

***Cissampelos pareira* ethanolic extract modulates hormonal indices, lipid profile, and oxidative parameters in transient infertility-induced female albino rats**

§Oladimeji Samuel Olugbenga, Igbalaye Jimoh Olamilekan, Awote Olasunkanmi Kayode, Shodimu Basit Opeyemi, Oladeinde Daniel Toluwani, Omorowa Valencia Truth, Oluwole Sekinat Mosunmola, Akinyemi Yewande Adenike, Shobowale Aishah Yetunde, Jimoh Damilola, Balogun Subomi Thouleehath, Adekoya Elizabeth Aduragbemi

¹Biochemistry Department, Faculty of Science, Lagos State University, Ojo, PMB 001, Lagos, Nigeria

§Corresponding author: Oladimeji Samuel Olugbenga, Email: olugbenga.oladimeji@lasu.edu.ng

Abstract

Cissampelos pareira is a medicinal plant with conflicting local claims regarding its usage in the management of fertility disorder. This study investigates the effect of the ethanolic extract of *C. pareira* on fertility indices in female albino rats. Ethanolic extract of *C. pareira* was administered orally for 14 days to female rats placed in different groups. Levonorgestrel (0.14mg/g) was orally administered to certain rat groups for 7 days to induce transient infertility before other treatments. The rats were sacrificed at the completion of their respective oral administration route. Blood samples were collected through cardiac puncture for hormonal and lipid profiling, which were analysed using commercial standard enzyme-linked immunosorbent assay (ELISA) kit. Organs were harvested for the assessment of oxidative parameters. Phytochemical screening reveals the presence of alkaloid, saponin, glycoside, tannin, steroid and flavonoid in the extract. The extract has no significant effect on progesterone, significantly reduced testosterone ($p<0.05$), but increased prolactin and estradiol concentrations ($p<0.05$). No significant hormonal difference in groups pre-administered levonorgestrel. The extract significantly elevated serum triglyceride, but reduced cholesterol level ($p<0.05$), even in groups pre-administered with levonorgestrel. The extract significantly reduced H_2O_2 and SOD activities ($p<0.05$) in the ovary and kidney respectively. Following levonorgestrel pre-administration, the extract significantly reduced H_2O_2 and GSH in the liver and kidney, elevated SOD activities in the liver, ovary and brain ($p<0.05$). This study demonstrated the antifertility properties of the ethanolic extract of *Cissampelos pareira*, shown by its effects of the hormonal and lipid profile, with moderate antioxidant effect.

Keywords: *Cissampelos pareira*, Reproductive hormones, Oxidative parameters, Lipid profiles, Fertility

Received October 2,2022; **Revised** April 23, 2023; **Accepted** May 8, 2023

<https://dx.doi.org/10.4314/br.v21i2.4> This is an Open Access article distributed under the terms of the Creative Commons License [CC BY-NC-ND 4.0] <http://creativecommons.org/licenses/by-nc-nd/4.0>.
Journal Homepage: <http://www.bioresearch.com.ng>.
Publisher: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.

INTRODUCTION

Infertility is established in a couple's inability to conceive after 12 months of unprotected regular intercourse. Accumulating evidence shows that the prevalence of human infertility has increased over the past decades, making it an important issue in the world (Noh *et al.*, 2020). About 3 to 7 % of all heterosexual couples, worldwide, have an unresolved problem of infertility (Mustafa *et al.*, 2019). Infertility can arise due to female, male or mixed problems, though diagnostic glitches make it difficult to ascertain the extent of the female's contribution. Study points toward female problems as the commonest single defined cause of infertility (Oladimeji *et al.*, 2014). Female infertility can arise due to failures at various steps, including ovulation, fertilization, embryo development, embryo transport, and implantation (Choi *et al.*, 2017).

Common major underlying factors of female infertility are hormonal imbalance and oxidative stress (Mustafa *et al.*, 2019, Noh *et al.*, 2020). Several reproductive functions such as oestrus cycle, folliculogenesis, fertilisation, implantation, milk production etc., are regulated under a timely interplay of paracrine and endocrine hormones of the reproductive system. Any disturbance in the equilibrium level of these hormones may lead to loss of a reproductive function and may cause infertility (Ogbuehi *et al.*, 2015). Cellular redox activity is a normal mechanism of male and female reproductive system (Alam *et al.*, 2019). Oxidative stress arises from an imbalance between reactive oxygen species (ROS) and protective antioxidants. Under normal circumstances, ROS production is moderated by antioxidants, which enable the organisms to cope with oxidative conditions and help the cell in repairing the damage caused by ROS (Wang *et al.*, 2017). It was reported that oxidative stress, at regulated levels, facilitates some physiological reproductive functions but if unmitigated it can initiate some pathological processes in the reproductive tract that contribute to infertility and poor pregnancy outcomes (Noh *et al.*, 2020). Oxidative stress, due to mitochondrial dysfunction, is the main cause for chromosomal segregation disorders, maturation and fertilisation failures, or oocyte/embryo fragmentation (Ishii *et al.*, 2014).

Series of plants have been screened worldwide as being used for treatment of various human ailments since ancient times (Ansari *et al.*, 2017). The use of plant extracts as fertility enhancer in humans is now on the increase, especially in

developing countries, due to their accessibility, availability and affordability. About 80% of people in Africa depend on traditional medicine (Oladimeji *et al.*, 2014). Moreover, plant products are regarded to be natural and are generally considered safer than synthetic drugs, sparking an increasingly shifting of attention to plant extracts as remedy for numerous medical conditions (Egba *et al.*, 2020). *Cissampelos pareira* (velvetleaf) is a popular medicinal climber-plant of the Menispermaceae family. Its major phytoconstituents are alkaloids, though also contains flavonoids, glycosides, fatty acids, terpenoids, saponins, pectin and reducing sugars (Hikal *et al.*, 2021). *C. pareira* has been used extensively in the traditional medicine for the treatment of numerous diseases such as ulcer, wound, rheumatism, fever, asthma, cholera, diarrhoea, inflammation, snakebite, malaria, rabies, and also recommended for blood purification (Kumari *et al.*, 2021). Outside Africa, parts or whole plant of *C. pareira* have been used locally to increase muscle strength and sexual vigour (Suresh *et al.*, 2016), as remedy for breast milk disorders and painful menstruation, to prevent miscarriage (Khare, 2008; Sudhakaran, 2012) and uterine haemorrhage (Bafna and Mishra, 2010). Though the aforementioned traditional usages indicate the fertility enhancing potentials of *C. pareira*, there are reports on traditional usages that suggest otherwise. The leaves and aerial parts have been used for birth control and to induce abortion in human (Tiwari *et al.*, 1982; Dangol and Gurung, 1991). The root has also been reported to be used traditional for birth control and as anti-fertility agent (Maurya *et al.*, 2004; Samanta *et al.*, 2015). Similarly, in Africa, reports on the fertility-related traditional usage of *C. pareira* are contrasting. The roots and/or leaves of *C. pareira* were reported to be used in preventing frequent abortions, treating pregnancy pain and other pregnancy-related problems (Hedberg *et al.*, 1983; Cecilia and Lucy, 2010), whereas juice extracted from the roots was used as contraceptive (Cecilia and Lucy, 2010). Moreover, several pharmacological studies have demonstrated the crude extracts of *C. pareira* to possess various activities such as antipyretic, anti-inflammatory, antiarthritic, antiulcer, antioxidant, antidiabetic, anticancer, antimicrobial, antioxidant, antivenom, antifertility, antimalarial and immunomodulatory (Kumari *et al.*, 2021).

Till date, despite the popular traditional usage of *C. pareira* in the management of fertility related conditions, scientific study of this plant to this effect remains fragmentary, as a few, findings from scientific studies indicated the antifertility

properties of *C. pareira* (Samanta *et al.*, 2015; Ganguly *et al.*, 2007), the traditional usage and claim of *Cissampelos spp.* as a pro-fertility agent persists, hence, the need for more scientific investigation for verification of these claims and usages. We studied the effects of the ethanolic extract of *C. pareira* on reproductive hormones, oxidative markers and lipid profiles in female albino rats.

MATERIALS AND METHODS

Collection of Plant Samples

Cissampelos pareira leaves were collected from a local farm of Agbara, Ogun state, Nigeria. It was properly identified and authenticated, LUH 8560 at the Herbarium unit of Botany Department, University of Lagos, Nigeria. The leaves were air-dried in the absence of sunlight for few weeks.

Extraction of *Cissampelos pareira*

Air-dried and powdered leaves of *Cissampelos pareira* (20g) were extracted using Soxhlet extraction method and ethanol as solvent. Powdered *Cissampelos pareira* was placed into the thimble of the Soxhlet extraction apparatus chamber using 250ml of 95% ethanol. The thimble was loaded into the main chamber of the Soxhlet extractor and the sample was extracted for 20 hours. The ethanolic extract was concentrated under vacuum in a rotary evaporator to yield semi-solid mass (19.6g) which was vehicle out by 50ml sunflower oil into a beaker.

Phytochemical Screening

Phytochemical studies of the ethanolic leaf extract were carried out by qualitative methods using commonly employed precipitation and coloration reaction to identify the major natural chemical groups such as: alkaloids, flavonoids, tannins, glycosides, saponin, terpenoids and steroid as proposed by Nandagoapalan *et al.* (2016).

Acquisition and Acclimatization of Animals

Female albino rats numbering 39, weighing between 60g-100g were obtained from animal

house of the department of Biochemistry in Babcock University, Ogun state. The rats were allowed to acclimatize for two weeks and fed with commercial rat feed under hygienic and favourable conditions with the availability of water and maintained under a 12 h light/12 h dark cycle following the Animal Care Ethics of the Biochemistry Department, Lagos State University. All experimental procedures were in adherence to all international and national standards and were examined and approved by the Lagos State University ethics committee.

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 120g and divided into three groups each containing three rats (Chinedu *et al.*, 2013). The dosages tested for were: a. 32g/100g for group 1(1ml), b.16g/100g for group 2(0.5ml), c.8g/100g for group 3(0.25ml).

The animals were maintained under the same natural condition and observed for 96 hours for symptoms of toxicity and mortality. All dosage administered were discovered to be non-lethal.

Animal Grouping and Experimental Design

After chronic toxicity testing, the animals were grouped accordingly:

- Group 1 and 2 served as control: The rats received 1ml of distilled water and sunflower oil respectively.
- Group 3 were induced with infertility using 0.14mg/g of levonorgestrel.
- Group 4 were orally fed with extracts only (0.7g/100g).
- Group 5 were orally fed with 0.14mg/g of levonorgestrel for 7days and later received 0.7g/100g of extract for 14 days.
- Group 6 were orally fed with 0.14mg/g of levonorgestrel for 7 days and later received 2.13g/100g of extract for 14 days.

Table 1: Grouping of animals

S/N	GROUPS
1	Control + distilled water
2	Control + Oil
3	Levonorgestrel (Infertile)
4	Extract only
5	Infertile + low dose extract
6	Infertile + high dose extract

Collection of Blood and Organ

The animals were sacrificed after 2 weeks of treatment with extract. The rats were anaesthetized with chloroform and the blood was collected through cardiac puncture. The organs (brain, ovary, liver and kidney) were collected and placed in physiological saline solution (Chen *et al.*, 2018).

Estimation of Reproductive Hormones

The reproductive hormones testosterone, progesterone, prolactin and estradiol were estimated using ELISA technique based on the principle of a solid-phase enzyme-linked immunosorbent assay. Thawed serum or plasma samples were assayed for testosterone, progesterone, prolactin and estradiol using (PerkinElmer, USA) kits. The assay procedure was according to manufacturer's description. Hormone concentrations were calculated using a standard curve of the absorbance of the standards against their concentrations and the results expressed as ng/ml.

Determination of Oxidative Stress Parameters Superoxide Oxide Dismutase (SOD) Activity

The SOD activity was measured according to the method of (Misra and Fridovich, 1972) and as reported by (Rahal *et al.*, 2014).

Reduced Glutathione (GSH) Concentration

The absorbance of the reaction mixture was read at 412 nm against a reagent blank. Glutathione concentration in each sample was extrapolated from GSH standard curve, according to the method of (Ruch *et al.*, 1989).

Hydrogen Peroxide (H₂O₂) Scavenging Activity

The ability of plant extracts to scavenge hydrogen peroxide was estimated according to the method

of (Ruch *et al.*, 1989). Briefly, a solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (20–60 lg/mL) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging activity was calculated as follows:

$$\% \text{ H}_2\text{O}_2 \text{ scavenged} = ((A_i - A_t) / A_i) \times 100$$

where A_i represents the absorbance of control and A_t is the absorbance of test samples.

Lipid Profile

Determination of Blood Cholesterol

The cholesterol level was determined after enzymatic hydrolysis and oxidation. The procedure was according to the kit's manufacturer (Randox) (Lai *et al.*, 2006).

Determination of Blood Triglyceride

The triglycerides level was determined after enzymatic hydrolysis with lipases by colorimetric method. The assay procedure was according to the kit's manufacturer (Randox) (Odetola *et al.*, 2006).

Statistical Analysis

Data were analysed by one-way analysis of variance (ANOVA) to test for significant differences among the group of rats using Graph pad prism v.8.4.2 and data were expressed as mean \pm standard error of mean. Bars having letters are significantly different with amount of * showing the levels of significance, taking $p < 0.05$ as the significant level.

RESULTS

Phytochemical screening of *Cissampelos pareira*

Table 1 below shows the qualitative phytochemical constituent of *Cissampelos pareira* leaf indicating the presence of phenol, flavonoid, alkaloid, steroid, tannin, saponin and terpenoid.

Effect of *Cissampelos pareira* on Reproductive Hormones

Figure 1 shows the serum progesterone, testosterone, estradiol and prolactin concentrations as measured in the different rat groups. No significant difference in the serum progesterone concentration across the groups was observed. However, testosterone concentration reduced significantly in the extract only group compared with the distilled water and oil only control groups. There was significant increase in serum estradiol concentration in the extract only group compared with both the distilled water and oil only control groups, whereas, serum estradiol concentration in the ILD and IHD groups decreased significantly in comparison with the distilled water, oil only and infertile control groups. Serum prolactin concentration increased significantly in the COO, CI, EO and ILD groups compared with the control distilled water group. Likewise, a significant increase in serum prolactin concentration was seen in the ILD group compared with the CI group. Conversely, administration of high dose of extract after the induction of infertility (IHD) resulted in a significant decrease in serum prolactin concentration when compared with the oil only (COO) and levonorgestrel (CI) controls, as well as with the group treated with low dose of extract after infertility induction (ILD), as presented in Figure 1.

Effect of *Cissampelos pareira* on Oxidative Stress Parameters

Figure 2 shows the SOD, GSH and H₂O₂ activities as measured in various organs; liver, kidney, ovary and brain of different rat groups. H₂O₂ activities was observed to show a statistical decrease in the control groups CDW, COO as compared to the levonorgestrel CI group and a statistical increase in the control group CI (levonorgestrel) as compared to the IHD group in the liver and kidney. However, in the ovary, H₂O₂ activities was observed to show a statistical decrease in the control groups CDW as compared with COO group and a statistical increase in the control group COO as compared with CI group (levonorgestrel) and EO group. No significant difference in H₂O₂ activities across the group in the brain. The GSH concentration in the liver shows a significant decrease in the control group COO as compared to the control group CI and a statistical increase in the control group CI as compared to the ILD group. Likewise, in the kidney, there is a significant decrease in the control groups CDW and COO as compared to the control group CI and a significant increase in control group CI (levonorgestrel) as compared to ILD and IHD groups. Whereas, both in the ovary and brain, there is no significant difference in GSH activity across the groups. The SOD activity in the liver shows a significant decrease in the control groups CDW, COO and CI as compared to the ILD and IHD groups. Also, there is a decrease in the control group CDW as compared to the ILD and IHD groups and a statistical increase in the control group COO as compared to the ILD and IHD groups in the kidney. Whereas in the ovary, there is a significant decrease in the control group CI (levonorgestrel) as compared to the ILD. In the brain, there is a significant decrease in the control groups CDW, COO as compared to the IHD group as presented in Figure 2.

Table2. Phytochemicals present in the ethanolic extract of *Cissampelos pareira* leaf.

Phytoconstituents	Inference
Alkaloid	Present
Saponin	Present
Glycoside	Present
Tannin	Present
Steroid	Present
Flavonoid	Present

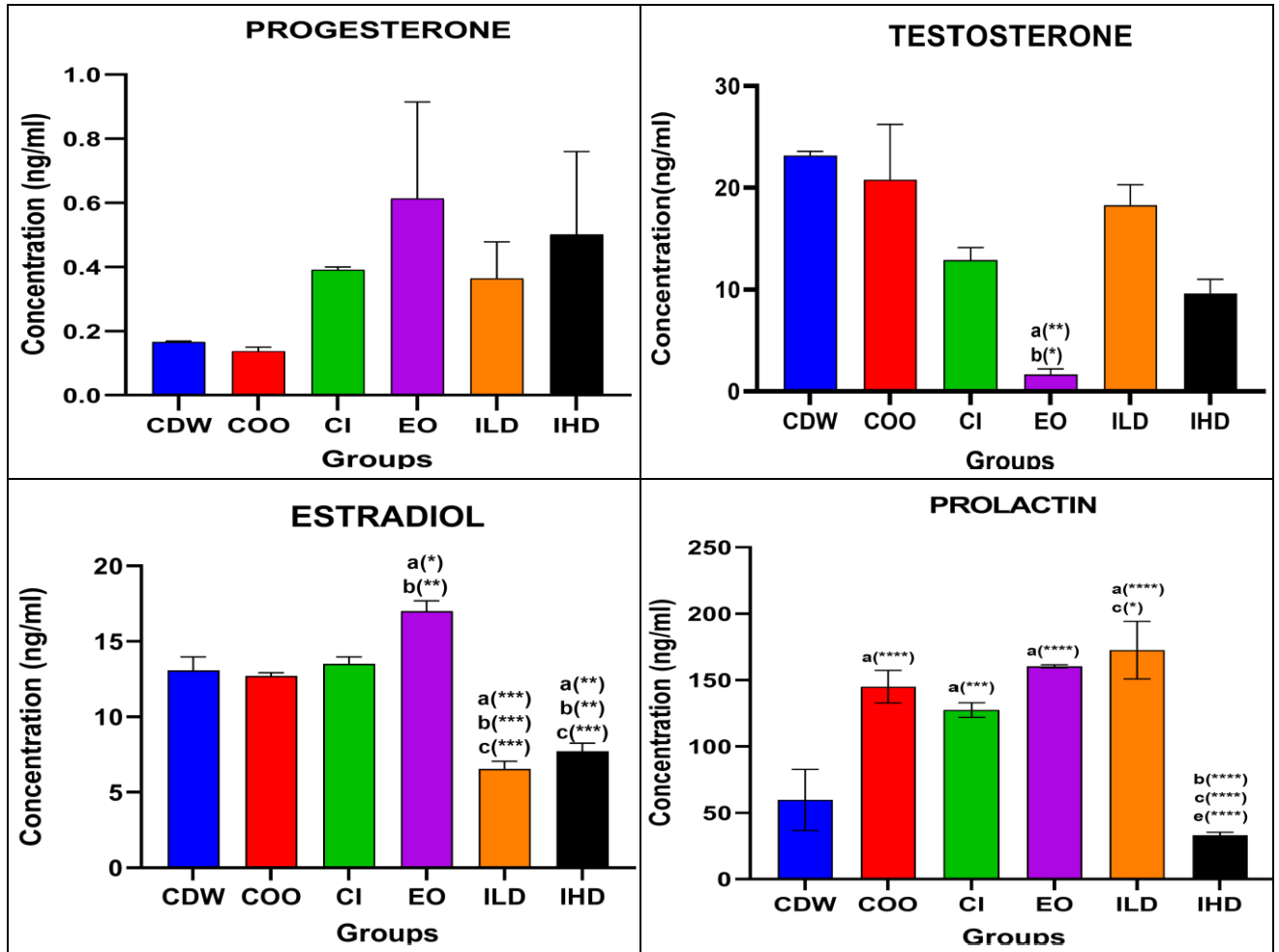


Figure 1. The concentration of serum sex hormones (progesterone, testosterone, estradiol and prolactin) in female albino rats as determined in the control and treatment groups.

a, b, c, e shows a comparison with the CDW, COO, CI and ILD group respectively.

(*), (**), (***), (****) indicates a significant relationship at $p < 0.05, 0.01, 0.001$ and 0.0001 respectively.

- Control Distilled Water (CDW)
- Control Oil Only (COO)
- Control Infertile (CI)
- Extract Only (EO)
- Infertile Low Dose (ILD)
- Infertile High Dose (IHD)

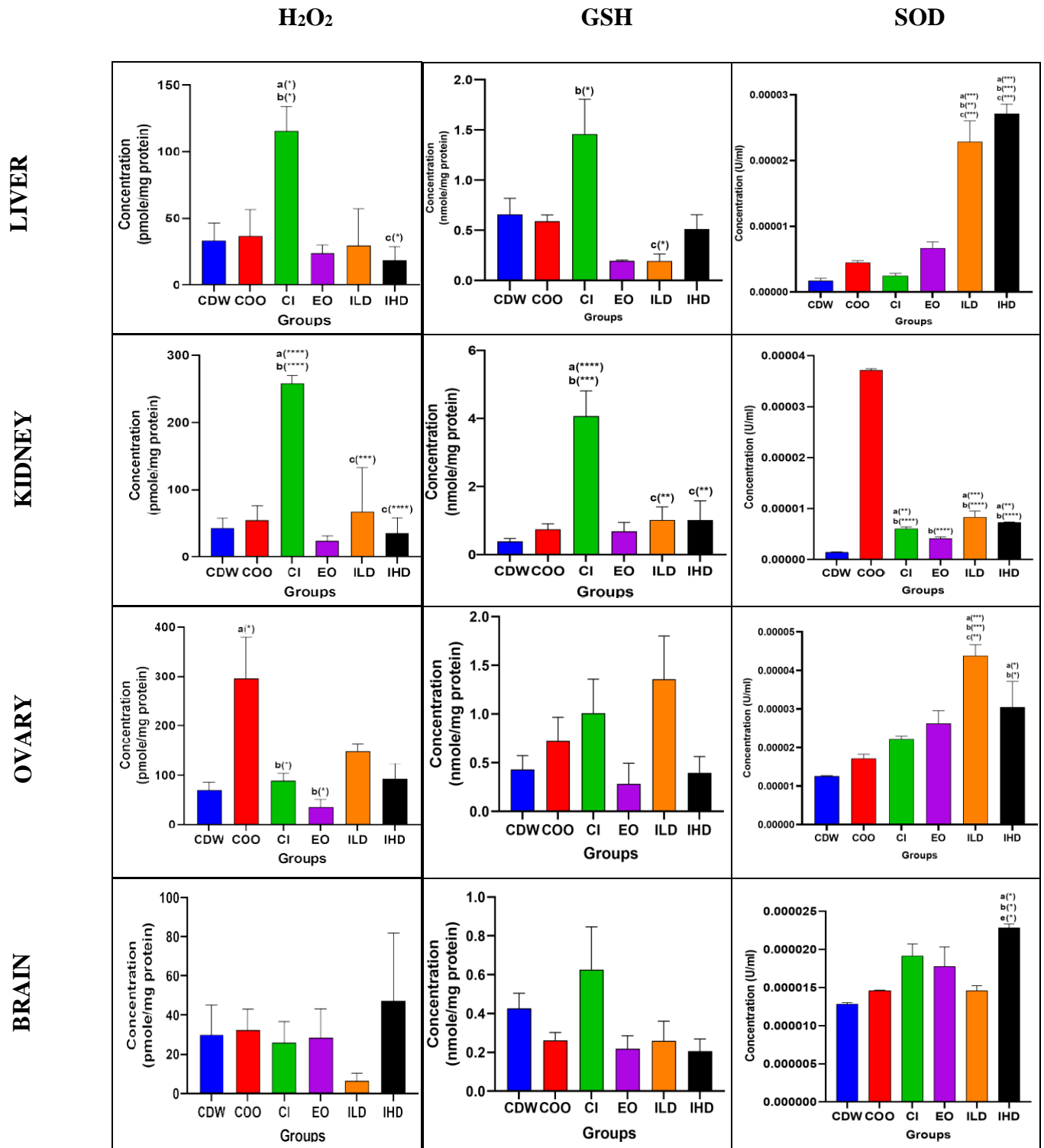


Figure 2. The concentration of oxidative stress markers (hydrogen peroxide – H₂O₂, glutathione – GSH and superoxide dismutase - SOD) as determined in the liver, kidney, ovary and brain of female albino rats across the control and treatment groups.

a, b, c, shows a comparison with the CDW, COO, CI and ILD group respectively.

(*), (**), (***) and (****) indicates a significant relationship at p < 0.05, 0.01, 0.001 and 0.0001 respectively.

- Control Distilled Water (CDW)
- Control Oil Only (COO)
- Control Infertile (CI)
- Extract Only (EO)
- Infertile Low Dose (ILD)
- Infertile High Dose (IHD)

Effect of *Cissampelos pareira* on Lipid Parameters

Figure 3 shows the Triglyceride and Cholesterol level as measured in the different rat groups. In the triglyceride levels, there is a significant decrease in the control group CDW as compared

to the IHD group, also a significant decrease in the control groups COO and CI as compared to the ILD and IHD groups. Likewise in the cholesterol levels, there is a significant increase in the control groups CDW, COO and CI as compared to the ILD and IHD groups as presented in Figure 3.

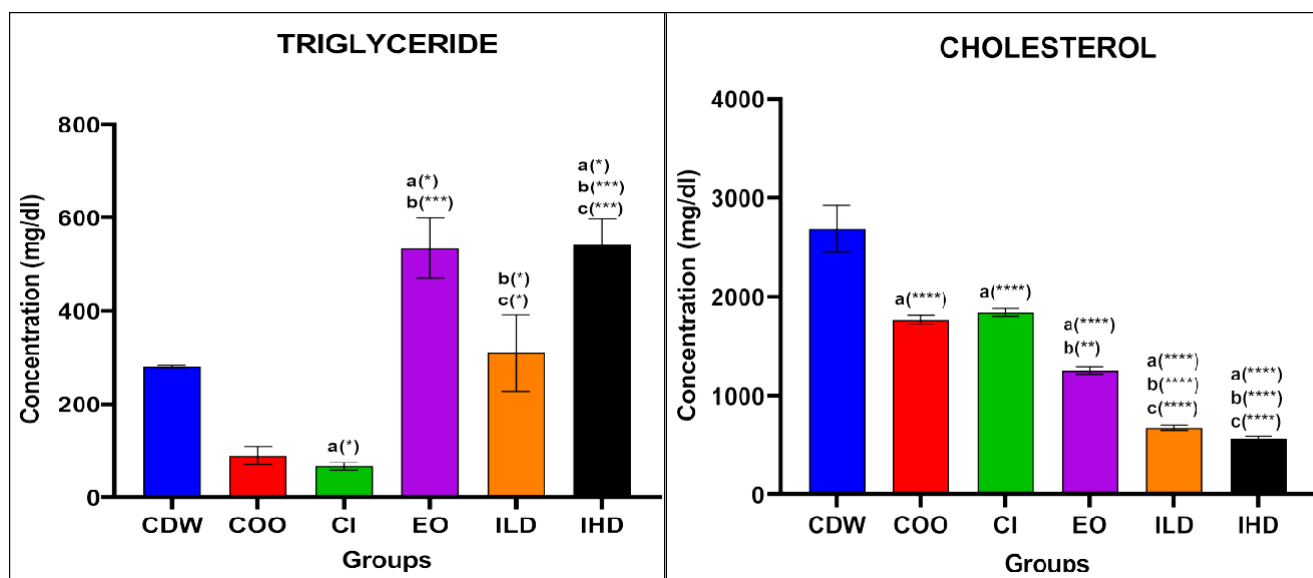


Figure 3. The concentration of serum triglyceride and cholesterol in female albino rats as determined in the control and treatment groups.

^{a, b, c} shows a comparison with the CDW, COO and CI group respectively.

(*), (**), (***) (****) indicates a significant relationship at $p < 0.05, 0.01, 0.001$ and 0.0001 respectively.

- Control Distilled Water (CDW)
- Control Oil Only (COO)
- Control Infertile (CI)
- Extract Only (EO)
- Infertile Low Dose (ILD)
- Infertile High Dose (IHD)

DISCUSSION

Plants contain bioactive compounds that possess therapeutic properties, hence their usage in the treatment of various human diseases or disorders. Many of these bioactive constituents of plants can modulate the reproductive system and are being exploited for their potentials in the treatment and management of fertility related disorders. *C. pareira* was selected for this study due to claims by local medicine practitioners of its usage in the treatment of reproductive problems. Local practitioners, majorly, use water to extract bioactive compounds from these plants because it is safe, cheap and readily available. However, certain important active phytoconstituents do not extract in water due to their hydrophobic chemical

nature. Hence, we employed ethanolic leaf extract of *C. pareira* in our study. Furthermore, to investigate the impact of *C. pareira* extracts on contraceptive drugs, some of the experimental rats were pre-administered with levonorgestrel, an oral contraceptive, before treatment with the plant extract.

In this study, the phytochemical screening of the ethanolic leaf extract of *C. pareira* revealed the presence of all tested phytochemicals (alkaloids, flavonoids, tannins, terpenoids, saponins, glycosides and steroids). These secondary metabolites are known to show medicinal activities, as well as exhibit physiological effects (Wink, 2015). Therefore, the medicinal roles of this plant could be related to such identified bioactive compounds.

Disruption of reproductive hormone levels or signalling can result to fertility disorders. In this study, the effect of the ethanolic leaf extract of *C. pareira* on serum reproductive hormone levels in female rats was investigated. We found that the administration of the plant extract has no major effect on serum progesterone, however, was responsible for a significant decline in testosterone level. Testosterone is the most abundant biologically active female hormone having a role in female sex drive and libido (Glaser and Dimitrakakis, 2013), hence, females with lower-than-normal testosterone level may experience hypoactive sexual desire disorder. Conversely, we found that estradiol and prolactin concentrations were significantly elevated by the plant extract. Estrogen acts in a feedback mechanism to influence the production of FSH from the pituitary gland (Yakubu et al., 2008). When FSH is sufficiently suppressed by increased estrogen, follicular growth will be minimum and this hinders ovulation (Ogbuehi et al., 2015). Likewise, studies have shown that increased prolactin reduces libido (Wieck and Haddad, 2003) and can suppress the secretion of FSH and gonadotropic-releasing hormones (GnRH), leading to hypogonadism and sexual dysfunction in male and female (Grattan et al., 2007). Prolactin increase may disturb ovulation and cause the loss of menstrual periods which will hinder conception (Zhaira et al., 2019; Ogbuehi et al., 2015). Our findings indicate that the ethanolic leaf extract of *C. pareira* has anti-fertility properties. Reports from some similar studies corroborate our findings. Luangpirom et al. (2010) reported, amidst other findings, that the administration of high dose of *C. pareira* leaf extract caused a significant elevation of plasma prolactin and reduction of follicle stimulating hormone (FSH) in male mice. Elsewhere, *C. pareira* leaf gel was demonstrated to possess antifertility properties by increasing serum prolactin and decreasing testosterone levels, causing impairment of spermatogenesis and reduced sperm quality in male mice, whereas, it inhibits ovulation and embryo implantation by increasing prolactin concentration in female mice (Luangpirom et al., 2015). In another study, the methanolic extract of *C. pareira* was reported to produce antifertility effect by causing increase in serum estradiol and decrease in progesterone levels, as well as reduction of implants in female rats (Jhuma, 2016).

In addition, this present study investigated the effect of levonorgestrel on serum sex hormone concentrations in the female rats. Levonorgestrel

Bio-Research Vol.21 No.2 pp.1961-1972 (2023)

is a progestogen whose mode of action is to prevent the fertilization of eggs in the woman's uterus by thickening the uterine wall (Frag et al., 2015). Here, we found that levonorgestrel has no remarkable effect on progesterone, testosterone, estradiol and prolactin levels. Subsequent treatment with the ethanolic leaf extract of *C. pareira* has no observable impact on the progesterone and testosterone levels, however, estradiol concentration was reduced while the effect on prolactin level is inconsistent. Our findings suggest a possible antagonistic interaction between levonorgestrel and *C. pareira* extract on serum estradiol concentration. However, there is no available report to compare our findings with currently.

Furthermore, since studies have shown that reactive oxygen species have a physiological and pathological role in the female reproductive tract (Alam et al., 2019; Noh et al., 2020), the present study investigated the effect of the ethanolic extract of *C. pareira* on GSH level, SOD and H₂O₂ activities in some organs of the female rats. We found that the extract has no observable effect on the measured parameters in the brain and liver of the rats. In the kidney, H₂O₂ and GSH levels were unaffected; however, SOD activity was reduced by the extract. Whereas in the ovary, the extract caused the reduction of H₂O₂, although GSH level and SOD activity were unaltered. These observations demonstrate that the ethanolic extract of *C. pareira* possess mild antioxidant properties in the kidney and ovary of the female rats. This agrees with the findings of Amresh et al. (2007) who reported that ethanolic extract of *C. pareira* scavenged H₂O₂ and significantly reduced gastric lipid peroxidation and SOD activity. The extract also increased GSH level, glutathione peroxidase and glutathione-S-transferase activities in benzo(a)pyrene-induced oxidative stress in mice (Amresh et al., 2007). Furthermore, in this study, we observed in the rats that were pre-administered with levonorgestrel prior to *C. pareira* extract treatment that the extract exhibits antioxidant properties by scavenging H₂O₂ in the liver and kidney. However, reduction of GSH level in the liver and kidney and elevation of SOD activities in the liver, ovary and brain suggest a possible negative synergistic interaction between levonorgestrel and the phytoconstituents of *C. pareira* extract that led to the generation of superoxide anions, triggering the increase in SOD activities.

More so, this present study evaluated the effect of the ethanolic leaf extract of *C. pareira* on serum triglyceride and cholesterol concentrations in the

female rats. The extract elevated serum triglyceride concentration and lowered the cholesterol level. In addition, this study found that levonorgestrel has no effect on the serum triglyceride and cholesterol concentrations, however, subsequent treatment with *C. pareira* extract caused a dose-dependent increase in serum triglyceride and decrease in serum cholesterol concentration. High blood level of triglyceride is associated with and precedes the onset of pre-eclampsia – a hypertensive pregnancy condition (Gallos et al., 2013), however, cholesterol level has been demonstrated to be elevated in pregnancy (Bartels and O'Donoghue, 2011). Thus, these findings further suggest the antifertility potential of *C. pareira* extract. Though the serum lipid alterations may not be attributed to the observed disruptions of sex hormones, as it has been found in some studies that irregularities in sex hormone concentrations are uncorrelated with serum lipid profiles in females (Yasui et al., 2008; Bizoń et al., 2021). In agreement with our findings, Surendran et al. (2011) investigated the hepatoprotective activity of aqueous-ethanolic root extract of *C. pareira* against CCl₄-induced hepatic damage in male Sprague Dawley rats. They found that at 200 and 400 mg/kg dose levels of *C. pareira*, cholesterol level declined and the triglyceride level was elevated (Surendran et al., 2011). Meanwhile, Thavamani et al. (2014) reported that the methanolic extract of *C. pareira* decreased both cholesterol and triglyceride concentrations in Dalton's lymphoma ascites bearing mice.

CONCLUSION

Our findings indicate that the ethanolic leaf extract of *Cissampelos pareira* possess antifertility activities, demonstrated by its induction of increased prolactin and estradiol basal concentration while causing the reduction of testosterone level in the female albino rats. A further indication of the extract's potential antifertility action was its elevation of triglyceride levels, which has been linked to a higher risk of preeclampsia. Hence, there is need for caution in the use of *Cissampelos spp.* by local medicines as fertility enhancers. Nevertheless, the extract showed a modest antioxidant capacity by scavenging H₂O₂ in the kidney and ovary. Additionally, it was discovered that the extract combined with levonorgestrel in certain instance to exert more negative impacts on the hormonal, lipid, and oxidative stress indices. It is noteworthy, however, that this research used ethanolic extract of *C. pareira* as opposed to the aqueous extract,

which is commonly used by local practitioners. Also, this study focused on the leaf extract while local users would commonly make use of extract from different parts of the plants. Further research should be conducted to evaluate the molecular mechanisms of the extract's action and the prospective development and use as an oral contraceptive. Likewise, there should be further analysis on *C. pareira* extract, comparing different extraction medium and different parts of the plant.

Conflict of interest

Authors have no conflict of interest to declare.

Author contribution

OSO designed the study and wrote the protocol. IJO and AOK performed the statistical analysis. IJO, SBO and ODT wrote the first draft of the manuscript. OVT, OSM, AYA and SAY managed the analyses of the study. JD, BST and AEA performed literature review. All authors read and approved the final manuscript.

REFERENCES

- Alam, F., Khan, T. A., Amjad, S., & Rehman, R. (2019): Association of oxidative stress with female infertility-A case control study. *JPMA. The Journal of the Pakistan Medical Association*, **69**(5): 627.
- Amresh, G., Rao, C. V., & Singh, P. N. (2007): Antioxidant activity of *Cissampelos pareira* on benzo (a) pyrene-induced mucosal injury in mice. *Nutrition Research*, **27**(10): 625-632.
- Ansari, A. S., Sevliya, K., Mohammad, I., Badar, A., & Lohiya, N. K. (2017): Plants for female fertility regulation: a review. *Journal of Pharmacology and Toxicology*, **12**(2): 57-75
- Bafna, A., & Mishra, S. (2010): Antioxidant and immunomodulatory activity of the alkaloidal fraction of *Cissampelos pareira* linn. *Scientia pharmaceutica*, **78**(1): 21-32.
- Bartels, Ä., & O'Donoghue, K. (2011): Cholesterol in pregnancy: a review of knowns and unknowns. *Obstetric medicine*, **4**(4): 147-151.

- Bizoń, A., Franik, G., Niepsuj, J., Czwojdzńska, M., Leśniewski, M., Nowak, A., Szykaruk-Matusiak, M., Madej, P. and Piwowar, A. (2021): The associations between sex hormones and lipid profiles in serum of women with different phenotypes of polycystic ovary syndrome. *Journal of Clinical Medicine*, **10**(17): 3941.
- Cecilia, P. M. N. and Lucy, L. M. (2010): Medicinal plants used for contraception and pregnancy-related cases in Malawi: A case study of Mulanje District. *Journal of Medicinal Plants Research*, **4**(20): 2121-2127.
- Chen, P., Chen, F., and Zhou, B. (2018): Antioxidative, anti-inflammatory and anti-apoptotic effects of ellagic acid in liver and brain of rats treated by D-galactose. *Scientific reports*, **8**(1): 1-10.
- Chinedu, E., Arome, D. and Ameh, F. S. (2013): A new method for determining acute toxicity in animal models. *Toxicology International*, **20**(3): 224.
- Choi, H. J., Chung, T. W., Park, M. J., Jung, Y. S., Lee, S. O., Kim, K. J. and Ha, K. T. (2017): Water-extracted tubers of *Cyperus rotundus* L. enhance endometrial receptivity through leukemia inhibitory factor-mediated expression of integrin $\alpha V\beta 3$ and $\alpha V\beta 5$. *Journal of ethnopharmacology*, **208**: 16-23.
- Dangol, D. R., & Gurung, S. B. (1991): Ethnobotany of the Tharu tribe of Chitwan district, Nepal. *International Journal of pharmacognosy*, **29**(3): 203-209.
- Egba, S. I., Okonkwo, C. O., Omeoga, H. C., and Ekong, I. E. (2020): Comparative modulation of the reproductive system by ethanol leaf extracts of *Asystasia gangetica* and *Anthocleista vogelli* in male wistar rats. *European Journal of Medical and Health Sciences*, **2**(3): 287.
- Gallos, I. D., Sivakumar, K., Kilby, M. D., Coomarasamy, A., Thangaratinam, S. and Vatish, M. (2013): Pre-eclampsia is associated with, and preceded by, hypertriglyceridaemia: a meta-analysis. *BJOG: An International Journal of Obstetrics & Gynaecology*, **120**(11): 1321-1332.
- Ganguly, M., Borthakur, M. K., Devi, N. and Mahanta, R. (2007): Antifertility activity of the methanolic leaf extract of *Cissampelos pareira* in female albino mice. *Journal of Ethnopharmacology*, **111**(3): 688-691.
- Hedberg, I., Hedberg, O., Madat, P. J., Mshigeni, K. E., Mshiu, E. N. and Samuelsson, G. (1983): Inventory of plants used in traditional medicine in Tanzania. II. Plants of the families Dilleniaceae—Opiliaceae. *Journal of Ethnopharmacology*, **9**(1): 105-127.
- Hikal, W. M., Alhawiti, L. S., Mahmoud, A. A., Tkachenko, K. G. and Said-Al Ahl, H. A. (2021): *Cissampelos pareira*: A potential source of medicine to treat malaria. *International Journal of Mosquito Research*, **8**(5): 37-43.
- Ishii, T., Miyazawa, M., Takanashi, Y., Tanigawa, M., Yasuda, K., Onouchi, H., Kawabe, N., Mitsushita, J., Hartman, P. S. and Ishii, N. (2014): Genetically induced oxidative stress in mice causes thrombocytosis, splenomegaly and placental angiodysplasia that leads to recurrent abortion. *Redox Biology*, **2**: 679-685.
- Jhuma, S. (2016): Phytochemical and pharmacological investigation of *Cissampelos pareira* root and stem and *Thevetia peruviana* leaves for antifertility activity. PhD, Uttarakhand Technical University.
- Khare, C. P. (2008): Indian medicinal plants: an illustrated dictionary. *Springer Science & Business Media*.
- Kumari, S., Bhatt, V., Suresh, P. S. and Sharma, U. (2021): *Cissampelos pareira* L.: A review of its traditional uses, phytochemistry, and pharmacology. *Journal of Ethnopharmacology*, **274**: 113850.
- Lai, Chen, & Cheng. (2006): Chromium yeast supplementation improves fasting plasma glucose and LDL-cholesterol in streptozotocin-induced diabetic rats. *International Journal For Vitamin And Nutrition Research*, **76**(6): 391-397.
- Luangpirom, A., Junaimuang, T. and Somsapt, P. (2015): Antifertility properties of *Cissampelos pareira* Linn. leaf gel in male and female mice. *International Journal of Phytomedicine*, **7**(1): 112-118.
- Luangpirom, A., Sirisarn, W., & Pontaisog, J. (2010): Antifertility activity of the aqueous leaf extract of *Cissampelos pareira* in male albino mice. *Animal Biology & Animal Husbandry*, **2**(2): 59 – 64.
- Maurya, R., Srivastava, S., Kulshreshtha, D. K. and Gupta, C. M. (2004): Traditional remedies for fertility regulation. *Current medicinal chemistry*, **11**(11): 1431-1450.
- Misra, H. P. and Fridovich, I. (1972): The role of superoxide anion in the autoxidation of

- epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, **247**(10): 3170-3175.
- Mustafa, M., Sharifa, A. M., Hadi, J., Illzam, E. and Aliya, S. (2019): Male and female infertility: causes, and management. *IOSR Journal of Dental and Medical Sciences*, **18**: 27-32.
- Nandagoapalan, V., Doss, A. and Marimuthu, C. (2016): Phytochemical analysis of some traditional medicinal plants. *Bioscience Discovery*, **7**(1): 17-20.
- Noh, S., Go, A., Kim, D. B., Park, M., Jeon, H. W. and Kim, B. (2020): Role of antioxidant natural products in management of infertility: a review of their medicinal potential. *Antioxidants*, **9**(10): 957.
- Odetola, A. A., Akinloye, O., Egunjobi, C., Adekunle, W. A. and Ayoola, A. O. (2006): Possible antidiabetic and antihyperlipidaemic effect of fermented parkia biglobosa (jacq) extract in alloxan-induced diabetic rats. *Clinical and Experimental Pharmacology and Physiology*, **33**(9): 808-812.
- Ogbuehi, I. H., Ebong, O. O. and Obianime, A. W. (2015): A preliminary study on the effect of *Abrus precatorius* Linn on reproductive parameters in female *Rattus norvegicus*, wistar strain. *European Journal of Medicinal Plants*, **7**(3): 156.
- Oladimeji, S. O., Igbalaye, J. O. and Coleshowers, C. L. (2014): Immunological biomarkers determined in female rats administered with pro-fertility extract of *Anthocleista vogelii*. *Journal of Natural Sciences Research*, **4**(8): 113-123.
- Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S. and Dhama, K. (2014): Oxidative stress, prooxidants, and antioxidants: the interplay. *BioMed Research International*, **2014**: 1 – 19.
- Rush, J. W. and Sandiford, S. D. (2003): Plasma glutathione peroxidase in healthy young adults: influence of gender and physical activity. *Clinical Biochemistry*, **36**(5): 345-351.
- Samanta, J., Bhattacharya, S., & Rana, A. C. (2015): Evaluation of *Cissampelos pareira* root for antifertility activity. *Journal of Pharmacological Research*, **9**(2): 134-138.
- Sudhakaran, M. V. (2012): Histo-morphological, fluorescent and powder microscopic characterization of *Cissampelos pareira* Linn. *Pharmacognosy Journal*, **4**(34): 57-68.
- Surendran, S., Eswaran, M. B., Vijayakumar, M., & Rao, C. V. (2011): In vitro and in vivo hepatoprotective activity of *Cissampelos pareira* against carbon-tetrachloride induced hepatic damage. *Indian Journal of Experimental Biology*, **49**(12): 939 – 945.
- Suresh, M., Irulandi, K., Siva, V. and Mehalingam, P. (2016): An ethnobotanical study on medicinal plants in southern Western Ghats of Virudhunagar district, Tamil Nadu, India. *International Journal of Ayurvedic Herb Medicine*, **6**(4): 2321-2329.
- Thavamani, B. S., Mathew, M., & Dhanabal, S. P. (2014): Anticancer activity of *cissampelos pareira* against dalton's lymphoma ascites bearing mice. *Pharmacognosy Magazine*, **10**(39): 200.
- Tiwari, K. C., Majumder, R., & Bhattacharjee, S. (1982): Folklore information from Assam for family planning and birth control. *International Journal of Crude Drug Research*, **20**(3): 133-137.
- Wang, S., He, G., Chen, M., Zuo, T., Xu, W. and Liu, X. (2017): The role of antioxidant enzymes in the ovaries. *Oxidative Medicine And Cellular Longevity*, **2017**: 1 – 14.
- Wink, M. (2015): Modes of action of herbal medicines and plant secondary metabolites. *Medicines*, **2**(3): 251-286.
- Yasui, T., Uemura, H., Irahara, M., Arai, M., Kojimahara, N., Okabe, R. and Sato, H. (2008): Associations of endogenous sex hormones and sex hormone-binding globulin with lipid profiles in aged Japanese men and women. *Clinica Chimica Acta*, **398**(1-2): 43-47.
- Zhaira, D., Nafisa, A. and Ikram, N. (2019): A mosaic of risk factors for female infertility in Pakistan. *Journal of Rawalpindi Medical College*, **23**(2): 80-84.