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Assessment of Some Reproductive Hormones in Acute Lead-Acetate-Induced toxicity in Male and Female Albino Rats Treated *Hypoestes rosea* Leaf

F. K. Uwikor [a*], B. E. Echonwere-Uwikor [b] and O. E. Chukwuigwe-Igbere [b]

[a] Ema Dominion College of Health Sciences and Technology, Port Harcourt, Nigeria.
[b] Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author FKU designed the study, performed the statistical, experimental analyses and managed the literature searches, author BEE wrote the protocol, while Author OEC wrote the first. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To assess the levels of some gonadal hormones in lead-acetate-induced Albino Rats treated with *Hypoestes rosea* Leaf.
Study design: Experimental study.
Place and Duration of Study: Department of Pharmacology, University of Port Harcourt and Department of Medical Laboratory Science, Rivers State University, Port Harcourt, between October, 2019 and March, 2020.
Methodology: A total of 140 albino Wistar rats were used for this study. The animals were divided into 26 groups (6 post-treatment and 6 pre-treatment groups for female rats and the same number for male rats and 2 groups as male and female controls). Each group contained 5 rats for both sexes in both pre- and post-treatments groups, except the positive control groups that had 10 animals for both sexes. The study was carried out for 21 days. The negative control groups received rat feed only, extract control (EC) group received 100mg/kg bwt/day for 21 days, positive control (PC) group received 60mg/kg b.wt per day of lead acetate for 7 days. The other 3 groups received 100mgkg, 200mg/kg and 300mg/kg b.wt respectively for 14 days either as pre-treatment or

**Corresponding author: E-mail: freduwikor1@gmail.com;*

post treatment, for both sexes of the albino rats. At the end of the experimental periods, the rats were sacrificed under chloroform anesthesia and samples were taken through the jugular vein. Reproductive hormone levels were estimated using the Enzyme linked immunosorbent assay methods. Statistical analysis was done using Statistical Analysis System (SAS), STAT 15.1 and p values less than 0.05 were considered statistically significant.
Results: Results showed lead acetate had significant negative effect ($p<0.05$) on most reproductive hormones, except LH and TSH ($p>0.05$). The plant in a dose dependent pattern was able to significantly ($p<0.05$), reverse the effect of lead acetate in the Post treatment phase and also protect the endocrine system from the deleterious effect of lead acetate in the pre-treatment phase. Testosterone was significantly reduced ($p<0.05$) in all the treatment groups that had the extract.
Conclusion: Our study showed that dose dependent AEHR extract significantly reduced the impact of lead intoxication on some hormones.

Keywords: Gonadal hormones; lead-acetate; albino rats; Hypoestes rosea Leaf.

1. INTRODUCTION

Since the dawn of history, man has relied so much on medicinal plants for health and food needs. The traditional use of medicinal plants for curing and preventing illnesses, including the promotion of both physical and spiritual well-being among human beings is as old as man's existence [1]. Over one third of the population in developing countries lack access to essential medicines. The provision of safe and effective traditional medicine therapies could become a critical tool to increase access to healthcare [2]. Most herbal drugs are consumed without adequate research on their toxic effects and activities; hence mechanism of herbal drugs and its activities need extensive scientific research and study.

Medicinal plants are sometimes referred to as being phytoestrogenic or phytoprogesteronic. This is because some plants have molecular structures similar to the hormones estrogen (phytoestrogenic) and progesterone (phytoprogesteronic). They can occupy the receptor sites in the body that would normally be taken up by these hormones [3]. Specific physiological activity of *Hypoestes rosea* as herbal remedy in fertility treatment in relationship to its effect on the fertility hormones and oxidative stress has not been fully studied.

Infertility is a global problem, but the highest prevalence is in low resource countries, particularly in Sub-Saharan Africa [4]. Increasing cases of infertility has become a global challenge, more over infertility due to hormonal abnormalities. Due to the economic predicament of some countries, the people resort to the traditional herbal system for primary health care. In Africa, particularly West Africa, new drugs are not often affordable, thus up to 80% of the population use medicinal plants as remedies [5]. Poverty is on the rise in developing countries, a resultant effect of this is the total dependence on herbal remedies, which becomes very necessary for research on these herbal remedies to improve knowledge and access to refined alternatives. Among the methods used to treat male and female infertility problems, medicinal plants have been used as extracts [6].

Infertility as a result of hormonal complications is a very common problem faced in our environment. The major causes of infertility in Nigeria are sexually transmitted infections and hormonal abnormalities. The most important cause of infertility is hormonal imbalance. Infertility is a major cause of marital crises and divorce in our environment in recent times. With the increasing cost of infertility treatment, which includes in vitro fertilization (IVF), a lot of couples and individuals resort to herbal remedies to help boost their chances of conception because of its affordable cost. People in Nigeria like in most of the developing countries lack regular access to essential medicines, for these people, faith in and popularity of traditional methods have not decreased because modern medicine is unlikely to be a tenable treatment alternative because of high cost [7].

Heavy metals toxicity is a major threat to public health throughout the world. Considerably, Lead exposure is clearly common and capable of inducing permanent damage to various organs. Several evidence has shown the risk of lead toxicity on male reproductive system [8]. Lead induces reduction in number of primordial follicles and increases the number of atretic follicles in ovaries of mice, while it also damages the endometrium [10]. Some studies have found

lead to be a cause of reproductive toxicity in both men and women. In men, it causes a reduction in libido, infertility, as well as a reduction in sperm count and vigor, while in women there is an increase in the incidence of stillbirth and miscarriage. The aim of this study was to assess the levels of some gonadal hormones in lead-acetate-induced Albino Rats treated with *Hypoestes rosea* Leaf.

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification, Extraction and Preparation

Fresh leaves of *Hypoestes rosea* were collected from Sime in Tai (4^0 42' 59.99"N. 7^0 17' 60.00"E) Local Government Area of Rivers State in Nigeria in November 2018. They were deposited at the forest herbarium of the Forestry Research Institute of Nigeria, Ibadan where it was identified by Dr Osiyemi Seun as *Hypoestes rosea* Beauv, with a Herbarium number of FHI 112295.

2.1.1 Preparation of aqueous extracts *Hypoestes rosea* leaf

Extract was prepared using the method of Janardan, [11]. The identified leaves were air dried in a room away from sunlight. It was ground using a blender. The pulverized powdered material was macerated with distilled water in a maceration jar for twenty-four hours. During this period of maceration, the contents are well agitated. They are subsequently filtered with Whatman's No 1 filter paper severally until a very clear filtrate is obtained. The filtrate is transferred to an evaporating dish, which is then poured into a tall column. Cold water is added until the powdered material is completely immersed. It is allowed to stand for 24 hrs so that water-soluble ingredients attain equilibrium in the water. The enriched aqueous extract is concentrated in multiple-effect evaporators until it becomes completely dry. The dry extract is weighed, kept in the fridge until ready for use**.**

2.1.2 Calculation of Dose of *Hypoestes rosea* Leaf for Administration

The aqueous extract for the experimental animals was prepared according to the organization of economic corporation and developments guideline [12], using the calculations based on the method of [12].

The vehicle for the dissolution of the extract for administration was distilled water [13].

Calculations.

A uniform 1ml was used for all animals.

$$\text{Dosage in mg} = \frac{\text{Body weight of animal (g)}}{1000} \times \text{dose (mg)}$$

For a rat of 120g receiving 100g/kg body weight $= \frac{120}{1000} \times 100 = 12\text{mg/ml}$.

2.2 Reagent Acquisition and Preparation

Lead acetate 99.5 % purity for this research was bought from Tianjin Kermel chemical reagent co. ltd, China – 022-28545263 through their agent in Nigeria Hysec Services. It was confirmed to be pure lead acetate by the Chemistry department of the Rivers State University, Port Harcourt, Nigeria. The reagents for the analysis of the reproductive hormones were imported from Elabscience Biotechnology incorporated USA, Monobind Incorporated USA and Perfemed Incorporated USA.

2.3 Experimental Animals

A total number of 140 albino rats made up of seventy male rats and seventy female rats with an average weight 150-180g were procured for the research work. All animals were procured from the Animal House Physiology department of the Faculty of Basic Medical Science of the University of Port Harcourt. The animals were kept in a well-ventilated cage, where they were fed with Grower's Mash. Rats were allowed free access to feed and water *ad libitum.* They were divided into their different groups and allowed to acclimatize for two weeks.

All animals were handled in conformity with the conditions outlined by the National Academy of Science [14-16].

2.4 Experimental Design

2.4.1 Grouping and Treatment

A total of 140 rats previously acclimatized for two weeks weighing between 150-180g rats equal in both sexes of 70 each were divided into 26 groups comprising of 5 rats in each group except the positive controls group that had 10 rats each. The process of the experiment involved induction of some rats with 60mg/kg body weight of lead acetate for 7days to alter their hormones and subsequent treatment with 3 different doses of *Hypoestes rosea* by oral gavage for the pre-

treatment phase, while post treatment phase had treatment with the extract for a period of 14 days, then subsequent induction with lead acetate for 7days for both sexes.

The group that was used as negative control had the normal rat feed only. Positive control received lead acetate only, extract control received 100mg of the extract only. The 3 doses for the treatment groups were 100mg/kg body weight, 200mg/kg body weight and 300mg/kg body weight of the extract respectively as pre-treatment and post-treatment by oral gavage. In the two stages of the experiment the positive control rats were given 60mg/kg body weight of lead acetate for 7days, were fasted overnight and sacrificed on the 8th day, while all others in the pre-treatment started their different doses of extract on the 8th day and continued until the 21st day when they were fasted overnight and sacrificed on the 22nd day.

The post treatment group had their varying doses of the extract from day 1 till day 14, when they were commenced on 60mg/kg body weight of lead acetate up to the 21st day when they were fasted and sacrificed. Euthanasia was under diethyl ether anesthesia. On sacrifice, blood was taken from the jugular vein for reproductive hormone parameters into lithium heparin bottles. The blood for reproductive hormone parameters was spurn at 3000rpm for 10mins in a Wisperfuge centrifuge (Model 1384).

2.5 Experimental Analysis

2.5.1 Quantitative Determination of Estradiol (E2)

Method: Enzyme Immunoassay. ELISA technique as described by the manufacturer. Catalog Number 10009.

2.5.2 Quantitative Determination of Testosterone

Method: Enzyme Immunoassay. ELISA technique as described by the manufacturer. Catalog Number 10007.

2.5.3 Quantitative Determination of Progesterone

Method: Enzyme Immunoassay. ELISA technique as described by the manufacturer. Catalog Number: 10005

2.5.4 Quantitative Determination of Follicle Stimulating Hormone (FSH)

Method: Sandwich-ELISA method as described by the manufacturer. Catalog Number E-EL-R0391

2.5.5 Quantitative Determination of Rat Luteinizing Hormone (LH)

Method: Sandwich-ELISA method as described by the manufacturer. Catalog Number: E-EL-R0026

2.5.6 Quantitative Detection of Rat Prolactin (PRL)

Method: Sandwich-ELISA method as described by the manufacturer. Catalog Number: E-EL-R0052

2.6 Statistical Analysis

The statistical software used for the analysis and graphics presentation was the Statistical Analysis System (SAS), STAT 15.1, developed by SAS Institute, North Carolina State University, USA. Data are presented as Means ± SEM, comparison of mean values of groups that are more than two was done using analysis of variance (ANOVA), and the Turkey test of multiple comparison was used to test for variance within and across groups. Variation between two groups was done using the Student t-test analysis. P values less than 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

The parameters used in the assessment of the effect of the aqueous extract of *Hypoestes rosea* leaf on the reproductive hormones were prolactin, progesterone, testosterone, estradiol, luteinizing hormone, follicle stimulating hormone, testosterone and estradiol ratio. These hormones control changes like release of egg from ovulation, and thickening of uterine wall lining. Unexplained infertility may result if the balance of these hormones is distorted. Therefore, hormones play an important role in the ethiopathogenesis of unexplained infertility.

Table 1. Effects of various concentrations of aqueous extract of *Hypoestes rosea* on the reproductive hormones (PRL, FSH, LH) of female rats in the treatment phases and experimental groups

Treatment Phase (Female)	Experimental Group	PRL (ng/mL)	FSH (ng/mL)	LH (mIU/mL)
		Mean ± SEM	Mean ± SEM	Mean ± SEM
Post-Treatment	EC	50.42±0.369#	63.30±0.672#	19.88±1.716
	NC	53.21±0.552#	52.86±1.312#	21.28±1.407#
	PC	77.68±0.877	44.48±1.500	13.16±1.328
	AEHR (100 mg/kg)	58.16±0.571#	45.18±2.138	15.30±2.155
	AEHR (200 mg/kg)	56.48±0.479#	50.02±1.599#	14.18±1.911
	AEHR (300 mg/kg)	52.00±0.375#	55.16±0.881#	12.94±1.651
	P-value	<0.001	<0.001	0.006
	F-Value	413.950	23.882	4.343
Pre-Treatment	EC	50.42±0.369#	63.30±0.672#	19.88±1.716
	NC	53.21±0.552#	52.86±1.312#	21.28±1.407
	PC	77.68±0.877	44.48±1.500	13.16±1.328
	AEHR (100 mg/kg)	56.88±0.578#	50.24±0.569#	18.98±0.940
	AEHR (200 mg/kg)	54.54±0.347#	55.76±1.023#	17.08±0.801
	AEHR (300 mg/kg)	50.72±0.771#	66.26±1.597#	13.86±0.564
	P-Value	<0.001	<0.001	0.198
	F-Value	334.551	47.863	1.600

Abbreviations: SEM: Standard Error of Mean; PRL: Prolactin; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone. Experimental Groups: EC: Extract Control, NC: Negative Control, PC: Positive Control, Aqueous Extract of Hypoestes rosea at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR (300 mg/kg). Sex: Female, Male; Treatment Phases: Pre-Treatment, Post treatment. N for each level mean=5.Significance Level: =p < 0.05. # - significant at p < 0.05 when compared with PC

Table 2. Effects of various concentrations of aqueous extract of *Hypoestes rosea* on the reproductive hormones (PRL, FSH, LH) of male rats in the treatment phases and experimental groups

Treatment Phase (Male)	Experimental Group	PRL (ng/mL)	FSH (ng/mL)	LH (mIU/mL)
		Mean ± SEM	Mean ± SEM	Mean ± SEM
Post-Treatment	EC	47.52±0.730#	69.72±0.905#	19.28±0.538
	NC	49.62±0.791#	57.76±2.394#	20.06±0.443
	PC	71.16±0.391	29.90±0.957	18.14±0.570
	AEHR (100 mg/kg)	48.88±1.717#	54.76±1.933#	17.28±0.536
	AEHR (200 mg/kg)	47.42±0.310[3]	56.04±0.982#	16.21±0.525
	AEHR (300 mg/kg)	40.10±0.916#	39.84±1.815#	15.46±0.993
	P-Value	0.060	<0.001	<0.001
	F-Value	178.147	1.140	7.964
Pre-Treatment	EC	47.52±0.730#	69.72±0.905#	19.28±0.538
	NC	49.62±0.791[3]	57.76±2.394#	20.06±0.443
	PC	71.16±0.391	29.90±0.957	18.14±0.570
	AEHR (100 mg/kg)	53.82±0.543[3]	54.76±1.933#	17.78±0.758
	AEHR (200 mg/kg)	51.38±0.124#	56.02±0.981#	17.00±0.791
	AEHR (300 mg/kg)	46.10±0.633[3]	39.84±1.815#	14.78±0.875#
	P-Value	<0.001	0.371	<0.001
	F-Value	2.786	78.001	7.140

*Abbreviations: SEM: Standard Error of Mean; PRL: Prolactin; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone. Experimental Groups: EC: Extract Control, NC: Negative Control, PC: Positive Control, Aqueous Extract of Hypoestes rosea at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR (300 mg/kg). Sex: Female, Male; Treatment Phases: Pre-Treatment, Post treatment. N for each level mean=5.Significance Level: =p < 0.05. # - significant at p < 0.05 when compared with PC treatment phases by experimental groups, each parameter means ± SEM with different superscripts are significantly different at p<0.05. Significance Level: *=p<0.05; ***=p<0.001; ns=Not Significant (p>0.05).*

Table 3. Effects of various concentrations of aqueous extract of *Hypoestes rosea* on the reproductive hormones (PROG, TESTO, ESTRAD, T: E2) of female rats in the treatment phases and experimental groups

Treatment Phase (Female)	Experimental Group	PROG(ng/mL)	TESTO (ng/mL)	ESTRAD (pg/mL)	T: E2
		Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean± SEM**
	EC	3.96±0.254	0.294±0.022#	10.96±0.649	0.252±0.017#
	NC	7.96±0.739#	0.508±0.039#	9.66±0.388	0.496±0.031#
	PC	3.30±0.126	0.128±0.010	8.46±0.824	0.144±0.007
Post-Treatment	AEHR (100 mg/kg)	3.46±0.287	0.288±0.010#	8.86±0.893	0.356±0.036#
	AEHR (200 mg/kg)	3.80±0.138	0.230±0.008#	9.76±0.443	0.268±0.013#
	AEHR (300 mg/kg)	7.56±0.423#	0.166±0.010	12.10±0.468	0.180±0.021
	P-Value	<0.001	<0.001	0.001	<0.001
	F-Value	30.564	45.940	4.467	30.815
	EC	3.96±0.254	0.294±0.022#	10..96±0.649	0.252±0.017#
	NC	7.96±0.739#	0.508±0.039#	9.66±0.388	0.496±0.031#
	PC	3.30±0.126	0.128±0.010	8.46±0.824	0.144±0.007
Pre-Treatment	AEHR (100 mg/kg)	4.02±0.169	0.322±0.013#	9.46±0.340	0.288±0.004#
	AEHR (200 mg/kg)	5.02±0.086#	0.276±0.009#	11.06±0.311	0.198±0.006
	AEHR (300 mg/kg)	8.22±0.146#	0.228±0.022#	13.06±1.101#	0.126±0.013
	P-Value	<0.001	<0.001	0.005	<0.001
	F-Value	40.393	33.558	5.834	72.496

Abbreviations: SEM: Standard Error of Mean; Prog: Progesterone; Testo: Testosterone; Estrad: Estradiol. Experimental Groups: EC: Extract Control, NC: Negative Control, PC: Positive Control, Aqueous Extract of Hypoestes rosea at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR (300 mg/kg). Sex: Female, Male; Treatment Phases: Pre-Treatment, Post treatment. N for each level mean=5.Within and across sex and treatment phases by experimental groups, each parameter means ± SEM with # superscripts are significantly different at p<0.05 with the mean of the PC group significance Level: =p<0.05. # - significant at p < 0.05 when compared with PC

There were significant increases in the FSH mean value of pre-treatment group in both sex of the albino rats, when compared to the positive (lead only) group. The pre-treatment with aqueous extract of *hypoestes rosea* was able to reverse the damage caused as well as enhance recovery from the damage by the administration of lead acetate. The significantly reduced mean value of FSH in lead induced rats in this study is in agreement with previous study by other researchers; where they found the effect of lead acetate reduce significantly the FSH of albino rats. Our study agrees with Foster, [17], who found suppression in the FSH of male monkeys exposed to lead poisoning. In 2004, it was also reported that a significant reduction in the FSH values occurred in albino rats exposed to varying doses of lead acetate. In a related study by Mokhtari & Zanboori, [18], their findings agreed with ours as albino rats treated with lead acetate also showed decreased FSH values. However, our study does not agree with another study by Thoreux-Manlay et al. [19] that found no difference in the FSH value of mice induced by lead acetate. our results are also not in agreement with Ayinde et al. [20], who in his study reported a remarkable increase in the level of FSH of rats treated with lead acetate, these results however may be as a result of the dose used in the different inductions compared to the body weight as lead toxicity is dose dependent response relationship, hence its toxic effect varies [21]. It was also found that the toxic effect of lead acetate at the hypothalamic-pituitary axis to be dose dependent.

The findings of our study show there was significant reduction in the value of luteinizing hormone of both male and female rats induced with lead acetate only induction of both male and female albino rats (Tables 1 & 2). This further confirms the devastating effect of lead acetate on the hypothalamic-pituitary gonadal axis. Luteinizing hormone in human of fertile age, it plays a central role in follicle development and spermatogenesis stimulating the production of steroid hormones and mediating proliferative signals [22]. The increase in LH stimulates ovulation and development of the corpus luteum. Gonadotrophin – releasing hormone triggers the LH surge that precedes ovulation, the sharp decline in the LH is capable of causing Anovulation in the PC female rats. LH stimulates testosterone secretion by the Leydig cells, implying a low LH will adversely affect testosterone levels. Testicular function is under the control of Gonadotropins. The reduction in the LH levels of animals exposed to lead acetate

is an indication of enzyme inhibition in steroidogenetic pathway [22], disruption of hypothalamic secretion of hormones and spermatogenesis has been associated with lead toxicity.

The results of our finding are in agreement with the work of Thoreux-Manlay et al. [19], who reported a decrease in LH of rats treated with lead acetate. The sharp decrease in the LH in lead-exposed rats, indicate impaired Leydig cell function. However, our studies are not in agreement with Ayinde et al. [12], who in their study found that there was no difference in the LH values of lead acetate induced rats and the controls.

In our study, the LH value in the post treatment phase of the study for the female albino rats showed no statistical significance among the various groups. Our findings show that even though the value was not statistically significant, there was a remarkable increase away from the decline in the value of the positive control, the group that received AEHR100 showed better LH values when compared to the PC. Incidentally even though the value was higher than PC group, it was lower than the NC group and also not statistically significant.

The result of our study at this point is in agreement with Riaz et al. [24] that found a statistically no significant increase in the serum LH of rats treated with *Zingiber officinale* (Ginger). The result of the pre- treatment phase of the study for the female albino rats showed statistical significance with the rats treated with AEHR leaf, the protective property of the plant was best felt at the rat groups that received AEHR100, and their values were closest to the Normal control.

Table 4. Effects of various concentrations of aqueous extract of *Hypoestes rosea* on the reproductive hormones (PROG, TESTO, ESTRAD, T: E2) of male rats in the treatment phases and experimental groups

Treatment Phase (Male)	Experimental Group	PROG(ng/mL)	TESTO (ng/mL)	ESTRAD (pg/mL)	T: E2
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean± SEM
Post-Treatment	EC	2.62±0.276#	1.706±0.092#	9.46±0.506	1.720±0.132#
	NC	3.12±0.146#	2.266±0.312#	10.06±0.541	2.136±0.282#
	PC	7.82±0.581	0.954±0.113	8.66±0.339	1.052±0.124
	AEHR (100 mg/kg)	6.92±0.169	1.212±0.132	8.76±0.336	1.308±0.136
	AEHR (200 mg/kg)	5.30±0.544#	0.882±0.046	9.06±0.236	0.918±0.041
	AEHR (300 mg/kg)	4.02±0.404#	0.846±0.041	9.46±0.665	0.858±0.057
	P-Value	<0.001	<0.001	0.399	<0.001
	F-Value	28.448	13.798	1.289	11.411
Pre-Treatment	EC	2.62±0.276#	1.706±0.092#	9.46±0.506	1.722±0.132#
	NC	3.12±0.146#	2.266±0.312#	10.06±0.541	2.136±0.282
	PC	7.82±0.581	0.954±0.113	8.66±0.339	1.052±0.124
	AEHR (100 mg/kg)	6.92±0.169	1.212±0.132	8.86±0.246	1.294±0.142
	AEHR (200 mg/kg)	5.30±0.544#	0.882±0.046	9.06±0.663	0.936±0.071
	AEHR (300 mg/kg)	4.02±0.404#	0.844±0.040	9.86±0.770	0.836±0.102
	P-Value	<0.001	<0.001	0.301	<0.001
	F-Value	28.448	13.780	1.075	10.387

Abbreviations: SEM: Standard Error of Mean; Prog: Progesterone; Testo: Testosterone; Estrad: Estradiol. Experimental Groups: EC: Extract Control, NC: Negative Control, PC: Positive Control, Aqueous Extract of Hypoestes rosea at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR (300 mg/kg). Sex: Female, Male; Treatment Phases: Pre-Treatment, Post treatment. N for each level mean=5.Within and across sex and treatment phases by experimental groups, each parameter means ± SEM with # superscripts are significantly different at p<0.05 with the mean of the PC group significance Level: =p<0.05. # - significant at p < 0.05 when compared with PC

The results of our finding show that the administration of lead acetate to the albino rats caused a decline in the value of their testosterone (Tables 3 & 4). Testosterone is a male hormone that has significant impact on spermatogenesis [25]. It is secreted by the leydig cells of the testis, the adrenals and the ovaries, it is the most important androgen secreted into the blood. A low sperm count may indicate a problem with testosterone levels. In females, testosterone is implicated in cases of polycystic ovary syndrome, where it is slightly elevated [26]. Lead toxicity is reputed to target the hypothalamic pituitary axis. The Leydig cells appear to be a target of lead exposure [19]. Studies have shown that lead accumulates preferentially in the epididymis and other accessory glands and Leydig cells appear to be its primary target [27]. Leydig cells and interstitial cells are the cells that secrete testosterone. It has been shown that in lead poisoning, a large concentration of lead is deposited in the testis. Lead has been found to cause hypospermia, lowered testosterone levels and testicular atrophy in male lead battery workers [28]. A significant decrease in the serum testosterone indicates the decreased steroidogenesis. The findings of our study agrees with the work done by Al-Chalabi *et al*. [29] on the ability of lead to decrease significantly the testosterone level of rats exposed to lead acetate.The findings of our study is not in agreement with Hachfi et al. [30] that found no difference in the testosterone level of rats exposed to low level of lead toxicity for 30 days in comparison with the control rats, the findings in their study could be as a result of the dose as the rats were exposed to low level of lead acetate in their drinking water.

The result of the acute and sub chronic effects of treatment in the pre-treatment and post treatment phase of our study in both sex of the albino rats shows a reduction in the mean of the testosterone level of the albino rats in a dose dependent manner. In the acute treatment phase, the male albino rats in both phases had mean values lower than the Positive control. The mean of testosterone of the albino rats that had no extract were NC group significantly higher than all other groups. At the sub chronic stage of the study in both sex and phases, the mean of all groups that had the extract was lower than the PC group, hence exposure of the rats to the extract over time showed continuous reduction in the level of the testosterone of both sex of the albino rats in a dose dependent manner with the higher dose groups showing lower mean values. Some plants rich in alkaloids have been shown to have negative effect on the testosterone level, considering that *hypoestes rosea* is rich in Alkaloids.

The result of our study showed that the administration of lead acetate to both sexes of the albino rats caused a significant drop in the T:E2 of all the albino rats (Tables 3 &4). Previous studies have not looked at T:E2 level in lead acetate induced rats. The result of our study showed that the administration of AEHR caused a dose dependent decrease in the mean T:E2 ratio of both sexes of the albino rats. The mean T:E2 ratio of the albino rats in both sexes was only slightly increased above the mean of PC group at a dose of AEHR 100mg/kg in the pre and post treatment phases of the study. Doses of 100mg and 200mg of AEHR rather showed declining mean values of T:E2 for both sexes of the albino rats. The mean values of T:E2 at a dose of AEHR 300 was significantly lower than the PC group in both sexes. The observation in this study may be as a result of the effect of AEHR on testosterone as earlier discussed in our previous findings. The result of our findings is in agreement with the previous findings of Lucky & Festus, [31] on the effect of *Cnidoscolus aconotifolius* on the pituitary-gonadal axis of male Wistar rats.

In our findings, the mean progesterone of the albino rats on administration of progesterone showed a significant decrease in the value. This is seen in the PC group. Progesterone is the dominant hormone responsible for the luteal phase, and deficiency results in failure of implantation of the embryo. Progesterone has a central role in reproduction, being involved in ovulation, implantation, and pregnancy. Progesterone in the uterus and ovary causes the induction of ovulation, even the facilitation of implantation, and maintenance of early pregnancy are controlled by progesterone. Progesterone helps to regulate the monthly menstrual cycle [31]. Decrease in serum progesterone level by lead acetate administration in this study suggests impaired endometrial function, which disrupts normal secretion of special protein required to nourish an implanted fertilized egg and prenatal development [32]. It is also an indication that lead acetate affects the ovaries, as it is secreted majorly by the corpus luteum. Lead interferes at several sites in the steroids biosynthesis via Hypothalamus-pituitary –gonadal axis and affects ovarian morphology, which leads to impairment of fertility in female.

The pretreatment and post treatment with different doses of AEHR at the acute and sub chronic phase of the study showed a reversal on the effect of the lead acetate for all the groups, the effect AEHR was observed to be dose dependent in all phases and stages of the study. The most significant increase was noticed at the Sub-chronic stage of the experiment that showed treated animals with significantly higher progesterone values. The increase in serum levels of progesterone of AEHR treated rats observed in this study is an indication that the extract ameliorated the damage caused to endometrium function, thus promoting reproduction.

The findings of our study show the mean estradiol level of the lead acetate only PC group was significantly lower than all other groups, these shows that the administration of lead acetate caused a sharp drop in the estrogen level of the albino rats. Estrogens are the primary female sex hormones and play important roles in both reproductive and non-reproductive systems. They are responsible for the development of many female secondary sexual characteristics; stimulate the growth of ovarian follicles and proliferation of endometrium during menstrual cycle [22]. Estradiol, the predominant form of estrogen, also plays a critical role in male sexual function. Estradiol in men is essential for modulating libido, erectile function, and spermatogenesis. The massive drop in the estrogen level shows lead has a toxic effect on endometrium. Lead acetate causes a significant decrease in steroid hormones synthesis and release from ovary of ovarian hormones estrogen and Progesterone. This reduction in the ovarian secreted hormones is supposed to cause a positive feedback mechanism on hypothalamus or pituitary gland which would have stimulated the release of Gonadotropic releasing hormones that will cause an increase in LH and FSH. The values of both hormones in the PC group are still low as a result of the action of lead acetate on the hypothalamic pituitary gonadal axis. The pretreatment and post treatment of both sex of rats with AEHR at the acute and sub chronic stages of the study showed significant increase in the mean serum of both sex in a dose dependent pattern, the extract caused a total reversal of the effect of the lead acetate on the estrous cycle. The effect increased as the duration of the study increased, being significantly higher than the negative control. The dose dependent mean value increase caused the mean value to rise as the dose of the extract increase. the elevation of the mean estrogen of the treated groups shows the ability of AEHR to ameliorate the negative effect of lead acetate.

The result of our study shows that exposure of the albino rats to lead acetate caused a remarkable rise in the level of their prolactin. This shows that lead acetate has the ability of affecting the dopinergic system. The dopaminergic and serotoninergic systems, respectively, are involved in the physiologic regulation of prolactin [33]. Previous studies have shown that lead affects the hypothalamic pituitary axis. Hyperprolactinemia is implicated in a lot of cases of infertility. Hyperprolactinemia can be regarded as one of the very common endocrine disorder that causes increased secretion on the hypothalamic-pituitary axis, it occurs predominantly in young women (20-30%) and can lead to several abnormalities one of which includes infertility [34]. Even though prolactin is required for breast development and lactogenesis, when it is secreted in excess it becomes a reproductive disorder. Hyperprolactinemia tends to suppress the ovulatory cycle by inhibiting the secretion of follicle stimulating hormone (FSH) and gonadotropic-releasing hormones, which are necessary for ovulation [35]. Men with hyperprolactinemia may present with erectile dysfunction, decreased libido, infertility, gynecomastia, decreased bone mass [36]. Women may present with decreased libido, infertility, oligomenorrhea/amenorrhea and galactorrhea. Men may present with decreased libido, infertility, gynecomastia or impotence [36]. Our study shows that acute and sub chronic exposure to aqueous extract of *hypoestes rosea* leaf has the ability to reduce the prolactin. This activity can be attributed to the presence of flavonoids in the phytonutrients of the plant, Ye*et al*, 2010 had earlier reported the anti hyperprolactinemia activity of casticin a flavonoid that inhibited prolactin *invivo* and *invitro*.

4. CONCLUSION

Our study shows that dose dependent AEHR extract significantly reduced the impact of lead intoxication on some hormones.

NOTE

The study highlights the efficacy of "traditional medicine", “herbal” which is an ancient tradition, used in some parts of india. This ancient concept should be carefully evaluated in the light of

modern medical science and can be utilized partially if found suitable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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