

Effect of white African mineral hair dye on the activities of phosphatases and malondialdehyde level in selected tissues of albino rats

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Abstract

The effect of white African mineral dye Yombofita (YF) on the activities of alkaline phosphatase (ALP), acid phosphatase (ACP) and malondialdehyde (MDA) levels in the skin, liver, kidney and serum of albino rats was investigated. The chemical analysis of the dye was first carried out using solubility test, pH determination and X-ray fluorescence (XRF) elemental analysis. Six different concentrations (0.05, 0.15, 0.25, 0.5, 0.75 and 1.00%) of the dye were prepared using hydrogen peroxide (30 volume) as solvent. A total of 80 albino rats (*Rattus norvegicus*) were used for the study. The rats were divided into 8 groups of 10 each and were maintained on commercial feed for the period of the experiment i.e. 30 days. In group 1, the control group, the animals were applied distilled water on their heads, whereas in group 2 the vehicle i.e. hydrogen peroxide was applied. In groups 3 to 8 various concentrations of YF (white) dye ranging from 0.05, 0.15, 0.25, 0.5, 0.75 to 1.00% was applied respectively. At the end of the experiment, blood samples were collected and portions of the selected tissues were excised for the determination of ALP and ACP activities. The MDA level was also determined in the skin of experimental animals. The results revealed a significant decrease ($p < 0.05$) with increasing concentration of the dye in the activities of ALP and ACP in the kidney and skin but not in the liver while the level of MDA in the skin increased significantly ($p < 0.05$) with increasing concentration of the dye.

Keywords: Dye, phosphatases, malondialdehyde, tumour, cancer, skin, hydrogen peroxide

Introduction

In Nigeria, the use of hair dye is fast gaining popularity especially among ladies and old men. Young ladies engage in the use of dyes mainly for fashion while old men use dyes to mask

their grey hair. Dyes are basically of two types namely natural dyes and synthetic dyes. Natural dyes include plant dyes, animal and mineral dyes. Plant dyes can be obtained from roots, flowers, leaves, fruits and bark of plants, such as wood and indigo. Some animal sources of dyes include insects, such as cochineal, found on cacti in Mexico, lac found in India and Iran, and kermes, found on oak trees near the Mediterranean. Mineral dyes come from ocher (yellow, brown, red), limestone or lime (white), manganese (black), cinnabar and lead oxide (red) azurite and lapis lazuli (blue), and malachite (green) [1].

The use of synthetic dyes became paramount in the mid-nineteenth century when the demand for handmade rugs increased in the west. The need for easy to use and less expensive dyes with wider range of colours led to the development of synthetic dyes in Europe, especially in Germany. The first synthetic dye, Fuchsine (magenta aniline) was developed in 1850. Other synthetic aniline dyes, such as chrome dyes were later developed.

Hair dyes can be either temporary or permanent dyes with varying chemicals, which may be poisonous to the users. Temporary dyes contain silver (Ag), mercury (Hg), lead (Pb), arsenic (As), bismuth (Bi), pyrogallol, and alcohol while permanent dyes contain naphthylamine, phenylenediamines, toluenediamines, and other aromatic compounds. Most people dye their hair without considering the side effects of the chemical constituents of the dye. The decision to change hair colour has recently become more complicated due to some recent studies, which linked hair colouring with an increased risk of contracting certain cancers [2]. Several other studies on dyes have been carried out by various researchers, which implicated dyes as carcinogens [3–5]. It has also been reported that hair dyes may be hazardous to the health of children due to the presence of lead in the form of lead acetate, as exposure to such lead-containing hair dyes can cause neurological problems [6]. Other symptoms, such as local skin irritation can be associated with toxic substances in the hair dyes [4].

The present study is therefore aimed at exploiting the activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) as well as malondialdehyde (MDA) concentration as possible means of understanding the toxicological effect and the mechanism of action of white African mineral dye (Yombofita, YF), which is commonly used in Nigeria for dyeing hair. The observed decrease in the activities of ALP and ACP in skin and kidney accompanied by an increase in the enzyme activities in the serum suggests cellular damage of these tissues by the dye. The increased level of MDA in skin suggests higher degree of lipid peroxidation.

Materials and methods

Source of the dye

The white rock-like mineral dye (YF) was obtained from Emir's Market in Ilorin, Nigeria. The original source of the dye was traced to the southern part of Ghana.

Solubility test

The solubility of the dye was investigated in 14 different solvents using the method of Annie et al. [7].

Elemental analysis of the dye

The dye was crushed and ground in a Tungsten Carbide Spex Mill followed by other procedures specific for the method of analysis [8]. The dye was then analysed for trace

elements using energy dispersive X-ray fluorescence (EDXRF) spectrophotometer. Quantification of the concentrations of detectable elements was done using a modified version of emission transmission method [9].

Preparation of different concentrations of the dyes

Six different concentrations of the dye were prepared (0.05, 0.15, 0.25, 0.50, 0.75 and 1.00% w/v) using hydrogen peroxide (30 volume) as the dissolving solvent. For example, 0.05% concentration was prepared by dissolving 0.05 g of the dye in a little quantity of hydrogen peroxide in a beaker, transferred into a 100-mL volumetric flask and made up to the mark with hydrogen peroxide.

Experimental animals

A total of 80 albino rats (*Rattus norvegicus*) of both sexes with an average weight of 26.8 g were used for the study. The rats were divided into eight groups of 10 rats each as follows:

- Group 1: Rats on whose heads were applied 2 mL distilled water (Control)
- Group 2: Rats on whose head were applied 2 mL of dissolving solvent (H_2O_2)
- Group 3: Rats on whose heads were applied 2 mL of 0.05% conc. white dye
- Group 4: Rats on whose heads were applied 2 mL of 0.15% conc. white dye
- Group 5: Rats on whose heads were applied 2 mL of 0.25% conc. white dye
- Group 6: Rats on whose heads were applied 2 mL of 0.50% conc. white dye
- Group 7: Rats on whose heads were applied 2 mL of 0.75% conc. white dye
- Group 8: Rats on whose heads were applied 2 mL of 1.00% conc. white dye

The different concentrations were applied every three days and the experiment lasted for 30 days. All the rats were housed in separate metabolic cages and fed *ad libitum* with commercial rat chow and water. At the end of the 30-day experimental period, the animals were anaesthetized with chloroform and blood was collected by cardiac puncture using needle and syringe. The blood sample was immediately spun using a centrifuge at 500 g for 5 min to obtain the serum, which was collected using a Pasteur's pipette. Portions of the liver, kidney, and skin of the head where the dye was applied were dissected out. The activities of ALP and ACP were then assayed in the selected tissues of the rats using the method of Wright et al. [10]. MDA concentration in the skin was also estimated using the thiobarbituric colorimetric method of Olusi [11].

Results and discussion

Solubility test

The result of the solubility test of the dye in 14 different solvents is summarized in Table I. It is of interest to note that the dye is soluble in almost all the solvents, with the solutions having different colours. Almost all colours appear to be unstable as they changed after 24 h. The dye was found to be soluble in both polar and non-polar solvents. The pH of the resulting aqueous solution shows that the dye is slightly alkaline. The solubility of the dye in both polar and non-polar solvents probably explains the penetrating ability of the dye into different surfaces. This is an indication that the dye may penetrate the skin and possibly affect other internal organs of the body.

Table I. Solubility of white dye (YF) in different solvents with observed colour change after 24 h.

S/No	Solvent	Solubility	Colour of solution	Colour after 24 h
1	Deionized water	Soluble	Colour less	Golden red
2	H ₂ O ₂ in water	Soluble	Dark brown	Yellowish red
3	Acetone	Soluble	Light brown	Brown
4	Benzaldehyde	Soluble	Wine red	Golden yellow
5	Acetaldehyde	Soluble	Reddish brown	Brownish black
6	Ethanol	Soluble	Faint brown	Dark brown
7	Pet. ether	Insoluble	Colourless	Residue
8	Conc. HNO ₃	Soluble	Colourless	Golden brown
9	Conc. HCl	Sparingly soluble	Cloudy whitish mixture	White sediment
10	Methanol	Soluble	Light brown	Dark red
11	Diethyl ether	Soluble	Colourless	Red
12	Ethylacetate	Soluble	Colourless	Red
13	Acetic acid	Soluble	Faint yellow	Faint yellow
14	Aniline	Soluble	Brown	Dark brown

pH of aqueous solution is 8 : 6.

Table II. Elemental analysis of white dye (YF) by XRF technique.

Element	White dye (YF)
Potassium	0.43 (P)
Calcium	0.26 (P)
Titanium	0.07 (P)
Vanadium	345
Chromium	207
Manganese	175
Iron	125
Cobalt	66.0
Nickel	53.0
Copper	35.0
Zinc	26.0
Arsenic	21.0
Lead	31.0
Bromine	13.0
Rubidium	9.00
Strontium	9.00
Yttrium	9.00
Zirconium	6.00
Niobium	30.0
Molybdenum	5.00

(P) indicate values in percentage, others are in ppm.

Elemental XRF analysis

The result of XRF analysis of white dye (YF) is summarized in Table II. Twenty elements were recorded with their concentrations ranging from major to ultra-trace levels. The major elements included potassium (K) and sodium (Na).

Minor elements were Ti, V, Cr, Zn, Mn and Fe, trace elements Co, Ni, Cu, As, Pb and Zr, and the ultra trace elements included Rb, Sr, Y and Mo. The result of XRF reveals

that the dye may possess a certain degree of deleterious effect due to the presence of toxic metals, such as As, Pb, Cr, Co and Zn. Some of the elements detected in the dye are heavy metals, which have been found to be potent carcinogens. Studies have shown that heavy metals, such as chromium (Cr), lead (Pb), arsenic (As) and copper are potent carcinogens [12]. This probably accounts for the implication of heavy metals in the etiology of cancer.

Specific activities of ALP and ACP

The measurement of the activities of various enzymes in tissues and body fluid is a significant and well-known aid in disease investigation and diagnosis [13]. Such measurement can also give an insight into the state of cellular tissue damage as a result of assault by a drug or other chemicals.

Alkaline phosphatase and ACP are found in specific regions of the cell, thus giving an indication of the sequence of cell damage, if any, arising from the administration of chemical compounds. The status of the two enzymes in tissues following administration of chemical compounds will give an indication of the state of tissue cell membrane [14,15].

The specific activities of ALP and ACP in the skin, serum, liver and kidney of rats following the application of white dye for 30 days are presented in Tables III and IV respectively. The data revealed a significant reduction ($p < 0.05$) in the specific activity of ALP in skin and kidney, as the concentration of the dye increased. A similar trend of result was obtained for the activity of ACP in the skin and kidney (Table IV). This may in part be attributed to damage in the tissues studied. The ALP is a "marker enzyme" for the plasma membrane and endoplasmic reticulum [16]. Therefore damage to the membranes especially in the skin may adversely affect the activity of ALP. The reduction in ALP activity could also be attributed to inhibition of the enzyme by the dye or inactivation of the enzyme molecules *in situ* [17].

There was a significant increase ($p < 0.05$) in the specific activities of ALP and ACP in the serum of rats following the application of white dye (Tables III and IV). This could be as a result of leakage of the enzymes from an affected tissue probably the kidney where a significant decrease ($p < 0.05$) in enzyme activity was observed. Normally enzyme will not always be found in the serum except when there is damage to one or more organs or tissues

Table III. Specific activity of ALP in selected tissues of rats following the application of white dye (YF).

Concentration of dye	Specific activity $\mu\text{mol/mg protein/min}$			
	Skin	Serum	Liver	Kidney
Control	8.54 ± 0.09^a	0.72 ± 0.01^a	4.89 ± 0.05^a	120.50 ± 3.56^a
Solvent	7.25 ± 0.08^b	1.04 ± 0.02^b	4.86 ± 0.04^a	110.66 ± 4.31^b
0.05%	5.64 ± 0.05^c	1.87 ± 0.01^c	4.87 ± 0.04^a	102.61 ± 3.56^c
0.15%	4.31 ± 0.03^d	3.06 ± 0.04^d	4.88 ± 0.04^a	91.52 ± 2.51^d
0.25%	2.56 ± 0.03^e	4.24 ± 0.01^e	4.90 ± 0.05^a	90.38 ± 2.56^d
0.50%	1.70 ± 0.02^f	5.27 ± 0.03^f	4.86 ± 0.05^a	82.10 ± 3.50^e
0.75%	1.18 ± 0.01^g	6.02 ± 0.02^g	4.89 ± 0.05^a	76.52 ± 2.36^f
1.00%	0.43 ± 0.01^h	8.12 ± 0.05^h	4.87 ± 0.04^a	70.10 ± 2.13^g

Each value is a mean of ten determinations $\pm$ SEM.

^{a, b, c, d, e, f, g, h} Column values with different superscript letters are significantly different ($p < 0.05$).

Table IV. Specific activity of ACP in selected tissues of rats following the application of white dye (YF).

Concentration of dye	Specific activity $\mu\text{mol/mg protein/min}$			
	Skin	Serum	Liver	Kidney
Control	9.87 ± 0.10^a	1.83 ± 0.02^a	62.86 ± 4.01^a	150.58 ± 4.05^a
Solvent	8.15 ± 0.09^b	2.15 ± 0.01^b	63.42 ± 3.21^a	141.60 ± 2.56^b
0.05%	6.46 ± 0.06^c	2.98 ± 0.02^c	63.51 ± 3.06^a	134.50 ± 2.33^c
0.15%	5.13 ± 0.05^d	4.17 ± 0.04^d	63.50 ± 3.21^a	121.36 ± 3.01^d
0.25%	3.65 ± 0.03^e	5.35 ± 0.05^e	64.01 ± 3.07^a	109.38 ± 1.98^e
0.50%	2.77 ± 0.03^f	6.38 ± 0.04^f	64.01 ± 3.12^a	101.12 ± 2.01^f
0.75%	2.08 ± 0.01^g	7.13 ± 0.03^g	64.05 ± 3.21^a	93.56 ± 2.36^g
1.00%	1.34 ± 0.01^h	9.23 ± 0.06^h	64.07 ± 3.22^a	80.59 ± 1.89^h

Note: Each value is a mean of ten determinations $\pm$ SEM.

^{a, b, c, d, e, f, g, h} Column values with different superscript letters are significantly different ($p < 0.05$).

Table V. Concentration of MDA in the skin of rats following the application of white dye (YF).

Concentration of dye	Concentration of MDA (mmol mL^{-1})
Control	0.75 ± 0.01
Solvent	0.85 ± 0.02
0.05%	0.87 ± 0.02
0.15%	1.20 ± 0.03
0.25%	1.48 ± 0.02
0.50%	1.62 ± 0.03
0.75%	1.85 ± 0.02
1.00%	2.06 ± 0.03

of the body. Therefore enzyme from diseased tissues or organs may become manifested in the serum resulting in increased activity since they must have leaked from the diseased tissue. Increased activities of serum enzymes have been reported in conditions of tissue damage due to such diseased conditions and from the use of several chemicals and drugs [13]. This is often accompanied by a corresponding decrease in enzyme activity in the affected tissue or organ.

The specific activities of ALP and ACP in the liver of experimental rats (Tables III and IV) revealed that there is no significant difference ($p < 0.05$) in the activities of the enzymes in all the experimental groups. This is an indication that the liver may not be affected by the application of the dye at least for the 30 days during which the experiment lasted. The reason for this could be attributed to the fact that the liver has a major role of detoxification and hence capable of handling some of the harmful compounds present in the dye.

MDA levels

The concentration of MDA in the skin of albino rats treated with white dye is presented in Table V. The data obtained revealed that the level of MDA increased by 174.7% ($p < 0.05$) in the animals treated with 1.00% concentrated dye. During oxidative stress, MDA and/or other aldehydes are formed in biological systems. These can react with

amino acids and DNA and introduce cross linkages between proteins and nucleic acids, resulting in alterations in replication, transcription [18] and leading to tumour formation. MDA is a product of lipid peroxidation and the level of serum MDA is a measure of the extent of lipid peroxidation. Higher level of MDA suggests a higher degree of lipid peroxidation. Elevated level of MDA was observed in the skin of animals treated with white dye (YF) suggesting oxidative stress, which could be an indication of skin tumour formation as observed in the work of Dhawan et al. [19]. The workers observed an increase in MDA level following the application of 9,10-dimethylbenz[a]anthracene/12-O-tetradecanoylphorbol-13-acetate (DMBA/TPA), which is a potent carcinogen on the skin of albino rats. High level of lipid peroxidation is indicative of the generation of reactive oxygen species (ROSs), which are suggested as causative factors in carcinogenesis and tumour promotion and have been implicated in the etiology and pathophysiology of many human diseases [20]. They induce strand breaks in DNA and also oxidative modification of DNA bases leading to mutagenic and carcinogenic effects [21].

Conclusion

The dye has been found to reduce the activities of ALP and ACP in the skin and kidney of the rats. The decrease in the activities of ALP and ACP may not be unrelated to the presence of some highly toxic metals, such as As, Pb, Cr, Co and Zn in the white mineral dye. The high concentration of MDA observed in the skin of animals especially at high concentration of the dye may be indicative of the formation of tumour. Further studies are still in progress especially related to the link between dye and cancer of the bladder. We also intend to isolate, purify, and characterize the organic compounds that may be present in the dye.

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