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Oxidative Stress Biomarkers and Antioxidant Status in Rabbits following Topical Application of Herbal Cosmetics

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Abstract

The aim of this study was to evaluate oxidative stress biomarkers and antioxidant status in rabbits following topical application of herbal cosmetics. Three herbal oils were 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 anesthesia. Blood samples were collected for investigation of biochemical parameters at intervals of, 30days, 60days and 90days respectively. GraphPad Prism version 7.0 was used for statistical analysis and $p<0.05$ was considered statistically significant. The results of malondialdehyde (MDA), Total Antioxidant Capacity (TAC), Lipid Peroxidation Index (LPI) and C-Reactive Index (CRP) at day 30 and 60 in sample A, B and C showed no significant difference compared to control. However, at day 90 the mean values of MDA, LPI, TAC and CRP were statistically significant $p<0.05$ in the samples compared to control. The results showed that heavy metals present in the cosmetics can induce oxidative stress by stimulating reactive oxygen species production, enhancing lipid peroxidation and depleting antioxidant reserves. Therefore, the resulting oxidative damage may contribute to the pathogenesis of chronic diseases, including cardiovascular disease.

Keywords: Oxidative Stress, Biomarkers, Antioxidant Status and Rabbits

Introduction

The use of cosmetics is a common practice particularly among women. However, concerns have been raised regarding the presence of heavy metals as contaminants in several cosmetic products. Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As) and chromium (Cr) have been detected in skin-lightening creams, lipsticks, eye liners and facial powders at concentrations that sometimes exceed regulatory limits [1], [2]. Chronic dermal exposure to these products represents a potential route of systemic metal absorption, with implications for oxidative stress and associated health outcomes.

Oxidative stress occurs as a result of an imbalance between the production of reactive oxygen species and the ability of biological systems to detoxify these reactive intermediates or repair the resulting damage. Heavy metals induce oxidative stress through multiple mechanisms. Although some metals like Pb and Cd are not redox-active, they deplete antioxidant reserves by binding to sulfhydryl groups of glutathione and antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase [3]. Other metals such as Hg and Cr can participate in Fenton-like reactions, leading to the generation of highly reactive hydroxyl radicals. The resultant increase in reactive oxygen species causes lipid peroxidation, protein oxidation and DNA damage [4].

Several studies have reported elevated biomarkers of oxidative stress in individuals exposed to heavy metals through cosmetic use. [5] analyzed facial cosmetics sold in Nigeria and found Pb, Cd and Hg levels above WHO permissible limits. The authors suggested that prolonged dermal application could lead to systemic accumulation and subsequent oxidative tissue injury. Similarly, [6] reported significant levels of Pb and Cd in lipsticks and eye pencils marketed in Nigeria, highlighting the potential for chronic low-dose exposure.

Experimental and occupational studies provide further mechanistic evidence. Cadmium exposure has been shown to increase malondialdehyde, a marker of lipid peroxidation, while decreasing superoxide dismutase, catalase and glutathione activities in liver and kidney tissues [7]. Lead toxicity is associated with increased reactive oxygen species generation and depletion of antioxidant enzymes, resulting in hepatocellular damage [8].

Exposure to heavy metals is strongly associated with increased oxidative stress in humans. Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) are redox-active or indirectly generate reactive oxygen species through depletion of antioxidant defenses. Once absorbed through dermal, inhalation or ingestion routes, these metals accumulate in target organs including the liver, kidney and vascular endothelium, where they disrupt cellular redox balance [4]. Several studies have consistently reported elevated levels of oxidative biomarkers in individuals with chronic heavy metal exposure, suggesting a key mechanistic link between metal toxicity and tissue damage.

The primary pathway involves the induction of oxidative stress and lipid peroxidation. Lead and cadmium, for example, do not directly participate in Fenton reactions but they inhibit antioxidant enzymes and bind to sulfhydryl groups in glutathione and proteins, thereby increasing free radical generation [3]. This leads to attack on polyunsaturated fatty acids in cell membranes and the formation of lipid peroxidation products. Malondialdehyde and 4-hydroxynonenal are the most widely used biomarkers of lipid peroxidation and have been reported to be significantly elevated in subjects exposed to Pb and Cd [7].

Concurrently, heavy metal exposure results in depletion of antioxidant defense systems. Glutathione, superoxide dismutase, catalase and glutathione peroxidase constitute the first line of cellular defense against reactive oxygen species. Cadmium and lead have been shown to down-regulate the activity of these enzymes, creating an imbalance between pro-oxidants and antioxidants [9].[1] noted that chronic use of cosmetics contaminated with Pb, Cd and Hg could contribute to systemic oxidative burden due to gradual metal accumulation in the body.

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:

Determination of malondialdehyde, total antioxidant capacity and C-reactive protein were carried out using Enzyme Linked Immunosorbent Assay. Lipid peroxidation Index was calculated using a formular developed by Christopher & Steven (1999).

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 mean values of oxidative stress markers and C-reactive protein during 30 days, 60 days and 90 days exposure is presented in Table 1, 2 and 3respectively.

Table 1: Mean ± SD of Oxidative Stress Markers and C-Reactive Protein during 30 days Exposure

Samples	MDA (nmol/L)	TAC (mol/L)	LPI	CRP (mg/dL)
Control	3.28 ±0.50	1.11 ±0.04	2.95 ±0.44	2.73 ±0.13
A	2.65 ±0.40	1.09 ±0.03	2.42 ±0.33	2.73 ±0.13
B	3.38 ±0.67	1.01 ±0.03	3.06 ±0.64	2.65 ±0.26
C	3.38 ±0.57	1.11 ±0.03	3.05 ±0.50	3.00 ±0.08
F-value	1.646	0.2292	1.518	2.247
P value	0.2311	0.8742	0.2601	0.1352

Keys: MDA= malondialdehyde, TAC= total antioxidant capacity, LPI= lipid peroxidation index, CRP=C-reactive protein.

Table 2: Mean ± SD of Oxidative Stress Markers and C-Reactive Protein during 60 days Exposure

Samples	MDA (nmol/L)	TAC (mol/L)	LPI	CRP (mg/dL)
Control	3.68 ±0.85^{a}	1.10 ±0.02^{a}	3.34 ±0.78^{a}	2.75 ±0.26^{a}
A	4.15 ±1.01^{ac}	1.01 ±0.05^{b}	4.12 ±0.94^{a}	8.50 ±0.71^{b}
B	5.60 ±0.86c	0.96 ±0.04^{b}	5.87 ±0.94^{bc}	10.20 ±0.81^{b}
C	4.30 ±0.57	1.01 ±0.06^{b}	4.25 ±0.39^{ac}	11.98±1.65^{b} ^{c}
F-value	3.857^{ac}	8.018	7.14	64.70
P value	0.0383	0.0034	0.0052	<0.0001

Keys: MDA= malondialdehyde, TAC= total antioxidant capacity, LPI = lipid peroxidation index, CRP = C-reactive protein. Mean ± SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Table 3: Mean ± SD of Oxidative Stress Markers and C-Reactive Protein during 90 days Exposure

Samples	MDA (nmol/L	TAC (mol/L)	LPI	CRP (mg/dL)
Control	3.25 ±0.91[a]	1.11 ±0.03[a]	2.93 ±0.84[a]	3.03 ±0.45[a]
A	6.35 ±1.15[b]	0.91 ±0.05[b]	7.01 ±1.39[b]	16.38 ±0.84[b]
B	7.13 ±1.30[b]	0.83 ±0.06[b]	8.59 ±0.89[b]	18.55 ±0.31[c]
C	5.85 ±0.74[b]	0.90 ±0.04[b]	6.51 ±0.87[b]	19.58 ±1.09[c]
F-value	12.01	27.89	21.9	429
P value	0.0008	<0.0001	<0.0001	<0.0001

Keys: MDA= malondialdehyde, TAC= total antioxidant capacity, LPI= lipid peroxidation index, CRP=C-reactive protein. Mean ± SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

DISCUSSION

The findings of the present study revealed that at day 30 and 60, the mean values of malondialdehyde (MDA), lipid peroxidative index (LPI), total antioxidant capacity (TAC) and C-reactive protein (CRP) in sample A, B and C was not statistically significant compared to control. However, there was a marked increase in malondialdehyde (MDA), lipid peroxidative index (LPI) and C-reactive protein (CRP) with a concomitant decrease in total antioxidant capacity (TAC) in sample A, B and C compared to control. This pattern indicates an imbalance between pro-oxidants and antioxidants, which is a hallmark of oxidative stress and it suggest that the use of cosmetics containing heavy metals is associated with increased oxidative stress.

This observation is consistent with previous reports that identified heavy metals as contaminants in cosmetic products. [1] and [2] reported elevated concentrations of heavy metals in facial cosmetics sold in Nigeria, often exceeding WHO permissible limits. Chronic dermal application of these products provides a route for systemic absorption and gradual accumulation of metals in target organs such as the liver and kidney. Once absorbed, these metals induce oxidative stress through two major pathways. First, redox-inactive metals like Pb and Cd bind to sulfhydryl groups in glutathione and antioxidant enzymes, thereby depleting cellular antioxidant defenses [3]. Second, metals such as Hg and Cr can catalyze Fenton-like reactions that generate highly reactive hydroxyl radicals, leading to damage of lipids, proteins and DNA [4].

The significant elevation in MDA observed in cosmetic users in this study reflects increased lipid peroxidation. MDA is a stable end-product of polyunsaturated fatty acid oxidation and has been widely used as a biomarker of oxidative damage. [7] also reported increased MDA levels following cadmium exposure, which they attributed to mitochondrial dysfunction and reactive oxygen species generation. Similarly, the reduction in TAC in the present study suggests that prolonged cosmetic use overwhelms the antioxidant defense system. This finding aligns with [8] who demonstrated that lead exposure impairs antioxidant enzyme activity in experimental models.

CONCLUSION

This study revealed that heavy metals promote oxidative stress by stimulating reactive oxygen species production, enhancing lipid peroxidation, and depleting antioxidant reserves. The resulting increase in oxidative biomarkers such as MDA, TAC, LPI and CRP provides measurable evidence of metal-induced cellular injury.

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 declares 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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