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SKIN LIGHTENING CREAM EXPOSURE ON SERUM ANTIOXIDANTS STATUS AND SELECTED HEAVY METALS IN SPRAGUE DAWLEY RATS

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ABSTRACT

Introduction: Skin lightening creams have been used mostly by women in developing countries for over a decade. The preference to having a fair skin is wide. These creams have been shown to contain hydroquinone. **Objectives:** This study is to investigate the effects of skin lightening creams on antioxidant enzymes, heavy metal levels and histology of the skin and liver in rats. **Methodology:** Skin lightening creams were purchased and applied to rats' skins for six weeks. Heavy metal levels were assessed in the serum, together with protein levels and antioxidant enzyme activities. Histopathological tests were also carried out in rats skin, liver and ovaries. **Results:** Application of skin lightening creams to rat skin led to a deposition of high concentrations of mercury and arsenic (above the Maximum Permissible Levels by the WHO), high levels of serum protein and increase in the concentrations of antioxidant activities. Histological examination of the skin and liver showed lesions in group 7 of the rats. **Conclusion:** The usage of skin lightening creams over a long period of time could have adverse health effects.

KEYWORDS: Heavy metals, Skin lightening creams, Antioxidants, Histology, Serum protein.

INTRODUCTION

Throughout Africa, fairness is regarded as having a high social status and beauty. The practice of using whitening creams to have a lighter skin has been rooted in the past. This ideology of associating fairness with beauty encourages most women in Africa with dark skin to bleach their skins. Skin lightening is being practiced in alarming levels all over the world (Lewis, 2009; Voegborlo, 2008; Mahe, 2004; Harada, 2001). The colonial legacy is one of the factors that led to this belief. The preference to have fair skin has escalated the sales of skin lightening products. These products are readily available and easily accessible nationwide in Nigeria. They are marketed as Fading Creams, Skin Whiteners, Fairness Creams, Skin Toners, Skin Lighteners etc (Hunter, 2011). Although, both men and women practice skin lightening, the highest rate of this practice is found in women. Lots of African women desire their black skin toned or bleached.

Most of these skin lightening creams contain different kinds of toxic chemicals such as mercury, hydroquinone, kojic acid etc. These can adversely affect human health (Amponsah *et al,* 2014). Compounds containing mercury have been used in germicidal soaps, teething powders, skin lightening creams etc (Dyall–Smith and Scurry, 1990). Compounds containing mercury can be absorbed through the skin (Tlacuilo-Parra, 2001). Toxicity to the neurologic, dermal and renal systems have been reported in literature (Sah, 2012; ATDR, 2002), Due to the toxicity of mercurial compounds, the US Foods and Drug Administration, banned the use of these compounds in skin preparations, except as a preservative in very low concentrations (USEPA, 2002). However, skin lightening creams containing mercury and other toxic elements are available in countries all over the world (Perry, 2006). Most of these creams sold in the Nigerian market are imported from UK, USA, Europe, and Italy.

The aim of this study is to investigate the concentrations of heavy metals in the serum and organs of rats, after exposure to skin lightening creams and also to assess any damage to the antioxidant activities in the rats.

METHODOLOGY

Purchase of Skin Lightening Creams

Different brands of skin lightening creams were purchased from reputable retail shops and markets in Lagos State, Nigeria. Samples were transported to the laboratory and labeled.

Purchase of laboratory animals

Forty female albino (Sprague Dawley) rats (115.18±6.40g) were purchased from the Laboratory

Animal Centre, College of Medicine, University of Lagos. They were housed in plastic cages and equally grouped into eight groups. They were acclimatized for one week and fed with rat chow (obtained from Pfizer Livestock Feeds, Nigeria PLC) and water *ad libitum*.

Experimental Design
A small section of the rat's skin in the dorsal region was shaved with a sterile razor blade and a minute amount of the skin lightening cream was applied, every morning at 8.00am, for a duration of four weeks. During this period, the rats were also fed with animal feed. During the four weeks of application of different creams to different groups of rats, the rats were also observed.

Ethical Consideration
All experimental procedures were conducted in accordance with the guide for the care and use of laboratory animals with the Local Animal Care and Ethics Committee.

Collection of Samples
After four weeks, the rats were sacrificed under light anesthesia. Blood samples were collected from their veins with a sterile needle and different organs (liver, kidney, heart, ovary and the skin) were also collected. The blood samples were collected into universal sterile bottles and centrifuged at 3000g for 20mins to obtain the serum.

Protein Determination
This was determined according to the method of Lowry *et al*, 1951.

Digestion for Heavy Metal Analysis
Wet digestion was carried out on the blood samples and organs using Nitric acid. 50ml of concentrated HNO_3 was added to 1g of finely mashed tissue in a conical flask. The mixture was placed on a hot plate in a fume cupboard until the volume reduced to 20ml and the solution was clear. The digest was allowed to cool and filtered using a Whatman filter paper. The volume of the filtrate was made up to 100mls with distilled water. The digest was subjected to Atomic Absorption Spectrophotometer (AAS) to determine the heavy metals level. The following metals were analyzed using the AAS (Elman Perkins model): Arsenic, lead, mercury, cadmium, zinc, nickel, chromium, copper, iron and magnesium.

Antioxidant Assay
The following antioxidant activities were determined.

a Lipid peroxidation: Malondialdehyde (MDA) was determined using the method of Niehaus and Samuelson, 1968. 5 ml of the serum were treated with Trichloroacetic acid (TCA), to precipitate proteins and then vortexed for 30 sec. A clear supernatant was then obtained by centrifuging for 10 min at 3000rpm. One ml of the supernatant was added to 2ml of (1:1:1 ratio) TCA-TBA-HCL reagent (thiobarbituric acid 0.37%, 0.24N HCL and 15% TCA). The mixture was boiled at 100^0C for 15min and allowed to cool. The mixture was centrifuged at 3000rpm for 10min, at room temperature. The supernatant was removed and the absorbance read at 535nm against a blank. MDA was calculated.

b. Catalase Activity Determination
Catalase activity was determined according to Sinha *et al*, 1972. 2ml of Phosphate buffer (0.01M, pH 7.0) was added to 4mls of hydrogen peroxide (2M). The solution was mixed gently together.1ml of this mixture was taken and injected into 2ml of dichromate acetic acid. The reaction was stopped. This procedure was repeated at 60 sec intervals, using different test tubes. Each test tube was then heated for 10 minutes in a boiling water bath. The blue precipitate was decomposed and a green solution obtained. The mixture was cooled and absorbance read at 570 nm.

c. Determination of SOD activity (Superoxide Dismutase).
SOD activity was estimated according to the method described by Kakkar *et al*, 1984.
5ml of serum were mixed with 1.35ml of double distilled water, 1.2ml of sodium pyrophosphate buffer (pH 8.3), 0.1ml of phenazine methosulphate and 0.3ml of nitroblue tetrazolium. NADH solution was added to the mixture (0.2ml), to initiate the reaction and the mixture was then incubated at 39^OC for 90 seconds. The reaction was terminated by the addition of 1ml of glacial acetic acid. 4ml of n-butanol were added and the solution was centrifuged at 4000rpm for 10 min. The absorbance of the upper butanol layer was read at 560nm.

d. Determination of Reduced glutathione
The reduced glutathione (GSH) content of liver tissue was determined according to the method described by Sedlak and Lindsay (1968). 10% TCA was added to the homogenate and centrifuged. lml of the supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5-dithiobisnitro benzoic acid, DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH8.0). The absorbance was read at 412nm.

Histological Assay
The liver, ovary and skin from the different groups were dissected out and fixed in 10% Neutral Buffered Formalin (NBF). They were then subjected to histology according to the method of Bogdanovic *et al*, 2008.

Statistical Analysis
Data obtained were analyzed by student multiple comparison test of ANOVA.

RESULTS

Table 1: Names of the creams used in each group of the rats.

GROUPS OF RATS	CREAMS APPLIED
1	CLINIC CLEAR CREAM
2	HI WHITE CREAM
3	SOULMATE CREAM
4	PURE WHITE GOLD CREAM
5	CARO WHITE CREAM
6	CAROTONE CREAM
7	CLEAR THERAPY CREAM
8	CONTROL

Table 2: Serum antioxidant activities in rats topically applied skin lightening creams.

Group	MDA µmol/min	SOD µmol/min/mg	Reduced Glutathione µmol/min	CAT µmol/ml/min
1	0.27± 0.01*	1.31±0.09	172.73±0.11*	417.07±0.34*
2	0.25±0.05	0.29±0.02	178.91±0.31*	416.63±0.51*
3	0.06±0.03	2.82±0.08*	11.83±0.08	381.18±0.47*
4	0.25±0.01*	0.60±0.02*	185.37±0.32*	533.92±0.17*
5	0.21±0.01	0.87±0.01*	49.33±0.09	694.97±0.36*
6	0.35±0.04*	0.13±0.03	171.92±0.64*	667.08±0.27*
7	0.21±0.03	0.33±0.07	32.40±0.05	429.76±0.37*
8 CONTROL	0.23±0.02	0.57±0.01	159.83±0.22	376.37±0.18

*P<0.05 ND=NOT DETECTED

Table 3: Concentrations of arsenic, mercuy and lead in blood samples of rats topically applied skin lightening creams.

Group	As mg/ml	Hg mg/ml	Pb mg/ml
1	0.12±0.01*	0.90±0.04*	ND
2	0.07±0.03*	0.07±0.02*	ND
3	0.06±0.01*	0.08±0.04*	ND
4	0.09±0.04*	0.11±0.03*	ND
5	ND	ND	ND
6	0.09±0.02*	0.08±0.03*	ND
7	0.10±0.01*	0.10±0.01*	ND
8 CONTROL	ND	ND	ND

*P<0.05 ND=NOT DETECTED

Table 4: Cadmium, chromium and nickel concentrations in organs of rats topically applied skin lightening creams.

ORGANS	Cd mg/g	Cr mg/g	Ni mg/g
GROUP 1: Liver	0.06±0.02	0.15±0.08*	0.06±0.03
Heart	ND	ND	0.12±0.08*
Kidney	0.14±0.07	ND	ND
GROUP 2: Liver	0.26±0.09*	0.07±0.02	0.06±0.04
Heart	0.09±0.01	0.07±0.01	0.06±0.02
Kidney	0.11±0.01*	0.07±0.02	ND
GROUP 3: Liver	0.09±0.03*	0.22±0.09*	0.06±0.01
Heart	ND	0.07±0.05	ND
Kidney	0.03±0.01	0.07±0.02	ND
GROUP 4: Liver	0.17±0.06*	ND	ND
Heart	0.06±0.03	0.15±0.07*	0.12±0.09*
Kidney	0.03±0.01	0.15±0.07*	ND
GROUP 5: Liver	0.17±0.09*	0.07±0.02	ND
Heart	0.11±0.07*	0.15±0.09*	0.12±0.07*
Kidney	0.11±0.05*	0.15±0.07*	ND
GROUP 6: Liver	0.26±0.08*	0.07±0.03	0.06±0.03

Heart Kidney	0.06±0.02 ND	0.15±0.08* ND	0.06±0.01 ND
GROUP 7: Liver Heart Kidney	0.07±-0.08 0.14±-0.02* 0.14±0.01	0.07±0.02 0.15±0.07* ND	ND 0.06±0.01 0.12±0.08*
CONTROL GROUP 8: Liver Heart Kidney	 ND ND ND	 ND ND ND	 ND ND ND

*P<0.05 ND=NOT DETECTED

Table 5: Concentrations of iron, zinc, copper and magnesium levels in organs of rats.

GROUPS/ORGANS	Zn (mg/g)	Cu (mg/g)	Mg (mg/g)	Fe (mg/g)
GROUP 1				
Liver	2.13±0.19*	0.99±0.07*	3.74±0.17*	0.03±0.01
Heart	0.86±0.08*	0.85±0.02*	1.68±0.19	0.11±0.04*
Kidney	1.60±0.13*	0.42±0.02	4.05±0.28*	0.01±0.01
GROUP 2				
Liver	0.86±0.06*	0.45±0.03	0.79±0.04	0.03±0.03
Heart	0.31±0.04	0.59±0.05	3.89±0.17*	0.06±0.01
Kidney	0.80±0.07*	0.51±0.07	4.20±0.21*	0.06±0.02
GROUP 3				
Liver	0.80±0.05*	0.56±0.07	3.68±0.15*	0.06±0.01
Heart	1.33±0.17*	0.48±0.02	0.58±0.07	0.03±0.01
Kidney	2.35±0.19*	0.31±0.02*	4.26±0.27*	0.14±0.02*
GROUP 4				
Liver	0.80±0.06*	0.51±0.07	4.42±0.22*	0.22±0.03
Heart	1.33±0.11*	1.13±0.15*	0.42±0.03	0.08±0.02
Kidney	2.35±0.17*	0.23±0.02*	2.52±0.16	0.03±0.01
GROUP 5				
Liver	0.62±0.04	0.23±0.05	3.32±0.15*	0.03±0.02
Heart	0.77±0.02	0.14±0.01	0.53±0.03	0.01±0.01
Kidney	2.35±0.16*	0.34±0.02*	2.68±0.14*	0.01±0.01
GROUP 6				
Liver	2.38±0.12*	0.34±0.04	2.95±0.11*	0.06±0.01
Heart	2.22±0.11*	1.18±0.15*	0.47±0.02	0.06±0.01
Kidney	2.35±0.23*	0.34±0.03	4.32±0.24*	0.08±0.02
GROUP 7				
Liver	2.38±0.17*	0.42±0.05	0.74±0.03	0.03±0.02
Heart	0.12±0.01	0.25±0.01	3.37±0.17*	0.03±0.01
Kidney	1.57±0.14*	1.07±0.12*	4.47±0.21*	0.06±0.01
CONTROL				
GROUP 8: Liver	0.03±0.01	0.10±0.01	1.00±0.03	0.02±0.01
Heart	0.02±0.02	0.18±0.02	0.16±0.05	0.01±0.01
Kidney	ND	ND	1.16±0.12	0.01±0.01
***P<0.05**	ND=NOT DETECTED			

Table 6: Concentrations of proteins in serum of rats topically applied skin lightening creams.

GROUPS	PROTEIN CONCENTRATION (mg/ml)
1	38.37±0.29
2	73.46±0.78*
3	48.20±0.39*
4	41.54±0.42
5	51.16±0.33*
6	58.78±0.61*
7	66.32±0.72*
8 (CONTROL)	40.66±0.27

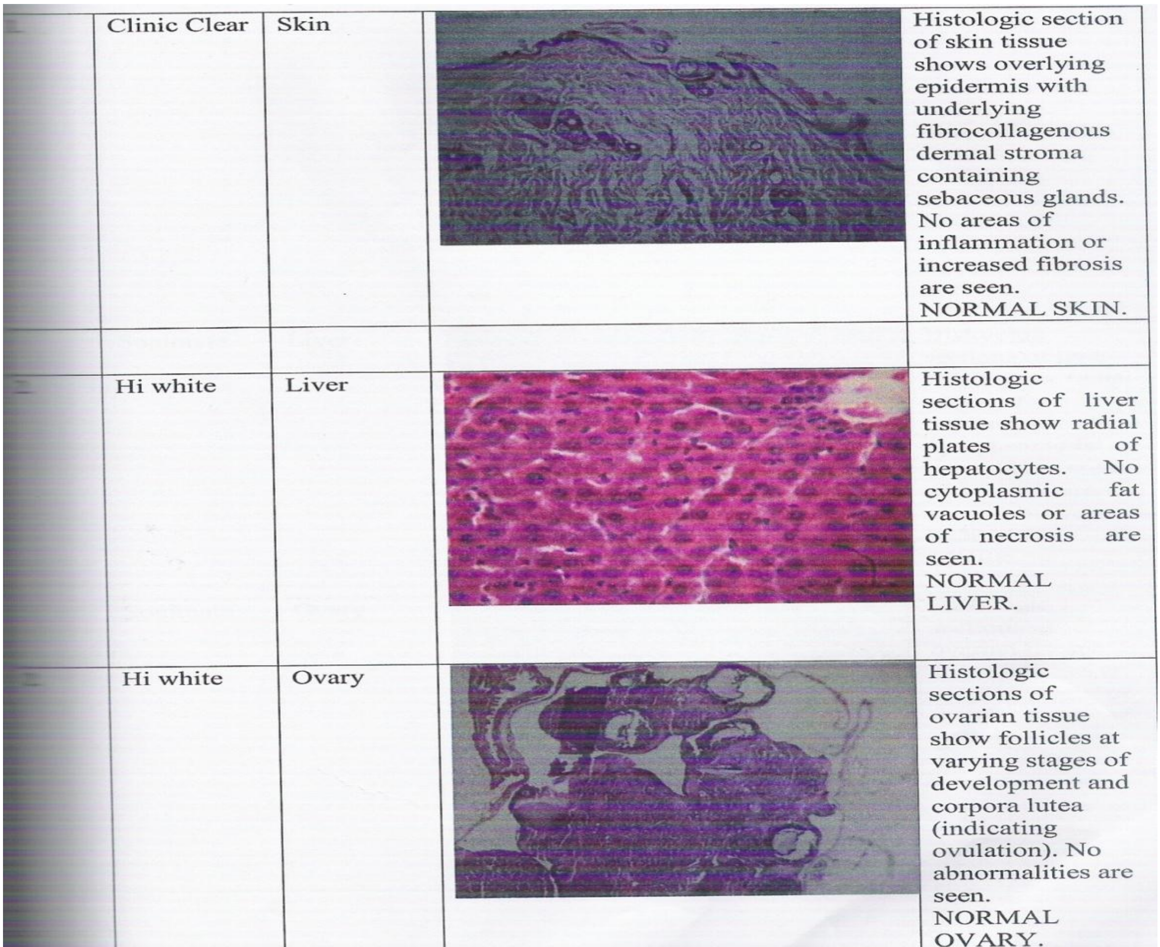
*P>0.05

APPENDIX
HISTOPATHOLOGICAL RESULTS

The histopathology test conducted gave the following results.

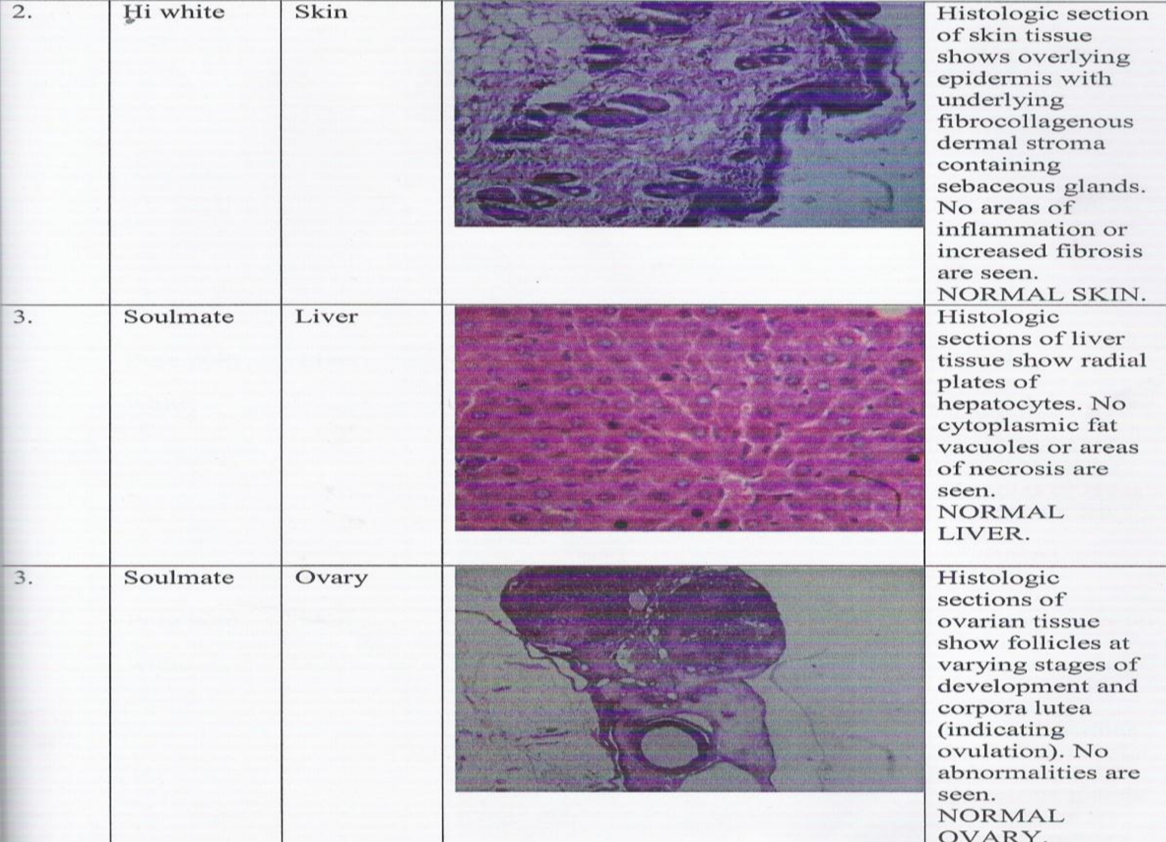
Group	Cream	Organ	Photomicrograph	Summary
1.	Clinic Clear	Liver		Histologic sections of liver tissue show radia plates of hepatocytes. No cytoplasmic fat vacuoles or areas of necrosis are seen. NORMAL LIVER.
1.	Clinic Clear	Ovary		Histologic sections of ovarian tissue show follicles at varying stages of development and corpora lutea (indicating ovulation). No abnormalities are seen. NORMAL OVARY.

Plate 1: Photomicrographs of liver and ovary of rats treated with Clinic Clear (group 1) cream showing normal tissues (x400).



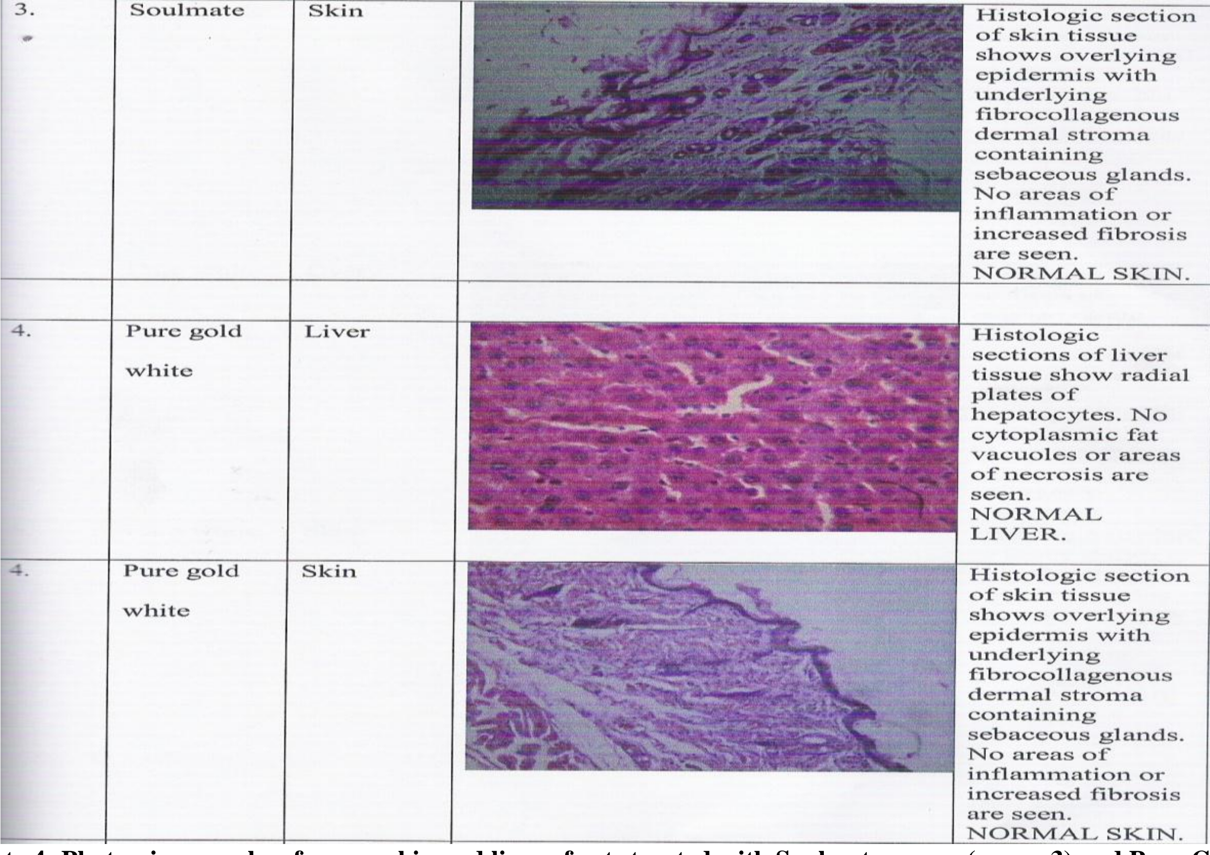
	Clinic Clear	Skin		Histologic section of skin tissue shows overlying epidermis with underlying fibrocollagenous dermal stroma containing sebaceous glands. No areas of inflammation or increased fibrosis are seen. NORMAL SKIN.
	Hi white	Liver		Histologic sections of liver tissue show radial plates of hepatocytes. No cytoplasmic fat vacuoles or areas of necrosis are seen. NORMAL LIVER.
	Hi white	Ovary		Histologic sections of ovarian tissue show follicles at varying stages of development and corpora lutea (indicating ovulation). No abnormalities are seen. NORMAL OVARY.

Plate 2: Photomicrographs of skin, liver and ovary of rats treated with Clinic Clear cream (group 1) and Hi White cream (group 2) (x400) showing normal tissues.



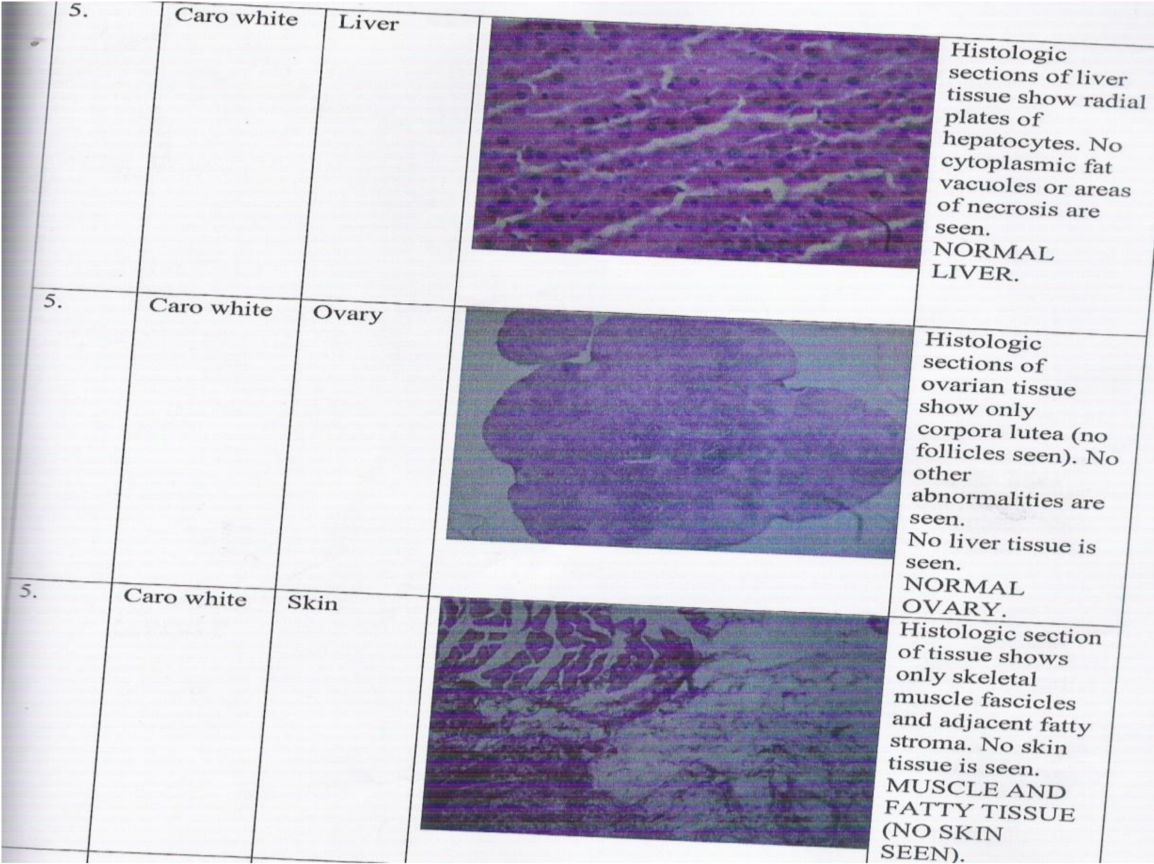
2.	Hi white	Skin		Histologic section of skin tissue shows overlying epidermis with underlying fibrocollagenous dermal stroma containing sebaceous glands. No areas of inflammation or increased fibrosis are seen. NORMAL SKIN.
3.	Soulmate	Liver		Histologic sections of liver tissue show radial plates of hepatocytes. No cytoplasmic fat vacuoles or areas of necrosis are seen. NORMAL LIVER.
3.	Soulmate	Ovary		Histologic sections of ovarian tissue show follicles at varying stages of development and corpora lutea (indicating ovulation). No abnormalities are seen. NORMAL OVARY.

Plate 3: Photomicrographs of skin, liver and ovary of rats treated with Hi White cream (group 2) and Soulmate cream (group 3) (x400) showing normal tissues.



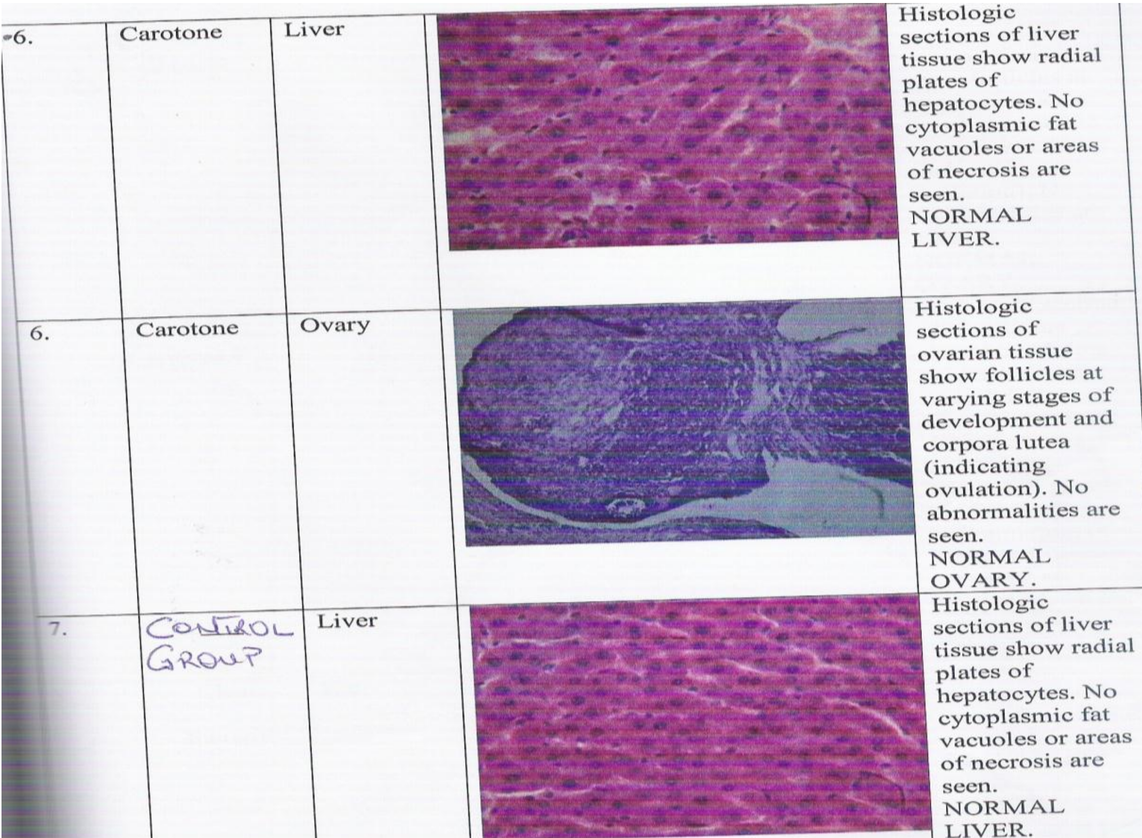
3.	Soulmate	Skin		Histologic section of skin tissue shows overlying epidermis with underlying fibrocollagenous dermal stroma containing sebaceous glands. No areas of inflammation or increased fibrosis are seen. NORMAL SKIN.
4.	Pure gold white	Liver		Histologic sections of liver tissue show radial plates of hepatocytes. No cytoplasmic fat vacuoles or areas of necrosis are seen. NORMAL LIVER.
4.	Pure gold white	Skin		Histologic section of skin tissue shows overlying epidermis with underlying fibrocollagenous dermal stroma containing sebaceous glands. No areas of inflammation or increased fibrosis are seen. NORMAL SKIN.

Plate 4: Photomicrographs of ovary, skin and liver of rats treated with Soulmate cream (group 3) and Pure Gold cream (group 4) showing normal tissues (x400).



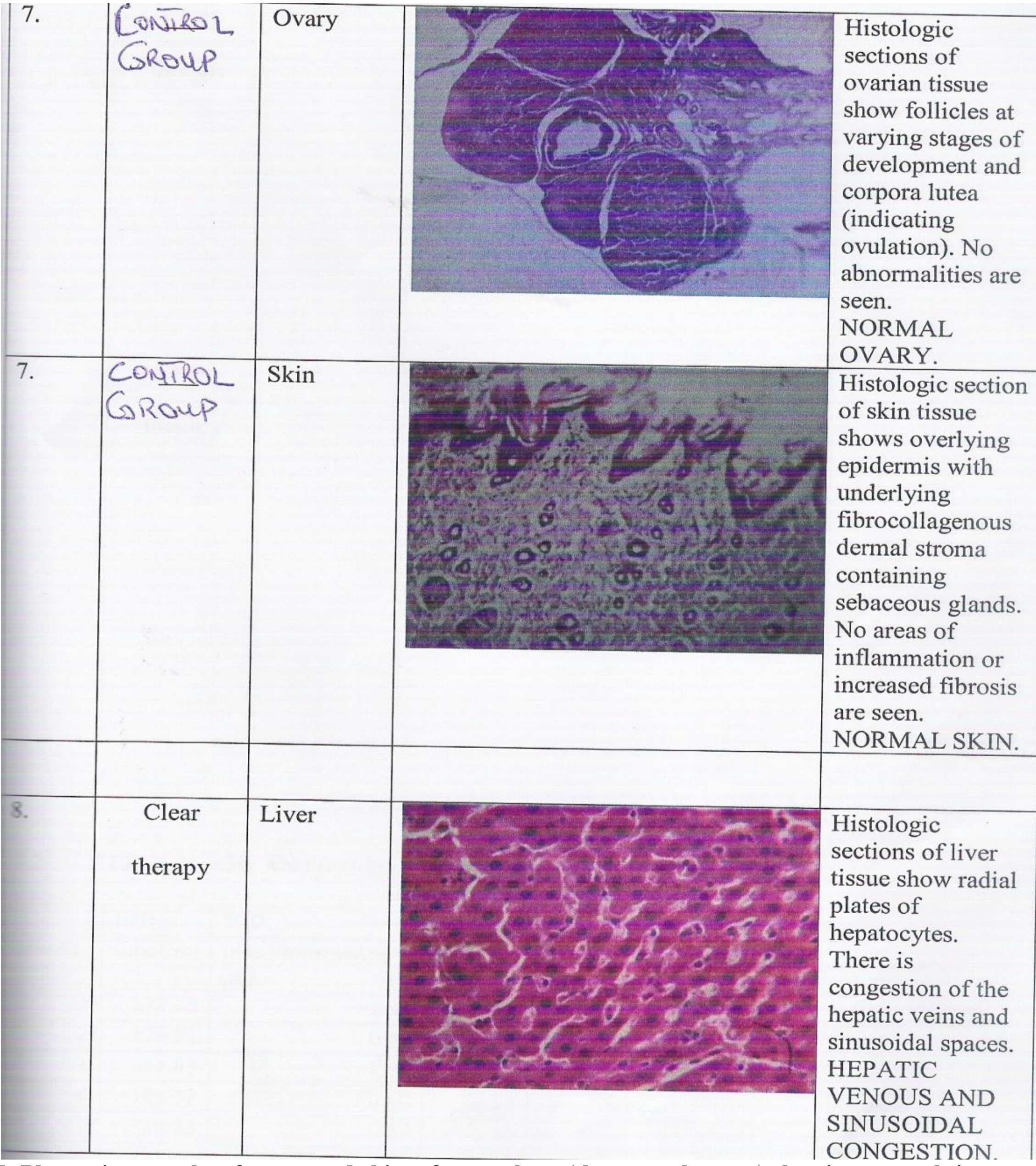
5.	Caro white	Liver		Histologic sections of liver tissue show radial plates of hepatocytes. No cytoplasmic fat vacuoles or areas of necrosis are seen. NORMAL LIVER.
5.	Caro white	Ovary		Histologic sections of ovarian tissue show only corpora lutea (no follicles seen). No other abnormalities are seen. No liver tissue is seen. NORMAL OVARY.
5.	Caro white	Skin		Histologic section of tissue shows only skeletal muscle fascicles and adjacent fatty stroma. No skin tissue is seen. MUSCLE AND FATTY TISSUE (NO SKIN SEEN).

Plate 5: Photomicrographs of liver, skin and ovary of rats treated with Caro White cream showing normal liver and ovary, but skeletal fascicles and adjacent fatty stroma for skin (x400).



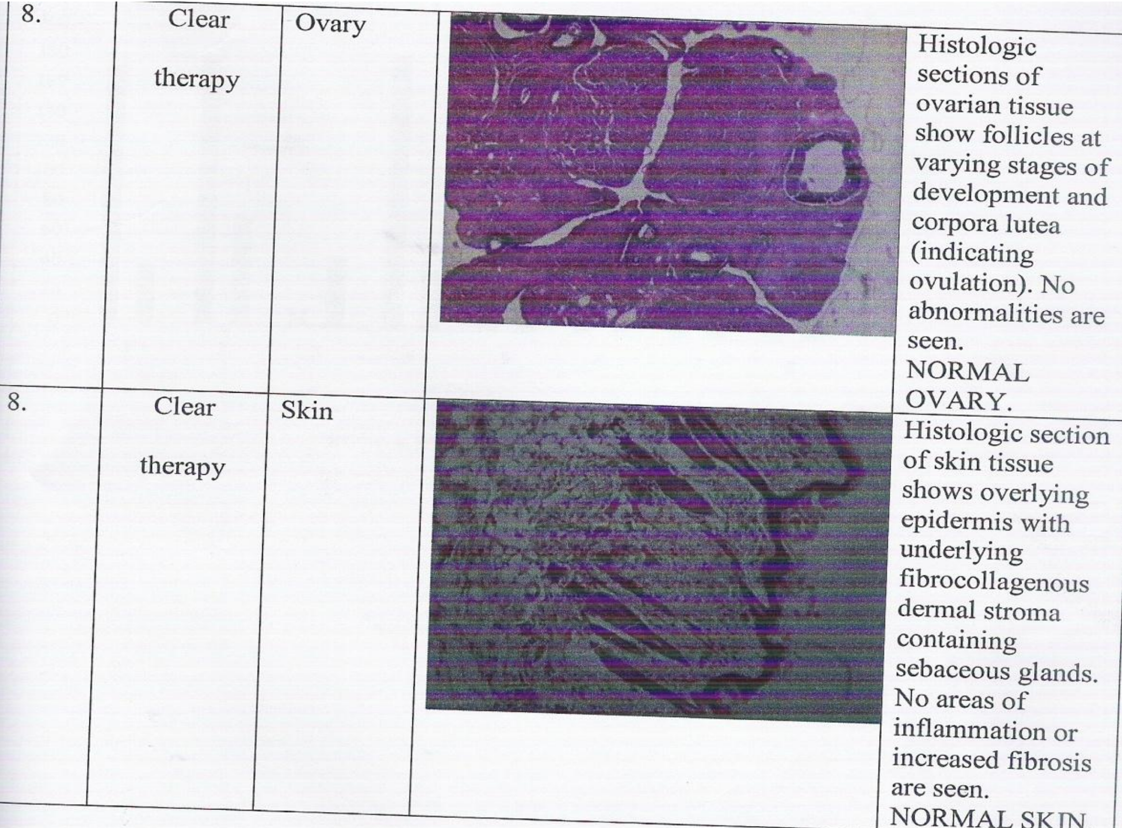
6.	Carotone	Liver		Histologic sections of liver tissue show radial plates of hepatocytes. No cytoplasmic fat vacuoles or areas of necrosis are seen. NORMAL LIVER.
6.	Carotone	Ovary		Histologic sections of ovarian tissue show follicles at varying stages of development and corpora lutea (indicating ovulation). No abnormalities are seen. NORMAL OVARY.
7.	CONTROL GROUP	Liver		Histologic sections of liver tissue show radial plates of hepatocytes. No cytoplasmic fat vacuoles or areas of necrosis are seen. NORMAL LIVER.

Plate 6: Photomicrographs of skin and ovary of rats treated with Carotone cream (group 6) and the liver of the control group, showing normal tissues (x400).



7.	CONTROL GROUP	Ovary		Histologic sections of ovarian tissue show follicles at varying stages of development and corpora lutea (indicating ovulation). No abnormalities are seen. NORMAL OVARY.
7.	CONTROL GROUP	Skin		Histologic section of skin tissue shows overlying epidermis with underlying fibrocollagenous dermal stroma containing sebaceous glands. No areas of inflammation or increased fibrosis are seen. NORMAL SKIN.
8.	Clear therapy	Liver		Histologic sections of liver tissue show radial plates of hepatocytes. There is congestion of the hepatic veins and sinusoidal spaces. HEPATIC VENOUS AND SINUSOIDAL CONGESTION.

Plate 7: Photomicrographs of ovary and skin of normal rat (the control group) showing normal tissues, and the liver of rat treated with Clear Therapy cream, showing hepatic venous and sinusoidal congestion (x400).



8.	Clear therapy	Ovary		Histologic sections of ovarian tissue show follicles at varying stages of development and corpora lutea (indicating ovulation). No abnormalities are seen. NORMAL OVARY.
8.	Clear therapy	Skin		Histologic section of skin tissue shows overlying epidermis with underlying fibrocollagenous dermal stroma containing sebaceous glands. No areas of inflammation or increased fibrosis are seen. NORMAL SKIN.

Plate 8: Photomicrographs of ovary and skin of rats treated with Clear Therapy cream showing normal tissues (x400).

DISCUSSION

The serum mercury concentration ranged from 0.07mg/ml - 0.11mg/ml in all the rats applied skin lightening creams, while arsenic concentration ranged from 0.06mg/ml to 0.12mg/ml. Mercury, arsenic and lead were absent in the serum of group 5 (Caro White group). All other groups of rats contained significantly high levels of arsenic and mercury in comparison with the control group. These levels are high and toxic even to the rats. In a human being of 70kg, mercury, exposure should not be more than 1.0mg/g maximum limit set by the United States Food and Drug Administration (USFDA, 2009). Weldon *et al* (2000) together with Dyall-Smith and Scurry (1990), reported mercury poisoning which was associated with the use of beauty creams.

In Ghana, Agorku *et al* (2016), reported concentrations of mercury as ranging from (0.001 to 0.327µg/g). These levels were lower than the USFDA maximum limit of 1.0µg/g. Similar results were reported by Voegborlo *et al* in 2008. Previous studies by Al-Saleh and Al-Doush (1997), reported high mercury concentrations in creams obtained from the Saudi Arabian market.

In Tanzania, Kinabo in 2003, reported high concentration of mercury in creams above the USFDA limit. The concentration of mercury ranged from 0.16-25.30µg/g in cosmetic creams. Chakera *et al,* (2011) reported 2 cases of biopsy-proven membrane glomerulonephitis (MGN) associated with elevated serum and urine mercury levels in females who used skin lightning creams containing mercury. In the first case, the patient had impaired renal function and proteinuria. The second patient had heavy proteinuria, periorbital oedema and nephrotic syndrome. Both patients had high levels of serum and urine mercury levels and they both confirmed to have used skin lightning creams. Soo *et al* (2003), reported nephritic syndrome in a woman that used a skin lightening cream. The rats used in this study had high concentrations of proteins greater than the control group with the exception of Group 1 (Clinic Clear group). They also have high levels of serum mercury and arsenic levels. This is in accordance with Chakera *et al* studies in 2011.MGN has been associated with morbidity and about 30% of the patients who have it, develops progressive renal impairment (Chakera *et al*, 2011).

From this present study, histological examinations of the liver and skin tissue of rats exposed to skin lightening creams showed only skeletal muscle fascicles and adjacent fatty stoma in group 5 skin (Caro White group). No skin was seen at all. This indicates that the normal skin has been damaged. Mahe *et al* (2013), reported skin diseases associated with the use of bleaching products in women from Dakar, Senegal. Histological examination also revealed congestion of hepatic veins and sinusoidal spaces in the liver tissue of group 7 (Clear Therapy cream group). The liver and skins of the other groups remained normal after histopathological examinations.

Currently, there is little or no literature available on effects of skin lightning creams on liver histology.

The application of skin lightening creams to these rats also led to the deposition of high levels of cadmium and chromium in the liver, heart and kidneys of the rats in comparison with the control. Chromium ranged from 0.07mglml to 0.22mglml while cadmium raged from 0.03mg/ml to 0.26mg/ml. The highest cadmium level was found in group 6 liver (Carotone group) while the lowest cadmium level was found in the group 3 and group 4 kidney. The highest chromium level was found in group 3 liver (Soul Mate group) while the lowest level was found in group 4 liver (Pure White Gold group). Lead was not detected in all the samples analyzed. Significantly high nickel concentrations were found in the hearts of groups 4 and 5 rats.

Li *et a*l (2010) reported membranous nephropathy in people exposed to mercury over a duration of 2-60 months. The urinary mercury concentrations in the study were 1.5-50 times higher than the reference values. The patients showed proteinuria and some had nephrotic syndrome. Light microscopy revealed thickened glomerular basement membrane and mildly proliferative mesangial cells. Acute tubulointerstitial injury occurred in 3 patients. Mercury has been showed to lead to toxic effects in kidneys, nerves and gastrointestinal tracts. Exposure causes acute and chronic renal lesions, while long term exposure leads to membranous nephropathy.

Effect on protein levels

All the rats in all the groups (with the exception of group 1: Clinic Clear group) had significantly elevated levels of protein in the serum in comparison with the control group. Protein levels ranged from 41.54-73.46mg/ml. High levels of protein in the blood could be as a result of inflammation, infection, cancer, dehydration, chronic kidney disease or chronic liver disease. Mercury poisoning has been associated with elevated levels of protein in the urine (Chan, 2011).

EFFECTS ON ANTIOXIDANTS

Effect on SOD activity

Exposure of the rats to skin lightning creams, led to elevated levels of SOD concentration in groups 1, 3, 4 and 5, in comparison with the control. Activities of SOD ranged from 0.13 – 2.82µmol/min/mg. The highest level of SOD activity in the serum was found in group 3 (Soul Mate group). Increase in SOD activities in the brain has been reported in rats fed with diet containing permethrin (Otitoju *et al*, 2008). SOD activities in the brain increased significantly in all the experimental groups when compared with the control group. They also found out that the activity of SOD was age and concentration dependent. The high levels of SOD found in the serum of experimental rats used in this study is an indication of oxidative stress.

Effect on catalase activity

In all the groups of the experimental rats, catalase activity in the serum, increased significantly ($p>0.05$). High levels of mercury and arsenic caused significant increase in the reactive oxygen species (ROS levels) in the serum of the rats accompanied by an increase in SOD and catalase activities. The study compares favorably with a study in 2009 by Patlolla *et al*, which found high levels of SOD and catalase activities in rat exposed to hexavalent chromium.

Effect on malondialdehyde activity (MDA)

In the rats exposure to skin lightening creams, groups 1, 2, 4 and 6 had high levels of MDA in comparison to the control group. Elevated MDA contents in liver and kidney has been reported in rats exposed to hexavalent chromium (Patlolla *et al*, 2009).

Effect on reduced glutathione levels

Four groups of the rats (groups 1, 2, 4, and 6) showed significantly high levels of reduced glutathione in the serum of the rats exposed to mercury and arsenic in the skin lightening creams. Exposure of the rats to some skin lightening creams led to high levels of mercury and arsenic in the blood of the exposed rats. This caused significant increase in the levels of reactive oxidative species in the serum, accompanied by increases in the reduced glutathione levels in the rats. From this study, it can be seen that mercury and arsenic poisoning through the application of skin lightening creams induces oxidative stress and the rats, in an attempt to bring about a defense against the mercury and arsenic induced oxidative stress, enhanced their antioxidant enzyme activities. This lead to the increased levels of antioxidant activities measured in the rats' serum. Heavy metals have been showed to generate reactive oxygen species (ROS) (O'Brien *et al,* 2003). Excessive quantities of ROS generated by these reactions can cause injury to cellular proteins, DNA, lipids etc. This can lead to oxidative stress (Nordberg and Arner, 2001).

Antioxidant enzymes are frequently used as market of oxidative stress (Gutteridge, 1995; Gutteridge and Quinlan, 1983). Among these biomarkers, SOD, glutathione peroxidase and catalase are important for the preservation of homeostasis for normal cell function. CAT acts by metabolizing hydrogen peroxide to water and free oxygen (Knight, 1997; Bagchi *et al*, 1997; Bagchi *et al*, 1995). SOD scavenges superoxide and converts it to hydrogen peroxide where it is maintained at a safe concentration by the glutathione system. Glutathione peroxidase protects membrane lipids from oxidative damage (Kantola *et al*, 1988). Catalase activity is expected to rise in response to tissue trauma. The increase in oxidative enzymes is an adaptive response to oxidative stress.

CONCLUSION

The continuous usage of these creams may pose a health threat to the populace since accumulation of mercury and arsenic can cause serious damage to organs in the body.

REFERENCES

1. Agency for Toxic Substances and Diseases Registry (ATSDR). (2002). *Toxicological profile for mercury.* United States: US Department of Health and Human Services.
2. Agorku ES, Kwaans-Ansah EE and Opoku F. (2016). Mercury and hydroquinone content of skin toning creams and cosmetic soaps and the potential risks to the health of Ghanian women. *Springerplus*, 5: 319-330.
3. Al-Saleh I and Al-Doush I. (1997). Mercury content in skin lightening creams and potential hazards to the health of Saudi women. *J. Toxicol Environ Health, 51*: 123-130.
4. Amponsah D, Sebiawu GE and Vooegborlo R. (2014). Determination of amount of mercury in some selected skin lightening creams sold in the Ghanaian Markets. *Int. J. Eng. Res. Technol*, 3(6): 344-350.
5. Bagchi D, Hassoun EA, Bagchi M, Muldon D, Stohs SJ. (1995). Oxidative stress induced by chronic administration of sodium dichromate (CrVI) to rats. *Comp Biochem Physiol*, 110C: 281-287.
6. Bagchi D, Vuchetich PJ, Bagchi M, Hassoun EA, Tran MX, Tang L, Stohs SJ. (1997). Induction of oxidative stress by chronic administration of sodium dichromate (chromium VI) and cadmium chloride (cadmium II) to rats. *Free Rad Biol Med*, 22: 471-478.
7. Bogdanovic M, Janeva AB and Bulat P. (2008). Histopathological changes in rat liver after a single high dose of aluminium. *Arch. Industrial Hygiene Toxicol, 59:* 97-101.
8. Chakera A, Lasserson D, Beck LH, Roberts ISD and Winearls CG. (2011). Membranous nephropathy after use of UK manufactured skin creams containing mercury. *An International J. of Medicine*, 104(10): 893-896.
9. Chan TY. (2011). Inorganic mercury poisoning associated with skin lightening cosmetic products. *Clin. Toxicol. (Phila), 49*(10): 886-891.
10. Dyall -Smith DJ and Scurry JP. (1990). Mercury pigmentation and high mercury levels from the use of a cosmetic cream. *Med. J. Aust, 153*: 409-415.
11. Gutteidge JMC and Quinlan GJ. (1983). Malondialdehyde formation from lipid peroxides in thiobarbituric acid test. The role of lipid radicals, iron salts and metal chelator. *J. Appl Biochem*, 5: 293-299.
12. Gutteridge JMC. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin. Chem, 41*, 1819-1828.
13. Harada M, Nakachi S and Tasaka K. (2001). Wide use of skin lightening soaps may cause mercury poisoning in Kenya. *Sci. Total Environ*, 269(1-3): 183-187.
14. Hunter ML. (2011). Buying racial capital: Skin - bleaching and cosmetic surgery in a globalized world. *Journal of Pan African Studies*, 4(10): 142-164.
15. Kakkar PS, Das B and Viswanathan PN. (1984). A modified spectrophotometric assay of superoxide dimutase. *Ind. J. Biochem. Biophys. , 21* (2), 130-132.
16. Kantola M, Sarranen M and Vanha PT. (1988). Selenium and glutathione peroxidase in seminal plasma of men and bulls. *J. Reprod. Fertil, 83*: 785-794.
17. Kinabo LD. (2003). Comparative analysis of mercury in human hair and cosmetic products used in Dar es Salam, Tanzania. *J. Pharm. Pharmacol, 2*: 23-45.
18. Knight JA. (1997). Reactive oxygen species and the neuro-degenerative disorders. *Ann. Clin. Lab. Sci, 27*: 11-25.
19. Li SJ, Zhang SH, Chan HP, Zeng CH, Zeng CX, Li LS et al. (2010). Mercury induced membranous nephropathy: Clinical and pathological features. *Clinical J. Am. Society of Nephrology*, 5: 439-444.
20. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem, 193*(1): 265-275.
21. Mahe A, Ly F and Gounongbe A. (2004). The cosmetic use of bleaching products in Dakar, Senegal: Socio-econimic factors and claimed motivations. *Sciences Sociales Et Sante*, 22(2): 5-33.
22. Mahe A, Ly F, Aymard G and Dangou JM. (2013). Skin diseases associated with cosmetic use of bleaching products in women from Dakar, Senegal. *British J of Dermatology*, 493-500.
23. Niehaus WG and Samuelsson B. (1968). Formation of malonaldehyde from phospholipid arachidonate during microsomal liupid peroxidation. *European J. of Biochemistry, 6*, 126-130.
24. Nordberg J and Arner ESJ. (2001). Reactive oxygen species, antioxidants and the mammalian thioredoxin system. *Free Radical Biology and Medicine, 31*: 1287-1312.
25. O'Brien TJ, Ceryak S and Patierno SR. (2003). Complexities of chromium carcinogenesis: Role of cellular response, repair and recovery mechanisms. *Mutat. Res*, 533: 3-36.
26. Otitoju O, Onwurah INE, Otitoju GTO and Ugwu CE. (2008). Oxidative stress and SOD activity in brain of rats fed with diet containing permethrin. *Biokemistri, 20*(2): 93-98.
27. Patolla AK, Barnes C, Yedjou C, Velma VR and Tchounwo PB. (2009). Oxidative stress, DNA damage and antioxidant enzyme activity induced by hexavalent Cr in Sprague-Dawley rats. *Environmental Toxicology, 24*(1): 66-73.
28. Perry I. (2006). Buying White beauty. *Cardozo J. Law Gend*, 12: 579-607.

29. Sah RC. (2012). Poisonous cosmeticxs, the problem of mercury in skin lightening creams in Nepal, Kathmandu. C*EPHED.* Technical Report.
30. Sedlak J and Lindsay RH. (1968). Estimation of total protein-bound and non protein sulfhydryl groups in tissue with Ellman'sreagent. *Analytical Biochemistry, 25:* 1192-1205.
31. Sinha AK. (1972). Colorimetric assay of catalase. *Analytical Biochemiistry, 47:* 389-394.
32. Soo YO, Chow KM, Lam CW, Lai FM, Szetoc CC, Chan MH et al. (2003). A whitened face woman with nephrotic syndrome. *Am. J. Kidney Dis*, 41(1): 250-253.
33. Tlacuilo-Parra A, Guevara-Gutierrez E and Luna-Encinas JA. (2001). Percutaneous mercury poisoing with a beauty cream in Mexico. *J. Am. Acad. Dermatol*, 45: 966-967.
34. United States Environmental Protection Agency (USEPA). (2002). *Mercury study report to Congress.* United States: USEPA.
35. United States Food and Drug Administration. (2009, May 21). Supporting information for toxicological evaluation. *National Toxicology Program*.
36. Voegborlo RB, Agorku ES, Buabeng-Acheampong B and Zogli E. (2008). Total mercury content of skin toning creams and the potential risk to the health of women in Ghana. *J. Sci. Technol*, 28(1): 88-94.
37. Weldon MM, Smolinski MS, Maroufi A, Hasty BW, Gilliss DL, Boulanger LL, Balluz LS and Dutton RJ. (2000). Mercury poisoning associated with a Mexican beauty cream. *West. J. Med*, 173: 15-18.
38. Zogli. (2008). Total mercury content of skin toning creams and the potential risks to the health of women in Ghana. *J. Sci. Technol*, 28(1): 88-94.