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Effects of an herbal formulation on lead acetate-induced hormonal imbalance in female albino rats

Ojoye N. Briggs and Kemzi N. Elechi-Amadi *

Department of Clinical Chemistry, Faculty of Medical Laboratory Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

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Abstract

Hormonal imbalance is a situation in which there is too little or too much of one or many hormones in the body, and this has negative effect on certain physiological processes including reproduction.

Aim: This study evaluated the effects of an herbal formulation on lead acetate-induced hormonal imbalance in female albino rats

Methodology: The herbal formulation was prepared by mixing 5 g each of bay leaf, scent leaf, clove and cheese wood, and making an aqueous extract using the mixture. Thirty-five (35) female albino rats were used for this study. They were grouped into 5 groups of 7 rats each as follows: group 1(negative control), group 2(positive control), Group 3 (treated with 500 mg/kg of herbal formulation as low dose), Group 4 (treated with 1000 mg/kg of herbal formulation as high dose), Group 5 (treated with 81 mg/kg of Vitamin E as standard drug). Rats in groups 2-5 were induced with hormonal imbalance using 60 mg/kg. Groups 3-5 were treated as indicated for 28 days.

Results: The mean hormonal levels in the rats in the treated groups were significantly higher ($P<0.05$) than the levels in the positive control group. However, the mean hormonal levels in the treated groups, were significantly lower ($P<0.05$) than the mean levels in the negative control group, except for testosterone levels in the Vitamin E group. The LH/FSH ratios were significantly higher ($P<0.05$) in the negative control group, compared to the other groups. Testosterone/estradiol ratio was significantly higher ($P<0.05$) in the negative control group, compared to the other groups, except the vitamin E administered group. The progesterone/estradiol ratio was significantly raised ($P<0.05$) in the positive control group compared to the other groups. The liver enzymes were significantly lower ($P<0.05$) in the groups treated with the herbal formulation and vitamin E, compared with the levels in the positive and negative control groups

Conclusion: These finding indicate that the herbal formulation and vitamin E exhibited beneficial effects in restoring hormone levels and attenuating inflammation. Therefore, the herbal formulation can be considered to possess ameliorative potential in hormonal imbalance.

Keywords: Hormonal Imbalance; Herbal Formulation; Lead Acetate; Reproductive Hormones; Ameliorative Potential

* Corresponding author: Kemzi N. Elechi-Amadi

1. Introduction

Hormones are chemical substances synthesized and secreted by endocrine glands, which control and regulate the physiological activities of most cells and organs in the body. These hormones can generally be classified as peptide hormones (insulin, somatotropin etc.), amino acid derived hormones (melatonin, adrenaline etc.), and steroid hormones (estrogen, testosterone and other reproductive hormones) [1].

The hormones involved in female reproductive physiology include luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogen, progesterone, prolactin and testosterone. These hormones are synthesized in the body within the hypothalamic-pituitary-adrenal (HPA) axis. The hypothalamus releases gonadotropin-releasing hormone (GnRH) in a pulsatile pattern, which in turn stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary. These hormones, LH and FSH, then act directly on ovarian cells, leading to the release of ovarian androgens (testosterone) and estradiol [2]. Thus, the hormones, LH and FSH, are both synthesized within the gonadotroph; a single cell type in the anterior pituitary [3]. These hormones are important factors in the normal functioning of the menstrual cycle.

In humans, the menstrual cycle consists of two phases, namely, the follicular (proliferative) phase, and the luteal (secretory) phase [4]. The follicular phase starts with the onset of menstrual flow and ends with ovulation, while the luteal phase starts from ovulation to a pregnancy (if the woman gets pregnant) or the start of another menses (if pregnancy does not occur). During late follicular stage, there is a significant rise in LH levels known as mid-cycle surge, which is caused by the feedback of high levels of estradiol on the pituitary gland. This triggers the release of dominant follicles. During ovulation there is a release of the mature/dominant follicle into the fallopian tube where it can be fertilized, leading to pregnancy outcomes. Once this follicle is released, the ovary forms a corpus luteum (starting the luteal phase) which functions to secrete progesterone (that functions to maintain the endometrial walls to carry the pregnancy), as well as small amounts of estradiol. If pregnancy does not occur, the corpus luteum atrophies into corpus albicans and stops producing progesterone and estradiol, therefore their levels fall, leading to the shedding of the endometrial linings which is another menstruation [2]. Thus, the major hormone in the follicular phase is estradiol while progesterone is the major hormone in luteal phase.

In the follicular phase, low levels of estrogen and progesterone causes the hypothalamus to release gonadotropin-releasing hormone (GnRH), which in turn causes the anterior pituitary to release LH and FSH. Consequently, the action of LH causes the ovary to release testosterone while that of FSH causes the release of estrogens from the ovary. Furthermore, FSH is responsible for the maturation and release of the follicles. At the later end of the follicular phase, the level of estradiol rises while the levels of LH and FSH fall due to a negative feedback mechanism [2].

Hormonal imbalance is a condition in which the endocrine glands produce hormones either too little or in excess [5]. In other words, hormonal imbalance sets in when there is too little or too much of hormones in the body. Given their physiological importance, slight changes in the quantities of hormones can cause serious alterations that may lead to medical conditions that will require treatment [6]. Hormonal imbalance is becoming very common in the society [7], although some hormonal imbalances are temporary while others may be chronic [5]. It has been reported that hormonal imbalance is more common in women than in men [8].

There are concerns about the increasing number of endocrine disruptors globally. These endocrine disruptors can be found in items and substances used regularly, such as personal care products, drinking water, canned foods, pesticides, cooking utensils, drugs, cleaning products, etc. [9]. These products contain substances like phthalates, bisphenol A, dioxin etc, which are known endocrine disruptors. Endocrine disruption has a negative effect on the reproductive hormones. This study evaluated the effects of an herbal formulation on hormonal imbalance in female albino rats.

2. Materials and methods

2.1. Experimental Animals

Thirty-five (35) female albino rats, weighing 100-110 g were used for this study. They were purchased from the Animal House of the Pharmacology department, Rivers State University, Port Harcourt, Nigeria. They were put in compartmentalized cages, and exposed to 12-hour light/dark cycle. They were allowed unhindered access to feed and water.

2.2. Herbal Formulation

The herbal formulation was prepared using bay leaf (*Laurus nobilis*), scent leaf (*Ocimum gratissimum*), clove (*Syzygium aromaticum*) and cheese wood (*Alstonia boonei).* These herbs were bought from the local market from herb sellers. The formulation was prepared by blending 5 g each of the herbs and then extracting the aqueous extract of the mixture by dissolving 20 g of the mixture in 100 ml of water using Soxhlet extractor.

2.3. Standard Drug

The standard drug used for this study was Vitamin E (a product of Puritan's Pride Inc., USA) and purchased from a local pharmacy in Port Harcourt.

2.4. Acute Toxicity Study

Acute toxicity of the herbal formulation was determined using the Fixed Dose Procedure, with slight modification. Nine (9) rats were divided into 3 groups (A, B and C) of 3 rats each, and were given 500 mg/kg, 1000 mg/kg and 3000 mg/kg of the herbal formulation respectively. The rats were observed for signs of toxicity for 48 hours. After observation for 48 hours, there were no signs of toxicity, hence the formulation was considered safe up to a dose of 3000 mg/kg. For the study, 500 mg/kg and 1000 mg/kg were adopted and used as low and high doses respectively.

2.5. Experimental Design

Thirty-five (35) rats were divided into 5 groups of 7 rats each as follows:

- Group 1 (Negative control).
- Group 2 (Positive control).
- Group 3 (Treated with 500 mg/kg of herbal formulation as low dose).
- Group 4 (Treated with 1000 mg/kg of herbal formulation as high dose).
- Group 5 (Treated with 81 mg/kg of Vitamin E as standard drug).

Rats in groups 2-5 were induced with hormonal imbalance using 60 mg/kg of lead acetate according to the method of Nwogu *et al* [10]. Then, groups 3-5 were treated as indicated for 28 days. On the 29th day, after an overnight fast, the rats were sacrificed after being anaesthetized using chloroform. Blood samples were collected via cardiac puncture into plain bottles for the biochemical analyses. All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

2.6. Reagents and Biochemical Analyses

The hormones Testosterone, Estradiol, Progesterone, Prolactin, Follicle stimulating hormone, and Luteinizing hormone were determined quantitatively using a rat-specific Sandwich-enzyme linked immunosorbent assay (ELISA) method [11], as described by Elabscience biotechnology company limited (China). The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the Reitman-Frankel method [12], as modified by Randox laboratories limited (UK). Alkaline phosphatase (ALP) was determined using the Colorimetric endpoint method [13] as modified by Randox laboratories limited (UK). Semi-quantitative phytochemical analysis was done on the herbal formulation using classical methods [14].

2.7. Statistical Analysis

Data from this study was analyzed using Minitab version 16. Results are expressed as mean ± standard deviation. Comparison of means of parameters was done using one-way ANOVA and Tukey Post hoc with $P \leq 0.05$ considered statistically significant.

3. Results

Table 1 Semi-quantitative Phytochemical Analysis of the Herbal Formulation

Phytochemical	Observation
Flavonoids	++
Tannins	+++
Phenols	+
Alkaloids	++
Steroids	++
Terpenoids	+++
Saponins	++

++ Absolutely detected, + Moderately detected, - Not detected

The phytochemical analysis of the herbal formulation showed the presence of the phytochemicals shown in Table 1 above.

Table 2 Effects of the Herbal Formulation on Hormones

Groups	LH (ng/mL)	FSH (ng/mL)	PROL (ng/mL)	Estradiol (ng/mL)	Prog (ng/mL)	Testo (ng/mL)
Group 1 (Negative Control)	1.58±0.77^{a}	1.32±0.72^{a}	1.35±0.67^{a}	2.63±0.35^{a}	7.65±0.59^{a}	0.88±0.17^{a}
Group 2 (Positive Control)	0.68±0.33^{b}	0.67±0.31^{b}	0.27±0.05^{b}	1.13±0.37^{b}	5.42±0.59^{b}	0.17±0.08^{b}
Group 3 (High Dose Formulation)	0.78±0.22^{c}	1.23±0.60^{c}	1.43±0.63^{c}	2.09±0.41^{c}	7.17±0.13^{a}	0.32±0.18^{c}
Group 4 (Low Dose Formulation)	0.76±0.44^{c}	1.22±0.27^{c}	0.94±0.19^{d}	1.56±0.60^{d}	6.58±1.42^{c}	0.18±0.08^{b}
Group 5 (Vitamin E)	0.73±0.39^{c}	1.20±0.46^{c}	1.67±0.45^{e}	2.30±0.47^{c}	6.13±1.31^{c}	0.88±0.21^{a}
p-value	0.008	0.015	<0.001	<0.001	0.006	<0.001
F-value	4.431	3.813	7.868	10.668	4.782	31.105

Values with different superscripts are significantly different from each other (P<0.05). Keys: LH-luteinizing hormone, FSH-follicle stimulating hormone, PROL-prolactin, Prog-progesterone, Testo-testosterone.

The results of the effects of the herbal formulation on the reproductive hormones are as shown in Table 2 above. The mean hormonal levels in the rats in the treated groups were significantly higher (P<0.05) than the levels in the positive control group. However, the mean hormonal levels in the treated groups, were significantly lower (P<0.05) than the mean levels in the negative control group, except for testosterone levels in the Vitamin E group.

Table 3 Effects of the Herbal Formulation on Hormone Ratios

Groups	LH/FSH	Prog/Estradiol	Testosterone/Estradiol
Group 1 (Negative Control)	1.43±0.93[a]	2.94±0.41[a]	0.32±0.18[a]
Group 2 (Positive Control)	1.14±0.60[b]	5.09±1.23[b]	0.12±0.09[b]
Group 3 (High Dose Formulation)	0.53±0.19[c]	3.54±0.75[c]	0.09±0.02[c]
Group 4 (Low Dose Formulation)	0.97±0.60[d]	4.56±1.59[d]	0.10±0.03[b]
Group 5 (Vitamin E)	0.47±0.31[e]	2.73±0.67[a]	0.37±0.17[a]
p-value	0.042	0.001	0.040
F-value	2.916	6.306	2.963

Values with different superscripts are significantly different from each other (P<0.05). LH-leutinizing hormone, FSH-follicle stimulating hormone, Prog-progesterone

The results of the effects of the herbal formulation on hormone ratios is shown in Table 3 above. The LH/FSH ratios were significantly higher (P<0.05) in the negative control group, compared to the other groups. Testosterone/estradiol ratio was significantly higher (P<0.05) in the negative control group, compared to the other groups, except the vitamin E administered group. The progesterone/estradiol ratio was significantly raised (P<0.05) in the positive control group compared to the other groups.

Table 4 Effects of the Herbal Formulation on Liver Enzymes

Groups	AST	ALT	ALP
Group 1 (Negative Control)	145.4±5.93[a]	65.22±11.98[a]	181.05±12.53[a]
Group 2 (Positive Control)	185.20±4.33[b]	109.20±11.85[b]	208.11±13.11[b]
Group 3 (High Dose Formulation)	135.30±6.11[c]	82.11±12.25[c]	163.32±15.89[c]
Group 4 (Low Dose Formulation)	133.75±4.43[c]	79.85±8.85[c]	178.45±8.54[d]
Group 5 (Vitamin E)	134.70±2.44[c]	89.50±11.99[c]	168.45±6.12[c]
p-value	<0.001	0.009	<0.001
F-value	58.150	11.501	88.980

Values with different superscripts are significantly different (P<0.05). AST- Aspartate transaminase, ALT- Alanine transaminase, ALP- Alkaline phosphatase

The results of the effects of the herbal formulation on the liver enzymes are shown in Table 4 above. The liver enzymes were significantly lower (P<0.05) in the groups treated with the herbal formulation and vitamin E, compared with the levels in the positive and negative control groups.

4. Discussion

This study evaluated the effects of herbal formulation on reproductive hormones in female rats induced with hormonal imbalance. The results from this study show that the mean values of the hormone parameters in treated groups were significantly higher (P<0.05) compared to the values in the positive control groups, but significantly lower (P<0.05) compared to the negative control. These results indicate that exposure to lead reduced all of the reproductive hormones, and that the herbal formulation and vitamin E at the doses used, exhibited ameliorative effects in the treated rats. Lead exposure can affect the female reproductive system in a number of ways, including impairment of menstruation, reduction of fertility potential, delaying conception time and altering the production and circulation of hormones, affecting pregnancy and its outcome [15]. Lead exposure has been associated with hormonal imbalance, which in turn causes reproductive impairment. Lead accumulation also negatively affects many endocrine glands [16]. Several pathways and processes associated with the reproductive system are affected, including the hypothalamic–pituitary axis thereby blunting thyroid-stimulating hormone, growth hormone, and follicle-stimulating hormone

(FSH)/luteinizing hormone (LH) responses to thyrotropin-releasing hormone, growth hormone–releasing hormone and gonadotropin-releasing hormone stimulation [17].

The reduction in hormone ratios in this study could probably be due to the inhibitory effect of the herbal phytochemicals on the synthesis of GnRH by the hypothalamus [18]. Herbal substances are known to contain phytoestrogens [19], and these phytoestrogens exert inhibitory action on gonadotropin release [20]. The beta-ring structure found in flavonoids are identical to the substrates for 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD), which are involved in androgen biosynthesis. Therefore, flavonoids reduce the actions of these enzymes by binding to androgen receptors, thereby reducing the levels of testosterone and also androstenedione [21].

The decreased levels of estradiol ratios in the treated rats can also be due to the binding of flavonoids to the estrogen receptors, thereby reducing the activity of aromatase which is involved in the conversion of testosterone of estrogen [21]. Akbaribazm *et al*. [22], in their study showed that plant parts are rich in phytonutrients such as polyphenols, which can regulate female endocrine system and treat disorders such as hyperprolactinaemia, hypothalamic dysfunction and other conditions. The results from this study therefore show that the herbal formulation and vitamin E supplementation was able to correct the hormonal imbalance that was caused by the lead acetate. This is probably due to the presence of phytochemicals in the formulation. Phytochemicals and herbal formulations have been reported to have effects on sex and reproductive hormones [23, 24].

The liver enzymes in the rats treated with the herbal formulation and vitamin E were significantly lower than the levels in the positive control group. This is probably because the herbal formulation attenuated the inflammation that resulted from the lead acetate. Herbal formulations have been reported to have the potential to attenuate inflammation [25].

5. Conclusion

This study has shown that the herbal formulation used was able to ameliorate hormonal imbalance in albino rats. There were significant decreases in the levels of liver enzymes in the treated rats, showing that the herbal formulation attenuated the inflammation in the treated rats. This is probably due to the presence of phytochemicals in the herbal formulation.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

All the animal experiments were conducted according to the ethical norms approved by the Institutional Ethical Committee.

Authors' Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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