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Effects of Some Herbal Cosmetics on Sex Hormones of Rabbits

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ABSTRACT

Heavy metals have been observed as competitor for receptor sites in hormones thereby resulting in infertility problems. Herbal cosmetics have been observed to contain heavy metals which interfere with the receptor sites of hormones. Three herbal cosmetics sold in Port-Harcourt were evaluated for heavy metals and rabbits exposed to them for 30 -90days. The aim is to determine the effect of heavy metals on the sex hormones of the rabbits. Three herbal oil namely All things natural (Emi herbal oil), Kakiva (Kakiva herbal oil) and Amal (botanical herbal oil) as well as forty –eight rabbits were purchase from a market in and animal house in Port Harcourt respectively. The rabbits were exposed to the oil for 30, 60 and 90 days and scarified for blood samples. The sex hormones of the rabbits evaluated using standard methods. Data were analyzed using statistical package for social sciences (SPSS) to compare means. There were no significant differences in the sex hormones in 30days and 60days. However, there was a significantly lowered progesterone and estrogen after exposure 90days. It is therefore pertinent to conclude that herbal cosmetics contain heavy metals which may alter the sex hormones pattern which leads to luteal phase dysfunction.

1. INTRODUCTION

Cosmetics have been used as ornamental substances to improve the outlook either facial or body depending on the manufacturer. They can be chemically or naturally formulated depending on the raw materials used in its production. Herbal cosmetics are formulated from herbal substances which make them natural. These herbal products have gained popularity in recent times due to beliefs that they are harmless because of the natural ingredients used in their manufacturing. However, this has not been the case because there are reported cases of contamination either from the process or the environment. Thompson and colleagues (2022) in their study reported cadmium, arsenic, lead and mercury as components of herbal cosmetics. This was attributed to environmental contamination or process contamination.

Metals have been observed to induce changes in reproductive hormones by binding to the receptor site (Pollack *et al*., 2011).Earlier study (Gallagher et al.,2010) observed that metals alter hormone levels of both premenopausal and postmenopausal women. Choe and co-workers (2003) in an

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earlier study observed that lead and mercury exact estrogenic changes in experimental animals. Also, Stocia and colleagues (2000) in their study reported that cadmium obstruct the hormone binding domain of the estrogen receptor –α which ultimately affect transcription process. This position was affirmed by Zhang and colleagues (2008) in their study that lead and mercury can initiate estrogen receptor to interact with amino acids at the receptor binding site. Evidence from a recent study in Port-Harcourt on herbal cosmetics revealed that they contain heavy metals (Thompson *et al*., 2022). However, the effect of these herbal cosmetics on the reproductive hormone has not being fully exploited. Therefore, this study aims to assess the effect of some herbal cosmetics (*All things natural* (Emi herbal oil), *Kakiva* (Kakiva herbal oil) and *Amal* (botanical herbal oil) sold in Port – Harcourt metropolis in rabbits exposed to these herbal cosmetics.

2. MATERIALS AND METHODS

2.1.Experimental animals

A total of forty (68), Two-month-old, New Zealand white rabbits (Oryctolagus cuniculus) that weighed between 1.2 - 1.5kg were used for this study. Four (4) rabbits as baseline control, while the remaining sixty-four (64) rabbits were divided into three (3) groups (A, B, and C) of twelve (12) rabbits each with matched control.

The rabbits were kept in a spacious and well-ventilated cage at room temperature, under natural circadian rhythm, and allowed to acclimatize for fourteen (14) days. They were housed in standard cages and allowed access to feed and water *ad libitum* in the animal house. All the animals received humane treatment according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institute of Health.

2.2.Procurement and Determination of Toxicants of Herbal Cosmetics

Three (3) types of commonly used herbal hair oils were purchased from a Supermarket in Port Harcourt and labeled as product A, B and C, respectively.

Sample preparation for the determination of lead, cadmium, arsenic, copper and zinc adopted the method by Chauhan *et al.* (2010) . The oils were digested and digested samples were tested for lead, cadmium, copper, and zinc using an atomic absorption spectrophotometer.

2.3. Dosage Calculation of Volume of Oil Used

Based on organisation for economic cooperation and development (OECD) guideline for volume selection (0.5ml/kg) of the herbal cosmetic was applied to 5cm by 5cm scrapped dermal Forsa of the rabbits in each group. Matched control for each of the groups was four, and they were given feed and tap water ad-libitum only. Blood samples were collected from the rabbits at intervals, 0 day, 30days, 60days and 90days.

2.4. Sample Collection and Storage

At days zero, thirty, sixty and ninety, respectively, four rabbits from each group were sacrificed under chloroform anaesthesia. The blood samples were collected and used for determination of sex hormones of the rabbits.

Five (5ml) of the blood was emptied into a plain container for the determination of reproductive hormone. The samples in the plain container were allowed to stand for 30 minutes to clot then the serum was separated using a bench centrifuge. The serum samples were then stored frozen at -20°C, until the time of determination of the parameters.

2.5. Biochemical analysis

All biochemical analysis was carried out using standard methods. Follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen and progesterone using enzyme linked immunosorbent assay (ELIZA) method. Ratio of FSH to LH was also calculated. In all analysis, manufacturer`s instructions were adhered to strictly.

2.6. Statistical analysis

Data analysis was done using statistical package for social sciences (SPSS), IBM Chicago version 25. Difference in means ± SD was done using analysis of variance (ANOVA) with confidence interval at 95% and level of significance at ≤0.05.

3. RESULTS

3.1. Results Presentation

The chemical analysis of the cosmetics shows that they contain cadmium, lead, arsenic, copper and zinc at varying proportions as presented in table 1. The percentage of prevalence of the heavy metals in these herbal cosmetics is presented in figure 1-3 below.

Table 1: Components of heavy metals presents in the various herbal cosmetics

Sample	Cadmium	Lead	Arsenic	Copper	Zinc
A (mg/kg)	2.370	13.390	21.090	1.190	5.11
B (mg/kg)	1.58	11.38	30.088	1.080	5.76
C(mg/kg)	2.260	18.060	45.660	1.700	5.910

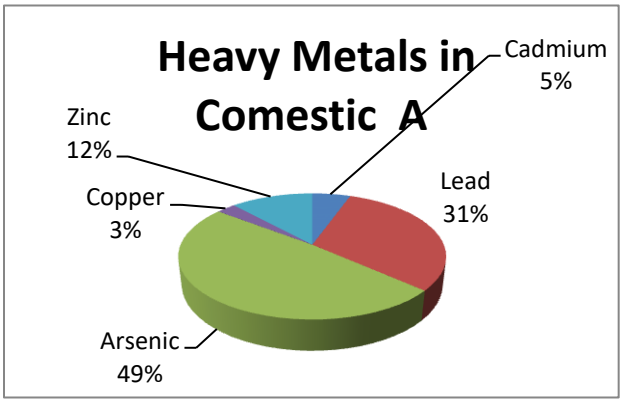

Figure 1: Percentage of heavy metals in comestic A

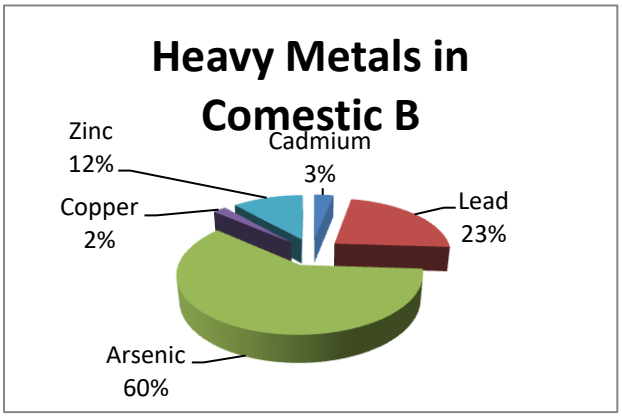

Figure 2: Percentage of heavy metals in comestic B

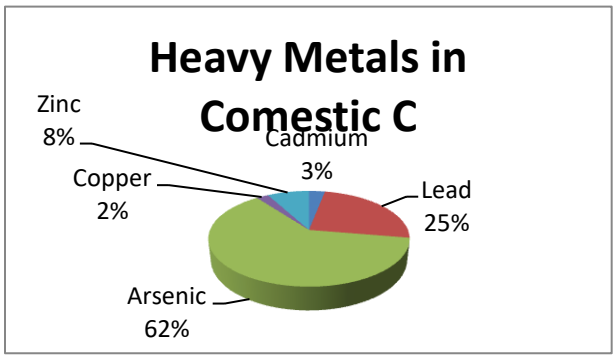

Figure 3: Percentage of heavy metals in comestic C

There was no significant difference observed (p>0.05) in FSH when 30days, 60days, 90days exposure of rabbits with sample A was compared with control rabbits. Likewise, there was no significant difference (p>0.05) in LH of 30days, 60days, 90days exposure of rabbits with sample A when compared with control rabbits. This is also same for estrogen but there was a significantly lower (p<0.05) progesterone in 90days exposure with sample A than 60days, 30days exposure and those without exposure. Likewise, there was a significantly lowered (p<0.05) progesterone in 60days exposure when compared with 30days exposure with sample A and control rabbits. Also, there was asignificantly lowered (<0.05) progesterone in 30days exposure with sample A than control rabbits without exposure. However, there was no significant difference (p>0.05) observed in the FSH/LH ratio in 30days, 60days and 90days exposure to sample A when compared with control rabbits as shown in table 2 below.

Table 2: Effect of Duration of Exposure on Some Reproductive Hormones in Rabbits administered Sample A

S/N	Parameters	Control	30 Days	60 Days	90 Days	F-value	P-value
1	FSH (IU/mL)	1.60±0.06	1.61±0.06	1.48±0.08	1.51±0.12	2.396	0.1192ǂ
2	LH (IU/mL)	1.14±0.06	1.18±0.14	1.14±0.10	1.24±0.10	0.8168	0.4711ǂ
3	Estrogen (	24.45±2.13	29.50±3.70	24.24±4.11	21.75±4.04	3.254	0.0598ǂ
4	Progesterone	2.27 ± 0.22^{a}	1.78 ± 0.13^{b}	0.60 ± 0.34^{c}	0.27 ± 0.03^{c}	80.12	<0.0001*
5	FSH/LH ratio	1.41±0.10	1.38±0.20	1.30±0.08	1.22±0.13	1.568	0.2484ǂ

Keys: FSH= follicle stimulating hormone, LH= luteinizing hormone. Mean ± SD of experimental groups with different superscripts are significantly different from each other at p<0.05.

There was no significant difference observed (p>0.05) in FSH when 30days, 60days, 90days exposure of rabbits to sample B was compared with control rabbits. Likewise, there was no significant difference (p>0.05) in LH of 30days, 60days, 90days exposure of rabbits to sample B when compared with control rabbits. But there was a significantly lower (p<0.05) estrogen in 90days when compared with 60days, 30days exposure to sample B and control rabbits without exposure, Also, there was a significantly lower (p<0.05) estrogen in 60days exposure than 30days exposure to sample B and control rabbits without exposure. Likewise, there was a significantly lower (p<0.05) estrogen in 30days exposure to sample B than control

rabbits with exposure to sample B. There was a significantly lower ($p<0.05$) progesterone in 90days exposure with sample B than 60days, 30days exposure and those without exposure. Likewise, there was a significantly lower ($p<0.05$) progesterone in 60days exposure to sample B when compared with 30days exposure with sample B and control rabbits. Also, there was a significantly lower (<0.05) progesterone in 30days exposure with sample B than control rabbits without exposure. However, there was no significant difference ($p>0.05$) observed in the FSH/LH ratio in 30days, 60days and 90days exposure to sample B when compared with control rabbits as shown in table 3 below.

Table 3: Effect of Duration of Exposure on Some Reproductive Hormones in Rabbits administered Sample B

S/N	Parameters	Control	30 Days	60 Days	90 Days	F-value	P-value
1	FSH (IU/mL)	1.60±0.06	1.52±0.10	1.56±0.05	1.48±0.09	1.563	0.2496ǂ
2	LH (IU/mL)	1.14±0.06	1.22±0.08	1.20±0.12	1.16±0.07	0.8226	0.5062ǂ
3	Estrogen	24.45±2.13	26.08 ± 3.39^{b}	23.25 ± 1.71^{a}	20.50 ± 2.38^{a}	3.823	0.0392*
4	Progesterone	2.27 ± 0.22^{a}	1.79 ± 0.13^{b}	0.84 ± 0.11^{c}	0.30 ± 0.02^{a}	164.1	<0.0001*
5	FSH/LH ratio	1.41±0.10	1.25±0.06	1.31±0.14	1.28±0.12	1.661	0.2278ǂ

Keys: FSH= follicle stimulating hormone, LH= luteinizing hormone. Mean ± SD of experimental groups with different superscripts are significantly different from each other at p<0.05

There was no significant difference observed ($p>0.05$) in FSH when 30days, 60days, 90days exposure of rabbits with sample C was compared with control rabbits. Likewise, there was no significant difference ($p>0.05$) in LH of 30days, 60days, 90days exposure of rabbits with sample A when compared with control rabbits. This is also same for estrogen but there was a significantly lower ($p<0.05$) progesterone in 90days exposure with sample C than 60days, 30days exposure and those without exposure. Likewise, there was a significantly lowered ($p<0.05$) progesterone in 60days exposure when compared with 30days exposure with sample C and control rabbits. Also, there was significantly lowered (<0.05) progesterone in 30days exposure with sample C than control rabbits without exposure. However, there was no significant difference ($p>0.05$) observed in the FSH/LH ratio in 30days, 60days and 90days exposure to sample C when compared with control rabbits as shown in table 4 below.

3.1 Discussion

Metals are chemical compound which are either find in the environment or products used by human. Heavy metals are found to interfere with hormones at the receptor binding site or indirect mechanism. Herbal cosmetics which are used as ornamental substances were evaluated for heavy metals and possibly assess the effect of these heavy metals on sex hormones of rabbits. Analysis of the herbal cosmetics revealed that they contain heavy metals such as cadmium, lead,

arsenic, copper and zinc in varying concentration. The rabbits were exposed to the herbal cosmetics for various days ranging from 30 -90days. There was no effect of heavy metals on sex hormones except progesterone and estrogen. Progesterone is lowered with increased exposure to the herbal cosmetics. This finding is in tandem with Pollack et al (2011) which observed that lead was positively associated with progesterone and positively but not statistically significantly associated with estradiol are consistent with a recent study of lead and inhibin B, a marker of follicular development in their study. However, Pollack *et al*., (2011) in same study also reported that FSH level decreases with increasing level of cadmium. The reduced progesterone in rabbits as a result of exposure to heavy metals as potential avenue for spontaneous abortion due to insufficient progesterone to maintain the integrity of the pregnancy which can be referred to as luteal phase deficiency (Czyzyk et al.,2017).

Table 4: Effect of Duration of Exposure on Some Reproductive Hormones in Rabbits administered Sample C

S/N	Parameters	Control	30 Days	60 Days	90 Days	F-value	P-value
1	FSH (IU/mL)	1.60±0.06	1.58±0.06	1.52±0.04	1.54±0.09	1.275	0.3272ǂ
2	LH (IU/mL)	1.14±0.06	1.22±0.15	1.24±0.11	1.20±0.14	0.5919	0.6321ǂ
3	Estrogen	24.45±2.13	26.35±2.63	25.60±2.81	25.50±3.11	0.1802	0.9078ǂ
4	Progesterone	2.27 ± 0.22^{a}	0.59 ± 0.09^{b}	0.88 ± 0.12^{c}	0.32 ± 0.04^{d}	163.2	<0.0001*
5	FSH/LH ratio	1.41±0.10	1.31±0.19	1.23±0.10	1.29±0.08	1.415	0.2865ǂ

Keys: FSH= follicle stimulating hormone, LH= luteinizing hormone. Mean ± SD of experimental groups with different superscripts are significantly different from each other at p<0.05.

Conclusively, it is therefore pertinent to state that these herbal cosmetics contain heavy metals such as cadmium and lead in quantity that has a negative effect on progesterone and estrogen which can results in luteal phase dysfunction leading to recurrent abortion.

Conflict of interest: None

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