ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2023; 8(1): 14 - 22

SJMLS - 8(1) - 002

Some Histological and Biochemical Evaluation on Commercial Hair Dyes

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Abstract
The use of hair dyes as a means of beautification is currently increasing. This study aimed at evaluating the toxicity of different commercial hair dyes on the histology of the skin and biochemical parameters of the liver of Albino rats. This study was carried out in the Pathology Department, University of Calabar Teaching Hospital Calabar and College of Medical Sciences, University of Calabar, Calabar between May, 2019 and October, 2019. Four different colours of hair dyes were obtained from a cosmetic store in Watt daily market in Calabar, Cross River State. Twenty male Albino rats with average weight of 55g were used for this study. The rats were randomly divided into five (5) groups of four (4) rats each. Group 1 to 4 served as the test groups while group 5 served as the control group. Different colours of hair dye were applied topically on selected area of the back of Albino rats daily for 30 days after which, the rats were sacrificed using chloroform inhalation procedure. The skin of the rat was harvested for histology while heart puncture was used to collect 5 milliliters of blood from each rat for biochemical analysis. Plasma Alkaline phosphatase (ALP) was determined using Kind and King's method. Plasma Alanine amino transferase (ALT) and Aspartate Amino transferase (AST) were determined by Reitman and Frankel method. Serum protein (TP) was determined by Biurette's method while bilirubin was estimated using Powell's method. Liver tissues were processed using routine paraffin wax tissue processing method for histological analysis. The serum levels of ALP, AST, TP, Total bilirubin (TB) and conjugated bilirubin (CB) in all the test groups were significantly increased when compared with the control (P=0.001). Histological evaluations indicated evidence of cellular injury in all the test groups. Prolonged use of commercial hair dyes caused adverse effects to the skin and liver as seen in the Albino rat used in this study.

Key words: Hair dyes, inflammation, keratinocytes

Introduction

A dye is a coloured substance that chemically binds to the substrate to which it is being applied (Kumar *et al*., 2021). Dyes are applied in an aqueous solution and usually need mordants to facilitate affinity for the material the substance are applied on. In the ancient times, all the dyes used were natural. Such dyes include Indigo and Alizarin (Luque *et al.,*2011). Dyes used in those early days were obtained from vegetable, mineral and animal sources (Kumar *et al.,* 2021). In recent years, the dye formulations developed are synthetic mostly made from petrochemical products (Booth, 2000). Although hair dying has been in existence for a long time, the use and application of hair dye in recent time has seen a marked increase. Dying of hairs is cosmetic procedure globally which is commonly practiced by individuals for different reasons irrespective of creed, age and gender (Rehman, *et al.,* 2019). Permanent hair dyes, semi-permanent hair dyes, demi-permanent hair colors and temporary hair color are the various hair dyes that are in use (Bryan, 2017; Rehman, *et al.,* 2019) Permanent hair dyes are the most important group of hair dyes and consist of two components; a developer

or oxidizing agent and ammonia (an alkalizing agent) that are mixed before use to generate the dye within the hair by chemical reactions (Soni, 2009). Temporary hair color comes in various forms which includes gels, foams, sprays, rinses, and shampoos, Temporary hair colors give brighter and more vibrant appearance then other types of hair dyes and they typically do not last for more than 24 hours (Rehman *et al*., 2019). Semi-permanent hair dyes contain basic or cationic dyes with low molar mass which has a high affinity for hair keratin. The hair dyeing process does not involve oxidation reaction, but it may contain a toxic constituent such as P-phenylenediamine or other agents (Rehman *et al*., 2019).

The major constituents of commercial hair dyes are hydrogen peroxide, ammonia and p-phenylenediamine or a similar agent called **toluene-2,5-diamine** (Materials Research Laboratory, 2021). Hydrogen peroxide is known as developer or oxidizing agent. It comes in varying forms and strengths. It initiates the colour forming process of the dye and creates lasting colour. It removes Sulphur from the hair. Hair tends to harden and lose weight due to the presence of Sulphur in hair dyes (Brain, 2000). Ammonia is an alkaline medium which enhances lightening of hair when it combines with hydrogen peroxide. Ammonia separates cuticle, allowing the hair dye to penetrate the cortex of the hair. Paraphenylenediamine (PPD) is the main colouring agent in hair dyes. PPD is found in almost all currently available permanent hair dyes and in some so-called natural dyes **(Katta, 2022**). Hair dyes also contain coupler, which are aniline derivatives. Coupling agents define the hair dye final colour (*Murata, et al*., 2006). These couplers are not coloured compounds but combines with the PPD and oxidizing agent to give different coloration.

Studies have reported that hair dying is associated with several adverse effects. MADESAFE (2019) reported that short term exposure to hair dyes containing PPD could lead to asthma, skin and eye, vertigo, convulsions, and coma. While long term exposure may cause toxic effects on the heart, liver, kidney (Al-Shaikh *et al.,* 2018) and on the neurons (MADESAFE, 2019). All the previous studies on the adverse effects of hair dyes were done using oral and subcutaneous exposure of hair dyes. Also no work has been done on the effect of hair dye on the histology of the skin. Hair dyes, when being applied topically, maybe absorbed through the skin to the blood. From the blood, the hair dye constituents may circulate up to the liver. Therefore, in this study, an attempt was made to evaluate the adverse effect of different hair dye colors on the histology of the skin and some biochemical functions of the liver using Albino rat model.

Materials and methods

Study Area

This study was carried in the College of Medical Sciences, University of Calabar and University of Calabar Teaching Hospital, Calabar, Nigeria between May, 2019 and October, 2019.

Hair Dye

A total of 4 colours of hair dyes were used in this study. The colours of the dyes used include; gold, ash, red and brown. The dyes were purchased from a popular market in Calabar called Watt market, Calabar Road, Cross River State.

Albino rats

A total of twenty (20) adult Albino rats average weight of 55g were used in this study. They were purchased from the Animal unit of the Department of Physiology, University of Calabar. They were housed in individual cages with steel net as its cover. These cages were covered with a welded mesh to allow proper ventilation. and were allowed free access to food (standard pellet diet) and water ad libitum. The animals were acclimatized to laboratory conditions for one week prior to the commencement of experiment which lasted for 30 days.

Ethical Clearance

Ethical approval for the use and sacrifice of experimental animals for research was obtained from the Ethical Committees in the Faculty of Basic Medical Sciences, College of Medical Sciences University of Calabar and that of the College of Medical Sciences, University of Calabar, Calabar, Cross River State.

Study design
The animals were randomly divided into five experimental groups designated Group 1, Group 2, Group 3, Group 4 and Group 5 serving as the control group. Each group comprised of four albino rats. Groups 1, 2, 3 and 4 were treated with the gold, ash, red and brown dyes respectively. The dyes were applied on a specific marked area on the back skin of the Albino rats. The animals were treated for a period of 30 days.

Dye administration
The different hair dye colors were applied topically on the selected areas on the back side of the Albino rats as shown in the table below:

Table 1.0: Summary of dye administration

Group1	Group2	Group3	Group4	Group5 (Control)
Gold dye	Ash dye	Red dye	Brown dye	No dye applied
Rat1	Rat1	Rat1	Rat1	Rat1
Rat2	Rat2	Rat2	Rat2	Rat2
Rat3	Rat3	Rat3	Rat3	Rat3
Rat4	Rat4	Rat4	Rat4	Rat4

Sacrifice of the Albino rats and sample collection
The experimental rats were sacrificed after 30 days of the experiment using the chloroform inhalation procedure. The skin of the experimental rats were harvested and fixed in 10% neutral buffer formal saline before processing. Five (5) milliliters of blood samples were collected by means of cardiac puncture into lithium heparin bottle for evaluation of, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels. Also analyzed for were Total protein (TP), Albumin (ALB), Total bilirubin (TB) and Conjugated bilirubin (CB). The blood specimens were spun at 4500 rpm for 10 minutes to obtain plasma which was transferred into another sets of labeled plain bottles and stored at -4ºC. After 24 hours of fixation, the liver tissues were processed immediately via dehydration of tissue in ascending concentrations of alcohol, cleared in xylene, and infiltrated with paraffin wax before embedding. Sections were cut and mounted on slides and stained with Hematoxylin and Eosin staining technique.

Sample analysis.
Sample analysis
The Histology samples were processed using routine manual paraffin wax tissue processing method and sectioned with a rotary microtome at 3µm. Tissue sections were stained with Haematoxylin and Eosin staining method. The stained slides were studied under the light microscope using x10 and x40 magnifications. The laboratory analysis of Alkaline phosphatase (ALP) was determined using spectrophotometer as described byKing Armstrong's method. Plasma Alanine amino transferase (ALT) and Aspartate Amino Transferase (AST) were also determined with spectrophotometer as described by Reitman and Frankel. Total protein and Total albumin was carried out using Biurette method. Total and conjugated bilirubin (TB, CB respectively) were estimated using H. Powell's method.

Statistical analysis. Mean values of AST, ALT, ALP, TP, ALB, TB and CB of the test and control groups were compared using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Results were considered significant at $P < 0.05$.

Results
As seen in table 2.0, the serum ALP and AST of all the test groups were significantly increased ($p<0.05$) when compared to the control group. There was no significant difference between the serum ALT of test groups when compared to the control group($p>0.05$). As seen in table 3.0, the serum TP, TB and UB of test groups were significantly increased when compared to the control group ($p<0.05$). There was no difference between the serum AB of test groups when compared to the control group ($p>0.05$).

Table 2.0: Serum ALP and AST values

Parameter (iu/l)	Groups					F-ratio	P value
	Control	1	2	3	4		
ALP	22.30±0.64	63.93±3.51	24.73±0.85	63.25±1.77	40.33±1.66	419.24	**0.001**
AST	62.25±1.71	65.25±2.50	67.75±4.57	62.50±5.20	68.00±2.94	31.203	**0.001**
ALT	19.50±7.59	21.25±2.22	27.50±5.92	25.50±5.69	25.25±2.22	1.620	**0.221**

Table 3.0: Serum TP, TB and CB values

Parameter (iu/l)	Groups					F-ratio	P value
	Control	1	2	3	4		
TP	5.98± 0.17	7.55±0.47	5.95±0.13	6.84±0.89	7.35±0.37	9.522	**0.001**
AB	3.80±0.29	3.80±0.18	3.85±0.26	4.58±1.03	3.60±0.39	2.009	**0.145**
TB	4.15±0.13	4.28±0.22	4.53±0.52	4.45±0.57	8.43±0.97	41.589	**0.001**
CB	1.43±0.13	1.50 ±0.34	1.48±0.24	1.40± 0.45	4.88±1.02	36.726	**0.001**

The histology of skin of control group.
The section of the skin shows the epidermis and dermis with its skin adnaxae structures which consists of the hair follicles, adipocytes and sebaceous glands. The epidermis is thin and intact, and the hair follicles are prominent. Bundles of irregularly arranged collagenous fibres. were noticed as shown in figure 1.

The effect of Gold coloured hair dye on the skin (group 2)
The section of the skin shows the epidermis and dermis with its skin adnaxae structures which consists of the hair follicles, adipocytes and sebaceous glands. The epidermis shows mild acanthosis. The hair follicles are prominent with mild hyperplasia of the lining of outer layer of the follicular cells. The dermis consists of bundles of irregularly arranged collagenous fibres. There was mild cellular injury as seen in figure 2.

The effect of Ash coloured hair dye on the skin (group 3)
The section of the skin shows the epidermis and dermis with its skin adnaxae structures which consists of the hair follicles, adipocytes and sebaceous glands. The epidermis shows mild erosion and it is thinned out. The hair follicles are prominent with mild hyperplasia of the lining of outer layer of the follicular cells. The dermis consists bundles of irregularly arranged collagenous fibres. There is mild cellular injury as seen in figure 3.

The effect of red coloured hair dye on the skin (group 4)
The section of the skin shows the epidermis and dermis with its skin adnaxae structures which consists of the hair follicle, adipocytes and sebaceous glands. The epidermis shows mild hyperkeratosis and acanthosis with vacuolation of the keratinocytes. The hair follicles are prominent. The dermis consists of thick bundles

of irregularly arranged collagenous fibres. There is scanty haemorrhage and sparse inflammatory cells within the dermis. There is moderate cellular injury as seen in figure 4.

The effect of brown coloured hair dye on the skin (group 5)

The section of the skin shows the epidermis and dermis with its skin adnaxae structures which consists of the hair follicles, adipocytes and sebaceous glands. The epidermis shows mild acanthosis with destruction of the skin adnaxae structures. There is moderate cellular injury as seen in figure 5.

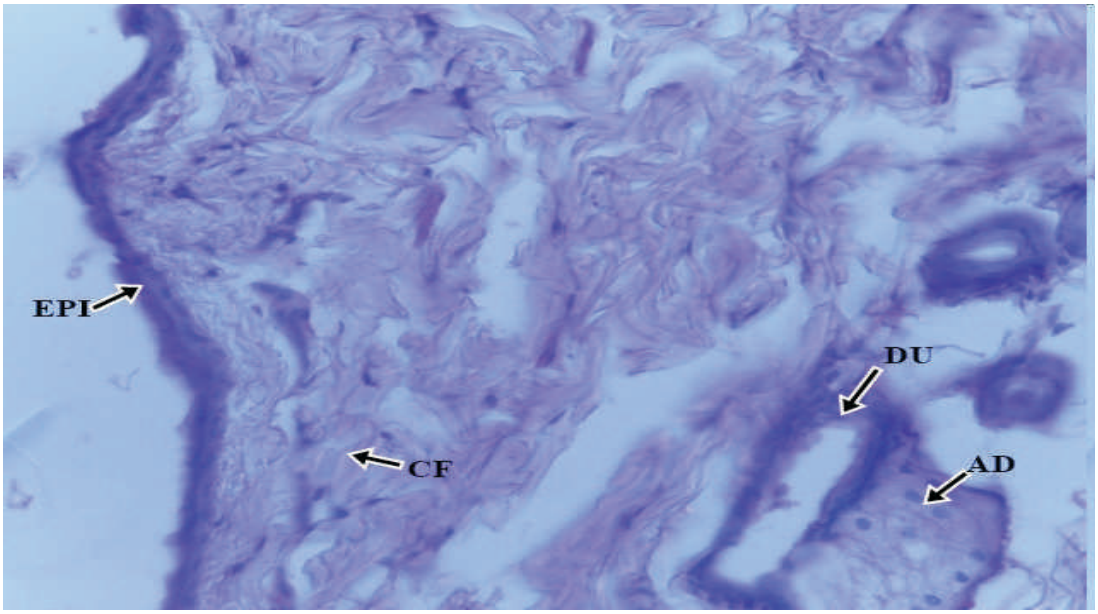

Figure 1 – Skin section stained with Haematoxylin and Eosin. Control group. X400 EPI, Epidermis; DU, Ducts; CF, Collagenous fibres; AD, Adipocytes.

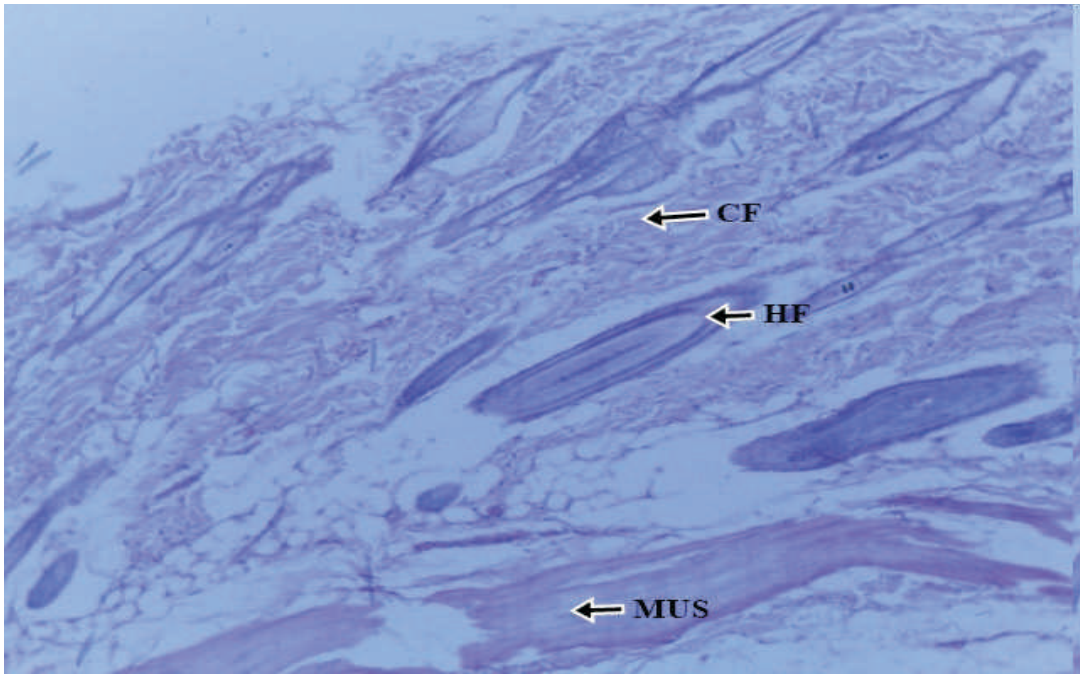

Figure 2 – Skin section stained with Haematoxylin and Eosin. Group 1. X400. CF, Collagen fibres; MUS, Muscle; HF, Hair follicle.

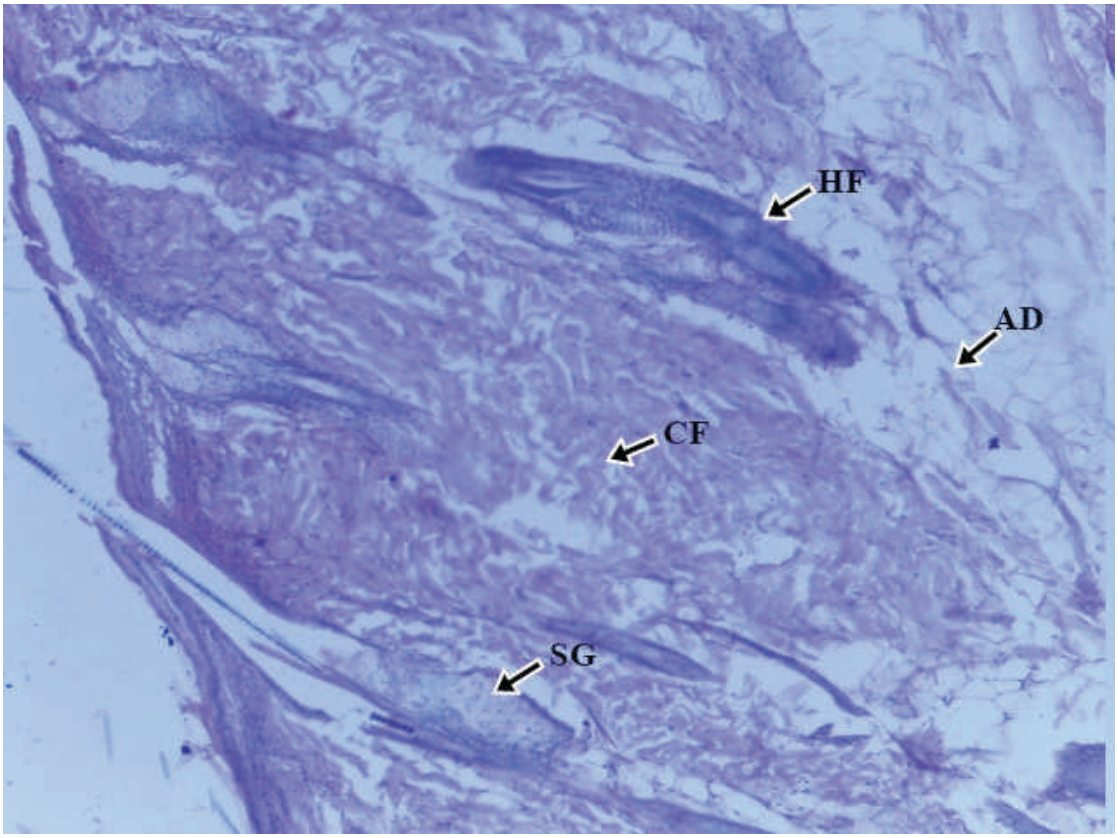

Figure 3 – Skin section stained with Haematoxylin and Eosin. X400
HF, Hair follicle; SG, Sebaceous gland; CF, Collagenous fibres; AD, Adipocytes.

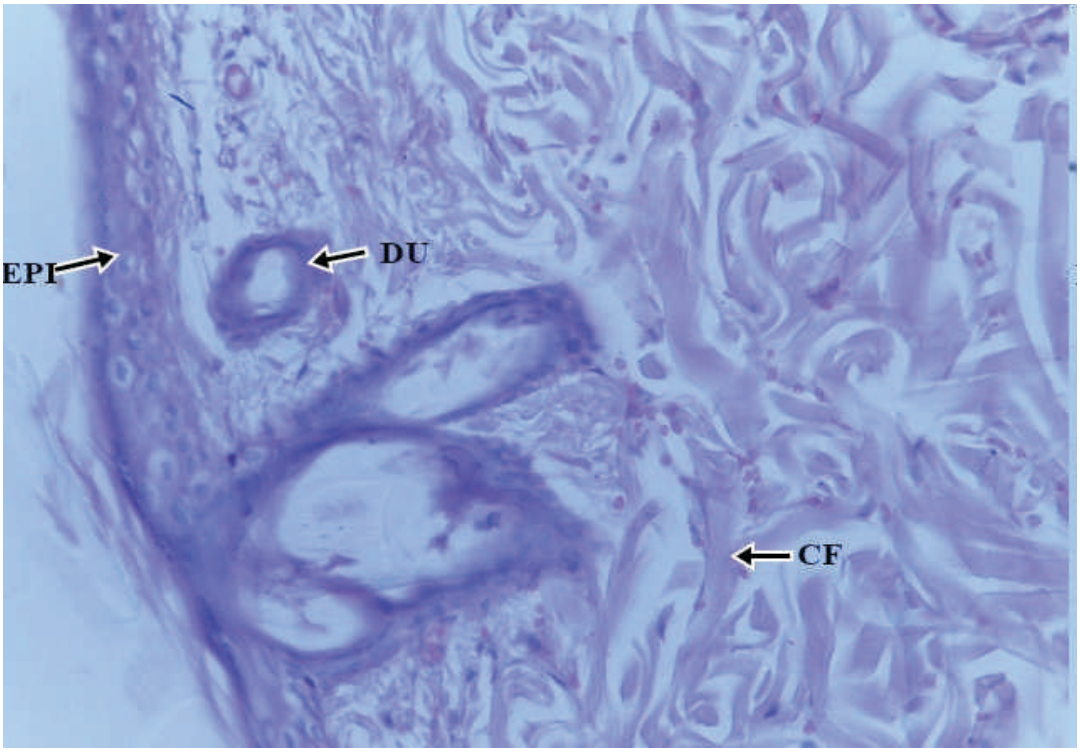

Figure 4 –Skin section stained with Haematoxylin and Eosin. X400
EPI , Epidermis; CF, Collagenfibres, DU; Ducts

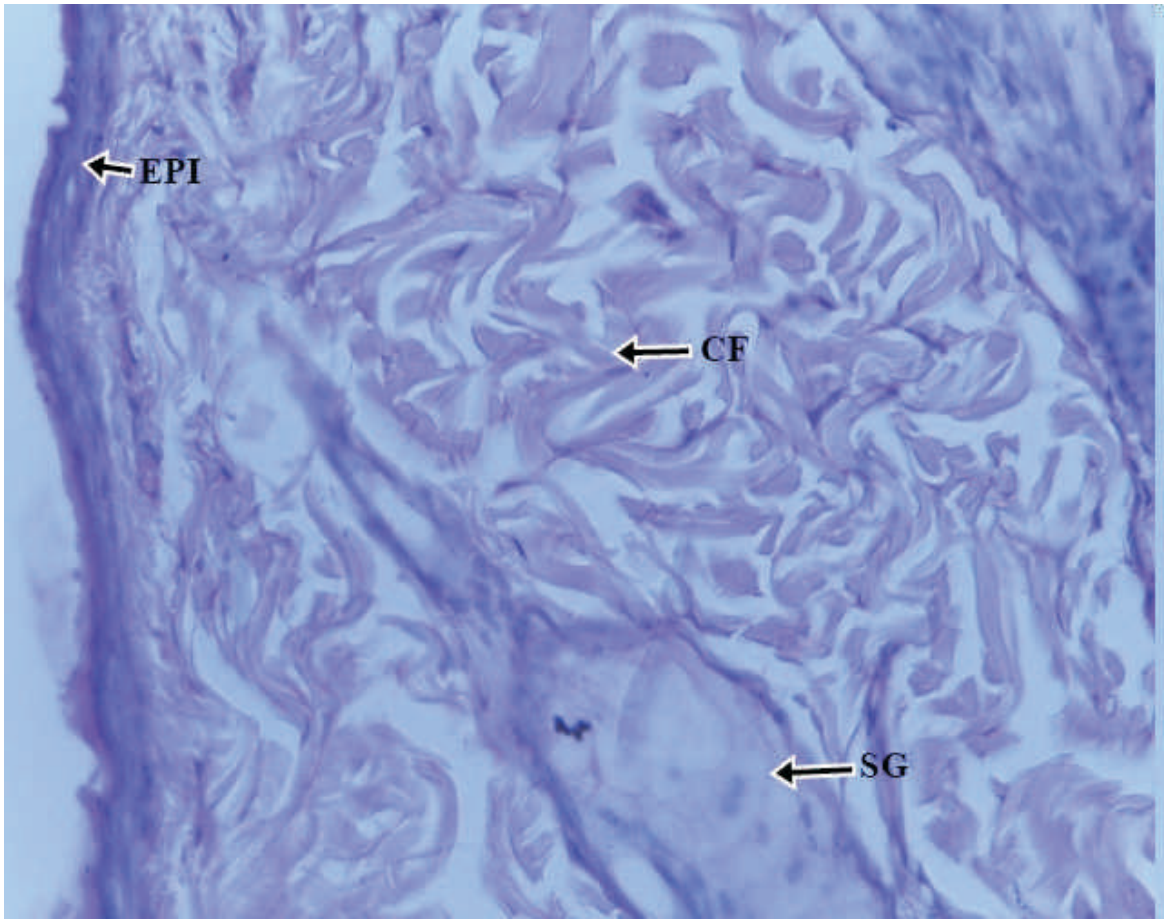

Figure 5 – Skin section stained Haematoxylin and Eosin. X400.
EPI, Epidermis; SG, Sebaceous gland; CF, Collagen fibres.

Discussion

Hair dyeing is an age- long cosmetic procedure practiced globally. The practice cuts across gender, race and age (Bolduc *et al*., 2001; Ahn *et al.,* 2002; Rehman *et al.,* 2018). Studies have suggested various side effects of hair dye which include skin irritation and allergy, liver disease, heart and kidney diseases (Sosted *et al.*, 2004; Bouillon *et al.,* 2005; Amy, 2013; Al-Shaikh *et al.,* 2018). A research done by MADESAFE (2019) also reported asthma, eye, vertigo, convulsions, and coma as some of the side effects of exposure to hair dyes. Singla *et al.* (2005) reported the presence of anaemia, leukocytosis, haemoglobinemia, haemoglobinurea, and liver necrosis after exposure to hair dye. Previous studies have made use of oral and subcutaneous exposure to hair dyes. Sayee (2022) in a different study reported coughing, wheezing, lung inflammation, throat discomfort and asthma attack after exposure to hair dye. In this present study, topical exposure of the hair dyes to selected area the animal was explored.

The result from this study have shown that the concentration of serum Alkaline phosphatase (ALP) and Aspartate Amino transferase of the four test groups were significantly raised ($P<0.05$) when compared with control. This outcome agreed with previous reports (Spector, 1955 and El-Amin *et al.,*2014) which indicated a significantly raised ALP and AST after oral and subcutaneous administration of hair dye in Albino rats. In their studies however, the concentration of serum ALT was significantly increased which disagreed with the report obtained from this present study, where concentration of serum ALT did not show any significant difference. The difference in the outcome of serum ALT in these studies may be attributed to difference in the route of exposure and the concentration of the hair dyes given to the animals.

Hair dyes when applied have the tendency to diffuse into the body through skin pores. The PPD content of the hair is carried by blood to the liver for metabolism. Continuous exposure of the liver increases its workload, leading to damage to the liver cell damage. The concentration of serum total bilirubin and conjugated bilirubin of the test groups were significantly raised in this study ($p<0.05$) when compared with the control group. This outcome is possibly due damaged hepatocytes, impairing the liver metabolic activities one of which is bilirubin conjugation, hence the accumulation of serum conjugated and increased serum total bilirubin. Another reason for significantly raised bilirubin is the presence of haemolysis due to continued exposure to PPD in hair dyes.

There was also a significantly raised concentration of serum total protein among the test groups ($0<0.05$) when compared with that of the control group. This outcome is at variance with report by El-Amin *et al*. (2014) who observed a significantly reduced total protein after oral and subcutaneous exposure of the rats to hair dyes. The reason for significantly raised total protein may be attributed the haemolysis due to the presence of PPD contained in hair dyes and possibly the effect of PPD on the bone marrow after a long exposure.

In this present study, the effect of hair dyes on the histology of the skin of the Albino rat was also evaluated. Conventionally, the hair dyes are applied on the hair. This necessitated topical hair dye applications used in this study. The histology of the animal skin from test group 2 and 3 after application of hair dyes showed prominent hair follicles with mild hyperplasia of the outer layer of the follicular cells, indicating the present of mild cellular injury. In groups 4 and 5, the histology showed epidermis with mild hyperkeratosis and acanthosis with vacuolation of the keratinocytes. There was also the presence of sparse inflammatory cells within the dermis and destruction of skin appendages. These features are consistent with moderate cellular injury. These observations were consistent with in Sosted *et al. (*2004) in Denmark and Bouillon *et al. (*2005) who observed defects on the skin due to continuous use of hair dyes. Bouillon *et al. (*2005) explained that irritation of the skin may occur when the active chemical component of the hair dyes penetrates through the stratum corneum and epidermis into the dermis. Long period of penetration of hair dye chemicals causes changes in the skin barriers which may be influenced by the thickness of the stratum corneum and the duration of exposure. Bouillon *et al*. (2005) also reported the presence of keratinocyte damage, inflammatory and non inflammatory toxic reactions in the histology of skin tissue exposed to hair dyes when they chemicals overcome the skin barriers.

Conclusion

The use of hair dyes as instrument of beautification is currently common. Some of the data obtained from this present study have proved that the way and manner the hair dyes are used should be a cause for concern global. In the olden days, hair dyes were mostly used by elderly people to cover gray hairs. Then another group of people that commonly use hair dyes are celebrities who use them temporarily mostly as costumes. But today, hairs dyes are used indiscriminately, and usage has cut almost all age group and both gender. This study has shown that regular use of synthetic hair dyes is injurious both to the histology of the skin and the biochemical functions of the liver of the Albino rats.

Acknowledgement

The authors acknowledged the invaluable contributions of Stanley Efewangbe, a Principal Medical Laboratory Scientist and Ogar Osim Ogar, a Senior Medical Laboratory Scientist and all Staff of the Histopathology Department, University of Calabar Teaching Hospital, Calabar.

Authors' Contribution

ASO conceptualized the topic. ASO, EIB, EA and AE designed this study. ASO and EIB performed the laboratory work. ASO and AE carried out the data analysis and drafted the manuscript. ASO is the guarantor of the paper.

Competing Interests

Authors have declared that no competing interests exist.

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Citation: Anyanwu, Stanley Obialor, Akwiwu, Euphoria C, Egbe, Agala and Eborty-Ike, Iwaeni Bassey. Some Histological and Biochemical Evaluation on Commercial Hair Dyes. ***Sokoto Journal of Medical Laboratory Science; 8(1): 14- 22.*** https://dx.doi.org/10.4314/sokjmls.v8i1.2.