

Original Research Article

doi: <https://doi.org/10.20546/ijcrbp.2019.604.001>

Hormonal assessment of *Sida rhombifolia* ethanolic leaf extract on micronor induced infertility in female rats

R. U. Ukpanukpong^{1*}, R. N. Uyabeme², B. S. Akinfesola², F. D. Adebisi⁴,
P. O. Aigbadumah³ and D. I. Basiru²

¹Department of Biochemistry, University of Calabar, Nigeria

²Department of Chemical Sciences, Biochemistry Unit, Joseph Ayo Babalola University, Osun State, Nigeria

³Faculty of Medical Sciences, University of Jos, Nigeria

⁴Department of Cell Biology and Genetics, University of Lagos, Nigeria

*Corresponding author

Article Info

Date of Acceptance:
18 February 2019

Date of Publication:
06 April 2019

Keywords

Body weight index
Cholesterol level
Estrogen
Luteinizing hormone
Phytochemical screening

ABSTRACT

The study investigated the hormonal activities of *Sida rhombifolia* ethanolic leaf extract on micronor induced infertility in female rats. *Sida rhombifolia* is a shrub from the family Malvaceae. Forty eight (48) female albino rats were randomly placed into six (6) study groups of eight (8) rats each. Positive control group (PC) was given clean water and food, micronor control group (MC) induced with micronor but not treated with *Sida rhombifolia*, *Sida rhombifolia* control groups; SRC, SRM₁, SRM₂, SRM₃ were induced with micronor and treated with ethanolic leaf extract of *Sida rhombifolia* at varying concentrations of 100mg/kg, 200mg/kg, and 400mg/kg body weight respectively for 14 days. Phytochemical screening, estrogen, luteinizing hormone, cholesterol and body weight indices were monitored in this research. Animals treated with *Sida rhombifolia* showed significant increase in estrogen and luteinizing hormone level ($P < 0.05$) compared to the micronor control at varying concentrations. All treatment groups showed no significant difference ($P < 0.05$) in cholesterol level compared positive control and micronor control.

Introduction

Medicinal plants are herbs that have medicinal properties. They have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and also defend its self against attack. Plant-derived chemicals that influence endocrine activities in human being and animals have received a great attention due to their likely therapeutic potentials as well as

adverse effects. Some of these plants are known to possess fertility effects through their action on hypothalamo-pituitary-gonadal axis or direct hormonal effects on reproductive organs resulting in inhibition or activation of ovarian steroidogenesis (Raja et al., 2010).

Over the years plants have been used for the management of infertility and it is gradually gaining acceptance due to its availability and affordability. The world health organization in 2001 estimated that 80% of the world population

uses medicinal plants in the treatment of disease and in African countries this rate is said to be much higher (Sofowora et al., 2013). It was also estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to meet their primary health care needs. Again, available report show that more than 300 district ethnic groups making up the Nigerian society has its own unique indigenous experiences and needs of its people.

Currently, it is estimated that traditional medicine is the only health care resources are affordable and accessible by one third of all Nigerians (Ishola et al., 2014). In Nigeria, traditional medicine is used to treat several health conditions including mental disorder, fractures, insomnia amongst other (Ishola et al., 2014). Medicinal plants contain substances used for the prevention and treatment of diseases or infections and other health disorders in human body. They are those plants whose chemical contents have some physiological effect on the body chemistry. Mankind has used plants in an attempt to cure disease and relieve physical suffering. The medicinal value of medicinal plants is due to substances found in the plant tissues that produce a definite physiological action on the human body.

The most important of these substances are the alkaloid, fixed oil, essential oil, tannins, resins, etc. It is obvious that some negative results obtained in the use of local plants as sources of medicine are basically due to over-dosage and lack of adequate knowledge of other detrimental byproducts (poisons) contained in some plants. Hence, most medicinal agent in relatively large doses destroys enzyme activities and in relatively small quantities stimulates reactions that generate good health (Sharma et al., 2010).

Plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into primary metabolites such as sugars and fats, which are found in most plants; and secondary metabolites which are found in a smaller range of plants serving specific functions (Meskin and Mark, 2002). Some secondary metabolites are toxins used to deter predators and others as pheromone that attracts insects for pollination. It is these secondary metabolites that possessed therapeutic

potentials in humans and could be refined to produce drugs like inulin from roots of Dahlias, quinine from Cinchona, morphine and codeine from poppy, and digoxin from foxglove (Meskin and Mark, 2002).

Infertility refers to inability to conceive after having regular unprotected sex. Infertility may be a biological inability of an individual to contribute to conception, or to a female who cannot carry pregnancy to full term (Unuane et al., 2011). In many countries infertility refers to a couple that has failed to conceive after 12 months of regular sexual intercourse without the use of contraceptive. Studies indicate that slightly over 50% of all cases of infertility are attributed to inability to conceive by female, while the rest are caused by either sperm disorders or unidentified factors. Female infertility can also be associated with age, smoking, alcohol, being obesity, over-exercising, under exercising, sexually transmitted infections, exposure to some obnoxious gases, mental stress and endocrine disorders (Templeton, 2000).

Materials and methods

Materials, equipments and chemicals

Drug: Micronor (BAYER WEIMAR GmbH and Co.KG Germany/Allemagne), ethanol (Fimlab), centrifuge (Sorvall RC-5B super speed, England), spectrophotometer (Spectrum lab 22PC England). ELISA kits were purchased from Randox Laboratories Limited (Antrim, United Kingdom BT294QY), analytical weighing balance (AXIS, model: BT200, Gdansk, Poland.). The animal pallets were purchased from Osogbo central market, Osun State.

Experimental animals

Forty eight (48) adult female albino Wistar rats weighing between 140-150g were purchased from a disease free stock of Ladoke Akintola University of Technology Osun State and used for the study. The animals were allowed to acclimatize for two weeks before the commencement of the experiment. The rats were randomly assigned on the basis of their weight into six study groups of eight (8) rats each. Normal feeds and clean running water were

given to the rats *ad libitum*. They were placed in a well-ventilated animal house of Joseph Ayo Babalola University Ikeji Arakeji Osun State at normal temperature of 30-35°C. The cages were cleaned regularly and the rats were treated according to the international guidelines for the care and use of laboratory animals (NIH, 2008).

Plant materials and identification

Fresh and healthy leaves of *Sida rhombifolia* were obtained from Federal College of Agriculture, Ijapo, Akure, Ondo State, Nigeria and used for the study. The leaves were taken to the Department of Botany, Obafemi Awolowo University, Ile-Ife for identification and authentication.

Preparation of plant extract

The *Sida rhombifolia* leaves were sorted out and thoroughly washed and allowed to air dry under laboratory condition for two months. The dried leaves were pulverized into powder with the use of an electric blender. The powdered form was stored in an air tight container for further analysis.

Extraction procedure

About 100 grams of the powdered leaves was soaked in 400ml of 98% ethanol-water (1:1) at room temperature for 3 days after which the mixture was filtered with a Whatman filter paper (No. 1). The filtrate was allowed to dry at room temperature in stainless plates after which it was scrapped out and kept in an air tight container. The dried extract was dissolved in water to make 100mg/kg, 200mg/kg and 400mg/kg and stored.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedure to identify the constituents as described by Sofowora (1993), Trease and Evans (1989) and Harbone (1973).

Induction of infertility

Infertility was achieved by administering 0.5 mg/kg orally of Micronor for 5 days. Female rats were allowed to co-habit with male rats for 30 days to ensure their infertility status before further treatment.

Experimental design

The grouping and treatment given to the rats in each of the groups were as follows:

- Group 1:** designated PC consisted of positive control rats (Normal female rats).
- Group 2:** designated as MIC consisted of Micronor Induced Control rats (Female rats administered Micronor only).
- Group 3:** designated as SRC consisted of extract control rats (Female rats administered 200 mg/kg of *Sida rhombifolia* ethanolic leaf extract only).
- Group 4:** designated SRM1 consisted of Micronor induced female rats administered 100 mg/kg of *Sida rhombifolia* ethanolic leaf extract.
- Group 5:** designated SRM2 consisted of Micronor induced female rats administered 200 mg/kg of *Sida rhombifolia* ethanolic leaf extract.
- Group 6:** designated SRM3 consisted of Micronor induced female rats administered 400 mg/kg of *Sida rhombifolia* ethanolic leaf extract.

Sacrifice of the animals and blood collection

At the end of the experimental period, rats in each study group were fasted overnight and sacrificed under anesthesia by cardiac puncture, blood was dispensed into specific sterile plain bottles for hormonal and cholesterol assay.

Hormonal assay: Luteinizing hormone

Principle: The LH Quantitative Test was based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a mouse monoclonal anti-ALPHA-FSH antibody for solid phase (micro titer wells) immobilization and another mouse monoclonal anti- β -LH antibody in the antibody enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45 minute incubation at room temperature, the wells were washed with water to remove unbound-

labeled antibodies. A solution of TMB Reagent was added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development was stopped with the addition of Stop Solution and the color was changed to yellow and measured spectrophotometrically at 450 nm. The concentration of LH was directly proportional to the color intensity of the test sample (Lequin, 2005).

Procedure: Desired numbers of coated wells were secured in the holder. 50µl of standard, specimen and control were dispensed into appropriate wells, 100 µl of Enzyme Conjugate Reagent was dispensed into each well after which mixtures were thoroughly mixed for 30 seconds. They were incubated at room temperature (18-25°C) for 45 minutes. The incubation mixture was removed by flicking plate contents into a waste container. The micro titer wells were rinsed and flicked 5 times with distilled or deionized water. The wells were sharply struck onto absorbent paper or paper towels to remove all residual water droplets then 100µl TMB reagent was dispensed into each well and gently mixed for 10 seconds. They were incubated at room temperature in the dark for 20 minutes then the reaction was stopped by adding 100µl of Stop Solution to each well. Wells were gently mixed for 30 seconds. It was ensured that all the blue color changes to yellow color completely. The optical density was read at 450nm with a micro titer plate reader within 15 minutes (Lequin, 2005).

Estrogen

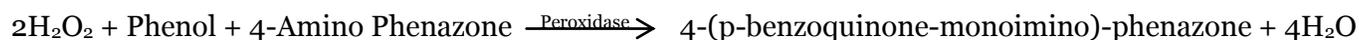
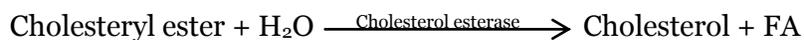
Principle: The Estrogen EIA was based on the principle of competitive binding between Estrogen in the test specimen and Estrogen-HRP conjugate for a constant amount of rabbit anti-Estrogen. In the incubation, goat anti-rabbit IgG-coated wells were incubated with 25 µl Estrogen standards, controls, patient samples, 100 µl Estrogen-HRP conjugate reagent and 50µl rabbit anti-Estrogen reagent at room temperature (18-25°C) for 90 minutes. During the incubation, a fixed amount of HRP-labeled Estrogen competes with the endogenous Estrogen in the standard, sample, or quality control serum for a fixed number of binding sites of the specific Estrogen antibody. Thus, the amount of Estrogen peroxidase

conjugate immunologically bound to the well progressively decreases as the concentration of Estrogen in the specimen increases. Unbound Estrogen peroxidase conjugate was removed and the wells washed. Next, a solution of TMB Reagent was added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 1N HCl, and the absorbance was measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled Estrogen in the sample. A standard curve was obtained by plotting the concentration of the standard versus the absorbance. The Estrogen concentration of the specimens and controls run concurrently with the standards was calculated from the standard curve (Lequin, 2005).

Procedure: All reagents were brought to room temperature (18-25°C) before use. Desired number of coated wells was secured in the holder. 25 µl of standards, specimens and controls were dispensed into appropriate wells and 100 µl of Estrogen-HRP Conjugate reagent was dispensed into each well, 50µl of rabbit anti-Estrogen reagent was also dispensed into each well. They were mixed thoroughly and incubated at room temperature (18-25°C) for 90 minutes. The micro wells were rinsed and flicked 5 times with distilled water and 100 µl of TMB reagent was dispensed into each well and gently mixed for 10 seconds. They were incubated at room temperature (18-25°C) for 20 minutes. Reaction was stopped by adding 100 µl of Stuck Solution to each well. Wells were gently mixed for 30 seconds. It was ensured that all the blue color changes to yellow color completely. Absorbance was read at 450 nm with a micro titer well reader within 15 minutes (Lequin, 2005).

Total cholesterol determination

Cholesterol was measured enzymatically, in serum or plasma, in series of coupled reactions that hydrolyze cholesteryl esters and oxidized the 3-OH group of cholesterol. One of the reaction by which products, H₂O₂ was quantitatively measure in a peroxidase catalyzed reaction that produces a colour. Absorbance is measured at 500nm. The colour intensity was proportional to cholesterol concentration. The reactions are as follows:



Statistical analysis

The data obtained were expressed as Mean \pm S.E.M (Standard Error of Mean). All results were mean of 8 determinant and statistical analysis was carried out using student's "t"-test. The values were considered at probability level of $P < 0.05$.

Results and discussion

This experiment evaluated the effect of the ethanolic leaf extract of *Sida rhombifolia* on body weight indices, luteinizing hormone (LH), estrogen levels and cholesterol concentration of micronor-induced infertile female albino Wistar rats. In this study, alkaloids, tannins, phlobatannin, saponin, flavonoids, anthraquinones, steroids, terpenes, phenol, chalcones, and cardiac glycosides were detected by qualitative analytic methods described by Odebiyi and Sofowora (1978). The results of the present study are given in Table 1 and Figs. 1 – 4.

Table 1. Phytochemical screening of ethanolic leaf extract of *Sida rhombifolia*.

S/N	Phytochemical	Observation
1	Alkaloids	+++
2	Tannin	+++
3	Phlobatannin	++
4	Saponin	+++
5	Anthraquinones	++
6	Steroids	++
7	Terpenes	+
8	Cardienolides	-
9	Phenol	+++
10	Chalcones	-
11	Cardiac glycosides	+++

+++ Present in appreciable amount; ++ Present in moderate amount; + Present in minute amount; - Completely absent.

Changes on body weight was used as an assessment of the response of an individual to therapeutic drugs and as an indication of the adverse effect of the drug (Teo et al., 2012; El Sanusi and El Adam, 2007). In this study, *Sida rhombifolia* had an increasing effect on the body weight in a dose dependent manner which may be

associated with the fact that *Sida rhombifolia* has an appetizing effect on the rats (Fig. 1) and they fed better during the experiment as earlier suggested by (Adeneye and Agbaje, 2008), rats treated with varying concentrations of ethanolic leaf extract of *Sida rhombifolia* (100, 200 and 400mg/kg) showed proportional increase in body weight. It could also be that *Sida rhombifolia* interferes with the lipid metabolism of experimental animals. However, it was generally observed that there was a relatively significant increase in the final body weight when compared to the control.

Micronor induces infertility by making vaginal fluid thicker to prevent sperm from reaching the ovum and also by changing the lining of the uterus to prevent attachment of a fertilized egg. The oral administration of ethanolic leaf extract of *Sida rhombifolia* showed a significant increase ($P < 0.05$) in the luteinizing hormone concentration in the extract treated groups when compared with the micronor control group (MC). This shows that the extract reversed the effect of the oral contraceptive (Micronor). The extract only group showed significant increase ($P < 0.05$) in LH level when compared with the positive control which implies that the extract boosted the fertility of normal animals (Fig. 2).

The significant increase in the concentration of LH seen in the extract treatment groups when compared with the positive and negative controls implies that the extract of *Sida rhombifolia* contains some phyto-estrogen properties. According to Zhao and Qing (2011) phyto-estrogens are natural compounds which are derived from plants and are similar to estrogens structurally and functionally. Zhao and Qing (2011) added that phyto-estrogens affect the hypothalamic-pituitary gonadal axis as well as the external genitalia. Furthermore, the possible phyto-estrogen components of the plant extract may have some stimulatory roles in the release of GnRH (gonadotropin releasing hormone) which is the principal regulator of LH and FSH secretion, aromatase and 17- α hydroxylase enzyme which are

necessary in estrogen and progesterone secretion thus increasing the secretion of gonadotropin.

In addition, the extract treatment groups showed a significant increase ($p < 0.05$) in the estrogen level when compared with the micronor control (MC) and there is a progressive increase in the estrogen levels of the treatment groups as the *Sida rhombifolia* concentration increases ($SRM_1 < SRM_2 < SRM_3$). The significant increase in the extract treated group may also be linked to the presence of phyto-estrogen in the plant extract (Fig. 3). Since

phyto-estrogens stimulate aromatase and increase conversion of testosterone to estrogen, they possess the ability to function competitively and bind to estrogen receptors, and elevate estrogen and progesterone levels (Mill and Bones, 2000).

There was a no significant difference ($P < 0.05$) in the cholesterol level of the extract treated group when compared to the positive and micronor control (Fig. 4), thus indicating that *Sida rhombifolia* does not affect hepatic and serum cholesterol level.

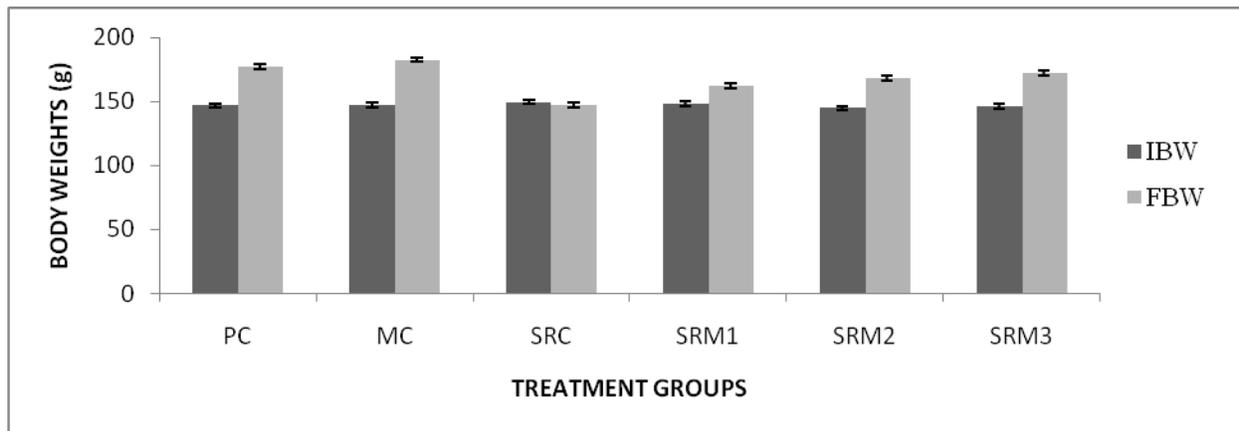


Fig. 1: A graph showing the Body weight of ethanolic leaf extract of *Sida rhombifolia*. Values expressed in Mean \pm SEM of 8 determinations. PC: Positive control; MC: Micronor induced control (Negative control). SRC: *Sida rhombifolia* control; SRM1: Micronor + *Sida rhombifolia* (100mg/kg); SRM2: Micronor + *Sida rhombifolia* (200mg/kg); SRM3: Micronor + *Sida rhombifolia* (400mg/kg); IBW: Initial Body Weight (Before the experiment); FBW: Final Body Weight (After the experiment); a: shows significant difference when compared to Positive control ($P < 0.05$); b: shows significant difference when compared to Micronor induced control ($P < 0.05$).

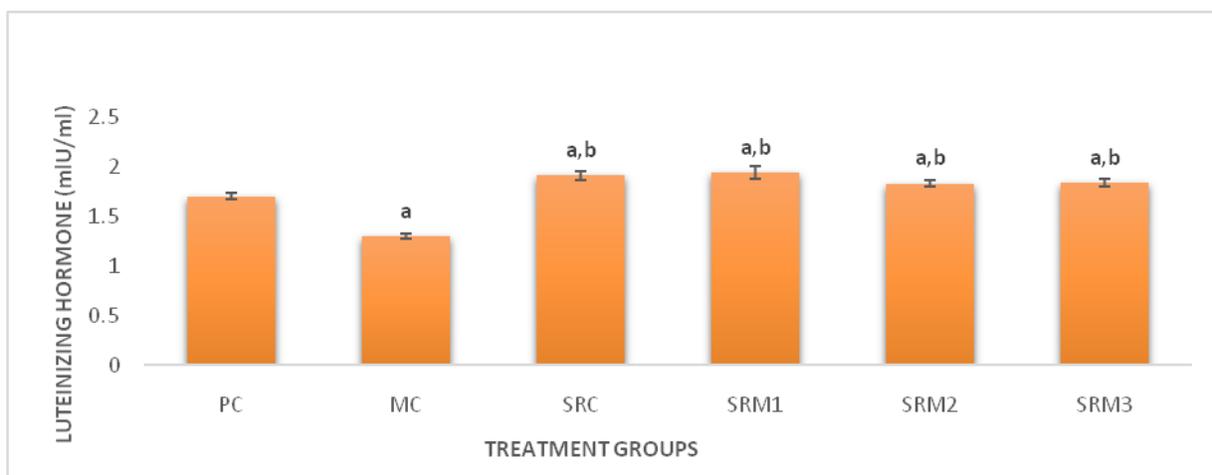


Fig. 2: A graph showing the Luteinizing hormone level of ethanolic leaf extract of *Sida rhombifolia*. Values expressed in Mean \pm SEM of 8 determinations. PC: Positive control; MC: Micronor induced control (Negative control); SRC: *Sida rhombifolia* control; SRM1: Micronor + *Sida rhombifolia* (100mg/kg); SRM2: Micronor + *Sida rhombifolia* (200mg/kg); SRM3: Micronor + *Sida rhombifolia* (400mg/kg); a: shows significant difference when compared to Positive control ($P < 0.05$); b: shows significant difference when compared to Micronor induced control ($P < 0.05$).

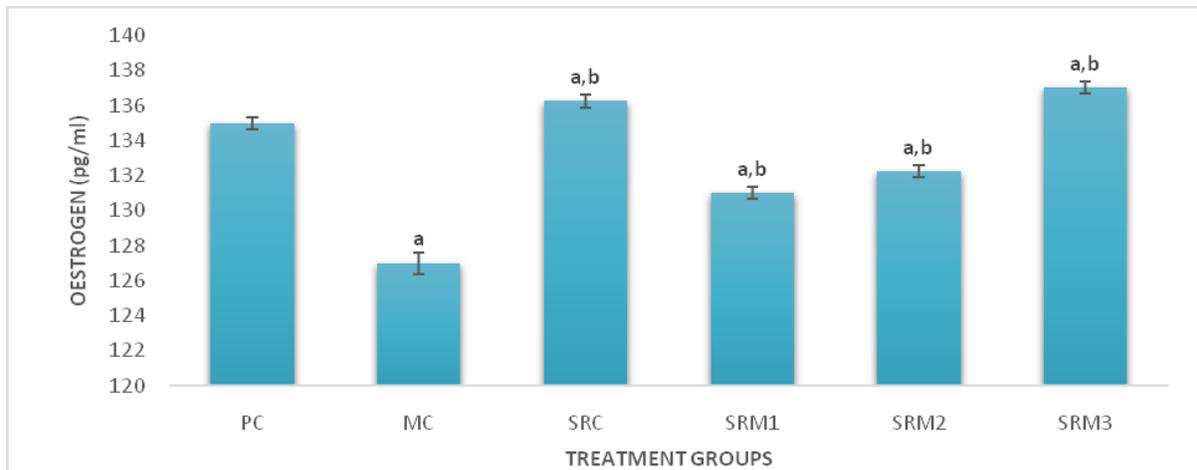


Fig. 3: A graph showing the estrogen level of ethanolic leaf extract of *Sida rhombifolia*. Values expressed in Mean \pm SEM of 8 determinations; PC: Positive control; MC: Micronor induced control (Negative control); SRC: *Sida rhombifolia* control; SRM1: Micronor + *Sida rhombifolia* (100mg/kg); SRM2: Micronor + *Sida rhombifolia* (200mg/kg); SRM3: Micronor + *Sida rhombifolia* (400mg/kg); a: shows significant difference when compared to Positive control ($P < 0.05$); b: shows significant difference when compared to Micronor induced control ($P < 0.05$).

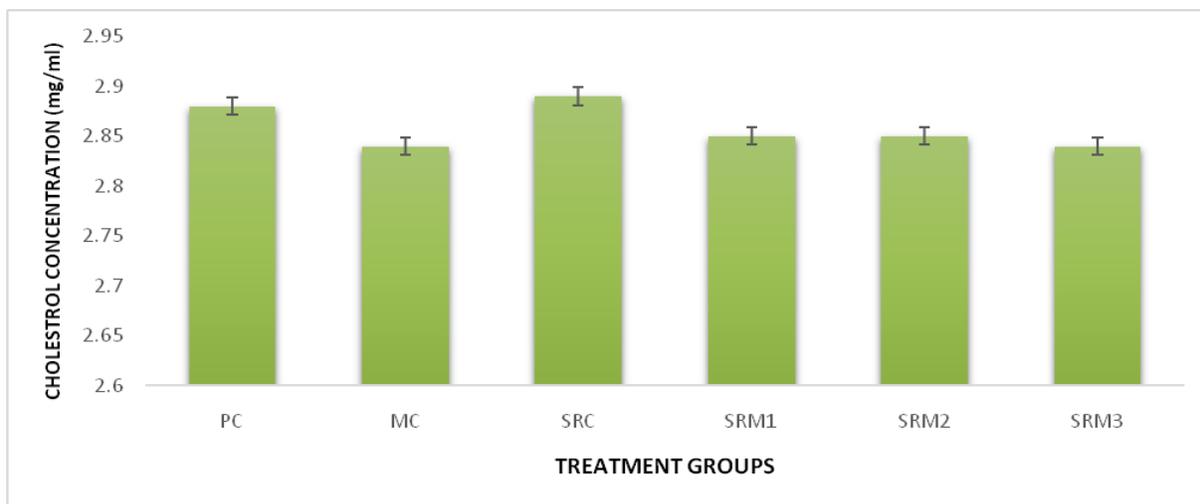


Fig. 4: A graph showing the cholesterol level of ethanolic leaf extract of *Sida rhombifolia*. Values expressed in Mean \pm SEM of 8 determinations; PC: Positive control; MC: Micronor induced control (Negative control); SRC: *Sida rhombifolia* control; SRM1: Micronor + *Sida rhombifolia* (100mg/kg); SRM2: Micronor + *Sida rhombifolia* (200mg/kg); SRM3: Micronor + *Sida rhombifolia* (400mg/kg); a: shows significant difference when compared to Positive control ($P < 0.05$); b: shows significant difference when compared to Micronor induced control ($P < 0.05$).

Conclusion

The biological effects of the ethanolic leaf extract of *Sida rhombifolia* obtained in this study supports the therapeutic use of *Sida rhombifolia* for the treatment of endocrine related infertility and facilitation of fertility. The extract increased the levels of luteinizing hormone and estrogen of rats at low, moderate and high doses, possibly due to the presence of phyto-estrogens in the leaf of *Sida rhombifolia*.

Conflict of interest statement

Authors declare that they have no conflict of interest.

References

- Adeneye, A. A., Agbaje, E. O., 2008. Pharmacological evaluation of oral hypoglycemic and antidiabetic effects of fresh ethanol leaves extract of *Morinda lucida*

- Benth. in normal and alloxan-induced diabetic rats. *Afr. J. Biomed. Res.* 11, 65-71.
- Fernandes, A.A., Novelli, E. L., Okoshi, K., Okoshi, M.P., Di, B.P., Muzio, J.F., Guimaraes, A., 2010. Influence of routine treatment on biochemical alterations in experimental diabetes. *Biomed. Pharmacother.* 64, 214-219.
- Harbone, F.B., 1973. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall Ltd., London. 279p.
- Ishola, I.O., Agbaje, E.O, Adeyemi, O., Shukla, R., 2009. Analgesic and anti-inflammatory effects of the methanol root extracts of some selected Nigerian medicinal plants. *Pharmaceut. Biol.* 52(9), 8-16.
- Lequin, R. M., 2005. Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). *Clin. Chem.* 51(12), 2415-2418.
- Meskin, G., Mark, S., 2002. *Phytochemicals in Nutrition and Health*. CRC Press, 123p.
- Mills, S., Bone, K., 2000. *Principle and Practice of Phytotherapy: Modern Herbal Medicine*. Edinburgh, Churchill Livingstone.
- NIH (National Institute of Health), 2008. *Guide for the Care and Use of Laboratory Animals*. 1(14), 82-86.
- Odebiyi, A., Sofowora, A. E., 1978. Phytochemical screening of Nigerian medicinal plants. Part III. *Lloydia*. 41, 234-246.
- Oladimeji, S.O., Aroyehun, A. B., 2015. The hormonal profile and some immune factors of induced infertile albino rats treated with the ethanolic leave extract of *Newbouldia* leaves. *Int. J. Med. Health Profess. Res.* 2(1), 1-8.
- Raja, A., Gajalakshmi, P., Raja, M., 2010. Drugs from the natural bio-sources for human disease. *Int. J. Pharmacol.* 6(4), 360-363.
- Sharma, R., Sharma, A., Chaudhary, P., Pearce, V., Vatsyayan, R., Singh, S. V., 2010. The role of lipid peroxidation in cellular responses to disulfuraphane, a promising cancer chemopreventive agent. *Biochemistry.* 49, 3191-3202.
- Sofowora, A., 1993. *Medicinal Plants and Traditional Medicine in Africa*. 2nd Edn. Spectrum Books Limited (Publisher), Ibadan, Nigeria. pp.134-156 and 77-87.
- Sofowora, A., Ogunbodede, E., Onayade, A., 2013. The role and place of medicinal plants in the strategies for disease prevention. *Afr. J. Trad. Compl. Alter. Med.* 10, 210-229.
- Templeton, A., 2000. Infertility and the establishment of pregnancy—overview. *Bri. Med. Bull.* 56, 577-587.
- Teo, E. Y., Lew, P. S., Feo, C. S., 2012. Public perception of obesity and bariatrics surgery in Singapore; A pilot study. *Singapore Med. J.* 53, 104-108.
- Trease, G. E., Evans, W.C., 1989. *Pharmacognosy*. 11th Brailliar Tiridel Can. MacMillian Publishers, USA.
- Unuane, D., Tournaye, H., Velkeniers, B., Popp, E. K., 2011. Endocrine disorders and female infertility. *Best Pract. Res. Clin. Endocrinol. Metab.* 25(6), 861-873.
- Zhao, E., Qing, M. U., 2011. Phytoestrogen biological actions on mammalian reproductive system and cancer. *Sci. Pharm.* 79(1), 1-20.

How to cite this article:

Ukpanukpong, R.U., Uyabeme, R. N., Akinfesola, B. S., Adebisi, F.D., Aigbadumah, P.O., 2019. Hormonal assessment of *Sida rhombifolia* ethanolic leaf extract on micronor induced infertility in female rats. *Int. J. Curr. Res. Biosci. Plant Biol.* 6(4), 1-8. doi: <https://doi.org/10.20546/ijcrbp.2019.604.001>