

## Original Research Article

# Aqueous *Hypoestes rosea* Leaf extract Ameliorates Lead-Acetate-Induced Thyroid Hormones Disruption in Albino Wistar Rats

### ABSTRACT

**Aim:** To evaluate the effect of *hypoestes rosea* leaf extract on thyroid hormones in lead-acetate-induced Albino Rats.

**Study design:** Experimental study.

**Place and Duration of Study:** Department of Pharmacology, University of Port Harcourt and Department of Chemical Pathology, University of Port Harcourt, between October, 2019 and March, 2020.

**Methodology:** A total of 140 albino Wistar rats were used for the 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). 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 in each sex. The negative control groups received rat feed only, EC group received 100mg/kg bwt/day for 21 days, PC group received 60mg/kg b.wt per day of lead acetate for 7 days. The other 3 groups received 100mg/kg, 200mg/kg and 300mg/kg b.wt respectively for 14 days either as pre-treatment or post treatment, for both sexes of the albino rats. At the end of the experimental periods, the rats were sacrificed under chloroform anaesthesia and samples were taken through the jugular vein. Thyroid hormone levels (thyroid stimulating hormone (TSH), tri-iodothyronine (T3) and tetra-iodothyronine (T4)) 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:** The results showed that the administration of lead acetate in male and female rats caused significant ( $p < 0.05$ ) fall in the levels of TSH, fT3 and fT4. 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.

**Conclusion:** This study shows that the consumption of AEHR by albino rats could help protect the endocrine system against endocrine disruptors.

**Keywords:** *Hypoestes rosea* Leaf, Thyroid Hormones, Lead-Acetate, Albino Rats.

### 1. INTRODUCTION

Hormones are substances that serve as vehicles for intracellular and extracellular communication. Historically, hormones have been defined as chemical substances that are produced by a gland in one part of the body, are secreted into the bloodstream, and act on a target organ elsewhere [1].

A hormone is a chemical substance produced in the body by an organ, cells of an organ, or scattered cells that has a specific regulatory effect on the activity of an organ or tissues [2]. Hence hormones can be seen as biologic messengers secreted by an organ or cells of an organ, and has effect on that organ thereby affecting the functionality of the cells of that organ (Autocrine). It may also be released or synthesized from one type of cell and binds to the cell receptors of other nearby cell, thereby eliciting hormonal signal of such nearby cells (Paracrine).

Evidence has shown that lead affects growth, thyroid and hormone homeostasis by interfering with the production, release, biological action and metabolism of relevant hormones [3]. Lead exposure affects the hypothalamic-pituitary-thyroid axis, with evidence on the effect of lead on the thyrotrope cells. Several researchers have noted higher concentrations of Thyroid stimulating hormone (TSH) in lead-exposed workers which may enhance the pituitary release of TSH [4]. Lead appears to increase the binding of Thyroid releasing hormone (TRH) to anterior pituitary receptors in rats [5], even though weak TSH responses to TRH have been found in lead exposed children, and in experimental in vitro studies on rat pituitary cells incubated with lead [6]. Occupational exposure to inorganic lead has been associated with impaired uptake of iodine by the thyroid gland and alterations in morphology of the thyroid tissue [7]. A negative correlation between blood lead and free T4 (FT4) levels in long term low-level lead exposure without significant changes in TSH and T3 levels in adolescents has also been reported. Similarly, serum T4 and estimated FT4 levels were found to regress negatively with blood lead level.

Herbal remedies have long been used to address problems with fertility. In fact, evidence of the use of herbal remedies for female and male fertility dates all the way back to 200 A.D. [8]. The use of alternative medicines in the treatment of infertility resulting from hormonal complications becomes a veritable tool in the hands of herbal practitioners without scientific backing, evidence and research findings. Several researches have indicated that exposure to heavy metals including lead is deleterious to body organs which may include neurological, hematological, gastrointestinal, reproductive, circulatory and immunological disorders [9][10].

The patronage for herbal remedies as a result of faith in and popularity of traditional methods have not decreased, because modern medicine is unlikely to be a tenable treatment alternative, primarily because of its high cost [11]. The importance of herbal medicines in the life of people in our climes cannot be overemphasized, the resurgence of herbal medicine is as a result of the increasingly expensive and unavailability of orthodox drugs to average income earners [12]. Odugbemi et al. [13] had earlier posited that one of the major challenges facing orthodox drugs in the treatment of ailments is the growing resistance of a lot of ailments to orthodox drugs. Medicinal plants are the inexpensive drugs for all categories of people in the world because of their less serious side effects compared to the synthetic ones [14]. The cost and availability of herbs and their utilization resources transferred from one generation to another keep the information alive and useful to all [14]. The aim of this study was to evaluate the effect of *hypoestes rosea* leaf extract on thyroid hormones in lead-acetate-induced Albino Rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection, Identification, Extraction and Preparation

Fresh leaves of *Hypoestes rosea* were collected from Sime in Tai (4° 42' 59.99"N. 7° 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, [15]. 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 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 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 [16], using the calculations based on the method of [16].

The vehicle for the dissolution of the extract for administration was distilled water [17].

Calculations.

A uniform 1ml was used for all animals.

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 =  $\frac{120 \times 100}{1000} = 12\text{mg/ml}$ .

## **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.

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 growers 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 [18-20].

## **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 24 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 8<sup>th</sup> day, while all others in the pre-treatment started their different doses of extract on the 8<sup>th</sup> day and continued until the 21<sup>st</sup> day when they were fasted overnight and sacrificed on the 22<sup>nd</sup> 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 21<sup>st</sup> day when they were fasted and sacrificed. Euthanasia was under diethyl ether anesthesia. On sacrifice, blood was taken from the jugular vein for oxidative stress markers into lithium heparin bottles. The blood for oxidative stress markers was spun at 3000rpm for 10mins in a Wisperfuge centrifuge (Model 1384).

## 2.5 Assay Methods

### 2.5.1 Quantitative Determination of Free Triiodothyronine (fT3) Concentration

**Method:** Accu-Bind Microplate competitive Enzyme Immunoassay, (ELISA technique as described by the manufacturer. Product Code:1325-300.

**Principle**

### 2.5.2 Quantitative Determination of the Free Thyroxine (fT4) Concentration

**Method:** Accu-Bind Microplate Enzyme Competitive Immunoassay (ELISA technique as described by the manufacturer. Product Code: 1225-300

### 2.5.3 Quantitative Determination of Rat Thyroid Stimulating Hormone (TSH)

**Method:** Sandwich-ELISA as described by the manufacturer. Catalog Number: E-EL-R0976

## 2.6 Statistical Analysis

The statistical software used for the analysis was the Statistical Analysis System (SAS), STAT 15.1, developed by SAS Institute, North Carolina State University, USA. Data are presented as Means  $\pm$  SEM, comparison of mean values of groups that are more than two was done using analysis of variance (ANOVA), and the Tukey 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

**Table 1: Acute effects of various concentrations of aqueous extract of *Hypoestes rosea* on the thyroid hormones (TSH, fT3, fT4) treatment phases and experimental groups (Female rats)**

Treatment Phase	Experimental Group	TSH (ng/mL)	fT3 (pg/dL)	fT4 (ng/dL)
		Mean $\pm$ SEM	Mean $\pm$	Mean $\pm$ SEM

		SEM		
Post-Treatment	EC	90.60±6.250	1.83±0.021 <sup>#</sup>	1.05±0.067 <sup>#</sup>
	NC	86.60±4.545	1.81±0.022 <sup>#</sup>	0.75±0.040 <sup>#</sup>
	PC	78.60±1.503	1.58±0.022	0.42±0.009
	AEHR (100 mg/kg)	93.60±1.778	1.81±0.018 <sup>#</sup>	0.97±0.039 <sup>#</sup>
	AEHR (200 mg/kg)	96.60±4.115	1.84±0.012 <sup>#</sup>	0.93±0.020 <sup>#</sup>
	AEHR (300 mg/kg)	96.60±5.627	1.86±0.012 <sup>#</sup>	0.89±0.060 <sup>#</sup>
	P-value	0.560	<0.001	<0.001
	F-Value	2.534	31.657	26.373
Pre-Treatment	EC	90.60±6.250	1.83±0.021 <sup>#</sup>	1.05±0.067 <sup>#</sup>
	NC	86.60±4.545	1.81±0.022 <sup>#</sup>	0.75±0.040 <sup>#</sup>
	PC	78.60±1.503	1.58±0.022	0.42±0.009
	AEHR (100 mg/kg)	96.60±3.776	1.80±0.026 <sup>#</sup>	0.99±0.051 <sup>#</sup>
	AEHR (200 mg/kg)	98.60±5.741 <sup>#</sup>	1.87±0.023 <sup>#</sup>	0.98±0.028 <sup>#</sup>
	AEHR (300 mg/kg)	98.60±2.768 <sup>#</sup>	1.85±0.028 <sup>#</sup>	0.96±0.039 <sup>#</sup>
	P-Value	0.022	<0.001	0.001
	F-Value	3.265	20.100	31.095

Abbreviations: SEM: Standard Error of Mean; TSH: Thyroid Stimulating Hormone (Thyrotropin)0; FT3: Tri-iodothyronine; FT4: Thyroxine. 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  $\pm$  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 2: Acute effects of various concentrations of aqueous extract of *Hypoestes rosea* on the thyroid hormones (TSH, FT3, FT4) treatment phases and experimental groups (Male rats)**

Treatment Phase	Experimental Group	TSH (ng/mL)	FT3 (pg/dL)	FT4 (ng/dL)
		Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM
Post-Treatment	EC	143.60±14.528 <sup>#</sup>	1.79±0.045	0.48±0.026
	NC	89.60±1.778	1.77±0.042	0.57±0.027
	PC	74.60±3.341	1.76±0.055	0.42±0.010
	AEHR (100 mg/kg)	82.20±1.800	1.77±0.029	0.77±0.100 <sup>#</sup>
	AEHR (200 mg/kg)	112.60±9.616 <sup>#</sup>	1.79±0.033	0.46±0.033
	AEHR (300 mg/kg)	121.80±5.352 <sup>#</sup>	1.81±0.030	0.44±0.025
	P-Value	<0.001	0.895	<0.001
	F-Value	12.030	0.955	7.784
Pre-Treatment	EC	143.60±14.528 <sup>#</sup>	1.79±0.045	0.48±0.026
	NC	89.60±1.778	1.77±0.042	0.57±0.027
	PC	74.60±3.341	1.76±0.055	0.42±0.010
	AEHR (100 mg/kg)	82.40±1.860	1.78±0.039	0.77±0.100 <sup>#</sup>
	AEHR (200 mg/kg)	112.60±9.616 <sup>#</sup>	1.81±0.020	0.46±0.033
	AEHR (300 mg/kg)	121.80±5.352 <sup>#</sup>	1.82±0.035	0.44±0.026
	P-Value	<0.001	0.209	<0.001
	F-Value	11.992	0.321	7.737

Abbreviations: SEM: Standard Error of Mean; TSH: Thyroid Stimulating Hormone (Thyrotropin)0; FT3: Tri-iodothyronine; FT4: Thyroxine. 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  $\pm$  SEM with # superscripts are significantly different at  $p<0.05$  with the mean of the PC group Significance Level: = $p<0.05$ .

**Table 3: Sub-chronic effects of various concentrations of aqueous extract of *Hypoestes rosea* on the thyroid hormones (TSH, FT3, FT4) treatment phases and experimental groups (Female rats)**

Treatment Phase	Experimental Group	TSH (ng/mL)	FT3 (pg/dL)	FT4 (ng/dL)
		Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM
	EC	80.60±1.288 <sup>def</sup>	1.79±0.019	1.06±0.048
	NC	84.20±3.184 <sup>def</sup>	1.81±0.036	0.76±0.084

Post-Treatment	PC	78.60±1.503 <sup>ef</sup>	1.58±0.022	0.42±0.009
	aEHR (100 mg/kg)	79.60±2.657 <sup>ef</sup>	1.81±0.022	0.99±0.023
	aEHR (200 mg/kg)	75.60±8.489 <sup>efg</sup>	1.86±0.018	0.94±0.032
	aEHR (300 mg/kg)	74.80±3.747 <sup>fg</sup>	1.89±0.013	0.92±0.078
	P-Value	0.295	<0.001	<0.001
Pre-Treatment	F-Value	1.305	21.905	22.444
	EC	80.60±1.288 <sup>ef</sup>	1.79±0.019	1.06±0.048
	NC	84.20±3.184 <sup>def</sup>	1.81±0.036	0.76±0.084
	PC	78.60±1.503 <sup>ef</sup>	1.58±0.022	0.42±0.009
	aEHR (100 mg/kg)	77.60±3.172 <sup>efg</sup>	1.86±0.045	1.02±0.023
Post-Treatment	aEHR (200 mg/kg)	75.60±3.265 <sup>efg</sup>	1.90±0.013	1.01±0.064
	aEHR (300 mg/kg)	74.00±4.848 <sup>fg</sup>	1.94±0.010	0.98±0.048
	P-Value	0.665	<0.001	<0.001
	F-Value	0.648	23.423	19.109

Abbreviations: SEM: Standard Error of Mean; TSH: Thyroid Stimulating Hormone (Thyrotropin); FT3: Tri-iodothyronine; FT4: Thyroxine. 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  $\pm$  SEM with # superscripts are significantly different at  $p<0.05$  with the mean of the PC group Significance Level:  $=p<0.05$ .

**Table 4: Sub-chronic effects of various concentrations of aqueous extract of *Hypoestes rosea* on the thyroid hormones (TSH, FT3, FT4) treatment phases and experimental groups (Male rats)**

Treatment Phase	Experimental Group	TSH (ng/mL)	FT3 (pg/dL)	FT4 (ng/dL)
		Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM
Post-Treatment	EC	78.60±4.179 <sup>ef</sup>	1.83±0.013	0.80±0.023
	NC	80.60±2.358 <sup>ef</sup>	1.84±0.015	1.11±0.127
	PC	74.60±3.341 <sup>fg</sup>	1.76±0.055	0.42±0.010
	aEHR (100 mg/kg)	68.60±6.266 <sup>g</sup>	1.79±0.013	1.14±0.012
	aEHR (200 mg/kg)	85.60±2.874 <sup>de</sup>	1.84±0.016	1.01±0.069
	aEHR (300 mg/kg)	114.60±2.874 <sup>b</sup>	1.86±0.024	0.84±0.066
	P-value	<0.001	0.218	<0.001
Pre-Treatment	F-Value	26.388	1.530	16.686
	EC	78.60±4.179 <sup>ef</sup>	1.83±0.013	0.80±0.023
	NC	80.60±2.358 <sup>ef</sup>	1.84±0.015	1.11±0.127
	PC	74.60±3.341 <sup>fg</sup>	1.76±0.055	0.42±0.010
	aEHR (100 mg/kg)	93.60±1.778 <sup>cd</sup>	1.82±0.022	1.00±0.051
	aEHR (200 mg/kg)	96.60±4.155 <sup>c</sup>	1.85±0.031	0.98±0.028
	aEHR (300 mg/kg)	96.60±5.627 <sup>c</sup>	1.87±0.027	0.96±0.039
Test statistics	P-Value	<0.001	0.153	<0.001
	F-Value	40.296	1.790	16.528
		0.0047**	0.9909 <sup>ns</sup>	0.7393 <sup>ns</sup>

Abbreviations: SEM: Standard Error of Mean; TSH: Thyroid Stimulating Hormone (Thyrotropin); FT3: Tri-iodothyronine; FT4: Thyroxine. 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  $\pm$  SEM with different superscripts are significantly different at  $p<0.05$ . Significance Level:  $*$ = $p<0.05$ ;  $***$ = $p<0.001$ ;  $ns$ =Not Significant ( $p>0.05$ ).

Serum levels of thyroid hormones, including T3, T4 and TSH, are commonly used as reliable indicators of the thyroid function in humans and experimental animals. All reactions necessary for the formation of T3 and T4 are influenced and controlled by pituitary gland. In our study, the administration of lead acetate to both sexes of the albino rats caused a significant drop in the mean value of thyroid stimulating hormone as seen in the mean value of the PC rats. The result of our study is in agreement with Sujatha et al. [21]. In their study, they found a significant decrease in the TSH value of rats induced with the same dose of lead acetate. In our study, we have observed that lead exposure caused a decline in TSH levels, which was statistically significant in the group that was treated with lead acetate.

Determination of TSH levels in clinical settings is the first step for the diagnosis of thyroid disorders since it is considered to be a more precise marker for identifying thyroid malfunctioning than the thyroid hormones themselves [22]. The decrease in fT3, fT4 and TSH values might be due to structural damage of thyroid follicular cells due to accumulation of lead in the thyroid gland and also effect on regulatory enzymes associated with hypothalamic pituitary thyroid (HPT) axis [23]. The results of our study are not in agreement with Pekcici et al. [24] that saw an increase in the mean TSH of lead exposed workers. This may have been as a result of their working on humans exposed to low level of lead toxicity from their work environment, while our work was on high dose lead on albino rats at acute exposure. Our result is also not in agreement with Krieg, [25] in his meta-analysis found occupational lead exposure does not have any effect at all on male thyroid hormones. This disparity in the findings is also as a result of low dose of exposure by the study subjects.

The result of our study also showed that the administration of AEHR to the female albino rats post-treatment with the AEHR at the acute phase showed a statistically significant dose dependent increase in the mean serum level of the female albino rats. This showed the plant was able to protect the thyroid against the effect of lead acetate post treatment exposure.

The result of acute pre-treatment exposure for the female albino rats showed a dose dependent increase in the mean level of TSH even though it was not statistically significant. This finding shows that AEHR could reverse the effect of lead poisoning on the TSH of female albino rats even though an exposure to lead acetate may affect the TSH value. The results of our finding are in agreement with Sujatha et al. [11] who in their study found out *Ossimum sanctum* leaf extract ameliorated the effect of lead acetate on male Wister rats in a dose dependent manner. This increase might be due to the antioxidant property of AEHR, as hyperthyroidism and hypothyroidism are associated with oxidative stress in human and animals. More so, experimental data have shown that many flavonoids could inhibit thyroperoxidase activity thus increasing TSH.

The result of the sub chronic post treatment and pretreatment with lead acetate and varying doses of AEHR for the female albino rats showed a dose dependent insignificant increase above the PC, the mean increase in the TSH was observed more at the lowest dose of AEHR 100mg/kg. There was reduction in the mean concentration of the female albino rats as the dosage increased.

This study showed that administration of various doses of AEHR on all sexes, phases and stages of the albino rats caused a significant increase in the fT4 of all the albino rats. The increase was dose dependent and duration dependent. The mean fT4 value in both sexes of the albino rats increased as the dose of AEHR decreased. The results of our findings agree with Sujatha et al. [11]. Our observation on the effect of AEHR on the thyroid hormones is the plant the effect of the plant on the different thyroid function hormones varied with sex and duration; it was also not consistent in pattern. Our various findings on the thyroid hormones and the variations in the activity of AEHR on the thyroid function could possibly be as a result of flavonoids, depending on the dose and time of treatment and species, flavonoids seem to differentially affect pituitary-thyroid axis [26]. Even though animals exposed to lead acetate may be at the risk of thyroid damage [27]. AEHR is able to substantially reverse this impact on the thyroid hormones.

The result of our findings shows that the administration of the extract in all doses, post treatment and pretreatment at the acute and sub chronic stage on the male albino rats showed significant increase of the TSH. The AEHR was able to significantly ameliorate the effect of the lead acetate on TSH of male albino rats treated with lead acetate prior to the plant and also the plant effectively and significantly protected the male albino rats from the negative hormonal effect of lead acetate induction on the TSH of the male albino rats.

The results of our finding is in agreement with Sujatha et al. [11] who in their study found out *Ossimum sanctum* leaf extract ameliorated the effect of lead acetate on male Wister rats in a dose dependent manner. This increase might be due to the antioxidant property of AEHR, as hyperthyroidism and hypothyroidism are associated with oxidative stress in human and animals.

The result of our findings showed that the administration of different doses of AEHR on all female albino rats at the post treatment and pre treatment phase in the acute and sub chronic stage of the study showed a significant dose dependent increase in the fT3 value away from the observed effect on the PC. The dose dependent increase in both phases and stages of the study increased as the dose of AEHR increased. The findings of our study are in agreement with Sujatha *et al.* [11].

The findings also indicated that the administration of different doses of AEHR on all male albino at the post treatment and pretreatment phase in the acute stage of the study showed no significance. The sub-chronic pretreatment and post treatment showed a significant dose dependent increase in the fT3 value away from the observed effect on the PC. Our findings show that a longer exposure of the male albino rats to AEHR was more effective and able to mitigate the effect of lead acetate on fT3.

#### 4. CONCLUSION

This study shows that the consumption of AEHR by albino rats could help protect the endocrine system against endocrine disruptors.

#### 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 DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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