Original Research Article

The Potential Effect of Henna (Stone Dye) Extract Paraphenylenediamine on Human Blood Cells, Liver and Renal Function

Abstract

Some of the dyes that are used for coloring hair and skin contain a chemical compound called paraphenylenediamine (PPD), which is potentially toxic. The study comprised 50 adult Sudanese women who were selected by stratified random sampling and working as henna artist. Urine samples were taken for detection of PPD while blood samples were taken for investigating kidney and liver functions by measuring its relevant hematological indices. In other hand Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) were used to detection of PPD in black henna (stone dye) samples and urine. The PPD was detected in the urine of all women who used the black henna its concentration varied according to duration of exposure being significantly higher in those with >10 years of exposure ($p \leq 0.01$). Similarly significant changes in liver function, kidney function and hematological indices were found in the group of women who used natural henna. This study concluded that natural henna is safe to use, while Black dye is potentially hazardous. It contains the high concentration of PPD, is the one most commonly associated with adverse effects.

Keywords: Henna, Paraphenylenediamine, Renal function, Liver function, Blood cells.

Introduction

Paraphenylenediamine (PPD) is an aniline-related aromatic diamine in the form of pinkish gray lumps of crystalline oxidation content, typically through exposure to air; it turns red, brown and finally black [1]. If the paste has black skin torso in less than half hour, it has PPD in it. If the paste is combined with peroxide, or the peroxide is wiped over the design to create the color, then it has PPD in it. In addition, the use of paraphenylenediamine is common, especially in tourist areas [2]. Since the blister reaction occurs 3 to 12 days after the application, and by that time most visitors have left and do not return to show how much harm the artist has done. This

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encourages artists to continue to hurt others, not understanding that they are causing serious damage. The great profit margin of "black henna" and the market for body art that resembles "tribal tattoos" further motivate artists to ignore danger [3]. The lethal dose of Phenylenediamine for man is estimate to be 109 mg/kg other studies revealed that the dose which eventually leads to death is estimated between 7-10 grams. On the other hand animal experiments showed that the oral lethal dose (LD) of PPD range to 100-250 mg/kg weight and subcutaneous LD (dose between100-200 mg/kg) [4]. Short-period exposure to high levels of PPD (acute effects) can lead extreme dermatitis, eye inflammation and tearing, asthma, gastritis, dizziness, tremor, convulsions, and coma in humans. Eczematous contact dermatitis can result from long-time exposure (severe influence) to humans [4].

Phenylenediamine is demonstrated to be mutagenic and carcinogenic in numerous studies [5]. However, in Sudan in spite of restrictions imposed on the import of PPD, it finds its way to the country through illicit channels [6]. It has been observed that the hair dye absorbed the through the skin, the results vary according to the metabolism of PPD, health, age of patients, and the time which it used [7]. This study was the first sophisticated scientific proof in Sudan that the black henna is a chemical and that there is no such plant, which gives black henna. It is not difficult to recognize and avoid para-phenylenediamine in henna. The main objective of this study to evaluate the toxicity of synthetic dye black henna or stone dye on blood cells, renal and liver functions.

Materials and methods

This was prospective cross sectional analytical study, fifty adult Sudanese women were comprised in the study, who were selected by stratified random sampling and working as henna artist in beauty salons .Urine samples were taken for detection of PPD while blood samples were taken for investigating kidney and liver functions by measuring its relevant hematological indices. In other hand Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) were used to detection of PPD in black henna (stone dye) samples and urine. Whereas blood counts were measured by Sysmex-KX 21 analyzer, and the renal and liver tests were done by Roche-Hitachi 904 analyzer

Chemical analysis

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TLC analysis and preparations

TLC analysis was conducted at The International Research Centre to detect the presence of PPD in natural and Black henna, using Soxhlet extractorapparatus (electro mag) Gallenkamp, U.

Preparation dyes samples for TLC:

0.2g of natural dyes and Black henna were extracted using Gallenkamp, U.K, and dissolved in 80% chloroform and stored till being used.2 drops from each sample were spotte don the TLC plate.

Preparation of the plates:

30 mg of silica gel were shaken with 60 ml distilled water for 2min using 250ml stoppered conical flask.The slurry was spread on plane making o.25mm thickness on glass 20× 20 cm plates. The coated plates were allowed to dry at room temperature. Then activated at 105 ºC for one hour. The hot plates were allowed to cool and stored until used

Preparation of 3 solvent solutions for TLC analysis of dyes samples

The three solvent systems were prepared by mixing Butanol, acetic acid and water with 40, 10 and 50% , hexane and acetone with 30, 90 % and acetone, ammonia 28% and chloroform with ratios 40, 2 and 40% respectively [8].

HPLC analysis

High performance liquid chromatography (HPLC) was done at central Lab. for Black henna to find the concentration of PPD in each dye sample, compared to the standard. The same analysis was used to check the concentration of PPD in 10 urine samples of Henna artists (B) compared to control urine sample using (SHEMADZU), Sof tware CLASS-VP Data system

Preparation of synthetic samples:

Stock concentration of Black henna was prepared by accurately weighing 50 mg of synthetic dye using sensitive balance into a 25-ml volumetric flask. Initially, dissolve the Black henna with about 20 ml of 10 N Sulphuric acid. After totally dissolved dilute to the mark

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with additional 10 N Sulphuric acids and thoroughly mix the solution. They were kept in a refrigerator till being used

Preparation of Urine samples:

Ten samples of urine of each were collected from women who practiced the henna process (Henna artist) and were stored in refrigerator, then treated with potassium hydroxide till the pH 9. All samples were extracted with chloroform in a separating funnel .Two clear layers were observed. The chloroform layer was collected and treated with aqueous sodium sulphate then kept in glass container tell being used for TLC and HPLC tests.

Hematological measurements:

5ml for each of Henna artist blood samples was prepared to measure complete blood count (CBC), hemoglobin concentration (Hb), red blood cells (RBCs) count, total white blood cells (TWBCs) count, Mean cell Hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), Lymphocytes, neutrophil and the mix (Eosinophil +Basophil) percentages Using Sysmex KX-21.

Biochemical measurements:

Plasma of Henna artist's blood samples was analyzed for renal function test to detect the levels of Blood urea, Creatinine, Uric acid, Serum potassium and Serum sodium. Liver function test was performed to chick Total bilirubin, Total protein, Albmin, Alanineaminotarnsferase: (ALAT/GPT) Asparteaminotrasferase (ASAT/GOT) and Alkaline PhosphaLiquid Using Roche/ Hitachi 904 analyzer.

Statistical analysis:

Data were analyzed by using Statistical Package for Social Science (SPSS) version 20 for window. Continuous and categorical variables were tested for significance using (T-test, One-way ANOVA, and Chi-square) respectively. P. value <0.005 was considered as statistically significant.

Ethical consideration:

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The study was approved by ethical committee of faculty research board and hospital. Verbal and written consent forms were obtained from each participant.

Results

A total of fifty henna artist women (hananat) participated in this research. In this study TLC analytical method, used three different solvent systems were used to confirm the results (Butanol: acetic acid: water, Hexane: Acetone and acetone, ammonia and chloroform). The presence of PPD in the Black henna as to standard as shown in figure 1(plate 1a, and 1b) and Henna artists urine samples figure 2 (plate 2). HPLC analytical method used to check the concentration of PPD in black henna comparing to the standard as was shown in Table (1).

HPLC analysis for detection of PPD in Black henna

The figure 3 (3a, 3b) shows the chromatograms of the PPD in hair dyes used by Sudanese women. The retention time for standard sample with concentrations 0.200/Cal. were found 1.600-1.611 minutes under the same conditions. Retention time of PPD of Black henna was found in the same range of standard (1.600-1.611).

The HPLC figure 2 showed the chromatograms of the PPD of 10 urine samples of Hananat who use Black henna during their job. one anther urine sample was taken from Sudanese woman who had no past history of Black henna used as control sample.

Hematological parameters:

As shown in Table 3 of this study the effect of the time exposures to PPD contented in black henna dye was significantly correlated with blood parameters. The tested groups A, B, C, D and E showed significant decrease ($P \leq 0.05$). Mainly hemoglobin (Hb) , Red blood cell counts (RBCs) compared to control group levels which were found to be 14.6 g/dl , ($5.0x 10^6$ / ul) respectively, while that of mean cell hemoglobin (MCH) and the mean cell hemoglobin concentration (MCHC) measurements of tested group A and B did not show significant difference ($P \leq 0 .05$) , whereas the tested C , D , and E showed significant decrease ($P \leq 0.05$) . On the other hand, The lymphocytes and Neutrophil percentages in the blood of tested B, C, D, and E showed significant increase ($P \leq 0.05$) compared to control group (30% ,50.%) with little decrease in group A.

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Renal function tests:

Renal problems in this study are considered to be the main risk that faced Sudanese women who are exposed to PPD for long time as the parameters showing renal function test namely: blood urea, creatinine, and uric acid measurements showed significant increase ($P \leq 0.05$) as compared to the control group with little decrease in creatinine of group A. For the electrolytes, Na level in tested group B, C, D and E showed significant increase ($P \leq 0.05$) , as compared to the control group (4 mmol/l), while group A and group B did not show significant difference to K ($P \leq 0.05$) as displayed in Table 4.

Liver function tests:

The parameters showing the liver function test namely: total bilirubin, total protein , and albumin of tested group C, D and E showed significant increase ($P \leq 0.05$) and plasma enzymes ALT, AST, and ALP for tested groups A, B, C, D and E are shown in Table 5 showed significant increase ($P \leq 0.05$).

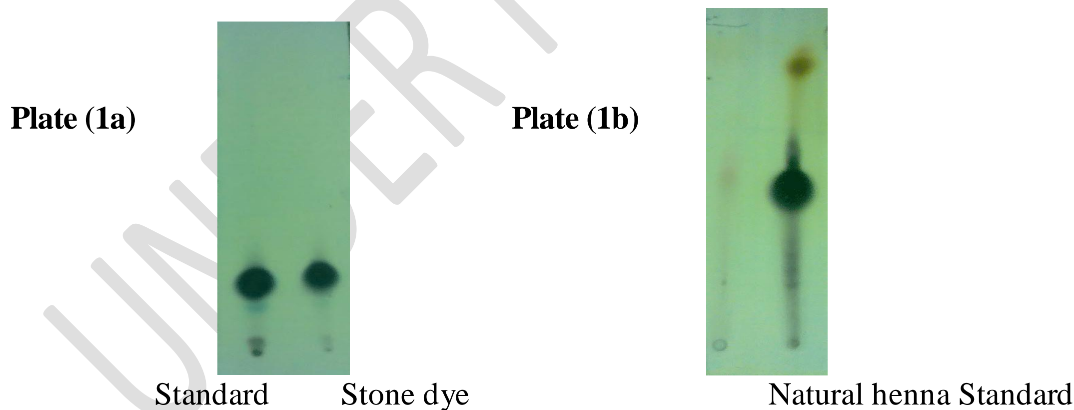

Figure 1: TLC plates (1a, 1b) showed two different solvent systems (Butanol: aetic acid: water &Hexane: Acetone)

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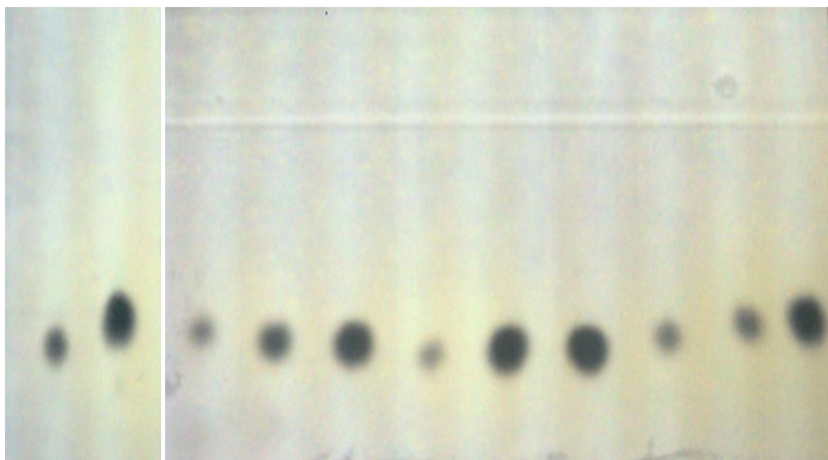

1 2 3 4 5 6 7 8 9 10 11

Key: (1–10) urine samples (11) standard

Figure 2: TLC plate (2) showing urine samples of Henna artists and standard spraying with Potassium Dichromate: Acetone: Ammonia (40:40:2) as solvent system

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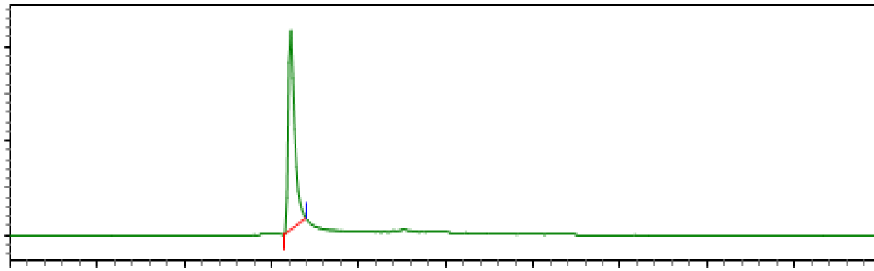
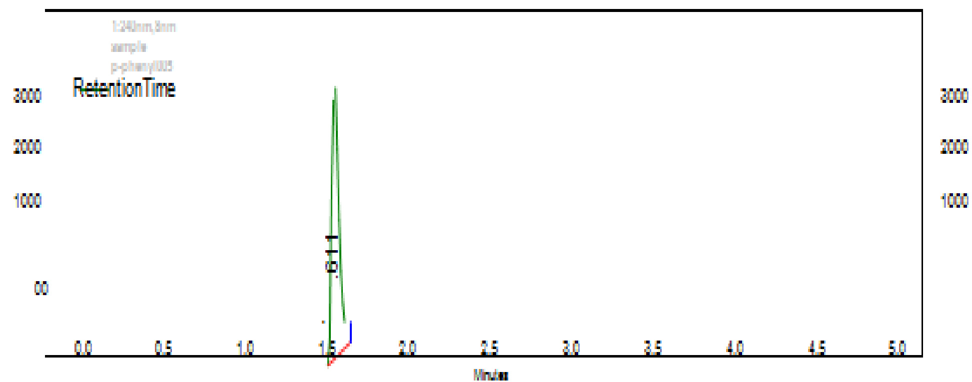


1:240nm,8nm

Name	RetentionTime	Area	concentration ESTD
p-phenylenediamine	1.611	5218564	0.200

(Fig 3a) Standard: (PPD)

Sample:Stone dye



1:240nm,8nm

Name	RetentionTime	Area	ESTD concentration
p-phenylenediamine	1.611	8823038	2.3٧٥mg/l

(Fig 3b): Black henna (stone dye)

Figure 3: (3a&3b) Chromatograms of synthetic black henna purchased from local Sudanese local market

Table (1) Synthetic dye Black henna (Stone dye) concentration percentages of the PPD

Sample	Concentration	Percentage
Standard (PPD)	2.450	97%
Black henna (Stone dye)	2.375	95%

Table 2: Hematological parameters according to dermal exposure time of PPD (Henna) in years (mean± SD)

Hematological parameters	Group F (n=10) control	Group A (n=8)	Group B (n=12)	Group C (n=16)	Group D (n=8)	Group E (n=6)
Hb (g/dl)	14.6±0	13.7±0.3*	11.7±0.3*	10.1±0.7*	8.8±0.5*	8.1±0.1*
RBCs (10^6/µl)	5.0±0	5.3±0.2*	5.3±0.3*	4.7±0.5*	4.2±0.1*	3.7±0.4*
TWBCs (10^3/µl)	8.5±0	7.1±0.4	7.0±0.5	8.4±0.5	9.7±0.3	12.1±0.6
MCH (pg)	27.0±0	25.2±2.2	25.7±0.5	23.7±1.6*	21.1±1.31*	20.1±1.3*
MCV (fl)	75.0±0	73.3±1.6	77.4±2.2	86.9±3.1	91.9±0.8	94.6±1.3
MCHC (g/dl)	32.0±0	33.4±1.3	33.7±0.8	31.2±2.1*	30.4±0.5*	28.4±0.4*
Lymph (10^3/µl)	3.0±0	2.8±2.9	3.8±2.6*	4.6±2.9*	5.2±2.0*	6.0±4.2
Neutrophil (10^3/µl)	5.0±0	4.4±2.1	4.2±5.4*	5.6±3.7*	6.3±1.9*	7.3±2.8*
Mix (10^3/µl)	1.5±0	1.1±0.4	1.1±0.9	1.5±0.7	1.7±1.4	2.3±1.7

A= 1-5 years, B=6-10 years, C= 11-15 years, D=16-20 years, E=21-25years, and F = 0year as control.

*$P \leq 0.05$

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Table 3: Renal function tests for henna artists according to different PPD dermal exposing periods (mean± SD)

Biochemical parameters	Group F (n=10) control	Group A (n=8)	Group B (n=12)	Group C (n=16)	Group D (n=8)	Group E (n=6)
Blood urea (mg/dl)	30±0	16.4±4.7	35.2±7.9*	51.7±2.3*	59±1.8*	68.4 ± 14.3*
Creatinine (mg/dl)	0.8±0	0.8±0.3	1.4±0.3*	1.8±0.1*	2.1±0.1*	2.5 ± 0.3*
Uric acid(mg/dl)	4.5±0	2.9±1.0	3.7±0.7*	6.4±0.8*	7.8± 0.5*	8.9 ± 0.5*
K (mmol/l)	4 ±0	3.4±0.3	3.6±0.5	4.9±0.6*	5.9±0*	7.1 ± 0.5*
Na (mmol/l)	140 ±0	143.4±4.1	152.7±3.6*	136.2±47.8*	141.3±4.1*	126 ± 4.1*

A= 1-5 years, B=6-10 years, C= 11-15 years, D=16-20 years, E=21-25years, and F = 0year as control.

*P≤0.05

Table 4: Liver function tests for henna artists according to different PPD dermal exposing periods (mean±SD)

Biochemical parameters	Group F (n=10) control	Group A (n=8)	Group B (n=12)	Group C (n=16)	Group D (n=8)	Group E (n=6)
T. Bilirubin (mg/dl)	0.5±0	0.4±0	0.5±0.1	0.8±0.2*	1.12±0.4*	1.4±0.2*
T. Protein (mg/dl)	7±0	8.3±0.3	7.7±0.3	7.1±0.5*	5.6±0.2*	3.9 ±0.6*
Albumin(mg/dl)	4±0	3.8±0.3	4.3±0.3	5.6±0.6*	6.8±0.2*	7.3±0.5*
ALT (u/l)	44±0	41.±4.1*	46.3±3.5*	50.8±2.1*	61.5±2*	72.7±4.1*
AST (u/l)	46±0	44.3±2.1*	38.5±1.8*	48.1±3.1*	54.5±2.1*	61.6±0.4*
ALP (u/l)	53±0	55.9±1.7*	42.4±3.1*	56.4±8.2*	100.1±13.4*	129.4±9.6*

A= 1-5 years, B=6-10 years, C= 11-15 years, D=16-20 years, E=21-25years, and F = 0year as control.

*P≤0.05

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Discussion

Several previous research concerned the study of PPD, most of which focused on the effect of this compound on the skin, the sensitivity of the skin and the toxic effect on the histological and functional properties of the various tissues. There are no adequate studies explaining the impact of PPD dermal exposure on the liver, renal function, and blood cells, and the duration of PPD exposure, as shown in this study.

In recent years, Henna tattoos have become increasingly popular and trendy among teenagers as they are thought to be a safe substitute to permanent tattoos. In several countries, temporary henna tattoos comprise not only henna but also other ingredients such as PPD, which also cause active sensitization [9].

In this research it has been found that there is a significant effect of PPD on blood cells, as well as there is an observed correlation between the time of exposure to PPD, hemoglobin and red cells count even if the exposure time is short, while the indices of red blood cells, lymphocytes, and neutrophils need even more exposure time, so that noticeable effect be obvious. This agreed with Garth, et al, Ashar found that duration of permanent dye used made hemoglobin problems and hematopoietic cancers. Acute poisoning with PPD caused leuokocytosis, anemia, secondary hemoglobinuria [10, 11].

In our current study, it was observed that the renal function changes in remarkably way with the time of exposure to PPD, when compared to the control samples, which is due to the toxic effect of PPD on kidney tissues, which leads to a decrease in its efficiency, which leads to the emergence of symptoms of renal failure by increasing all the criteria by which to measure renal efficiency. These findings are consistent with Kallel et al, who reported that the kidneys are especially susceptible to the toxic effects of PPD and that acute renal failure is a testimony to the magnitude of the intoxication and predicts bad prognosis [12]. In addition, our findings are consistent with Singla et al. and Filali et al., who identified two phases of severe harmful effects following acute PPD poisoning; the first phase typically occurs within 4–6 hours and manifests extreme facial and neck oedema, whereas the second phase occurs 12 hours after ingestion, in which rhabdomyolysis and acute renal failure are supervened [13, 14].

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Moreover, Ibrahim et al. found that kidney parts of the PPD-poisoned victims of acute renal failure had areas of extreme tubular necrosis. Furthermore, cellular ghosts had lost cellular information and sloughed into the lumen. In other way, the kidney parts of the majority of the victims displayed a mild to moderate level of tubular necrosis along with early glomerulonephritis in which the glomeruli demonstrated a distortion of the lobular structure with the thickening of the Bowman capsule 15. Ram et al. reported that renal biopsy in four Indian cases of PPD poisoning showed acute tubular necrosis in three of them and acute interstitial nephritis in the fourth [16].

Due to the toxicity of the liver cells, which contributes to a breakdown of the red blood cells within the hepatic tissue, which increases total bilirubin, as well as a decrease in the protein produced by the hepatic cells, the most widely used components are liver enzymes, which decreased significantly, even at a period of exposure not too long, due to their sensitivity and toxicity to the liver tissue.

Hepatic affection caused by paraphenylenediamine was reported microscopically by Ibrahim et al. who observed varying degrees of focal and diffuse necrosis. Hepatocytes demonstrated granular cytoplasm ballooning. The portal was expanded with large lymphatic spaces. Consequently, the bile duct lining epithelium demonstrated focal sloughing. The underlying mechanism of PPD-caused hepatic disease is not fully known. It can be due to the direct toxic action of PPD or its by-products on hepatocytes [15]. Bhagavathula et al. indicated that the liver is the organ responsible of PPD intoxication where it is metabolized [17]. PPD is an aromatic amine belongs to the group of substances that have toxic effects, typically after oxidative biotransformation, mainly in the liver. In addition, PPD initiates extra hepatic stimulation to release aminyl radicals. These reactive intermediates are rapidly disproportionate and covalently bound to thiol, leading in more toxic products than the parent compound due to their auto oxidizability [18].

Conclusion

It can be concluded that the following use of black Henna that contains PPD for a long time leads to more absorption through the skin and appears in the urine samples, and quantities that raise it are directly proportional to the period of use and dermal exposure, which leads to noticeable

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effects in the shape and number of blood cells and its effect extends to renal and liver functions. Among the recommendations that can be concluded is that Henna artists (Hananat) and hairdressers who are more susceptible to black henna should use protective plastic gloves to prevent direct skin contact, also see a doctor from time to time to reduce the medical effects of PPD on their bodies.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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