Research Article **ISSN: 2394 – 7403**

International Journal of Medicine and Health Profession Research

Journal home page: www.ijmhpr.com

THE HORMONAL PROFILE AND SOME IMMUNE FACTORS OF INDUCED INFERTILE FEMALE ALBINO RATS TREATED WITH THE ETHANOLIC LEAVE EXTRACT OF *NEWBOULDIA LAEVI*

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ABSTRACT

The plant kingdom offers a wide range of natural antioxidant and medicinal values. The pro-fertility potential of the ethanolic leaves extract of *Newbouldia laevis* were studied to ascertain its effects on some biochemical indices. Micronor, a synthetic progestin and NAC were administered orally to the respective groups of animals with 120mg/kg body weight for five (5) days to induce infertility before daily administration of the plant extract for 14 days. The phytochemical screening of the extract revealed the presence of Alkaloids Terpenoids, Saponins, Tannins, Flavonoids and phenols. The high concentration of prolactin compared with the low concentration of LH and Estradiol in the infertile rats group were significant at ($P<0.05$) compared with the low concentration in rats treated with the extracts only and after micronor treatment in a dose dependent manner. The low CD 4 and CD 8 counts in the rats group treated with extract only were significant compared with the counts after administration of the extract and following micronor treatment. The multi-factorial etiology of female infertility could be traced to hormonal dysfunction initiated by cellular susceptibility to immunological repression, which was shown to have been influenced in this study by the hormonal-regulation and immunological influence of *Newbouldia laevis*. The studies suggest that *Newbouldia laevis* leaf extract treatment can ameliorate the effect of hormone induced pathologies and can serve as a pro-fertility plant.

KEYWORDS

Newbouldia laevis, Prolactin, Estradiol Hormones, CD 4 and CD 8 counts.

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INTRODUCTION

According to World Health Organization (WHO) survey, developing nations are more interested than ever in making use of traditional indigenous resources in implementing their primary health care (PHC) programmes, (Sofowora, 2013)[1]. Thus, these traditional indigenous resources (plants) should be accurately identified, taxonomically classified and

botanically conserved in order to document and enhance the suitable contribution of traditional medicine to the primary health care. Fertility describes the potential for actual production of offspring while infertility is the inability to reproduce. According to the American society of Reproductive Medicine, infertility is the inability to attain pregnancy after engaging in regular unprotected sexual intercourse for at least one year (Olive, 2014)[2]. Infertility is a common condition affecting approximately 13% - 14% of all couples. Varied causes of infertility amongst men and women exist; therefore every cause is an entity in itself. Female factors are responsible for 40% - 50% of infertility cases while the others are due to male causes as well as combined female/male causes and unexplained infertility (Belloc *et al*. 2014)[3].

Over the years plants have been used for the management and treatment of infertility and it's gradually gaining grounds due to its availability and affordability. The world health organization in 2001 estimated that eighty percent 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, 2013)[1]. It was also estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to help 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 resource accessible to a third of all Nigerians (Ishola *et al*., 2014)[4].

In Nigeria traditional medicine is used to treat several health conditions including mental disorder, fractures, insomnia, and fertility (Ishola *et al*., 2014)[4]. Medicinal plants contain substances used for the treatment or prevention 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. From the earliest times, 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 or drugs are basically due to over-dosage and lack of adequate knowledge of other detrimental by-products (poisons) contained in some plants. This is so because, any medicinal agent which in relatively large dose destroys activity will in relatively small quantities stimulate it (Sharma *et al*. 2010)[5].

The knowledge of medicinal plants is passed on based on indigenous knowledge system (IKS) and orally by the traditional herbal practitioners from one generation to the next. There use varies from species to species as diseases vary from one form to another in various places.

By 2003, female infertility (age-dependent) affected 7% to 28% of women (Duckitt 2003)[6]. A demand for infertility services has increased substantially over the past decade due to the prevalence of infertility in the general population, (Oladimeji *et al*., 2013)[7]. Although infertility represents a small facet of the broad intellectual scope of reproductive endocrinologist's practice. The presence of oxidant and antioxidant systems in various reproductive tissues has evoked great interest in the role of oxidative stress in human reproduction (Agarwal and Allamaneni 2004, (Polak *et al*. 2013)[8,9].

Lipoproteins and polyunsaturated fatty acids produce lipid peroxidation products. Malondialdehyde (MDA) is a decomposition product of peroxidation of polyunsaturated fatty acid that is present in tissues and body fluids and can be measured to assess the stress levels, (Polak *et al*. 2013)[9].

The increased amounts of ROS in these patients are suggestive of a reduction in antioxidant defences, including GSH and Vitamin E, (Polak *et al*. 2013)[9] the low antioxidant status of the peritoneal fluid may be a determinant factor in the pathogenesis of idiopathic fertility.

Normal pregnancy is associated with high metabolic demand and elevated requirements for tissue oxygen. There is increased production of reactive oxygen species, (Fialova *et al*. 2006)[10] thus increasing oxidative stress and consequent decrease in

immunity to combat the oxidative stress. The objective of this study is to evaluate the effect of *Newbouldia laevis* on the hormonal profile and immunological parameters.

MATERIALS AND METHOD

Source of materials and Preparation of extracts

The leaves of *Newbouldia laevis* were collected from Ipaja, Ipaja L.G.A. The plant was botanically authenticated at the Herbarium, botany unit, Department of Biological Science, University of Lagos, Nigeria. A voucher specimen of the plant with (Ref No. LUH 5656) was deposited for reference.

Preparation of plant extract

The leaves were open-dried under the shade, cut into small pieces and pulverized into coarse powder (using wooden pestle and mortar) and stored until required for use. One hundred and sixty grams (160g) of the powdered were extracted with two litres of 70% ethanol-water (1:1) at room temperature for 24hrs. The extract was filtered with whatman filer paper (No.1) and concentrated under reduced pressure using a lyophilizer machine.

Phytochemical screening

The preliminary phytochemical screening of the crude ethanolic extract of *Newbouldia laevis* leaf was carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols (Markham *et al*. 1970), (Harborne, 1990), (Sofowora, 1996)[11-13].

Animals

White Wister albino rats of either sex weighing between 120- 140g were purchased from animal house, department of biological science, university of Ibadan, Ibadan Nigeria. They were kept in wire mesh cages in a well-ventilated room, allowed access to free food and water and kept for two weeks to acclimatize in the animal's house of the department of biochemistry, Lagos state university. The animals were fed on commercial standard animals feeds and were given water ad libitum. The animals were fasted from feeds for 12h before the commencement of each experiment, but were allowed water ad libitum. The guide for the care and use of laboratory animals procedures were followed in this study (Indian council of medical Research, 2001).

Micronor (Norethisterone) and N-acetyl-cysteine were purchase from alpha pharmacy Ltd at Ikeja.

Acute Toxicity Study

The lethal Doses (LD 50) of the plant extract were determined by method of (Lorke, 1983)[14] using 12 albino rats. Aqueous ethanolic leaf extract of *Newbouldia laevis* was administered orally to three (3) groups of 3 rats per group in a single dose at doses of 150, 250, 400mgkg^{-1} body weight while the control group received distilled water. Observation was made and recorded systematically one, two, four and six hours after administration. Finally, the number of survivors was noted after 48 hours for each group of animals. The toxicological effect was assessed on the basis of mortality and expressed as LD 50 and calculated using the limit test doses up and down procedure of organization for economic and cultural development.

Experimental design

White albino rats of both sexes weighing 120 - 140g were used for the study and they were evenly distributed into seven (7) groups of six rats each. The route of administration of extracts was via oral route with the aid of an oral canular tube. The group and doses administrated are summarized below.

Study:

Group 1: Control (Normal Female Rat).

Group 2: Positive control (Female rats fed with extract only).

Group 3: Induction control (Micronor Induced Female Rats)

Group 4: Induction Control (NAC induced Female Rats).

Group 5: Micronor induced female rats + 100mgkg^{-1} body weight of ethanolic leaf extract.

Group 6: Micronor induced female rats + 200mgkg^{-1} body weight of ethanolic leaf extract.

Group 7: NAC induced female rats + 100mgkg^{-1} body weight of ethanolic leaf extract.

Induction of infertility and administration of extract

The rats were fasted overnight prior to oral administration of (1:1) ml of Micronor and NAC to the respective group to induce infertility for five (5)

days. After the 5th day the animals were fasted for 12hrs before daily administration of the plant extract between 9:30 to 10:30 am. The treatment lasted for 14days in which the body weights of the rats were determined on day 15 before the animals were sacrificed by breaking the spinal cord at the end of the experiment after being starved for 12hrs.

Blood collection

Blood samples were collected from the optical sinus into clean dry centrifuge tube for serum, heparinized tube for the blood plasma, EDTA Vacuteiner bottle for immunological parameters and EDTA bottle for Haematology test. The blood sample in the centrifuge tube was left to clot at room temperature, then centrifuge for 10 minutes at 3000 RPM to separate the serum. Serum was carefully separated using a Pasteur pipette and kept frozen at (20^0C) until required.

Hormonal assay

Enzyme linked immunosorbent assay (ELISA) kits. A test that uses antibodies and colour change to identify a substance. The kit was manufactured by diagnostic automation INC 23961 craftsman road, suite D/EIF, Calabasas, USA was used for the assay of estradiol, luteinizing hormone, prolactin, and testosterone in the serum of the rats.

Immunological assay

Becton Dickinson's automated instrument was used for the assay of $CD4^+$ /CD8+ presents in the whole blood of the rats.

Statistical Analysis

Data were analyzed using One Way Analysis of Variance (ANOVA, SPSS Version 20) and expressed as mean Standard Error Mean (SEM). Differences between groups were regarded significant at P0.05 and post-hoc tests were then performed using the Tukey's test.

RESULTS

Phytochemical analysis

Freshly prepared extracts subjected to preliminary phytochemical screening test for various constituents revealed the presence of cardiac and steroidal glycosides, tannins, flavonoids, alkaloids, saponins present represented with the positive (+ve).

Effect of the ethanolic leaf extract of *Newbouldia laevis* on the female reproductive hormonal concentration

Reproductive hormones acts as effective biomarkers, micronor (norethindrone) alters the effective section of the pituitary gonadotropins and reproductive hormonal concentrations. The effects of various fractions of the leaf extract against micronor inducing infertility on hormonal concentration of luteinizing hormone (LH), Follicle stimulating hormone (FSH), Prolactin, Estradiol is summarized in Table No.2.

Micronor considerably ($p<0.05$) increase Prolactin thus resulting in decrease in Luteinizing Hormone and Estradiol. The serum concentration of these hormones (LH, Estradiol,) were restored ($p<0.05$) by oral administration of the leaf extract of *Newbouldia laevis* on the (LD (Micronor + Extract) and (HD (Micronor + Extract) compared to the control and infertile (Micronor) group.

Effect of the ethanolic leaf extracts of *Newbouldia laevis* on the immunological parameter of infertile albino rats induced with micronor (norethindrone).

CD4 CELLS

Figure No.1: Shows the graphical representation and the effects of the plant extract on the serum Triacylglycerol. The mean CD4 CELLS/ µl for the control (Distilled water) were 822.3 ± 1.45. Administration of micronor (Infertile group) only gave mean CD4CELLs/µl to be 851.3 ± 1.96, while that of Extract only give 622.5 ± 1.94. However, administration of N-acetyl cysteine (NAC-only) gave mean CD4 CELLS/ µl to be 638.7 ± 1.46.

When rats were administered with MICRONOR followed by treatment with extract at Lower dose (MICRONOR+EXTRACT), the mean CD4 CELLS/ µl was found to be 806.7 ± 1.45, while that of the higher dose treatment (MICRONOR+EXTRACT), gave 740.2 ± 1.45. The administration of NAC followed by treatment with extract (NAC+EXTRACT), revealed mean CD4CELLS/ µl to be 151.8 ± 2.22.

CD8 CELLS

Figure No.2: Shows the graphical representation and the effects of the plant extract on the serum Triacylglycerol. The mean CD8 CELLS/ µl for the

control (Distilled water) were 1059.3 ± 1.93. Administration of micronor (Infertile group) only gave mean CD8CELLs/ µl to be 803.7 ± 1.58, while that of Extract only give 813.5 ± 1.05. However, administration of N-acetyl cysteine (NAC-only) gave mean CD8 CELLS/ µl to be 966.7 ± 1.52.

When rats were administered with MICRONOR followed by treatment with extract at Lower dose (MICRONOR+EXTRACT), the mean CD8 CELLS/ µl was found to be 797.3 ± 1.71, while that of the higher dose treatment (MICRONOR+EXTRACT), gave 950.7 ± 1.51. The administration of NAC followed by treatment with extract (NAC+EXTRACT), revealed mean CD8CELLS/ µl to be 960.5 ± 1.52.

DISCUSSION

The effect of the ethanolic leaf extract of *Newbouldia laevis* on Prolactin, Luteinizing hormone and Estradiol level were evaluated as shown in (Table No.2). Rats administered with infertile (micronor) group shows significant increase ($P<0.05$) in Prolactin and significant decrease ($P<0.05$) in LH and Estradiol when compared to control animals. However, NAC-Only animals showed decreased Prolactin and resulting increase in LH and Estradiol but not significantly when compared to control. Administration of ethanolic extract of *Newbouldia laevis* (Extract only) showed significant ($P<0.05$) decrease in Prolactin and increase in the level of LH and Estradiol when compared to the infertile (micronor) group. The LD (Micronor + Extract) and HD (Micronor+ Extract) showed significant increase ($P<0.05$) in the level of LH and Estradiol and decrease in Prolactin when compared to the Infertile (Micronor) group. This shows that the extract caused a reversal of the effect of the oral contraceptive (Micronor) when compared to the infertile (micronor) and control group. However the ameliorating effect was not significantly higher when compared to the control animals. Maturation of pre-ovulatory follicles and ovulation are under the combined and balanced influences of ovarian and extra ovarian hormones. Imbalances or alterations in these hormones lead to irregularities in the ovarian functions and duration of oestrous cycle (Shivalingappa *et al*., 2002)[15]. Phytochemical screening has revealed many bioactive agents of plant extracts that could possibly affect the regulation of the oestrous cycle, conception and reproduction (Shivalingappa *et al*., 2002)[15].

This antioxidant activities of oestrogen are countered by progestin via the activation of NADPH oxidase and the inhibition of the expression and activity of the Mn SOD, Progestin antagonizes the vaso-protective effect of oestrogen on antioxidant enzymes expression and function, (Pincemail *et al*., 2007)[16].

Prolactin helps to initiate breast development by inducing lobule alveolar growth of the mammary gland. It also stimulates lacto-genesis. Dopamine serves as the major – inhibiting factor of Prolactin secretion (Fitzgerald and Dinan 2008)[17]. The enhanced level of Prolactin may be attributed to the effect of the progestin probably acting as a dopamine antagonist. High Prolactin levels tend to suppress the ovulatory cycle by inhibiting the secretion of LH and gonadotropic-releasing hormone (GnRH) (Fitzgerald and Dinan 2008)[17] which are necessary for ovulation. Such increase in Prolactin inhibits ovulation and promotes loss of menstrual periods which will hinder conception. The reversal effect of *Newbouldia laevis* in this study justifies the folkloric use as a pro-fertility plant.

The active component (Norethindrone) is a synthetic progestin with some anabolic, estrogenic and androgenic activities. Norethinndrone binds and activates nuclear progesterone receptors (PRs) in target tissues such as pituitary and reproductive system; Ligand - receptor complexes are translated to the nucleus where they bind to progesterone response elements (PREs) located on target genes, followed by various transcriptional events and histone acetylation. Physiological effects include the inhibition of luteinizing hormone (LH) release, an increase in the endometrial luteal-phase, and alterations in endo-cervical mucus secretion.

The administration of micronor (infertile group) showed no significant increase in CD4 cells but a statistical increase compared to the control animals while CD8 cells showed a significant decrease

(P<0.05) compared to the control animals. The effect of the ethanolic leaf extract of *Newbouldia laevis* (Extract Only) at lower dose of 100mg/kg only caused a significant decrease (P<0.05) in the CD4 cells and CD8 cells compared to the control and infertile group.

Similarly, the animals that received micronor followed by treatment with the extract at lower (100mg/kg.bw, LD (Micronor + Extract) and higher (200mg/kg.bw, HD (micronor + Extract) doses respectively showed no significant decrease in the CD4 cells when compared to the infertile group (micronor Only) and control groups while CD8cells (100mg/kg.bw, LD (Micronor + Extract) showed a significant decrease (P<0.05) compared to the control and (200mg/kg.bw, HD (Micronor + Extract) restored it .

Also the NAC + Extract group and NAC only showed statistical decrease in the CD4cells and CD8cells compared to the control while CD8cells, showed significant increase (P<0.05) compared to the infertile (micronor) group.

Newbouldia laevis leave extracts has been demonstrated as having an improvement of oxidative stress status from the investigation of the parameters assessed. The enhanced level of Prolactin attributed to the effect of the progestin tend to suppress the ovulatory cycle thus the reversal effect of the hormonal dysfunction thereby corroborating the influence of the extract of *Newbouldia laevis* as observed in the study to justify its folkloric use as pro-fertility plant.

Table No.1: Phytochemical constituents of the ethanolic leaf extract of *Newbouldia laevis*

S.No	Parameters	*Newbouldia laevis*	Tests
1	Alkaloid	+	Wagner's test
2	Terpenoids	+	Salkowski's test
3	Saponins	+	Foam test
4	Tannins	+	Alkaline reagent test
5	Flavonoids	+	Alkaline reagent test
6	Resins	+	Alkaline reagent test
7	Carbohydrate	+	Reducing Sugar test
8	Amino Acids	-	Ninhydrin test
9	Phenol	+	Ferric chloride

Table No.2: Effect of the ethanolic leaf extracts of *Newbouldia leavies* on the hormonal parameter of infertile albino rats induced with micronor (norethindrone)

S.No	Groups	Estradiol pg/ml	Luteinizing Hormone mIU/ml	Prolactin ng/ml
1	Control	1.546 ± 0.001	0.149 ±0.031	0.176 ±0.002
2	Infertile (micronor)	1.222 ±0.036	0.088 ±0.003	0.189 ±0.001
3	Extract only	1.546 ±0.001	0.151 ±0.015	0.120 ±0.008
4	LD(micronor+extract)	1.314 ±0.023	0.118 ±0.007	0.176 ±0.006
5	HD(micronor+extract)	1.546 ±0.001	0.071 ±0.001	0.136 ±0.002
6	NAC+ extract	1.546 ±0.001	0.398 ±0.077	0.106 ±0.001
7	NAC only	1.547 ±0.001	2.243 ±0.950	0.175 ±0.004

Values are mean ± standard error of the mean, n=6, Comparison was made between Control Vs Infertile group, Extract only, LD (Micronor+Extract), HD (Micronor + Extract), NAC + Extract, NAC only using (Dunnett Compare all column vs. control column)

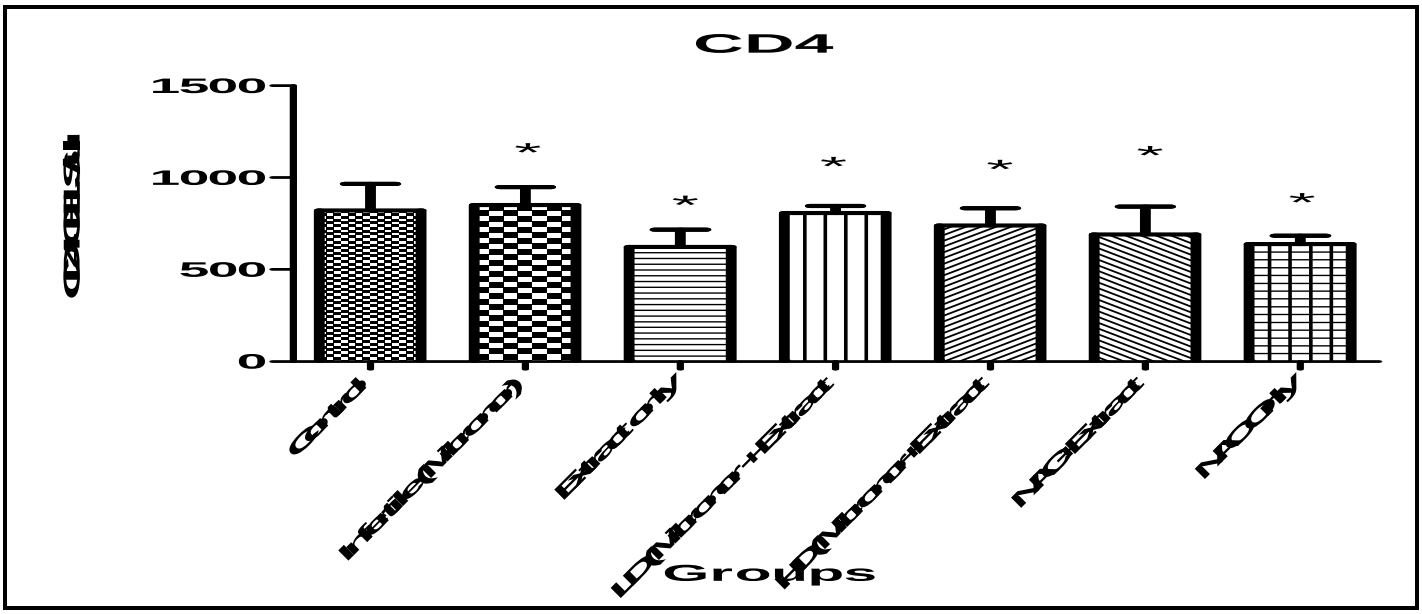

Figure No.1: Bar chart depicting the effect of the ethanolic leaf extracts of *Newbouldialaevis* on the CD4 cells of infertile albino rats. All data are expressed as mean ± S.D (n=6). $^{*}P < 0.05$

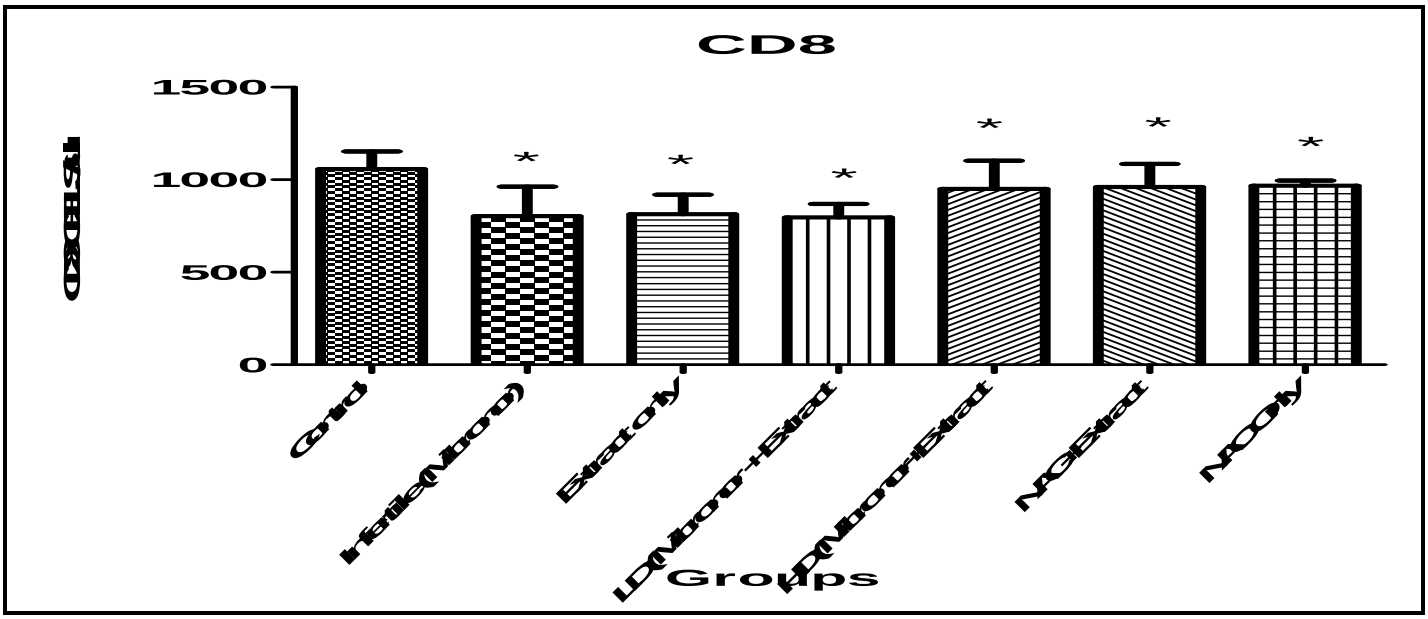

Figure No.2: Bar chart depicting the effect of the ethanolic leaf extracts of *Newbouldialaevis* on the CD8 cells of infertile albino rats. All data are expressed as mean ± S.D (n=6). $^{*}P < 0.05$

CONCLUSION

Scientific validations are being made globally to get evidences for traditionally used herbal plants and there still exist a large number of tropical trees with tremendous medicinal potentials but with no empirical proof to support claims of efficacy. The present study has provided an empirical basis for the use of *Newbouldia laevis* in traditional medicine practice.

ACKNOWLEDGEMENT

The authors are sincerely thankful to Department of Biochemistry, Lagos State University, Ojo for providing the facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Oladimeji S O and Aroyehun A.B. The Hormonal Profile and Some Immune Factors of Induced Infertile Female Albino Rats Treated with the Ethanolic Leave Extract of *Newbouldia Laevi*, *International Journal of Medicine and Health Profession Research*, 2(1), 2015, 1 - 8.