



# **The Effect of Heavy Metal Contaminants in Some Herbal Cosmetics on Cardiovascular Risk Indices of Rabbits**

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## **Authors' contributions**

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

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## **ABSTRACT**

**Background:** Cosmetics are ornamental products used in the improvement of the physical outlook of either the face or skin. These may be synthetic or natural depending on the source of production. Herbal cosmetics have gained prominence in our daily lives due to the belief that they are natural. Evidence has shown that they contain heavy metals which may alter biochemical parameters in the cause of usage. This study aims to evaluate the effects of heavy metals on the serum lipid profile and cardiovascular risk indices of rabbits.

**Materials and Methods:** 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 purchased from a market in an animal house in Port Harcourt respectively. The rabbits were exposed to the oil for 30, 60, and 90 days and sacrificed for blood samples. The lipid profile and cardiovascular risk indices

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were evaluated using standard methods. Data were analyzed using a statistical package for social sciences (SPSS) to compare means.

**Results:** There were no significant differences in the lipid profile and cardiovascular risk indices in 30 days. However, there was significantly higher total cholesterol, triglycerides, and low-density lipoprotein but lower high-density lipoprotein cholesterol after exposure in 60 days and 90 days.

**Conclusion:** It is, therefore, pertinent to conclude that herbal cosmetics contain heavy metals which may alter the lipid profile and expose users to cardiovascular risk.

*Keywords: Ornamental; natural; phytochemicals; cadmium; lipoprotein; Port Harcourt.*

## 1. INTRODUCTION

Cosmetics are generally a mixture of different chemical or natural compounds, produced from either synthetic or plant sources to improve the physical looks of the human face, skin, and hair [1]. There is a high demand for various types of herbal cosmetics. The highest need for them is in Europe, followed by Asia. The upsurge in the use of herbal cosmetics around the world is because they are safer for health than synthetic ones [2,3]. Herbal cosmetics are the new trend in the field of fashion and beauty and there is an increase in the use of herbal products due to the belief that it is safer than synthetic ones. Scientific research has now discovered that not all herbal products are safe, as per general belief, and that some are quite toxic, having several adverse effects. Various scientific studies show that these cosmetics can be polluted with heavy metals and contain phytotoxins or biotoxins [4]. As a result, the quality, safety, and efficacy of herbal products have become an essential concern for both consumers and health authorities throughout the world.

There are cases of reports involving severe adverse events after using some natural cosmetics. In a lot of these cases, the toxicity is due to contaminants and pollution. Herbal cosmetics can carry a risk of adverse effects if not adequately tested. Testing for toxicity in herbal products, therefore, is of utmost importance in herbal research. Risk assessment is a process involving the identification and quantification of the risk arising from the use of a specific product, in this instance herbal cosmetics. The risk is assessed through four different methods, which include, hazard identification, dose-response assessment, exposure assessment, and risk characterization [5].

“Currently, available evidence has demonstrated that systemic inflammation and immunological responses— derived from heavy metals coming

into contact with the epithelial lining and entering the systemic circulation—initiate a cascade of events leading to the acute and chronic effects of heavy metals on cardiovascular diseases (CVD)” [6]. “Based on our current knowledge, plausible pathophysiological mechanisms linking exposure to heavy metals and cardiovascular diseases include: increased systemic inflammation, which produces cardiovascular stress, activated platelets in the bloodstream, increasing the risk of acute thrombosis, as in myocardia infarctions and ischemic stroke, alterations of the autonomic nervous system and the autorhythmic cells in the sinoatrial node, which leads to decreased heart rate variability, and direct changes in the vascular cell types, including macrophages, endothelial and smooth muscle cells, thereby increasing CVD risk” [7].

Lead directly and indirectly inhibits glutathione synthesis and function, and depresses superoxide dismutase activity, a zinc metalloprotein in humans [8,9], and excess free radicals are atherogenic [10]. “Proatherosclerotic changes from lead exposure have also been associated with the inactivation of paraoxonase activity, which decreases the antioxidant effects of high-density lipoprotein” [11]. “Lead replaces calcium in various intracellular signaling reactions, including inhibiting the effect of calmodulin in the synthesis of NO, possibly explaining lead-induced hypertension” [12]. “Furthermore, lead exposure results in oxidative stress by upregulation of superoxide-generating enzymes, nicotinamide adenine dinucleotide phosphate [NAD(P)H], and hydroxyl radical production” [13]. “In rats, exposure to low lead levels compared with controls increased activation of nuclear factor- $\kappa$ B” [14]. “In addition to an increased risk of cardiovascular mortality, long-term exposure to low levels of lead has been associated with persistent hypertension in animal and human studies” [15]. In a prospective population study [16], “participants with higher blood lead concentration at baseline predicted impaired systolic left ventricular function a

decade later". A systematic review published in 2013 reported increasing evidence that "cadmium was significantly associated with CVD, and individually with coronary disease and peripheral arterial disease (PAD): CVD, 1.36 (95% CI, 1.11–1.66); CAD, 1.30 (95% CI, 1.12–1.52); and PAD, 1.49 (95% CI, 1.15–1.92), after controlling for smoking history" [17]. The paucity of safety data on some commonly used herbal cosmetics and the attendant implication of heavy metal contaminants underscores the imperativeness of this study. Therefore, this study aims to evaluate the effects of heavy metal contaminants on the lipid profile and cardiovascular risk of rabbits exposed to three herbal formulations.

## 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 a 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 products A, B, and C, respectively.

The oils were screened for phytotoxins: Alkaloids, terpenoids, saponins, and phenols using standard methods.

Sample preparation for the determination of lead, cadmium, arsenic, copper, and zinc adopted the method by Chauhan et al. [18]. The oils were digested and digested samples were tested for

mercury, lead, copper, and zinc using an atomic absorption spectrophotometer.

### 2.3 Dosage Calculation of Volume of Oil Used

Based on the organization 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, of 0 days, 30 days, 60 days, and 90 days.

### 2.4 Sample Collection and Storage

At days zero, thirty, sixty, and ninety, respectively, four rabbits from each group were sacrificed under chloroform anesthesia. The blood samples were collected and used for the determination of the heavy metal and lipid profiles of the rabbits.

Five (5ml) of the blood was emptied into a plain container for the determination of lipid parameters. 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. Serum Total Cholesterol and Triglycerides were analyzed using the enzymatic CHOD-PAP method of Trinder [19] as modified by Richmond [20] and HDL-Cholesterol was analyzed by the method of Burstein et al. [21]. LDL-Cholesterol and VLDL-Cholesterol were calculated using Friedewald's [22] equation. Cardiac Risk Ratio (CRR1) and (CRR11), atherogenic coefficient (Ac) were calculated [23] and atherogenic index of plasma (AIP) was calculated [24]. All test kits used were commercially available and products of Randox Laboratories UK. In all analyses, the manufacturer's instructions were adhered to strictly.

### 2.6 Statistical Analysis

Data analysis was done using the statistical package for social sciences (SPSS), IBM

Chicago version 21. The difference in means  $\pm$  SD was done using analysis of variance (ANOVA) with a confidence interval of 95% and a level of significance of  $\leq 0.05$ .

### 3. RESULTS

The results of the TC, HDL-C, LDL-C, VLDL-C, and TG concentrations of rabbits exposed dermally to samples A, B, and C for thirty (30) days are shown in Table 1.

After thirty (30) days of exposure of the rabbits to sample A, sample B, and sample C, the compared mean TC of the different groups showed no significant difference ( $p = 0.1206$ ) amongst the groups.

A similar outcome was observed for HDL ( $p = 0.9466$ ), LDL ( $p = 0.5363$ ), TG ( $p = 0.5765$ ), and VLDL ( $p = 0.6046$ ) respectively.

The results of the CRR-1, CRR-11, AIP, AAI, AC, and TG/HDL concentrations of rabbits exposed dermally to sample A, sample B, and sample C for thirty (30) days are depicted in Table 2.

After thirty (30) days of exposure of the rabbits to sample A, sample B, and sample C the mean CRR-1 of the different groups and control were compared. There were no significant differences amongst the various groups at  $p < 0.05$  [CRR-1 ( $p = 0.6449$ ), CRR-11 ( $p = 0.7364$ ), AIP ( $p = 0.2741$ ), AAI ( $p = 0.9815$ ), AC ( $p = 0.9815$ ) and TG/HDL ( $p = 0.7728$ )], respectively.

**Table 1. Mean  $\pm$  SD of lipoproteins during 30 days exposure**

S/N	Samples	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)	VLDL-C (mmol/L)
1	Control	2.45 $\pm$ 0.10	0.26 $\pm$ 0.02	1.65 $\pm$ 0.15	1.18 $\pm$ 0.13	0.54 $\pm$ 0.06
2	A	2.65 $\pm$ 0.14	0.27 $\pm$ 0.02	1.79 $\pm$ 0.13	1.33 $\pm$ 0.13	0.60 $\pm$ 0.06
3	B	2.49 $\pm$ 0.13	0.27 $\pm$ 0.01	1.66 $\pm$ 0.16	1.25 $\pm$ 0.17	0.57 $\pm$ 0.08
4	C	2.56 $\pm$ 0.06	0.27 $\pm$ 0.02	1.73 $\pm$ 0.14	1.23 $\pm$ 0.17	0.58 $\pm$ 0.08
5	F-value	2.381	0.12	0.7629	0.6881	0.6384
6	P-value	0.1206	0.9466	0.5363	0.5765	0.6046

Keys: TC= total cholesterol, HDL-C= high-density lipoprotein cholesterol, LDL-C= low-density lipoprotein cholesterol, VLDL-C=very-low-density lipoprotein cholesterol, TG= triglycerides

**Table 2. Mean  $\pm$  SD of cardioprotective and atherogenic risk ratios during 30 days of exposure**

S/N	Samples	CRR-1	CRR-2	AIP	AAI	AC	TG/HDL
1	Control	9.36 $\pm$ 0.73	6.31 $\pm$ 0.77	0.65 $\pm$ 0.05	11.50 $\pm$ 1.29	8.35 $\pm$ 0.73	4.49 $\pm$ 0.52
2	A	9.95 $\pm$ 1.10	6.78 $\pm$ 0.91	0.69 $\pm$ 0.06	11.25 $\pm$ 0.96	8.95 $\pm$ 1.10	4.98 $\pm$ 0.65
3	B	9.31 $\pm$ 0.32	6.19 $\pm$ 0.58	0.77 $\pm$ 0.14	11.25 $\pm$ 0.96	8.31 $\pm$ 0.32	4.88 $\pm$ 1.10
4	C	9.58 $\pm$ 0.77	6.50 $\pm$ 0.83	0.66 $\pm$ 0.06	11.25 $\pm$ 0.96	8.58 $\pm$ 0.77	4.59 $\pm$ 0.63
5	F-value	0.5708	0.4283	1.462	0.0566	0.5751	0.3740
6	P-value	0.6449	0.7364	0.2741	0.9815	0.6423	0.7728

Keys: CRR-1=cardiac risk ratio 1, CRR-11= cardiac risk ratio 11, AIP= atherogenic index of plasma, AC= atherogenic coefficient, AAI- anti-atherogenic index

**Table 3. Mean  $\pm$  SD of lipoproteins during 60 days of exposure**

S/N	Samples	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)	VLDL-C (mmol/L)
1	Control	2.43 $\pm$ 0.06 <sup>a</sup>	0.28 $\pm$ 0.01 <sup>a</sup>	1.62 $\pm$ 0.03	1.18 $\pm$ 0.13 <sup>a</sup>	0.54 $\pm$ 0.06 <sup>a</sup>
2	A	2.73 $\pm$ 0.07 <sup>b</sup>	0.24 $\pm$ 0.01 <sup>b</sup>	1.78 $\pm$ 0.05	1.58 $\pm$ 0.10 <sup>b</sup>	0.72 $\pm$ 0.03 <sup>b</sup>
3	B	2.56 $\pm$ 0.08 <sup>ab</sup>	0.25 $\pm$ 0.01 <sup>b</sup>	1.68 $\pm$ 0.06	1.38 $\pm$ 0.10 <sup>ab</sup>	0.63 $\pm$ 0.04 <sup>ab</sup>
4	C	2.60 $\pm$ 0.06 <sup>b</sup>	0.25 $\pm$ 0.01 <sup>b</sup>	1.67 $\pm$ 0.12	1.48 $\pm$ 0.17 <sup>b</sup>	0.67 $\pm$ 0.08 <sup>b</sup>
5	F-value	14.6	9.714	3.188	7.368	7.177
6	P-value	0.0003	0.0016	0.0629	0.0046	0.0051

Keys: TC= total cholesterol, HDL-C= high-density lipoprotein cholesterol, LDL-C= low-density lipoprotein cholesterol, VLDL-C= very low-density lipoprotein cholesterol, TG= triglycerides

The results of the TC, HDL-C, LDL-C, VLDL-C, and TG concentrations of rabbits exposed dermally to samples A, B, and for sixty (60) days are shown in Table 3.

The sixty (60) days of exposure of the rabbits to samples A, B, and C and the mean TC of the different groups and control, when compared, showed significant variations amongst the groups,  $p = 0.0003$ . The Tukey multiple comparison tests showed groups A and C significantly higher ( $p < 0.05$ ) than the control and group B. There is no significant difference ( $p > 0.05$ ) between groups A, B, and C.

Similarly, the compared mean HDL-C of the different groups and control were significantly different ( $p = 0.0016$ ). The Tukey multiple comparison tests showed that groups A and C were significantly lower than the control and group B. There is no significant difference between groups A, B, and C.

Furthermore, sixty (60) days of exposure of the rabbits to samples A, B, C, and control for mean LDL-C of the different groups with control was not significantly different  $p = 0.0629$ .

However, there were significant differences between the groups and control at  $p = 0.0046$ . The Tukey multiple comparison tests showed that groups A and C were significantly higher than the control and group B. There is no significant difference between groups A, B, and C.

More so there were significant differences amongst the mean VLDL-C of the different groups and control at  $p = 0.0051$ . The Tukey multiple comparison tests showed group A and group C significantly higher than the control and group B. There was no significant difference between groups A, B, and C.

Significant variations were observed amongst the mean CRR-1 of the different groups and control at  $p = 0.0001$ . The Tukey multiple comparison tests showed groups A, B, and C was significantly higher ( $p < 0.05$ ) than the control and group B. Group A was significantly higher than groups B and C. There was no significant difference between groups B and C.

Comparison of the means of the groups and control showed significant differences ( $p = 0.0002$ ). The Tukey multiple comparison tests showed groups A, B, and C significantly higher than the control. Group A is significantly higher

than groups B and C. There is no significant difference between groups B and C.

A comparison of the means of the groups and control for AIP showed a significant difference ( $p = 0.0024$ ). The Tukey multiple comparison tests showed significant variations amongst groups A, B, and C significantly higher than the control at  $p < 0.05$ . There was no significant difference between groups A, B, and C.

After sixty (60) days of exposure of the rabbits to samples A, B, and C, a comparison of the means of AAI for the groups and control showed significant differences ( $p < 0.0001$ ). The Tukey multiple comparison tests showed groups A, B, and C was significantly higher than the control. Group A was significantly higher than groups B and C. There was no significant difference between groups B and C.

Comparison of the means of AC for the groups and control showed significant differences ( $p < 0.0001$ ) between the groups. The Tukey multiple comparison tests showed groups A, B, and C, significantly higher than the control. Group A was significantly higher than groups B and C. There was no significant difference between groups B and C.

Comparison of the means of TC/HDL for the groups and control showed significant differences ( $p = 0.0032$ ) between the groups. The Tukey multiple comparison tests showed groups A, B, and C significantly higher than the control. There was no significant difference between groups A, B, and C.

The results of the TC, HDL-C, LDL-C, VLDL-C, and TG concentrations of rabbits exposed dermally to samples A, B, and C for ninety (90) days are shown in Tables 5 and 6.

Comparison of the means of TC for the groups showed significant differences ( $p = 0.0004$ ) between the groups. The Tukey multiple comparison tests showed groups A and B, abundantly higher ( $p < 0.05$ ) than the control. There is no significant difference ( $p > 0.05$ ) between groups A, B, and C.

Comparison of the means of HDL-C for the groups and control showed significant differences ( $p < 0.0001$ ) amongst the groups. The Tukey multiple comparison tests showed groups A, B, and C was significantly lower than the control. group A was significantly lower than groups B and C. There was no significant difference between groups B and C, with groups B and A.

**Table 4. Mean  $\pm$  SD of cardioprotective and atherogenic risk ratios during 60 days of exposure**

S/N	Samples	CR-1	CR-11	AIP	AAI	AC	TG/HDL
1	Control	8.85 $\pm$ 0.56 <sup>a</sup>	5.89 $\pm$ 0.27 <sup>a</sup>	0.63 $\pm$ 0.07 <sup>a</sup>	12.00 $\pm$ 0.82 <sup>a</sup>	7.84 $\pm$ 0.56 <sup>a</sup>	4.15 $\pm$ 0.27 <sup>a</sup>
2	A	11.39 $\pm$ 0.29 <sup>c</sup>	7.41 $\pm$ 0.19 <sup>c</sup>	0.81 $\pm$ 0.03 <sup>b</sup>	9.00 $\pm$ 0.00 <sup>bc</sup>	10.39 $\pm$ 0.29 <sup>c</sup>	6.11 $\pm$ 0.76 <sup>b</sup>
3	B	10.15 $\pm$ 0.58 <sup>b</sup>	6.67 $\pm$ 0.38 <sup>b</sup>	0.74 $\pm$ 0.04 <sup>b</sup>	10.50 $\pm$ 0.58 <sup>b</sup>	9.15 $\pm$ 0.58 <sup>b</sup>	5.46 $\pm$ 0.53 <sup>b</sup>
4	C	10.28 $\pm$ 0.04 <sup>b</sup>	6.62 $\pm$ 0.37 <sup>b</sup>	0.77 $\pm$ 0.05 <sup>b</sup>	10.00 $\pm$ 0.00 <sup>b</sup>	9.28 $\pm$ 0.04 <sup>b</sup>	5.85 $\pm$ 0.76 <sup>b</sup>
5	F-value	23.42	15.94	8.744	25	23.68	8.103
6	P value	<0.0001	0.0002	0.0024	<0.0001	<0.0001	0.0032

Keys: CRR-1=cardiac risk ratio 1, CRR-11= cardiac risk ratio 11, AIP= atherogenic index of plasma, AC= atherogenic coefficient, AAI- anti-atherogenic index. Mean  $\pm$  SD with different superscripts are significantly different from each other at  $p<0.05$

Comparison of the means of LDL-C for the groups showed no significant differences ( $p = 0.2863$ ) amongst the groups.

However, a comparison of the means of TG for the groups showed significant differences ( $p = 0.0001$ ) among the groups. The Tukey multiple comparison tests showed group A, group B, and group C were significantly higher than the control. Group A is significantly higher than group B. There is no significant difference between group C and group A with group C and group B.

Similarly, a comparison of the means of VLDL-C for the groups showed a significant difference ( $p = 0.0001$ ) between the groups. The Tukey multiple comparison tests showed groups A, B, and C were significantly higher than the control. There is no significant difference between groups A, B, and C.

Details of the results of the CRR-1, CRR-11, AIP, AAI, AC, and TG/HDL concentrations of rabbits exposed dermally to samples A, B, and C for ninety (90) days are shown in Table 6.

At ninety (90) days of exposure of the rabbits to samples A, B, and C the mean CRR-1 of the different groups and control showed significant differences ( $p = <0.0001$ ) between the groups. The Tukey multiple comparison tests showed groups A, B, and C significantly higher, than the control. Group A is significantly higher than group B and group C. There was no significant difference between group B and group C.

A comparison of the means of CRR-2 for the groups showed significant differences

( $p <0.0001$ ) amongst the groups. The Tukey multiple comparison tests showed groups A, B, and C and the control. There were no significant differences between groups A, B, and C.

Comparison of the means of AIP for the groups showed significant differences ( $p = 0.0015$ ) between the groups. The Tukey multiple comparison tests showed group A significantly higher than the control, group B and group C. There is no significant difference between the control, groups B and C.

Comparison of the means of AAI for the groups showed significant differences ( $p <0.0001$ ) amongst the groups. The Tukey multiple comparison tests showed groups A, B, and C were significantly different. Groups A and B, were abundantly higher than the control. Group C is significantly higher ( $p <0.05$ ) than groups B and C. There is no significant difference between groups B and C.

A comparison of the means of AC for the groups showed a significant difference ( $p <0.0001$ ). The Tukey multiple comparison tests showed groups A, B, and C significantly higher) than the control. Group A is significantly higher than group B and group C. There is no significant difference ( $p >0.05$ ) between groups B and C.

Comparison of the means of TG/HDL for the groups showed significant differences ( $P <0.0001$ ,  $F=31.7$ ). The Tukey multiple comparison tests showed group A and group C significantly higher than the control. Group A is significantly higher than group C. There is no significant difference ( $p >0.05$ ) between group B and group C with group B and control.

**Table 5. Mean  $\pm$  SD of lipoproteins during 90 days exposure**

S/N	Samples	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)	VLDL-C (mmol/L)
1	Control	2.46 $\pm$ 0.08 <sup>a</sup>	0.27 $\pm$ 0.01 <sup>a</sup>	1.65 $\pm$ 0.10	1.20 $\pm$ 0.14 <sup>a</sup>	0.55 $\pm$ 0.67 <sup>a</sup>
2	A	2.85 $\pm$ 0.07 <sup>b</sup>	0.22 $\pm$ 0.01 <sup>b</sup>	1.79 $\pm$ 0.07	1.88 $\pm$ 0.05 <sup>c</sup>	0.85 $\pm$ 0.02 <sup>b</sup>
3	B	2.67 $\pm$ 0.09 <sup>b</sup>	0.25 $\pm$ 0.01 <sup>bc</sup>	1.81 $\pm$ 0.12	1.35 $\pm$ 0.21 <sup>b</sup>	0.62 $\pm$ 0.10 <sup>ab</sup>
4	C	2.73 $\pm$ 0.10 <sup>b</sup>	0.25 $\pm$ 0.01 <sup>c</sup>	1.74 $\pm$ 0.16	1.65 $\pm$ 0.13 <sup>bc</sup>	0.75 $\pm$ 0.06 <sup>b</sup>
5	F-value	13.55	21.92	1.416	17.73	16.99
6	P value	0.0004	<0.0001	0.2863	0.0001	0.0001

Keys: TC= total cholesterol, HDL-C= high-density lipoprotein cholesterol, LDL-C= low-density lipoprotein cholesterol, VLDL-C= very-low-density lipoprotein cholesterol, TG= triglycerides.

Mean  $\pm$  SD with different superscripts are significantly different from each other at  $p <0.05$

**Table 6. Mean  $\pm$  SD of cardioprotective and atherogenic risk ratios during 90 days of exposure**

S/N	Samples	CR-1	CRR-2	AIP	AAI	AC	TG/HDL
1	Control	9.23 $\pm$ 0.64 <sup>a</sup>	6.18 $\pm$ 0.56 <sup>a</sup>	0.65 $\pm$ 0.06 <sup>a</sup>	11.50 $\pm$ 1.00 <sup>a</sup>	7.84 $\pm$ 0.56 <sup>a</sup>	4.50 $\pm$ 0.62 <sup>a</sup>
3	A	13.12 $\pm$ 0.54 <sup>c</sup>	8.21 $\pm$ 0.36 <sup>b</sup>	0.94 $\pm$ 0.04 <sup>bc</sup>	7.75 $\pm$ 0.50 <sup>c</sup>	10.39 $\pm$ 0.29 <sup>c</sup>	8.63 $\pm$ 0.57 <sup>b</sup>
2	B	10.79 $\pm$ 0.19 <sup>b</sup>	7.30 $\pm$ 0.40 <sup>b</sup>	0.75 $\pm$ 0.09 <sup>a</sup>	9.75 $\pm$ 0.50 <sup>b</sup>	9.15 $\pm$ 0.50 <sup>b</sup>	5.45 $\pm$ 0.82 <sup>ac</sup>
4	C	11.16 $\pm$ 0.55 <sup>b</sup>	7.10 $\pm$ 0.73 <sup>b</sup>	0.83 $\pm$ 0.02 <sup>ac</sup>	9.75 $\pm$ 0.50 <sup>b</sup>	9.28 $\pm$ 0.04 <sup>b</sup>	6.73 $\pm$ 0.48 <sup>c</sup>
5	F-value	39.75	9.747	17.58	21.48	23.68	31.7
6	P value	<0.0001	0.0015	0.0001	<0.0001	<0.0001	<0.0001

Keys: CRR-1=cardiac risk ratio 1, CRR-11= cardiac risk ratio 11, AIP= atherogenic index of plasma, AC= atherogenic coefficient, AAI- anti-atherogenic index. The mean  $\pm$  SD of experimental groups with different superscripts are significantly different from each other at  $p<0.05$



#### 4. DISCUSSION

It has been reported that these herbal cosmetics (All things natural (Emi herbal oil), Kakiva (Kakiva herbal oil), and Amal botanical herbal oil) contain heavy metals [25]. These heavy metals have been observed to accumulate in the body over time. These have resulted in lipids and lipoprotein alteration. The lipoprotein parameters showed significantly higher total cholesterol and significantly lower high-density lipoprotein compared with the control from day 60. Group A had the highest increase with group B being the least. At 90 days there were significantly higher total cholesterol, VLDL, and triglyceride levels with a significantly lower HDL level in all the experimental groups compared with the control, and no significant differences were observed between the groups. The results of the present study agreed with the report of Samarghandia et al. [26], which indicated that Cadmium administration significantly increased the serum levels of TG, TC, and LDL-C, but the reduction in the HDL-C.

The cardiovascular disease risk was assessed in the study, using AAI, CRI-1, CRI-11, AIP, and AC respectively. The result of this study showed a significantly higher CRR-1, CRR-2, AIP, AAI, AC, and TG/HDL compared with the control at day 60. Group A was greater than group C greater than group B for CRR-1, AIP, AAI, AC, and TG/HDL. Whereas for CRR-2 group A was greater than group B, and group B was greater than group C. Earlier studies suggested that “low levels of paraoxonase 1 activity occasioned by increased accumulation of Cadmium may be associated with an increased prevalence of CVD” [27,28].

The significant variations in the effects of three different herbal cosmetics on lipid levels and cardiovascular risk indices may not be unconnected with the high levels of lead, cadmium, and nickel found in this aligns with other related studies; residents with low compared with high cadmium exposure, high aligns and 24-hour urine cadmium were associated with an increased risk of cardiovascular and non-cardiovascular mortality [17,29].

Similarly, in rats, exposure to low lead levels compared with controls increased activation of nuclear factor- $\kappa$ B [14]. In addition to an increased risk of cardiovascular mortality, long-term exposure to low levels of lead has been

associated with persistent hypertension in animal and human studies [15]. In a prospective population study [16], participants with higher blood lead concentration at baseline predicted impaired systolic left ventricular function a decade later. Accumulation of cadmium, lead, and nickel has been documented to increase oxidative stress. This is attributed to catalyzing the formation of reactive oxygen species, thereby causing an increase in lipid peroxidation and glutathione depletion with protein-bound sulfhydryl groups. Excess free radicals are atherogenic in nature. Solenkova et al. [11,12], in separate studies, reported that “pro-atherosclerotic changes from lead exposure have also been associated with the inactivation of paraoxonase activity, which decreases the antioxidant effects of high-density lipoprotein. Lead replaces calcium in various intracellular signaling reactions, including inhibiting the effect of calmodulin in the synthesis of nitric oxide (NO), possibly explaining lead-induced hypertension”.

#### 5. CONCLUSION

It has been reported that these herbal cosmetics (All things natural (Emi herbal oil), Kakiva (Kakiva herbal oil), and Amal botanical herbal oil) contain heavy metals. These heavy metals (cadmium, lead, nickel) have been observed to accumulate over time and are responsible for alterations in lipid profile and cardiovascular risk indices. These alterations result in cardiovascular diseases as well as oxidative stress in individuals exposed to these heavy metals. It is therefore pertinent to engage in public awareness of the dangers inherent in these herbal cosmetics. Also, regulatory authorities should ensure registration of these products after proper evaluation of the contents to protect the public from imminent harm of these products.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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