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PREVALENCE OF HEAVY METALS IN HERBAL COSMETICS AND ASSOCIATED BIOCHEMICAL ALTERATIONS IN RABBITS

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ABSTRACT

The aim of this study was to evaluate the prevalence of heavy metals in herbal cosmetics and associated biochemical alterations in rabbits. Three herbal oils named sample A, B, C, and a total of 48 rabbits grouped into four were used for the study**.** Rabbits from each group were treated for thirty, sixty and ninety days respectively with 0.5ml/kg of Samples A, B, C and distilled water as control. At days thirty, sixty and ninety, respectively, four rabbits from each group were sacrificed under chloroform anaesthesia. Blood samples were collected to determine biochemical parameters at intervals of, 30days, 60days and 90days respectively. GraphPad Prism v.7.0 was used for statistical analysis and $p<0.05$ was considered statistically significant. The results showed sample A had 2.370mg/kg, 18.060mg/kg and 45.660mg/kg of cadmium, lead and arsenic respectively. while sample B had 1.580mg/kg, 11.38mg/kg and 21.090mg/kg with sample C having 2.260mg/kg, 13.390mg/kg and 30.088mg/kg respectively. Concentrations of lead, cadmium and arsenic exceeded the maximum allowable concentrations, whereas zinc and copper are found below the acceptable limit set by WHO. The results of the kidney function also showed that Potassium level was significantly higher ($p<0.05$*)* from day 30 for group A compare to control, group B and group C. This could be attributed to group A having a higher level of cadmium and arsenic compare to the other groups. The urea and creatinine result for group A was significantly higher by day 30 *($p<.05$)* compare to control with group B and group C. Whereas calcium became significantly lower at day 60. With chloride significantly higher *($p<.05$)* at day 60. The liver function results also showed, Serum alanine transaminase (ALT) significantly higher *($p<.05$)* at day 30, total protein and albumin became significantly lower at day 60. The results indicated that there are chances of cancer resulting from the use of these cosmetic

products due to the build-up of the heavy metals contained in the products. The result showed that the three different cosmetic brands had cadmium and arsenic levels above the acceptable limit for cosmetic products. The continued use of these products will release them slowly into the body of recipients and which will invariably damage the kidney and liver.

KEYWORDS: Heavy metals, Herbal cosmetics, Biochemical alterations and Rabbits

INTRODUCTION

Cosmetics are products used to improve or change the physical looks of the human face and skin by applying it on the face, hair, and skin. Cosmetics are generally a mixture of different synthethic or natural compounds, produced from either synthetic or plant sources. There are two major types of cosmetic products, which are classified based on the source of the raw material used for production. They include synthetic and herbal cosmetics. When chemical compounds are used in the creation of the beauty product, it is known as a synthetic cosmetic. While herbal cosmetics involves the use of parts or whole of plant material in the manufacturing of the beauty product. There is an all-encompassing variety of herbal cosmetics that are manufactured and commonly used for everyday purposes. These herbal cosmetics are produced from a plant source [1].

The upsurge in the use of herbal cosmetics is because they are believed to be safer for health than the synthetic ones [2]. However, scientific research has discovered that not all herbal products are safe, as per general belief, some are quite toxic, having several adverse effects, polluted with heavy metals and contain phytotoxins or biotoxins [3]. Plant-based cosmetics are generally regarded as safe because they are produced from natural plants. Notwithstanding, there are cases of severe side effects after their use and the toxicity has been traced to contaminants and adulteration from poor manufacturing processes. This resulted from using plants contaminated with heavy metals from the environment or soil. However, certain of the plants used in herbal cosmetics have inherent toxicity in them known as phytotoxins. Plants can neither evade herbivores by flight, nor do they have an immune system to fight invading microorganisms [4]. These Phytotoxins may also be toxic to humans such as alkaloids, terpenes, and phenolic [5].

The use of herbal substances has, unfortunately, resulted in several fatalities. These fatalities are related to problems associated with the failure of proper handling and manufacturing of the products. Lack of standardization, contamination with heavy metals, inadvertent and deliberate substitution of other herbs, and adulteration with synthetic chemicals also

contribute to the adverse health effect. There is a danger to the public without a proper evaluation of the possible harmful effect which could arise from the use of herbal cosmetics. Heavy metal contamination in herbal cosmetics is usually because of the absorption of these metals by plants, even if in minute quantities that lead to bioaccumulation of them in plant parts when such plants are used in the manufacturing of cosmetic products without removal. It is challenging to avoid heavy metal contamination in plants. However, it is possible to remove such contaminant from the plant part before it is used in the manufacturing of cosmetic products. The risk associated with heavy metals present in cosmetics needs to be studied to raise awareness of the adverse effect on humans. So that although expensive, manufacturers must assure the safety of their products by removing them [6].

MATERIALS AND METHOD

PROCUREMENT OF HERBAL COSMETICS

Three (3) types of commonly used herbal hair oils namely Allthingsnatural by Emi Herbal Oil, Amal Botanical Shea Oil and Kakiva herbal oil were purchased from a Supermarket in Port Harcourt and labelled product A (Allthingsnatural by Emi herbal oil), B (Amal Botanical Shea Oil and C (Kakiva Herbal Oil) respectively.

EXPERIMENTAL ANIMALS: A total of fifty-two (52), two-month-old, New Zealand white rabbits (*Oryctolagus cuniculus*) that weighed between 1.2 - 1.5 kg were used for this study. The rabbits were purchased from a breeder in Port Harcourt, Rivers State. Four (4) rabbits were used as baseline control, while the remaining fourty eight (48) rabbits were divided into three (3) groups (A, B and C) of twelve (12) rabbits each with matched control.

PLACE AND DURATION OF STUDY: This study was carried out at Animal House, Applied and Environmental Biology Department, Rivers State University, Port Harcourt, Rivers State, Nigeria, between April, 2020 and November, 2020.

STUDY DESIGN: Total of 48 rabbits grouped into four were used for the study**.** Rabbits from each group were treated for thirty, sixty and ninety days respectively with 0.5ml/kg of Samples A, B, C and distilled water as control. Blood sample of rabbits in all the groups was collected at intervals, 30, 60 and 90 days respectively.

SAMPLE COLLECTION

Prior to blood sample collection, the rabbits were anesthetized using diethyl ether then, surgical blade was used to cut the jugular vein of the rabbits, 5 ml of blood samples was collected and dispensed into plain bottle. The samples were allowed to retract, then spun for 15 minutes to separate sera for determination of biochemical parameters.

BIOCHEMICAL ANALYSIS:

The heavy metals were measured using an atomic absorption spectrophotometer. Renal function parameters and liver function parameters were also measured using Randox Laboratories United Kingdom reagent kits.

STATISTICAL ANALYSIS

Data are expressed as mean ± SD. Statistical differences between groups were computed using Graph pad prism 7.0 versions. Results were analyzed using one-way analysis of variance (ANOVA) and significance between groups was taken at $p < 0.05$.

RESULTS

The concentration of heavy metals in the oil samples are presented in Table 1

The heavy metal concentration of serum of rabbits exposed for 90 days to Sample A, B and C are presented in Table 2.

Table 3,4 and 5 shows the results of the Na^+, K^+, CL^-, KIM-1, urea and creatinine concentrations of rabbits exposed dermally to sample A, sample B, and sample C for thirty (30) days, sixty (60) days and ninety (90) days respectively.

Table 6,7 and 8 shows the results of the concentrations of liver function parameters in rabbits exposed dermally to sample A, sample B, and sample C for thirty (30) days, sixty (60) days and ninety (90) days respectively.

Table 1: Concentration of Heavy Metals in the Oil Samples.

S/N	OIL SAMPLES	Cadmiun (mg/kg)	Lead (mg/kg)	Arsenic (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)
1	A	1.580	11.38	30.088	1.080	5.760
2	B	2.260	18.060	45.660	1.700	5.910
3	C	2.370	13.390	21.090	1.190	5.110
USEPA LIMIT		0.5	0.5	2.5	0.5	18
WHO LIMIT		0.3	10	10	1	20

Key: Sample A= Allthingsnatural by Emi Herbal Oil, Sample B = Kakiva Herbal Oil, Sample C= Amal Botanical Herbal Oil

Table 2: Mean ± SD of Renal Function Parameters during 30 days Exposure.

Samples	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	KIM-1 (pg/mL)	Urea (mmol/L)	Creatinine (µmol/L)
Control	136.50 ±0.41^{a}	4.75±0.13^{a}	109.40±0.91	7.25 ±0.26^{a}	9.50 ±0.30^{a}	71.75±18.28^{a}
A	140.10 ±0.87^{bd}	5.32 ±0.36^{ac}	109.50±1.46	13.23 ±0.17^{b}	13.23 ±0.75^{bc}	97.00±0.82^{bd}
B	1.44.10 ±2.62^{c}	5.78±0.28^{b}	110.50±0.22	8.20 ±0.18^{c}	9.75 ±0.51^{d}	95.50±11.03^{e}
C	139.00 ±1.19^{ad}	5.41 ±0.35^{bc}	109.60±1.21	15.05 ±0.17^{d}	11.18 ±0.25^{c}	108.30±9.95^{e}
F_value	17.06	8.391	1.041	1419	45.45	6.781
P value	0.0001	0.0028	0.4098	<0.0001	<0.0001	0.0063

Keys: Na^+= sodium ion, K^+ = potassium ion, Cl^- =chloride ion, Ca^{2+} =ionized calcium, KIM-1= kidney injury molecule-1 Mean ± SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Table 3: Mean ± SD of Renal Function Parameters during 60 days Exposure.

Samples	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	KIM-1 (pg/mL)	Urea (mmol/L)	Creatinine (µmol/L)
Control	137.60 ±1.06^{a}	4.59 ±0.22^{a}	109.00 ±1.33^{a}	7.03 ±0.75^{a}	9.40 ±0.29^{a}	68.25 ±12.50
A	138.80 ±3.46^{a}	10.90 ±0.55^{b}	117.00 ±0.83^{bdf}	17.90 ±0.64^{b}	19.18 ±0.77^{b}	102.50 ±8.10
B	145.90 ±5.19^{b}	11.38 ±0.24^{b}	120.10 ±3.60^{cd}	10.20 ±1.18^{c}	11.65 ±3.20^{ac}	101.80 ±11.18
C	139.50 ±1.13^{a}	10.62 ±0.57^{b}	113.90 ±3.38^{af}	19.55 ±1.04^{b}	12.33 ±1.27^{c}	119.50 ±9.33
F-value	5.345	221.4	13.26	17.06	8.391	1.041
P value	0.0143	<0.0001	0.0004	0.0001	0.002	0.4098

Keys: Na^+= sodium ion, K^+ = potassium ion, Cl^- =chloride ion, Ca^{2+} =ionized calcium, KIM-1= kidney injury molecule-1. Mean ± SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Table 4: Mean ± SD of Renal Function Parameters during 90 days Exposure.

Samples	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	KIM-1 (pg/mL)	Urea (mmol/L)	Creatinine (µmol/L)
Control	140.60 ±3.13	4.77 ±0.35^{a}	109.70 ±0.76^{a}	7.53 ±0.70^{a}	9.25 ±0.61^{a}	76.50 ±3.42^{a}
A	144.90 ±2.49	14.03 ±0.51^{b}	119.20 ±4.76^{b}	21.03 ±3.06^{bd}	22.10 ±1.54^{b}	120.50 ±10.21^{b}
B	148.60 ±3.10	14.96 ±1.73^{b}	124.90 ±3.88^{cb}	13.73 ±0.79^{ab}	12.53 ±2.93^{ac}	124.50 ±15.93^{b}

C	145.00 ±4.96	12.25 ±0.95^{bc}	113.90 ±3.63^{a}	27.55 ±0.87^{cd}	13.55 ±0.87^{c}	130.50 ±3.00^{bc}
F-value	2.989	80.26	13.49	13.16	39.70	25.73
P value	0.0734	<0.0001	0.0004	<0.0004	<0.0001	<0.0001

Keys: Na^{+}= sodium ion, K^{+} = potassium ion, Cl^{-} =chloride ion, Ca^{2+} =ionized calcium, KIM-1= kidney injury molecule-1. Mean ± SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Table 5: Mean ± SD of Liver Function Parameters during 30 days Exposure.

Samples	ALT (I.U/L)	AST (IU/L)	ALP (IU/L)	Total Protein (g/L)	Albumin (g/L)	Glucose (mmol/L)
Control	74.50 ±0.66	71.75 ±12.61	84.50 ±11.03	62.75 ±2.75^{a}	30.75 ±1.71	8.65 ±0.56
A	105.80 ±24.72	73.25 ±5.74	83.00 ±7.53	59.25 ±3.30^{a}	31.75 ±2.75	8.30 ±0.41
B	88.00 ±12.11	65.75 ±10.28	94.00 ±21.23	49.50 ±3.11^{b}	29.00 ±2.16	8.20 ±0.18
C	87.00 ±13.54	78.50 ±7.59	87.75 ±17.23	50.00 ±4.16^{b}	30.50 ±2.08	7.88 ±0.52
F-value	2.683	1.24	0.4114	15.57	1.06	2.091
P value	0.0938	0.3384	0.7478	0.0002	0.4023	0.1549

Keys: AST= aspartate aminotransferase, ALT= alanine aminotransferase, ALP= alkaline phosphatase. Mean ± SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Table 6: Mean ± SD of Liver Function Parameters during 60 days Exposure.

Samples	ALT (I.U/L)	AST (I.U/L)	ALP (I.U/L)	Total Protein (g/L)	Albumin (g/L)
Control	79.50 ±7.14^{a}	75.25 ±6.19	76.00 ±8.21	59.75±3.69^{a}	25.75±3.86^{a}
A	123.80±24.5^{bc}	90.25±11.44	79.00 ±7.35	45.50 ±3.42^{b}	28.75 ±3.50^{a}
B	110.00±17.87^{a}	75.75 ±4.65	84.00 ±8.76	45.50 ±3.11^{b}	20.50 ±1.29^{ab}
C	130.80±11.96^{c}	82.25 ±6.85	86.25±10.34	47.00^{b}±2.94	22.00 ±2.58^{ac}
F-value	7.391	3.314	1.14	17.54	6.254
P value	0.0046	0.571	0.3724	<0.0001	0.0084

Keys: AST= aspartate aminotransferase, ALT= alanine aminotransferase, ALP= alkaline phosphatase. Mean ± SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Table 7: Mean ± SD of Liver Function Parameters during 90 days Exposure.

Samples	ALT (I.U/L)	AST (I.U/L)	ALP (I.U/L)	Total Protein (g/L)	Albumin (g/L)	
Control	82.25 ±5.85^{a}	78.25±11.53^{a}	86.00 ±9.76	59.25±2.22^{a}	25.25±2.06^{a}	
A	151.80±9.81^{b}	90.25±7.54^{b}	93.50±10.08	38.75±5.38^{b}	20.25±2.63^{ab}	
B	114.30±25.18^{a}	79.25±9.61^{a}	84.75±13.28	37.75±3.20^{b}	15.25 ±2.50^{b}	
C	142.00±20.12^{b}	84.25±6.55^{b}	88.25 ±8.42	36.00±3.56^{b}	16.00 ±3.56^{b}	

F-value	13.34	1.489	0.5381	33.71	11.27	
P value	0.0004	0.2673	0.6651	<0.0001	0.0008	

Keys: AST= aspartate aminotransferase, ALT= alanine aminotransferase, ALP= alkaline phosphatase. Mean ± SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

DISCUSSION

The effect of the three herbal hair oils (Allthingsnatural by Emi herbal oil, kakiva herbal oil and Amal botanical herbal oil) on the biochemical parameters of female rabbits were studied to ascertain if they were safe or toxic. Herbal products are generally assumed to be safe. Notwithstanding, there are cases of severe side effects after their usage and the toxicity has been traced to contaminants and adulteration from poor manufacturing processes and this resulted from using plants contaminated with heavy metals from the environment or soil [7].

The results of heavy metals analysis of the all threes oil samples are above the WHO acceptable limits of cadmium, lead, arsenic, and copper while zinc is within the acceptable limit [7]. This result agrees with [8] who stated that some cosmetics used in Nigeria seem to be laced with heavy metals beyond the acceptable limit established by Who Health Organization.

The effect of heavy metals on biochemical parameters such as kidney function and liver function is trace to the mechanisms of toxicity of heavy metals which involves the binding to oxygen, nitrogen, and sulphur ligands, that affect numerous enzymes and proteins in human body.

The result of renal function showed that Potassium level was significantly higher ($p< 0.05$) from day 30 for group A compare to control, group B and group C. It could be inferred that group A have a higher level of cadmium and arsenic compare to the other groups and this cause damage to the distal convoluted tubule and collecting duct there by increasing secretion of potassium. The urea and creatinine result for group A was significantly higher by day 30 ($p<0.05$) compare to control with group B and group C and chloride was significantly higher ($p<0.05$) at day 60. This result is consistent with previous studies by [9] that examined effects of herbal cosmetics renal parameters in rabbits.

The kidney is a major organ affected by cadmium exposure and chronic exposure is mostly by ingestion and dermal route [10]. Frequent exposure to cadmium and other heavy metals contributes to its excess accumulation in kidney, which results in damage to the kidney organ. Furthermore, the impact of these heavy metals exposure could be responsible for the abnormal increase in the renal function parameters as observed in this study.

There was a significant increase ($p<0.05$) in ALT activity in group A by day 30 compare to control. Also, ALT was significantly increased ($p<0.05$) in group B and C by day 90 compare to control. The AST enzyme activity was not affected by the three herbal oils as observed. ALT is known as an indicator of early liver damage and is more specific to the liver indicating its damage compare to AST. This is in tandem with the report by [11] where they reported increase in liver enzymes of rabbits exposed to cosmetics containing heavy metals. The results also revealed that ALT levels at 90 days showed the greatest increase of enzyme activity in group A compare to group C, while group B had the least increase in enzyme activity. This observation is a proof that heavy metals are present in the cosmetics. However, significant decrease ($p<0.05$) in total protein and albumin level was observed at day 60. This is an indication that the hepatocytes were severely impacted by the heavy metals in cosmetics since liver organ is the main site of plasma proteins synthesis. This is consistent with [12] that reported accumulation of heavy metal, hepatocellular injury, elevation of liver enzymes and decrease in protein and albumin levels of subjects exposed to herbal cosmetics.

CONCLUSION

The findings of this study indicate that heavy metal concentrations in the three brands of cosmetics exceeded the permissible limits set for cosmetic product. Chronic use of these products may pose a risk to vital organs, particularly the liver kidneys, as evidenced by significant alterations in biochemical parameters.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The Animal Welfare Act of 1985 of the United State of America for research and Institutional Animal Care and Use Committee (IACUC) protocol were strictly adhered to. All experiments have been examined approved by the appropriate ethic committee.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interest exist.

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