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Effects of Ethanolic Leaf Extract of *Spondias mombin* (IYEYE) Fed to Female Albino Rats on Hormonal Indices, Lipid Profile and Enzymatic Antioxidant Activity

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Abstract:
Background*: Almost all the part of the tree of Spondias mombin is used for various herbal remedies for different conditions all over the world.* ***Objective:*** *This study aims to determine the effects of ethanolic leaf extract of Spondias mombin on hormonal indices, lipid profile and enzymatic antioxidant activity on female albino rats.*
Materials and Methods*: Thirty female albino rats, weighing 60-70g were randomly assigned into six groups of five rats per group. Group 1(control) received 1ml distilled water only; Group 2(control) received 1ml oil only; Group 3 received 0.14mg/g levonogestrel only; Group 4 received 0.7g/100g of S. mombin leaves extract; Group5 received 0.14mg/g levonogestrel for 7days and later 0.7g/100g of S. mombin leaves extract for 14days; Group 6 received 0.14mg/g levonogestrel for 7days and 2.2g/100g of S. mombin leaves extract for 14days. Infertility was orally induced in the rats using oral cannula with the administration of 0.14mg/g of levonogestrel. Qualitative analysis of epinephrine, enzymatic antioxidant activity and lipid profile analysis were carried out using standard procedures. Hormonal assay was carried out using Enzyme–Linked Immunoabsorbent Assay (ELISA) kit.*
Results*: Data analysis revealed that there is a significant (P<0.05) increase in the mean level of serum progesterone prolactin, SOD activity (in the brain, liver, kidney and ovary), and a significant decrease in cholesterol and triglyceride levels of extract-treated groups (low and high dose) compared to the control groups*
Conclusion: *Spondias mombin ethanolic extract can increase the serum level of progesterone, prolactin, and SOD antioxidant activities in the brain, liver, kidneys and ovary in female rats as well as, acting as having a good lipid lowering potential in action.*
Key Word*: Spondias mombin; Infertility; Reproductive hormones; Enzymatic Antioxidants; Epinephrine;*

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I. Introduction

Fertility is the natural capability to produce offspring. Nutrition, sexual behavior, culture, instinct, timing, economy, way of life, and emotions all have a role in human fertility (Schaffnit and Sear, 2014). Reactive oxygen species generated via various endogenous systems (Ighodaro *et al*., 2018), have been implicated in the development of premature rupture of fetal membranes (Srivastava *et al.*, 2017) and evidence suggested that oxidative stress may be associated with pre-eclampsia (Opichka *et al*., 2021). Major processes involved in female reproduction such as ovulation, menstruation, embryo implantation and pregnancy also implicates hormones and inflammatory mechanisms (Vannuccini et al., 2016).

The naturally occurring illness alleviating chemicals compounds in plants has resulted in significant upsurge in their use worldwide. The World Health Organization (WHO) estimated that 80% of the earth's inhabitants rely solely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts' active components (Boveris *et al*., 2008). Their acceptability and use is as a result of their being safe for consumption with very little or no side effect, cost less, direct or indirect source of around 25% of all contemporary medicines and easy accessibility globally (Craig and David, 2007, Partap *et al*., 2012). Nonetheless, despite some medicinal plants' significant therapeutic benefits, several of its compounds have been discovered to be potentially poisonous, mutagenic, carcinogenic, and teratogenic (Ferreira-Machado *et al*., 2014). Cardiovascular toxicities, neurotoxicity, diarrhea, cramping, dermatitis, and allergic reactions have all been documented as severe side effects (Lather *et al*., 2011).

Spondias mombin known by several local names in Nigeria, including Okhikghan (Bini), Iyeye (Yoruba), Tsadermasar (Hausa), and Ijikara (Igbo) belongs to the Anacardiaceae family of angiosperms (Igwe *et al*., 2011). It is native to tropical America, but has been grown in various parts of the world, including Africa and Indonesia (Mattietto and Matta, 2011). *Spondias mombin* samples have been linked to a variety of biological activities (Ayoka *et al*., 2008) including anti-oxidant, antimicrobial and anti-inflammatory, hypoglycemic properties (Emeka and Funmilayo, 2011, Da Silva *et al*., 2012). *Spondias mombin* is also used to treat a variety of disorders that affect the female reproductive system. It is used to induce labor, relieve pain and bleeding during and after childbirth, prevent miscarriage, and treat uterine/vaginal infections, among other things (Igwe and Oikeh, 2015). *Spondias mombin* extracts have been found in vivo to reduce anxiety levels (Asuquo *et al*., 2013), in addition to anti-dopaminergic and anti-seizure properties (Ayoka *et al*., 2008).

II. Material And Methods

This study was carried out at the Department of Biochemistry, Lagos State University, Nigeria from December 2020 to June 2021.

Collection of Plant Samples

Fresh leaves of *Spondias mombin* were collected from a local farm at waterside, Ojo, Lagos state, Nigeria in December 2020 and authenticated at the Herbarium unit of Botany Department, Lagos state University, Lagos State, Nigeria. Leaves were air dried for three weeks.

Ethanolic Extraction of *Spondias mombin*

The soxhlet extraction method was employed for the extraction of the ethanol extract of *Spondias mombin*. 20g of the dried and blended *Spondias mombin* leaves were placed into the thimble of the soxhlet extraction apparatus chamber using 250ml of 95% ethanol. This was followed by loading the thimble into the main chamber of the soxhlet extractor and the sample extracted for 12 hours. After extraction, the solvent was removed by using rotary evaporator, yielding 19.7g of the extract.

Phytochemical Screening

Qualitative phytochemical studies of the ethanolic leaf extract of *Spondias mombin* was carried out according to the method s of Nandagopalan *et al*., (2016) using commonly employed precipitation and coloration reaction to identify the major natural chemical groups such as alkaloids, flavonoids, saponin, terpenoids, glycosides and steroids.

Collection and Acclimatization of Animals

Forty (40) female albino rats, weighing between 60-70g were obtained from the animal house of the Department of Biochemistry, Babcock University, Ogun State, Nigeria. The rats were allowed to acclimatize for two weeks, fed with commercial rat feed and kept under hygienic and favorable condition by maintenance under a 12h light/12h dark cycle, with rat feeds and water available. After acclimatization for two weeks, the commencement of extract administration began.

Chronic Toxicity Test

A total of nine rats were randomly selected with average weight of 115g and divided into three groups each containing three rats. Extract doses of 1ml of 33g/100g, 0.5ml of 16g/100g and 0.25ml of 8g/100g body weight were administered to group 1, 2 and 3, respectively. The animals were kept under natural condition and observed for toxicity signs and mortality for 72 hours (Arome *et al*., 2013). All animals in the group were fed orally with their respective doses of extract for 14days.

Experimental Design

Forty (40) female Albino rats were randomly divided into six groups of six rats each for the two plants. Oral administration of 0.14mg/g levonogestrel for four days was followed by the administration of *S. mombin* extract (0.7g/100g and 2.2g/100g) once for 14 days. Group I (negative control) received only distilled water; Group II rats received oil only, Group III (positive control) received levonogestrel (0.14mg/g), Group IV received low dose *S. mombin* extract (0.7g/100g b.w), Group V received levonogestrel (0.14mg/g) for 7days and later low dose *S. mombin* extract (0.7g/100g b.w) while, Group VI received levonogestrel (0.14mg/g) for 7days and later high dose BME (2.2g/100g b.w).

Collection of Blood and Organs

Twenty-four (24) hours after the last administration of extract, the animals were fasted overnight and sacrificed. They were anaesthetized with chloroform and blood was collected through cardiac puncture. The organs (Liver, Kidney, Ovary and Brain) were collected and soaked in physiological saline solution (Chen *et al*., 2018).

Determination of Biochemical Parameters

Estimation of Reproductive Hormones

Quantitative assessment of reproductive hormones; progesterone, prolactin and testosterone levels, were estimated using standard ELISA technique based on the principle of solid-phase enzyme-linked immunosorbent assay using ELISA kits (Perkin Elmer, US) (Karir *et al*., 2003, Veena *et al*., 2008, Somade *et al*., 2017).

Estimation of SOD Activity

The Superoxide Dismutase (SOD) activity was measured according to the methods of Misra and Fridovich, (1972). 100µL of the cytosolic supernatant was added to a cuvette containing 1.25ml of 0.05M carbonate buffer of pH 10.2 and 150µL of epinephrine, then gently mixed by inverting the cuvette. The change in absorbance was recorded at 480nm for 3minutes using a spectrophotometer. The unit of SOD activity is defined as the quality of SOD needed in oxidation of the epinephrine.

$$SOD\ Aactivity = \frac{(change\ in\ absorbance/min) \times volume\ of\ assay\ mixture}{exteinction\ coefficient\ of\ adenochrome\ \times volume\ of\ sample}$$

(Rahal *et al*., 2014).

Estimation of Cholesterol

The cholesterol is determined after enzymatic hydrolysis and oxidation. The procedure was according to the manufacturer of kit (Randox) (Lai *et al*., 2006). The method was enzymatic end point method. Concentration of cholesterol in sample was calculated using;

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times concentration\ of\ standard$$

Statistical Analysis

Data were analyzed using GraphPad Prism (version 8.4.2). One-way analysis of variance (ANOVA) to ascertain the significant of difference among the groups of rats and data was expressed as mean ± standard error of mean. Bars having different alphabets were considered as cut off values or significant ($P < 0.05$).

III. Results

Table 1: Qualitative phytochemicals constituents in *Spondias mombin* ethanol leaf extract.

The result below shows the qualitative phytochemical constituent of *Spondias mombin* ethanol leaf extracts, which revealed the presence of saponins, terpenoids, glycosides, steroids, tannins, flavonoids and alkaloids.

PHYTOCONSTITUENTS	INFERENCE
Saponins	Present
Terpenoids	Present
Glycosides	Present
Steroids	Present
Tannins	Present
Flavonoids	Present
Alkaloids	Present

Figure 4.1 below shows progesterone concentration in different female rat groups. There was a significant increase in progesterone concentration in the infertile high dose group compared to all the control groups but, progesterone concentration decreased significantly ($p<0.05$) in the CDW and COO groups as compared to the CI group.

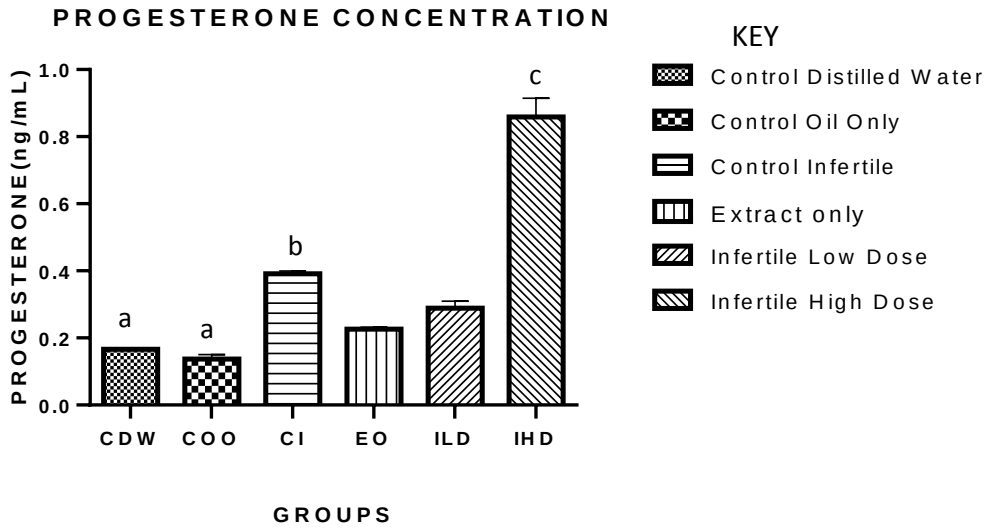


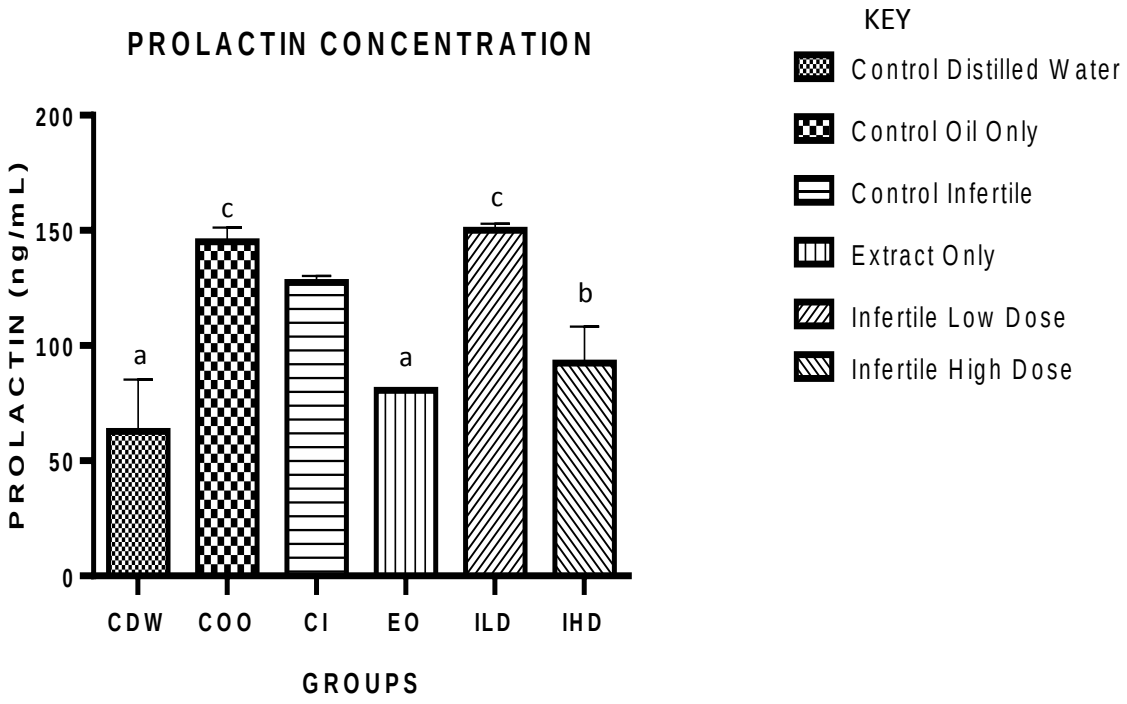
Figure 4.1: Progesterone Concentration in different female rat groups.

The result is expressed as mean ± SEM; n=5 animals in each group. Bars with different alphabets are statistically significant from each other.

The result is expressed as mean ± SEM; n=5 animals in each group. Bars with different alphabets are statistically significant from each other.

Figure 4.2 shows the prolactin concentration in different female rat groups. A significant ($p<0.05$) increase in prolactin concentration was seen in ILD and surprisingly in COO group compared to CDW, EO and IHD groups. Although, the IHD group also increased prolactin concentration compared to the CDW and EO group but significantly lower than the prolactin concentration in ILD group.

Figure 4.2: Prolactin Concentration in different female rat groups.



The result is expressed as mean ± SEM; n=5 animals in each group. Bars with different alphabets are statistically significant from each other.

Figure 4.3 shows SOD activity in the brain. As shown below, there was a significant ($p<0.05$) increase in the brain SOD activity in the ILD group compared to the control groups and IHD group. Also, SOD activity increased significantly in IHD compared to the control groups.

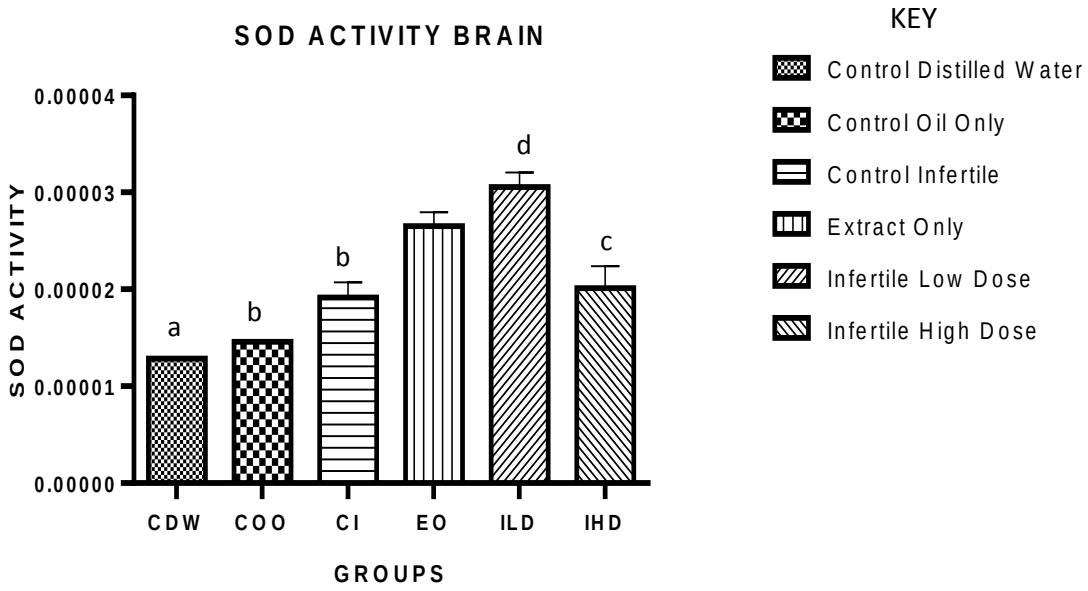


Figure 4.3: Superoxide dismutase (SOD) activity in the brain across the different groups.

The result is expressed as mean ± SEM; n=5 animals in each group. Bars with different alphabets are statistically significant from each other.

Figure 4.4 below shows the liver SOD activity across different groups. The activity of SOD in the liver increased significantly in the ILD group compared to all other groups. Also, treatment with the extract only (EO) significantly increased the liver SOD activity as compared to the control groups and IHD group.

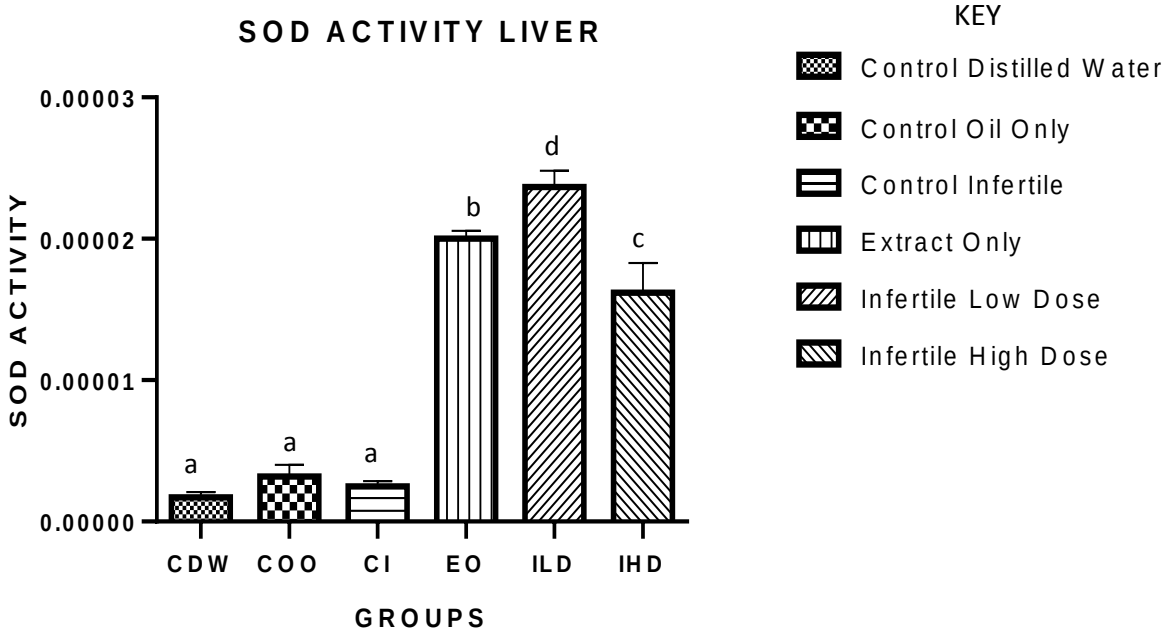


Figure 4.4: Superoxide dismutase (SOD) activity in the liver across the different groups.

The result is expressed as mean ± SEM; n=5 animals in each group. Bars with different alphabets are statistically significant from each other.

Figure 4.5 shows the activity of SOD in the ovary across different groups. The activity of SOD in the ovary increased significantly in the IHD and ILD groups compared to CDW, COO and EO groups. Also, treatment with extract only significantly increased the ovary SOD activity compared to CDW and CI groups.

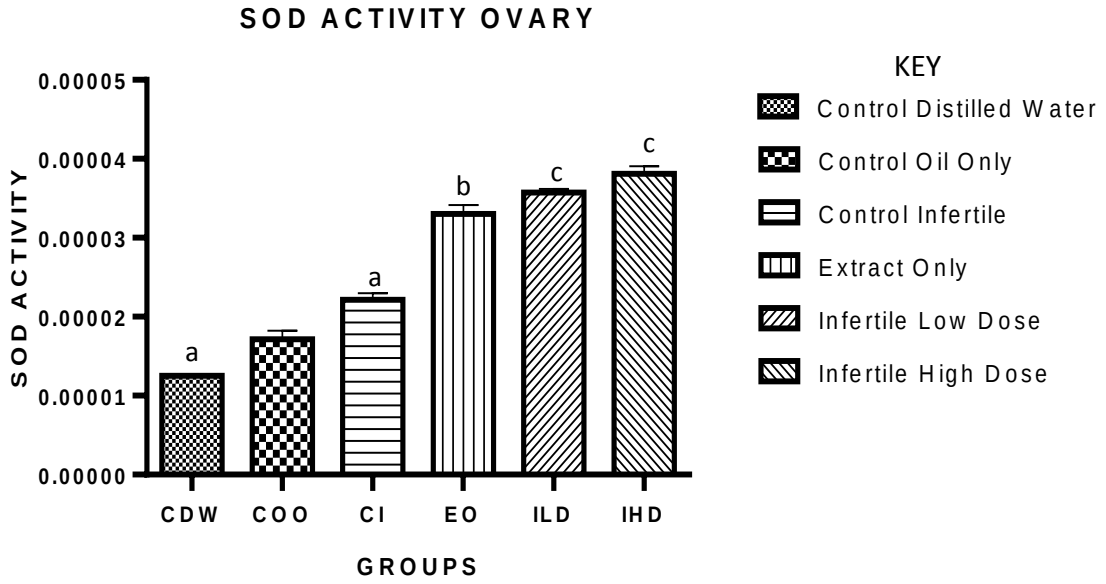


Figure 4.5: Superoxide dismutase (SOD) activity in the ovary across the different groups.

The result is expressed as mean ± SEM; n=5 animals in each group. Bars with different alphabets are statistically significant from each other.

Figure 4.6 shows the activity of SOD in the kidney across different groups. The activity of SOD in the kidney increased significantly in the ILD group compared to CDW, CI, EO and IHD groups. Although, the COO group surprisingly increased significantly in SOD activity compared to the treated groups.

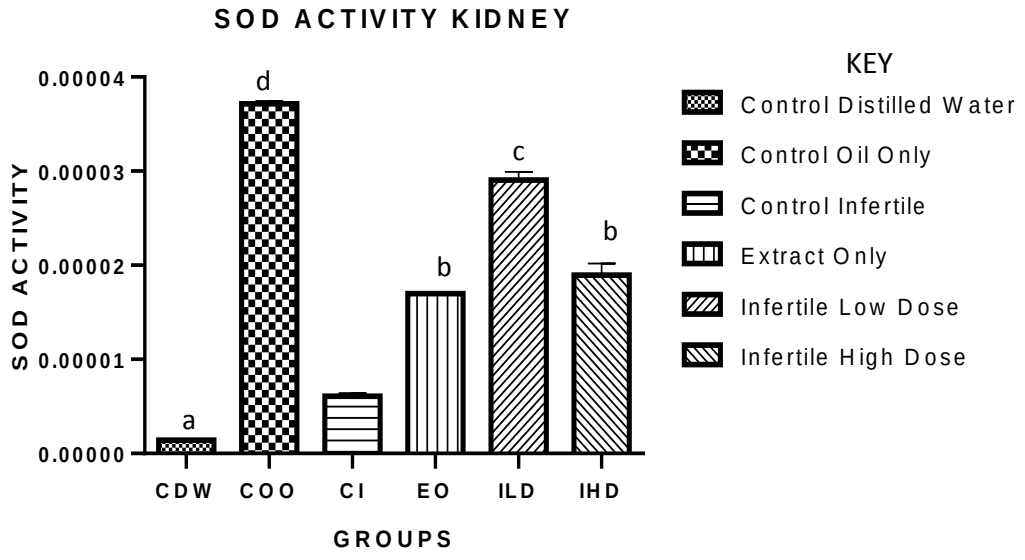


Figure 4.6 Superoxide dismutase (SOD) activity in the kidney across the different groups.

The result is expressed as mean ± SEM; n=5 animals in each group. Bars with different alphabets are statistically significant from each other.

Figure 4.7 shows the concentration of triacylglycerol in different female rat groups. ILD and IHD significantly decreased triglyceride level towards the control groups compared to when treated with extract only (EO) which showed an increased triglyceride level.

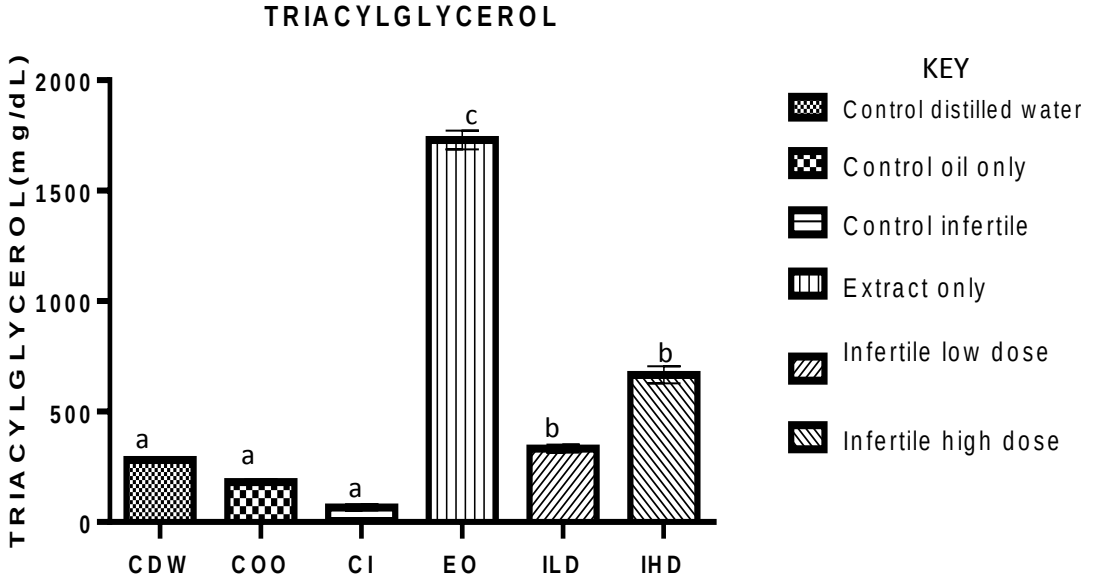


Figure 4.7: Concentration of Triacyglycerol in different female rat groups.

The result is expressed as mean ± SEM; n=5 animals in each group. Bars with different alphabets are statistically significant from each other.

Figure 4.8 shows the concentration of cholesterol in different female rat groups. ILD and IHD significantly decreased cholesterol level compared to the control groups.

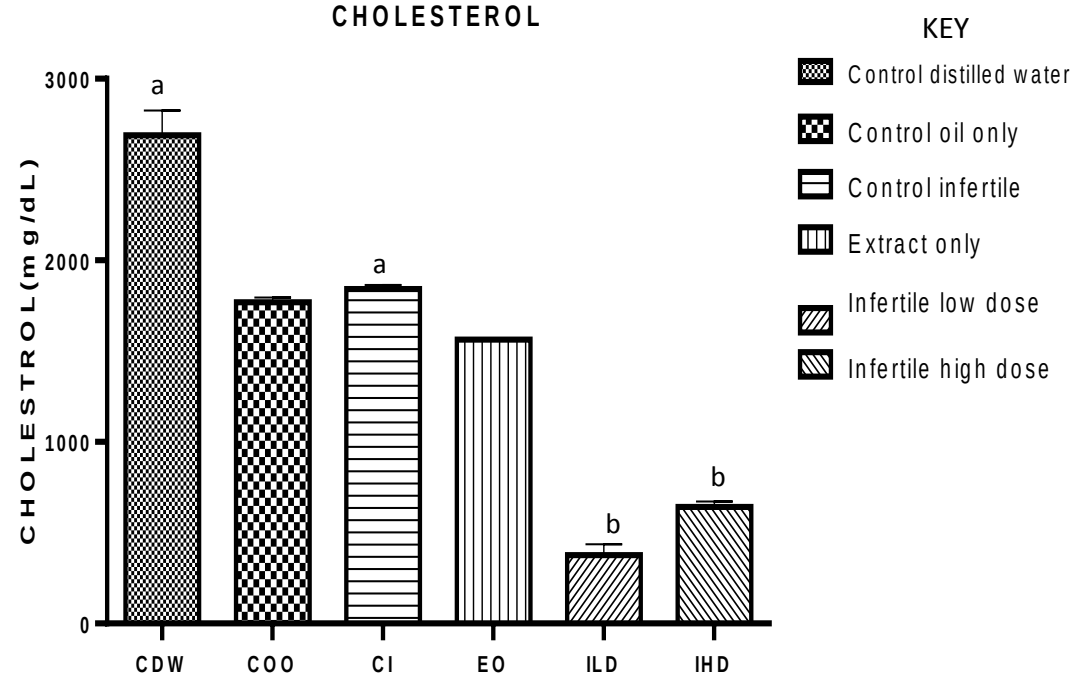
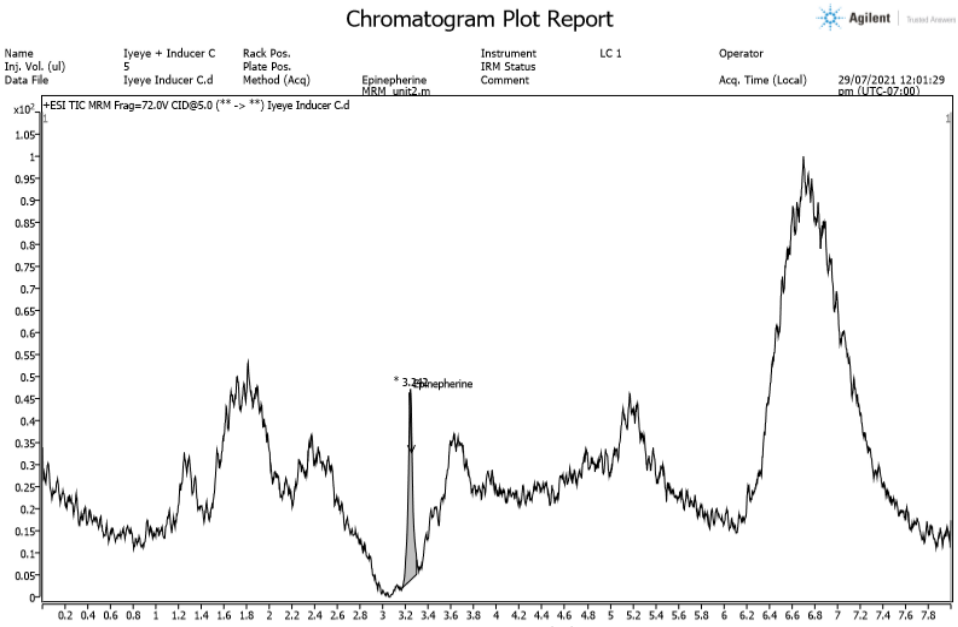


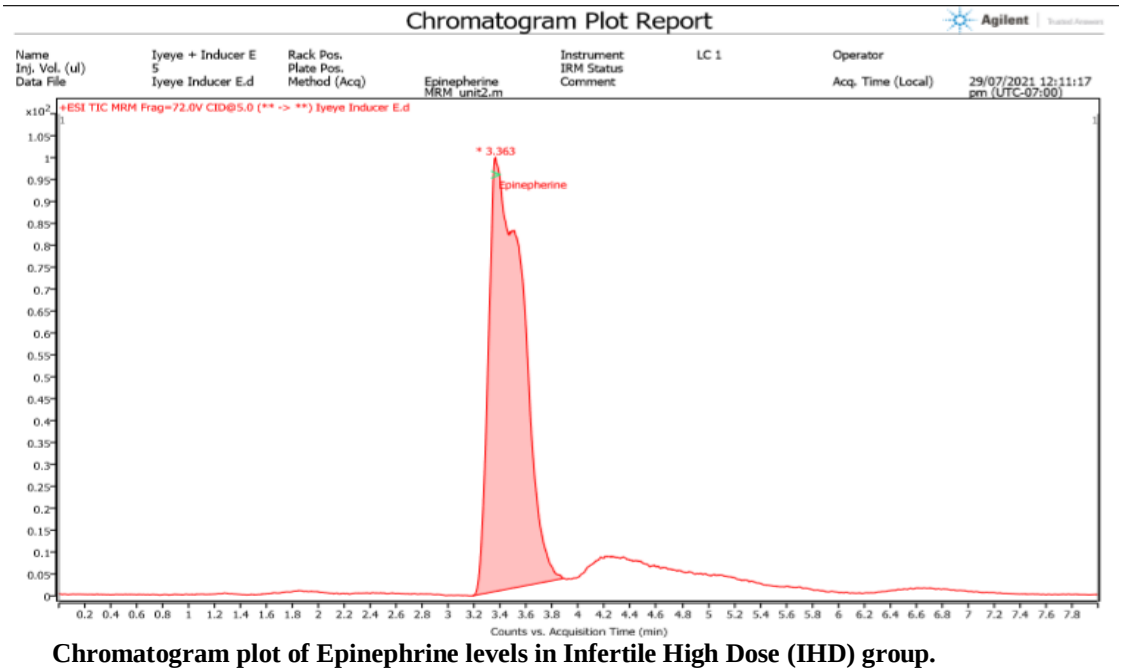
Figure 4.8: Concentration of cholesterol in different female rat groups.

The result is expressed as mean ± SEM; n=5 animals in each group. Bars with different alphabets are statistically significant from each other.

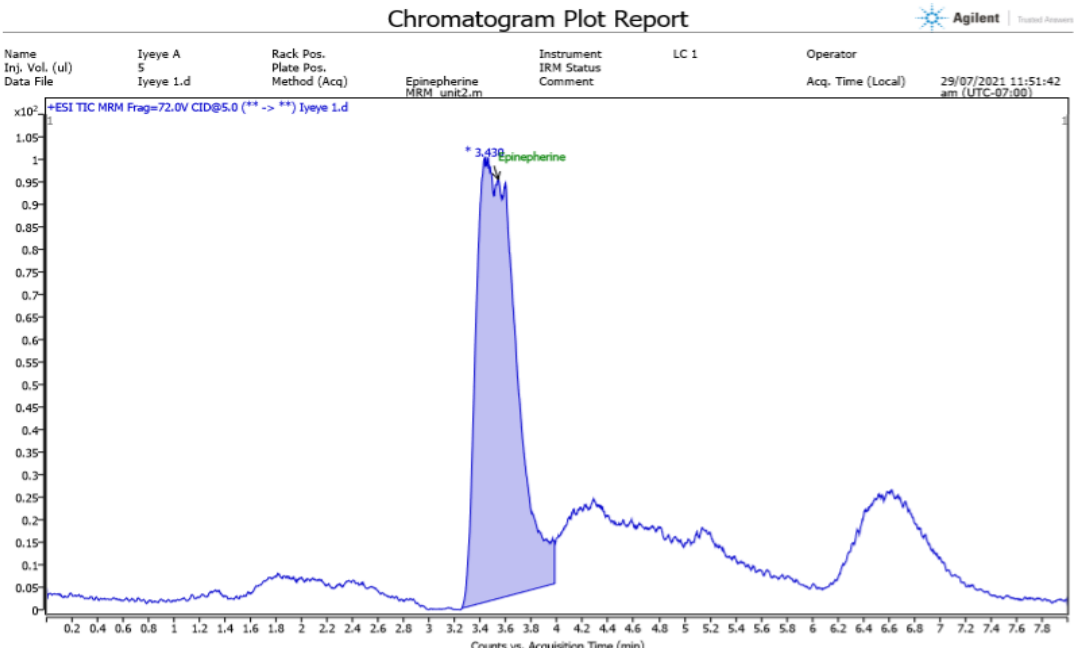
Figure 4.9 shows the qualitative analysis of epinephrine across different rat groups. A lower level of epinephrine was seen in the ILD (Infertile low dose) group compared to IHD (Infertile high dose) group which share almost the same level with EO (Extract only) and COO (Control oil only) groups, respectively. However, the CI (Control infertile) and CDW (Control distilled water) groups do not reveal the presence of epinephrine.



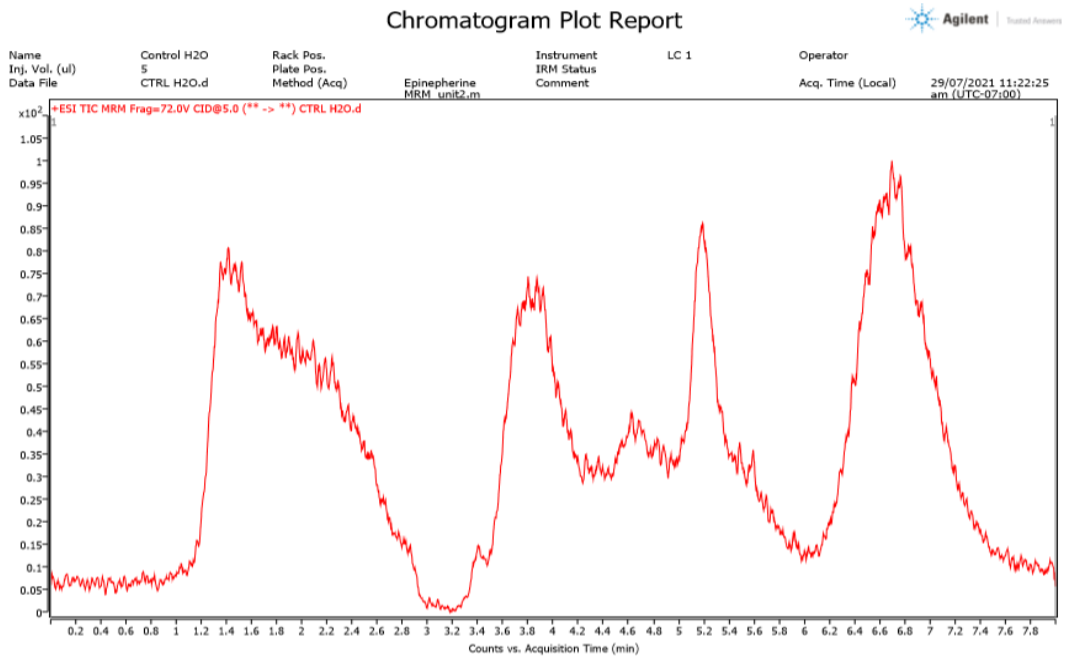
A: Chromatogram plot of Epinephrine levels in Infertile Low Dose (ILD) group.



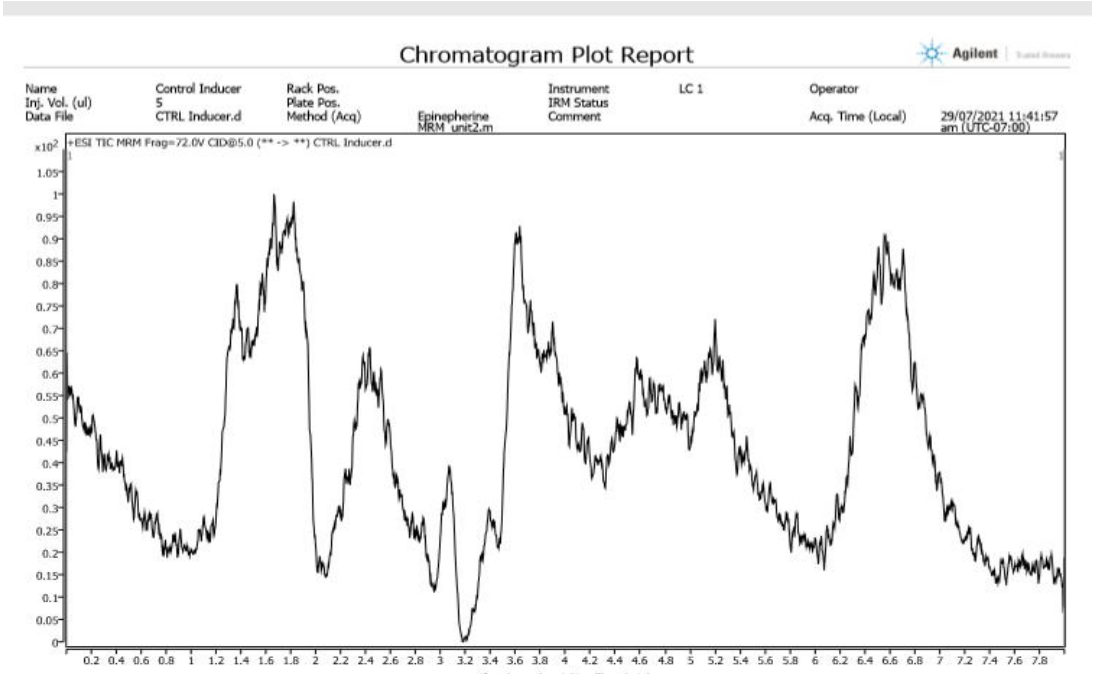
B: Chromatogram plot of Epinephrine levels in Infertile High Dose (IHD) group.



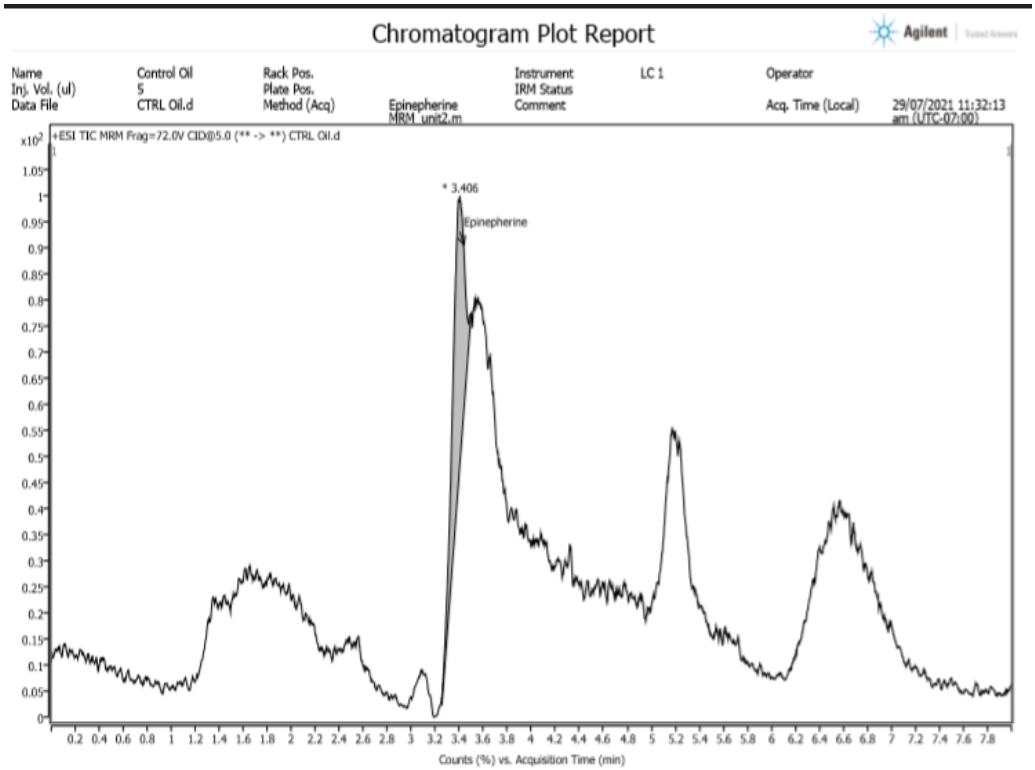
C: Chromatogram plot of Epinephrine levels in group treated with Extract Only (EO).



D: Chromatogram plot of Epinephrine levels in Control Distilled Water (CDW) group.



E: Chromatogram plot of Epinephrine levels in Control Infertile (CI) group.



F: Chromatogram plot of Epinephrine levels in Control Oil Only (COO) group.

Figure 4.9 (A-F): Qualitative analysis of Epinephrine across different rat groups.

IV. Discussion

Medicinal plants are found throughout the world with their abundance in the tropical areas. Plant use is increasingly gaining 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 (Agrawal and Pal, 2013, Mahomoodally, 2013, Thomford *et al*., 2015).

Spondias mombin is a common plant used in the complementary and alternative traditional medicine (CTM and ATM) for several disease treatment and management such as cancer, infertility, and other metabolic

disorders. Ayoka *et al*., (2005) suggested that the different phytochemical content present in different parts of *Spondias mombin* could be employed to treat infertility. Our study is in agreement with this finding because, secondary metabolites such as saponins, terpenoids, steroids, glycosides, tannins, flavonoids and alkaloids were found to be present in the ethanol leaf extract of *Spondias mombin*.

According to Freeman (2000), progesterone is an important part of infertility treatment since it supports implantation. It is essential in establishing and maintaining early pregnancy (Young, 2013, Ciampaglia and Cognigni, 2015), and female reproduction by acting as a regulator all along the female reproductive axis (Wetendorf and DeMayo, 2014). On the other hand, prolactin is a known polypeptide hormone synthesized in but not limited to lactrotrophs 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(Harvey *et al*., 2012). 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 (Shibli-Rahhal and Schlechte, 2011). In this present study, the ethanol leaf extract of *Spondias mombin* increased the level of progesterone and prolactin at different doses, high and low, respectively. This indicates that the ethanol extract of *S. mombin* leaf favored the production of progesterone and prolactin. This finding is in agreement with the report of Igwe *et al*., (2011) who also observed a significant increase in the level of progesterone before and after the administration of *S. mombin* extract compared to the control groups.

The disordered physiological processes in human sex cells have been associated with oxidative stress (Agarwal *et al*., 2003), which is seen as the consequence of an imbalance reactive oxygen species (ROS) production and degradation. Antioxidant biomarkers are useful in the determination of the extent and effect of oxidative stress. Superoxide dismutase(SOD), an antioxidant enzyme, is known to be a vital *in-vivo* antioxidant parameter that catalyzes the dis-mutation or partitioning of superoxide (O_2^-) radical into either conventional molecular oxygen (O_2) or hydrogen peroxide (H_2O_2) (Hayyan *et al*., 2016). The knowledge of SOD 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 (Ross *et al*., 2013). Our study revealed that the administration of the extract at different doses in the experimental groups increased SOD activity (in the brain, liver, ovary and kidney) significantly compared to the control groups. This indicates that these organs remain good markers of infertility and that, *S. mombin* extract could help to prevent injuries to these organs such as liver injury, and oxidative stress that can lead to infertility. Nwidu *et al*., 2018 also reported an elevated cellular SOD activity in carbon tetrachloride induced hepatotoxicity with the administration of *S. mombin* leaf and stem extracts.

Metabolic disorders such as obesity, and overweight has also been linked with infertility; thus, it is necessary to estimate lipid profile, and several plants such *Phragmenthera incana* has been screened for their effect on lipid profile (Awote *et al*., 2021). The *S. mombin* ethanolic extracts utilized in our study significantly suppressed triglyceride and cholesterol levels in the experimental animals, suggesting that *S. mombin* extract could also play a lipid lowering activity in action. Friday *et al*., 2020 also reported the hypolipidemic effect of *S. mombin* extract and its better synergic effect when combined with *Curcuma longa*.

V. Conclusion

In conclusion, our study has shown that *Spondias mombin* ethanolic leaf extract can increase progesterone and prolactin levels at different doses, and also possesses an antioxidant and lipid lowering activities which can help to reduce oxidative stress. This suggests that *Spondias mombin* ethanolic leaf extract has a very good therapeutic effect in the treatment of infertility. Further studies can be carried out on the epinephrine concentration.

References

[1]. Agarwal, A., R. A. Saleh and M. A. Bedaiwy (2003). "Role of reactive oxygen species in the pathophysiology of human reproduction." Fertility and sterility**79**(4): 829-843.

[2]. Agrawal, J. and A. Pal (2013). "Nyctanthes arbor-tristis Linn—A critical ethnopharmacological review." Journal of ethnopharmacology**146**(3): 645-658.

[3]. Arome, D., & Chinedu, E. (2013). The importance of toxicity testing. Journal of Pharmaceutical and BioSciences, 4, 146-148.

[4]. Asuquo, O., C. Fischer, O. Mesembe, A. Igiri and J. Ekom (2013). "Comparative study of aqueous and ethanolic leaf extracts of Spondias mombin on neurobehaviour in male rats." IOSR Journal of Pharmacy and Biological Sciences**5**(2): 29-35.

[5]. Awote, O. K., Igbalaye, J. O., Adeyemo, A. G., Awosemo, R. B., Omolaja, B. E., Thomas-Akinwale, K., & Ayanleye, O. E. (2021). Effect of Phragmanthera incana Leaves Extracts on Lipid Profile in Wistar Rats Fed High-Fat Diet. *International Journal of Formal Sciences: Current and Future Research Trends*, *12*(1), 23-32.

[6]. Ayoka, A., R. Akomolafe, E. Iwalewa and O. E. Ukponmwan (2005). "Studies on the anxiolytic effect of Spondias mombin L.(Anacardiaceae) extracts." African Journal of Traditional, Complementary and Alternative Medicines**2**(2): 153–165-153–165.

[7]. Ayoka, A., R. Akomolafe, O. Akinsomisoye and O. Ukponmwan (2008). "Medicinal and economic value of Spondias mombin." African Journal of Biomedical Research**11**(2).

[8]. Bazemore, R., Deo, R. Zanan, S. John, K. Khandagale and A. Nadaf (2018). γ-aminobutyric acid (GABA): biosynthesis, role, commercial production, and applications. Studies in natural products chemistry, Elsevier. **57:** 413-452.

[9]. Ben-Jonathan, N., K. Liby, M. McFarland and M. Zinger (2002). "Prolactin as an autocrine/paracrine growth factor in human cancer." Trends in Endocrinology & Metabolism**13**(6): 245-250.

[10]. Boveris, D., Subinay, M. Pal, U. K. Roy, T. Ghosh, L. Biswas, S. Sau, J. Ghosh, R. Rahut, S. Harlalka and S. Das (2008). "Assessment of oxidative stress in different areas of brain in streptozotocin induced diabetic Wistar rats."

[11]. Chen, P., F. Chen and B. Zhou (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.

[12]. Ciampaglia, W., & Cognigni, G. E. (2015). Clinical use of progesterone in infertility and assisted reproduction. *Acta Obstetricia et Gynecologica Scandinavica*, *94*, 17-27.

[13]. Craig, G. and J. David (2007). "Natural product drug discovery in the."

[14]. Da Silva, E., R. Nachbar, A. Levada-Pires, S. Hirabara and R. Lambertucci (2012). "Omega-3 fatty acids differentially modulate enzymatic anti-oxidant systems in skeletal muscle cells." Cell Stress and Chaperones**21**(1): 87-95.

[15]. Emeka, E. and D. Funmilayo (2011). "Hypoglycimic effects, biochemical and histological changes of Spondias mombin linn and Perinari ployandra benth seeds ethanolic extracts in alloxan induced rats." Journal pharm Toxicol**6**(2): 1001-1102.

[16]. Freeman, M. (2000). "Kanyicska B, Lerant A, and Nagy G." Prolactin: structure, function, and regulation of secretion. Physiol Rev**80**: 1523-1631.

[17]. Friday, S., Ojeka, S. O., Amadi, O., Ilochi, O. N., & Dapper, D. V. (2020). Comparative Study of the Effects of Methanolic Extracts of Spondias mombin Leaves and Curcuma longa Rhizomes on Serum Lipid Profile and Electrolytes in Alloxan Induced Diabetes in Male Wistar Rats. *Asian Journal of Advanced Research and Reports*, 1-9.

[18]. Harvey, S., C. Arámburo and E. Sanders (2012). "Extrapituitary production of anterior pituitary hormones: an overview." Endocrine**41**(1): 19-30.

[19]. Hayyan, S., Yu, J. Li, R. Gu, L. Yue, H. Wang, X. Zhan and B. Xing (2016). "Carotenoid and superoxide dismutase are the most effective antioxidants participating in ROS scavenging in phenanthrene accumulated wheat leaf." Chemosphere**197**: 513-525.

[20]. Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine*, *54*(4), 287-293.

[21]. Igwe, C., V. Onwuliri, G. Onyeze and C. Osuagwu (2011). "Spasmogenic activity of ethanolic leaf extract of Spondias Mombin Linn on isolated uterine muscle strips of rat: possible hormonal mechanism of action." Research Journal of Agriculture and Biological Sciences**7**(2): 228-233.

[22]. Igwe, C.U., Onwuliri, V.A., Onyeze, G.O.C., Osuagwu, C.G., (2011): Spasmogenic Activity of Ethanolic Leaf Extract of Spondias Mombin Linn on Isolated Uterine Muscle Strips of Rat: Possible Hormonal Mechanism of Action. Res. J. of Agri. and Biol. Science, 7(2): 228-233

[23]. Igwe, O., Ehimwenma S and E. I. Oikeh (2015). "Comparative studies on the phytochemical composition, phenolic content and antioxidant activities of methanol leaf extracts of Spondias mombin and Polyathia longifolia." Jordan Journal of Biological Sciences**147**(3427): 1-5.

[24]. Karir, T., N. Pal and N. Sivaprasad (2003). "Radioimmunoassay kit formulation and its validation for serum progesterone using progesterone radiotracer purified by gel filtration." Journal of radioanalytical and nuclear chemistry**256**(1): 127-131.

[25]. Lai, M. H., Chen, Y. T., Chen, Y. Y., Chang, J. H., & Cheng, H. H. (2011). Effects of rice bran oil on the blood lipids profiles and insulin resistance in type 2 diabetes patients. *Journal of clinical biochemistry and nutrition*, 1112120133-1112120133.

[26]. Lather, A., R. Valecha, K. Sharma and M. Garg (2011). "Worldwide potential of plants causing teratogenicity: An overview." Spatula DD**1**(2): 101-106.

[27]. Liu, F., M. Day, L. C. Muniz, D. Bitran, R. Arias, R. Revilla-Sanchez, S. Grauer, G. Zhang, C. Kelley and V. J. N. n. Pulito (2008). "Activation of estrogen receptor-β regulates hippocampal synaptic plasticity and improves memory." **11**(3): 334-343.

[28]. Mahomoodally, M. F. (2013). "Traditional medicines in Africa: an appraisal of ten potent African medicinal plants." Evidence-Based Complementary and Alternative Medicine**2013**.

[29]. Mattietto, R. and V. Matta (2011). Cajá (Spondias mombin L.). Postharvest biology and technology of tropical and subtropical fruits, Elsevier**:** 330-353e.

[30]. Misra, F., Ceballos-Picot, Iréne, J.-M. Trivier, A. Nicole, P.-M. Sinet and M. Thevenin (2002). "Age-correlated modifications of copper-zinc superoxide dismutase and glutathione-related enzyme activities in human erythrocytes." Clinical Chemistry**38**(1): 66-70.

[31]. Nandagoapalan V, Doss A and Marimuthu C, 2016. Phytochemical analysis of some traditional medicinal plants. Bioscience Discovery, 7(1): 17-20.

[32]. Nwidu, L. L., Elmorsy, E., Oboma, Y. I., & Carter, W. G. (2018). Hepatoprotective and antioxidant activities of Spondias mombin leaf and stem extracts against carbon tetrachloride-induced hepatotoxicity. *Journal of Taibah University Medical Sciences*, *13*(3), 262-271.

[33]. Odetola, A., O. Akinloye, C. Egunjobi, W. Adekunle and A. Ayoola (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.

[34]. Opichka, M. A., Rappelt, M. W., Gutterman, D. D., Grobe, J. L., & McIntosh, J. J. (2021). Vascular Dysfunction in Preeclampsia. *Cells*, *10*(11), 3055.

[35]. Partap, S., A. Kumar, N. K. Sharma and K. Jha (2012). "Luffa Cylindrica: An important medicinal plant." Journal of Natural Product and Plant Resources**2**(1): 127-134.

[36]. Rahal, A., A. Kumar, V. Singh, B. Yadav, R. Tiwari, S. Chakraborty and K. Dhama (2014). "Oxidative stress, prooxidants, and antioxidants: the interplay." BioMed research international2014.

[37]. Ross, A., Aikaterini, N. Aligiannis, I. P. Trougakos and A.-L. Skaltsounis (2013). "Natural compounds with anti-ageing activity." Natural product reports**30**(11): 1412-1437.

[38]. Schaffnit, S. B. and R. Sear (2014). "Wealth modifies relationships between kin and women's fertility in high-income countries." Behavioral Ecology**25**(4): 834-842.

[39]. Shibli-Rahhal, A. and J. Schlechte (2011). "Hyperprolactinemia and infertility." Endocrinology and Metabolism Clinics**40**(4): 837-846.

[40]. Somade, O. T., Ugbaja, R. N., & Adebayo, A. A. (2017). Effect of edible camphor administrations on levels of steroid and thyroid hormones in male wistar rats. *Am. J. Res. Med. Sci*, *1*(1), 27-34.
[41]. Srivastava, A., & Srivastava, A. (2017). Oxidative stress-mediated human diseases. In *Oxidative stress: diagnostic methods and applications in medical science* (pp. 141-151). Springer, Singapore.
[42]. Thomford, N. E., K. Dzobo, D. Chopera, A. Wonkam, M. Skelton, D. Blackhurst, S. Chirikure and C. Dandara (2015). "Pharmacogenomics implications of using herbal medicinal plants on African populations in health transition." Pharmaceuticals**8**(3): 637-663.
[43]. Vannuccini, S., Clifton, V. L., Fraser, I. S., Taylor, H. S., Critchley, H., Giudice, L. C., & Petraglia, F. (2016). Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. *Human reproduction update*, *22*(1), 104-115.
[44]. Veena, B. S., S. Upadhya, S. K. Adiga and K. N. Pratap (2008). "Evaluation of oxidative stress, antioxidants and prolactin in infertile women." Indian journal of clinical biochemistry**23**(2): 186-190.
[45]. Wetendorf, M. and F. J. DeMayo (2014). "Progesterone receptor signaling in the initiation of pregnancy and preservation of a healthy uterus." The International journal of developmental biology**58**: 95.
[46]. Young, S. L. (2013). Oestrogen and progesterone action on endometrium: a translational approach to understanding endometrial receptivity. *Reproductive biomedicine online*, *27*(5), 497-505.
[47]. Zhu, K. Y., Fu, Q., Leung, K.W., Wong, Z.C., Choi, R.C. and Tsim, K.W. (2011). "The establishment of a sensitive method in determining different neurotransmitters simultaneously in rat brains by using liquid chromatography–electrospray tandem mass spectrometry." Journal of Chromatography B**879**(11-12): 737-742.

Oladimeji S.O et. al. "Effects of Ethanolic Leaf Extract of Spondias mombin (IYEYE) Fed to Female Albino Rats on Hormonal Indices, Lipid Profile and Enzymatic Antioxidant Activity." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB), 8*(3), (2022): pp. 17-29.