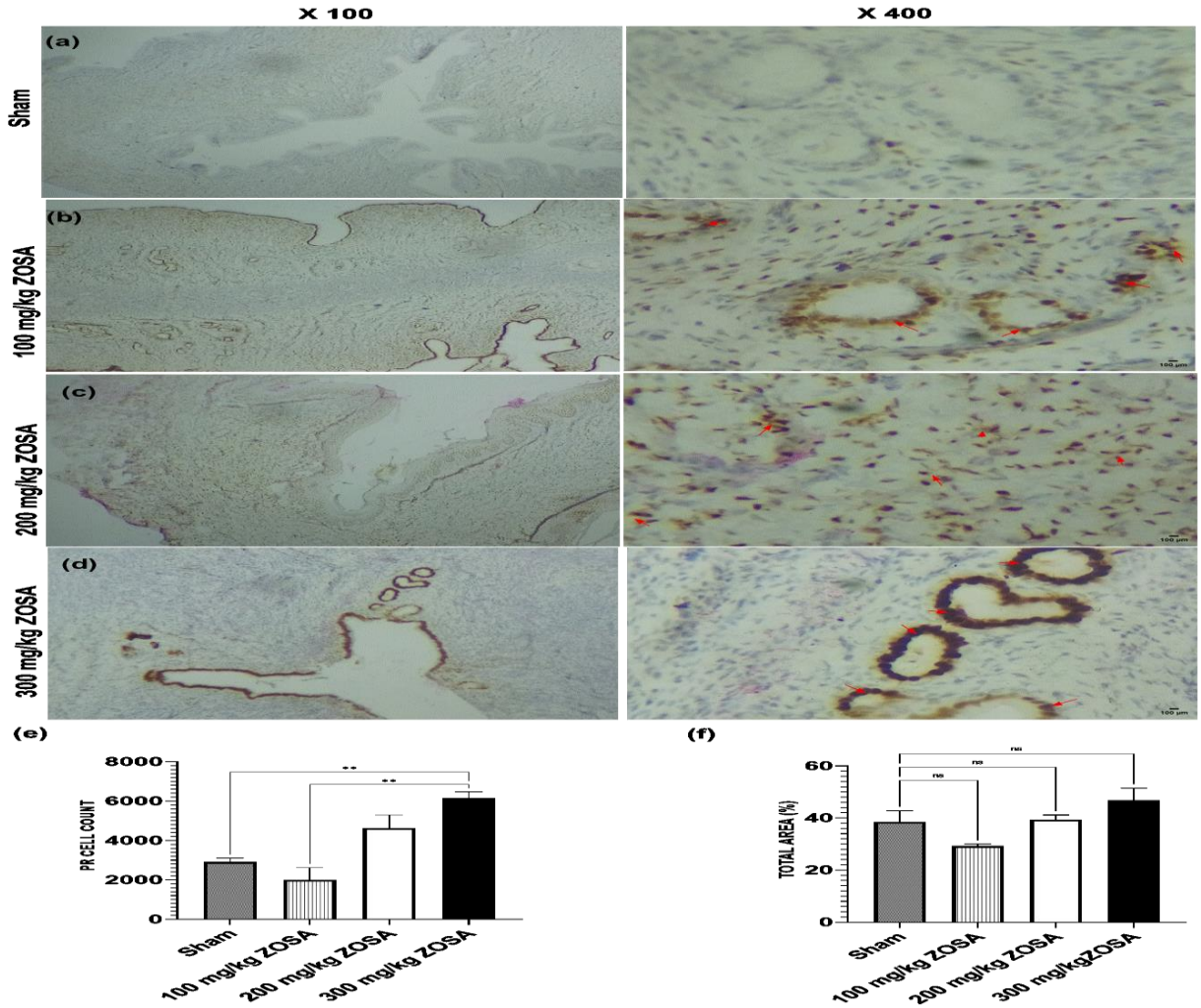

Figure 5: Representative photomicrographs of immunohistochemistry studies of progesterone receptor expressions in uterine sections of (a) Sham, (b) 100 mg/kg ZOSA, (c) 200 mg/kg ZOSA, (d) 300 mg/kg ZOSA, (e) PR cell count, (f) Total area (%). Arrowheads indicate positive cells. Magnifications at x100 and x400 and values expressed as Mean ± SD, n = 5. $^{*}p<0.05$, $^{**}p<0.01$. Abundant presence of progesterone receptors (red arrow) expression was seen notably at 300 mg/kg ZOSA relative to other groups. Abbreviations: ZOSA: Zingiber *officinale Syzygium aromaticum*, PR: Progesterone receptor, ZOSA: *Zingiber officinale Syzygium aromaticum*, ns: non-significant.

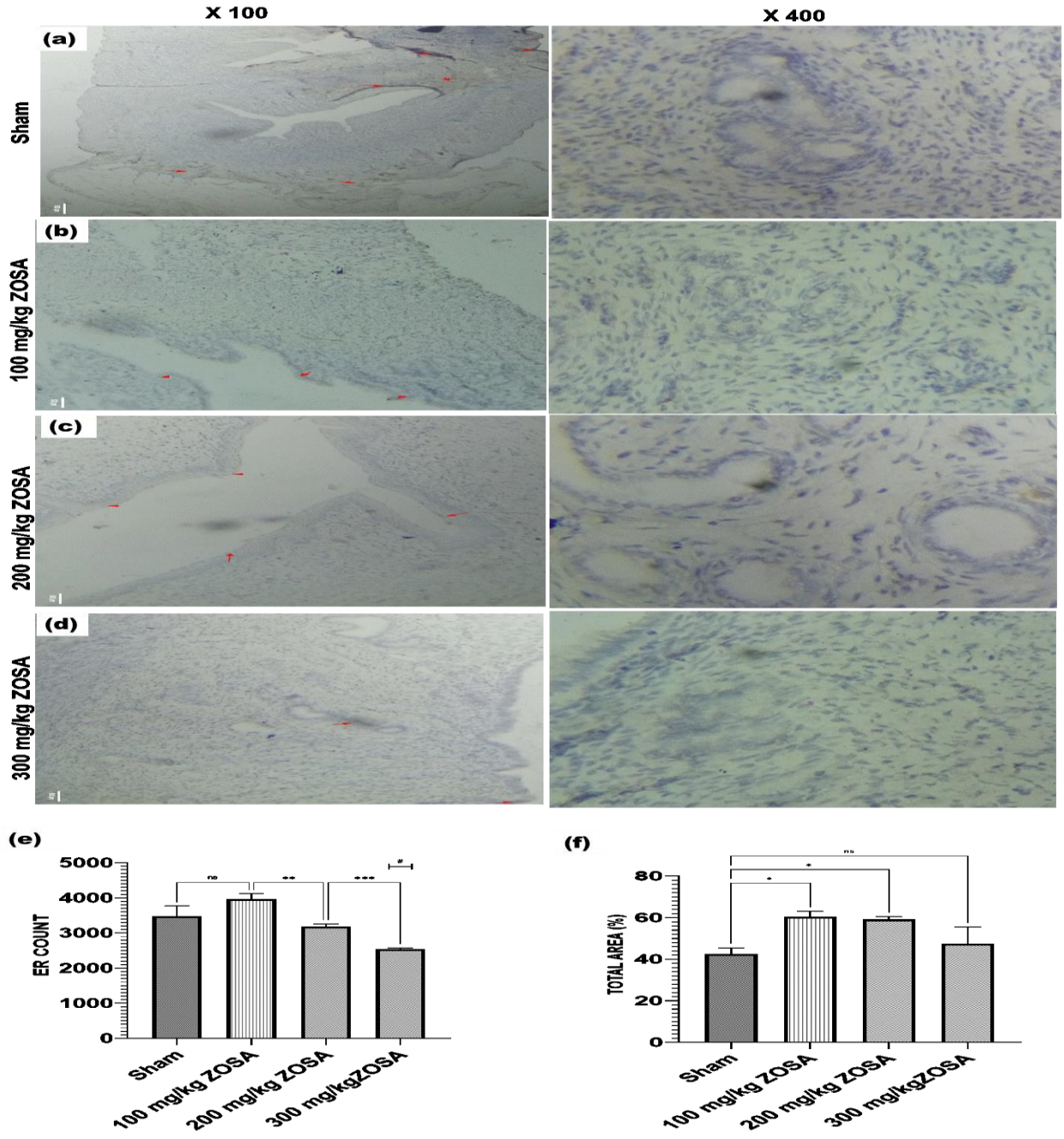

Figure 6: Representative photomicrographs of immunohistochemistry studies of estrogen receptor expressions in uterine sections of (a) Sham, (b) 100 mg/kg ZOSA, (c) 200 mg/kg ZOSA, (d) 300 mg/kg ZOSA. Arrowheads indicate positive cells. Magnifications x100 and x400. Estrogen receptors (red arrow) were scantily expressed in ZOSA groups relative to the Sham group. Abbreviations: ZOSA: *Zingiber officinale Syzygium aromaticum.* Immunohistochemistry of ER expression in (e) ER cell count, (f) Total area (%). Values are expressed as Mean ± SD, n = 5. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{\#}p<0.05$ vs Sham. Abbreviations: ns: non-significant, ER: Estrogen receptor, ZOSA: *Zingiber officinale Syzygium aromaticum*.

evidenced by immunohistochemistry (IHC) studies, where progesterone receptors exhibited strong expression in contrast to estrogen receptors. Data analysis from the IHC studies indicated significant variations in progesterone expressions ($p<0.05$–0.01) compared to estrogen ($p<0.05$–0.001). Previous studies [14-15] have individually demonstrated that both *Z. officinale* and *S. aromaticum* extracts elevate several female hormones. Yet, the synergistic effect of their co-therapy in diminishing estrogen levels has not been previously reported.

A dose-dependent relationship was observed in the influence of ZOSA extract on oxidative stress markers, with elevated levels of the antioxidants superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), and decreased levels of the oxidative stress biomarker malondialdehyde (MDA). Antioxidants are bioactive molecules capable of retarding,

inhibiting, or preventing the oxidation of free radicals [16-17], signifying a reduction in oxidative stress (MDA). Maintaining redox homeostasis is crucial, as its disruption can disrupt biological processes and lead to abnormalities, making the consumption of ZOSA beneficial.

However, a notable drawback of unprocessed herbal remedies, as evidenced in other studies, is their impact on biochemical parameters [1,18]. Similarly, biochemical parameters in our study exhibited alterations following the administration of ZOSA extract. For instance, liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in ZOSA-treated groups were elevated in a dose-dependent manner compared to the control group. Elevated liver enzymes serve as important indicators of potential hepatocellular damage and necrosis [18-19], with their serum levels postulated to correlate with the severity of such conditions [18-19]. Thus, this implies the potential hepatotoxicity of ZOSA at higher doses.

Regarding kidney function, a similar trend was observed, with elevated serum creatinine and urea levels, alongside reduced bicarbonate levels in the ZOSA-treated groups. Notably, there were no significant variations in the serum levels of sodium, potassium, and chloride. These alterations collectively suggest the potential for organ dysfunction or toxicity. The lipid profile of ZOSA revealed increases ($p<0.05$) in total cholesterol (TC) and very low-density lipoprotein (VLDL) relative to the control group. Elevated TC accompanied by increased VLDL levels is associated with a heightened susceptibility to dyslipidemia-related disorders [20]. Interestingly, while the elevation in serum high-density lipoprotein (HDL) was non-significant, it aligns with previous findings on the lipid-lowering properties of both *Z. officinale* [20-21] and *S. aromaticum* [22].

Histological examinations of the ZOSA-treated groups did not show any abnormal morphological changes, instead demonstrating positive effects on ovarian health, as evidenced by distinct follicular development and maturation processes, particularly with the presence of corpus luteum, indicating enhanced ovulatory processes [23]. Noteworthy changes were also observed in the uterus, including increased endometrial and glandular thickness, along with abundant endometrial glands.

Furthermore, the implications of ZOSA therapy extend beyond hormonal regulation and oxidative stress mitigation. The anti-inflammatory properties of *Z. officinale* and *S. aromaticum* have been acknowledged, suggesting their potential in managing inflammation-related conditions. Prior research has highlighted the anti-inflammatory effects of curcumin-like compounds in *Z. officinale*, which inhibit pro-inflammatory cytokines such as TNF-α and IL-1β [23-24]. This anti-inflammatory potential could be advantageous in conditions characterized by chronic inflammation, thereby enhancing overall reproductive health.

Nevertheless, further research is imperative to delve into the mechanistic pathways through which ZOSA extracts exert their effects. Longitudinal studies are essential to comprehensively evaluate their long-term effects on both hormonal profiles and organ function. Additionally, investigating the safety and efficacy of ZOSA in a broader population is crucial, considering the potential for individual variability in responses to herbal treatments.

CONCLUSION

Based on the results of this study, the ZOSA co-therapy appears to validate its social media acclaim and could potentially be advantageous for female fertility. Nevertheless, its consumption should be approached with care due to its impact on biochemical assays and estrogen expression.

AUTHORS' CONTRIBUTION

ESU, FJB, SCO, and **MOA** conducted the study and drafted the manuscript. **COF, SCO, FKO,** and **ESU** analysed the data. **ESU** and **FJB** reviewed and approved the final manuscript.

CONFLICT OF INTEREST

None.

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