

Modulatory Effect of *Baphia Nitida* Dye in Toluene Induced Cytogenotoxicity, Hematotoxicity and Histopathology in Dermal Exposed Wistar Rats

Chibuisi Gideon Alimba*¹, Khalid Olajide Adekoya², Adebayo Liasu Ogunkanmi², Bola O Oboh²

Received: 14.12.2014

Accepted: 05.01.2015

ABSTRACT

Background: There is unprecedented increase in the processing and packaging of many plant materials into food supplements, herbal medicine, skincare and cosmetic products for human needs. *Baphia nitida* is used for topical skincare products. Toluene, a toxic aromatic solvent, is increasingly being used in the production of these skincare and cosmetic products in many industries. This study assessed toluene toxicological profile and the ability of *Baphia nitida* dye to ameliorate toluene induced cytogenotoxicity, hematotoxicity and histopathological effects in rats.

Methods: Rats were treated with various concentrations; 0, 1000, 2000 and 5000 mg of the aqueous, ethanol and toluene processed *B. nitida* dye via dermal exposure for acute and sub-lethal toxicity. 96 h acute toxicity was assessed for the solvents. Micronuclei induction, alterations in hematological indices and erythrocyte morphology and skin histology were assessed after sub-lethal treatment.

Results: 96 h LD₅₀ of *B. nitida* processed dye for the three solvents were indeterminate. There was insignificant ($p>0.05$) alterations in the hematological indices and erythrocyte morphology, induction of micronucleated polychromatic erythrocyte and polychromatic and normochromatic erythrocyte ratio in the aqueous and ethanol processed *B. nitida* treated rats compared to their corresponding controls. Toluene induced significant ($p<0.05$) decrease in erythrocytes count, hematocrit and leucocytes, increased micronucleated PCE, decreased PCE/NCE ratio and induced necrosis, thick dermal layer and dispersed areolar tissues in treated rats. But, these effects were ameliorated by the *B. nitida* dye.

Conclusion: Camwood dye protects against toluene induced toxicity in rats. This suggests its relative safety in topical cosmetic and skincare production.

Keywords: Camwood Dye, Hematology, Histopathology, Micronucleus Test, Toluene, Rats.

IJT 2015; 1225-1234

INTRODUCTION

Medicinal plants, the reservoir of phytomedicine, contain phytochemicals and nutritional substances which serve as sources of therapeutics and food for human sustenance. World Health Organization (WHO) reported that about 80% of the world population relies on plant materials for their primary healthcare. This is due to their availability, low cost of acquisition, high curative properties and rare contamination of the environment [1, 2]. In recent times, there is unprecedented increase in the processing and packaging of many of these herbs into supplements, skincare and cosmetic products for human use [3]. Majority of the plants are yet to be examined for evidence of toxicity, one of the basic criteria set by WHO for

their use [4]. *Baphia nitida* Iodd (Camwood), a leguminous, shrubby, hard-wooded tree belonging to the sub-order Cesalpinieae and family Fabaceae [5], is a wide spread forest plant distributed around the globe and is common within the coastal region of Africa [6]. Leaves and bark from this plant are considered hemostatic and anti-inflammatory and are used for curing sores and wounds, while the dye from the bark is formed into red body cosmetics [7,8]. Honey-hunters rub their body with the dye to prevent bee-stings. It is also made into ointments which are applied against stiff and swollen joints, sprains and rheumatism [9]. The plant materials are increasingly being used as a major ingredient in the production of local cosmetic and skincare products like African black soap

1. Department of Zoology, University of Ibadan, Ibadan, Nigeria.

2. Department of Cell Biology and Genetics, University of Lagos, Akoka, Nigeria.

*Corresponding Author: E-mail: cg.alimba@ui.edu.ng

and ointments commonly used against microbial activities, and in the treatment of skin pathologies [6, 10, and 11]. This suggests its importance in the protection of the skin from chemical induced damage and microbial activities. Toluene is a common aromatic solvent used in the production of some cosmetic and skincare products, paints and other industrial products [12, 13]. Its use as solvent is becoming worrisome due to the toxic effects it exert on mammalian systems [14, 15]. When in contact with the skin, toluene is absorbed and readily distributed in the body through the circulatory system, and accumulates in tissues with high lipid content [12]. Human population studies showed that acute toluene intoxication produced euphoria and dis-inhibition which led to hallucinations, tinnitus, ataxia, confusion, nausea and vomiting [16, 17]. Similar studies using animal models showed that toluene exposure at low concentrations caused adverse changes in neurobehavioural and neurochemical functioning [15, 18]. Mattia et al. showed that toluene induced toxicity in mammalian systems was via oxidative stress, suggesting that the continuous use of toluene as solvent for skincare and cosmetic products and other industrial products may increase human exposure to toluene via absorption from the skin and/or inhalation [19].

The skin, the body's largest organ, is generally considered as impermeable protective barrier against mechanical, chemical, microbial and physical hazards. Hence, it is readily prone to damage from these substances. The ability of chemicals, radiations and microorganisms to penetrate the skin in sufficient quantities (transdermal route) may elicit the induction of systemic and genetic damage in mammalian systems. This study presents the 96 h acute (LD_{50}) and 28 days sub-lethal toxicity assessment of toluene in a dermal exposure in Wistar rats. Also, the ability of *B.nitida* dye to ameliorate toluene induced alterations in hematological indices and erythrocyte morphology, micronucleus induction in bone marrow cells, and skin histopathology in treated rats were evaluated.

MATERIALS AND METHODS

Baphia Nitida Plant Material Collection and Camwood Dye Preparation

Baphia nitida (Lodd) with fresh leaves barks and roots were collected from Ikorodo area

of Lagos State, Nigeria. They were identified, authenticated and the voucher number (LUH 2137) deposited at the Herbarium unit, Department of Botany, University of Lagos, Nigeria. The bark from the plant's stem was neatly removed, shredded and grounded into fine particles. The particles were air dried and sieved to remove fibers and threads and collect the red colored powder. The obtained powder was packaged in transparent polythene bags and stored at room temperature, in a moisture free compartment prior to experimental use. Camwood paste/dye was prepared from the dye in accordance with Soladoye [9]. Three doses; 1000, 2000 and 5000 mg of the powder were measured from the stock into 10 ml of toluene (purity = 99.98 %, Sigma St Louis, MO). Since most herbs or plant materials are usually prepared with water and ethanol, the three doses of camwood powder was also dissolved in distilled water and absolute ethanol (Sigma St Louis, MO) to serve as reference. The solutions were mechanically stirred at 2 hr intervals for 24 hr using a glass rod to produce red precipitated solutions labeled as distilled water prepared camwood dye, ethanol prepared camwood dye and toluene prepared camwood dye.

Animals

Healthy male Wistar rats with intact skin (8 – 9 weeks old; mean \pm SD weight; 205.37 \pm 2.14g) obtained from the animal house of the College of Medicine, University of Ibadan, Nigeria were used for the study. They were acclimatized for 2 weeks, maintained in laboratory conditions of 12 h dark and light cycle, temperature of 26 \pm 2°C and had access to drinking water and standard rodent chow (Ladokun feed Nigeria®) *ad libitum*. The animals were grouped into three according to the three solvents used in the preparation of the *B. nitida* into camwood dyes:

Group A rats were assigned to distilled water prepared *B. nitida* dye.

Group B rats were assigned to ethanol prepared *B. nitida* dye and

Group C rats were assigned to toluene prepared *B. nitida* dye.

The animal experiment conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1996) and approved by the ethical committee, College of Medicine, Idi-Araba, University of Lagos.

96 Hr Acute and 28 Day Sub-Lethal Repeated Dose Dermal Toxicity Study

24 hr preceding the experimental set up, 10 % of the fur at the dorsal region of the rat body was shaved for *B. nitida* prepared dye application [20]. 10 rats were randomly assigned to four different doses per group; 0, 1000, 2000, 5000 mg. The individual solvents used to prepare the *B. nitida* dye (0 mg), served as control for each group. The precipitated camwood dye solution was applied uniformly to make good contact with the surface of the shaved skin (figure 1). The treated and control rats were put in individual cage to avoid contacts and subsequent cleaning off the dye by other members of the group. In the case of drying out, the dye was applied repeatedly for at least three times daily to ensure continuous exposure [20]. During the sub-lethal toxicity study, 5 rats were randomly assigned to each dose of the various groups. Treated rats with re-grown furs at the site of application during exposure, were re-shaved to allow appropriate application of the dye [21]. Rats were observed twice daily (before and after treatment) for signs of clinical toxicity in the appearances of the skin and fur, eyes and mucous membrane, behavioural and breathing patterns, morbidity and mortality.

Hematological Analysis

At post exposure, rats were fasted overnight and blood was collected from the orbital plexus using heparinized 70 ml micro-hematocrit capillary tubes into EDTA bottles [22]. The recommended hemogram; erythrocyte count, hemoglobin content (HGB), percentage hematocrit (HCT), mean corpuscle hemoglobin concentration (MCHC), mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH),

platelets (PLT) and total white blood count (WBC) were determined using automated analyzer (Abbott Hematology Analyzer Cell-Dyn 1700, Abbott Laboratories, Abbott Park, Illinois, USA) [21,23]. Aliquot of the blood samples were used to make thin blood films on pre-cleaned slides (three slides per rat), air-dried, fixed in absolute methanol for 20 min and stained in 5 % Leishmann stain for 20 min [24]. 500 erythrocytes were scored per rat at x100 oil immersion to determine poikilocytosis (variations in red blood cell shapes) and acanthocytosis (variation in red blood cell sizes) in accordance with Christopher [25] and Cheesbrough [26].

Mammalian Bone Marrow Micronucleus Assay

Cytogenotoxic evaluation was done using the micronucleus test in accordance with Schmid [27] and Krishna and Hayashi [28]. Rats were sacrificed by cervical dislocation, and the femurs were surgically removed and bone marrow cells flushed into eppendoff tubes using Fetal Bovine Serum (FBS). Cells were centrifuged at 2000 rpm for 10 min, smears made on slides and cells fixed using absolute methanol for 30 min. Slides were air dried and stained with conventional May-Grunwald and Giemsa stains. All slides were coded and examined under a Leica bright field microscope at x100 oil immersion. 2000 cells per rat were scored for micronucleated polychromatic erythrocytes (MNPCE) [27] as genotoxicity index, while polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) (PCE/NCE) ratio obtained from 1000 erythrocytes is served as index of cytotoxicity [28].



Figure 1. Shows dermal application of camwood dye on the dorsal trunk of rat.

A: Rat treated with toluene (0 mg) without camwood dye.

B: Treated rat with aqueous, ethanol or toluene prepared camwood dye.

Histopathological Analysis

Skin tissues were collected from the shaved sites of both control and treated rats from the doses of each group and fixed in Bouin's solution. After 48 h of fixation, the tissue was dehydrated by passing through ascending order of ethyl alcohol-water concentrations, cleared in xylene and embedded in paraffin wax blocks using rotary microtome. Thin slices of the tissue sections of between 3 – 5µm thickness was stained with Hematoxylin-eosin (H-E), and mounted in neutral DPX medium for morphological microscopic examination at x400 magnification.

Statistical Analysis

Statistical analyses were conducted with Graphpad prism 5.0® and SPSS computer programs. Data are presented as mean ± SD. Data for the acute toxicity test were analyzed using probit analysis to determine the LD₅₀ for the aqueous (Group A), ethanol (Group B) and toluene (Group C) prepared *B. nitida* dyes in rats. One-way analysis of variance (ANOVA) was used to determine the differences ($p < 0.05$) in means from the treatments and the corresponding controls for each group. Comparison between treatments and corresponding controls for each group was determined using Dunnett multiple post-hoc test ($p < 0.05$).

RESULTS

Acute Dermal Toxicity Study

Table 1 shows the results of the 96 hr LD₅₀ dermal toxicity of camwood dye in rats. During the acute dermal toxicity test, there was no mortality in all the tested doses for the three solvent extracted *B. nitida* dyes in the three groups. Hence, the LD₅₀ for camwood dye preparations from water, ethanol and toluene was indeterminate. Although no mortality was observed during the acute and sub-chronic

toxicity studies, but toluene (0 mg) treated rats from group C presented dryness and skin irritation in the shaved region. Also, the shaved parts became thickened, fissured and reddish resembling skin burns without fur regeneration. There was increased tremor and dizziness among rats in this treatment groups. These clinical signs of toxicity were rarely observed in rats treated with water (0 mg) and ethanol (0 mg) from groups A and B respectively.

Hematological Analysis

Tables 2 and 3 showed the hematological indices and erythrocyte morphology data respectively obtained from rats in the various groups of treatments. There was insignificant ($p > 0.05$) alterations in the evaluated hematological parameters for both water and ethanol prepared *B. nitida* dye (1000, 2000 and 5000 mg) treated rats and the corresponding controls (0 mg) of the groups (A and B). In group C, toluene (0 mg) treated rats showed significant decrease in total erythrocyte count ($p = 0.0488$), hemoglobin concentration ($p = 0.0155$) and total leucocyte count ($p = 0.0457$) (Table 2). The values of these blood parameters in toluene prepared camwood dye in group C (1000, 2000 and 5000 mg) were brought to close range with the values observed in the aqueous and ethanolic treated groups (table 2). Similarly, rats in groups A and B (treated with aqueous and ethanol prepared camwood and the corresponding controls) did not show any significant ($p < 0.05$) alterations in their erythrocyte morphologies. Toluene (0 mg; Group C) treatment significantly ($p = 0.0155$) increased acanthocytes (crenated erythrocytes) in rats and this was reduced to the range observed in the aqueous and ethanol groups A and B, in toluene prepared *B. nitida* dye (1000, 2000 and 5000) treated rats (table 3). Figure 2(a-c) presents the erythrocyte morphologies scored in both the treatment and control rats.

Table 1. shows the 96 hr dermal acute toxicity evaluation of camwood dye preparation using water, ethanol and toluene solvents in rats.

Conc (mg/kg)	Water		Ethanol		Toluene	
	%mortality	%survivor	%mortality	%survivor	%mortality	%survivor
Control	0	100	0	100	0	100
1000	0	100	0	100	0	100
2000	0	100	0	100	0	100
5000	0	100	0	100	0	100
LD ₅₀	indeterminate		indeterminate		indeterminate	

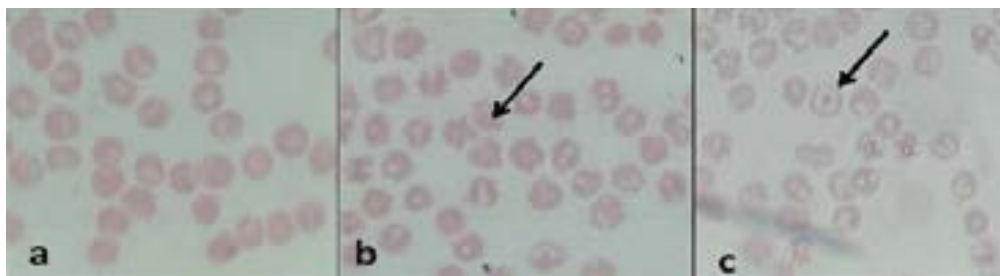


Figure 2. (a): Normal red blood cells in camwood dye treated rats.

(b): Abnormal red blood cell morphologies, mainly crenated cells (acanthocytes; arrowed) observed in toluene (0 mg) treated rats.

(c): Target cells (codocytes; Heinz bodies resembling Bull eyes; arrowed) observed in toluene (0 mg) treated rats.

Table 2. Haematological profile of rats treated with aqueous, ethanol and toluene formulated camwood dye.

Conc (mg/kg)	RBC ($\times 10^6/\mu\text{L}$)	HCT (%)	HGB (g/dL)	MCV (fl)	MCH (pg)	MCHC (g/dL)	PLT ($\times 10^6/\mu\text{L}$)	WBC ($\times 10^3/\mu\text{L}$)
Dist H₂O								
Control	7.10 \pm 0.38	40.47 \pm 1.78	11.80 \pm 0.67	57.50 \pm 2.43	16.70 \pm 1.18	28.03 \pm 0.83	727.0 \pm 91.0	13.43 \pm 1.67
1000	7.18 \pm 0.82	41.97 \pm 2.18	11.83 \pm 0.25	58.53 \pm 4.21	16.57 \pm 1.47	26.97 \pm 0.81	731.3 \pm 59.6	12.67 \pm 2.90
2000	7.22 \pm 0.40	42.73 \pm 2.95	11.87 \pm 0.50	59.17 \pm 0.98	16.43 \pm 0.35	27.80 \pm 0.79	754.7 \pm 14.0	11.47 \pm 2.48
5000	7.01 \pm 0.21	41.17 \pm 2.55	11.13 \pm 0.35	58.70 \pm 2.51	15.90 \pm 0.36	27.10 \pm 0.81	786.7 \pm 25.6	12.43 \pm 3.01
	(p=0.9566)	(p=0.3782)	(p=0.2437)	(p=0.3996)	(p=0.7658)	(p=0.4495)	(p=0.8708)	(p=0.1844)
Ethanol								
Control	7.53 \pm 0.20	43.37 \pm 3.10	12.23 \pm 0.76	67.30 \pm 2.67	15.43 \pm 0.60	22.39 \pm 1.07	928.7 \pm 14.4	13.93 \pm 3.61
1000	7.32 \pm 0.25	45.67 \pm 2.04	11.37 \pm 0.23	62.40 \pm 1.59	15.53 \pm 0.59	24.90 \pm 0.78	774.9 \pm 16.9	12.90 \pm 2.93
2000	7.07 \pm 0.37	46.40 \pm 1.45	11.74 \pm 0.71	65.67 \pm 2.89	16.27 \pm 0.50	24.83 \pm 0.69	643.0 \pm 13.0 ^a	13.73 \pm 1.04
5000	7.44 \pm 0.22	49.50 \pm 2.33	12.10 \pm 0.17	63.83 \pm 3.11	15.67 \pm 0.21	24.50 \pm 1.22	753.3 \pm 72.6	12.70 \pm 2.16
	(p=0.0562)	(p=0.0820)	(p=0.1971)	(0.5183)	(p=0.2499)	(p=0.2592)	(p=0.0972)	(p=0.0825)
Toluene								
Control	6.22 \pm 1.40	46.20 \pm 2.76	9.83 \pm 1.98	63.43 \pm 1.82	15.87 \pm 0.57	25.07 \pm 1.56	841.0 \pm 37.1	8.30 \pm 2.69
1000	7.38 \pm 0.11 ^a	49.70 \pm 3.84	11.70 \pm 0.82	68.67 \pm 4.12	15.83 \pm 0.87	23.07 \pm 0.15	765.0 \pm 91.1	12.50 \pm 2.11 ^a
2000	7.54 \pm 0.87 ^a	48.30 \pm 0.70	11.90 \pm 0.60	64.60 \pm 2.97	15.87 \pm 1.06	24.63 \pm 1.03	783.0 \pm 47.0	11.47 \pm 1.86 ^a
5000	7.42 \pm 0.04 ^a	49.27 \pm 0.85	10.77 \pm 0.15	65.70 \pm 1.77	16.77 \pm 0.15	25.47 \pm 0.85	531.0 \pm 45.3	12.30 \pm 1.00 ^a
	(p=0.0488*)	(p=0.0155*)	(p=0.1678)	(p=0.5098)	(p=0.3980)	(p=0.0870)	(p=0.4033)	(p=0.0457*)

End points represents mean \pm SD for 5 rats. RBC (Red blood cell); HGB (Haemoglobin); HCT (Haematocrit); PLT (platelet); WBC (White blood cell); MCV (mean corpuscular volume); MCH (mean corpuscular haemoglobin); MCHC (mean corpuscular haemoglobin concentration). Dist. H₂O (Distilled water). Superscripts differ significantly (^ap<0.05) from corresponding control using Dunnett's multiple post hoc test. Dist.H₂O = Distilled water.

Table 3. Erythrocyte morphological abnormalities in rats exposed to aqueous, ethanol and toluene formulated camwood dye.

Conc (mg/kg)	Target Cells	Acanthocytes	Tear drops	Schizocytes
Dist H₂O				
Control	2.11 \pm 0.52	2.65 \pm 0.11	0.57 \pm 0.15	0.61 \pm 0.01
1000	2.08 \pm 0.14	2.51 \pm 0.85	0.61 \pm 0.37	0.76 \pm 0.20
2000	1.92 \pm 0.25	2.12 \pm 0.75	0.50 \pm 0.51	0.73 \pm 0.11
5000	2.01 \pm 0.36	2.46 \pm 0.34	0.66 \pm 0.18	0.70 \pm 0.52
	(p=0.9566)	(p=0.3782)	(p=0.2437)	(p=0.3996)
Ethanol				
Control	2.53 \pm 0.30	2.74 \pm 0.18	2.39 \pm 0.39	1.51 \pm 0.05
1000	2.42 \pm 0.15	2.36 \pm 0.69	1.96 \pm 0.27	1.04 \pm 0.19
2000	2.07 \pm 0.36	2.11 \pm 0.72	1.87 \pm 0.81	1.13 \pm 0.27
5000	2.94 \pm 0.47	2.08 \pm 0.14	1.79 \pm 0.22	1.02 \pm 0.21
	(p=0.1052)	(p=0.8120)	(p=0.1971)	(0.5183)
Toluene				
Control	3.22 \pm 0.10	5.12 \pm 0.58	3.93 \pm 0.46	2.43 \pm 0.82
1000	2.19 \pm 0.41	3.65 \pm 0.36 ^a	2.47 \pm 0.69	2.07 \pm 0.12
2000	2.26 \pm 0.85	3.39 \pm 0.59 ^a	2.58 \pm 0.24	2.11 \pm 0.17
5000	2.13 \pm 0.49	3.42 \pm 0.47 ^a	2.02 \pm 0.55	2.02 \pm 0.39
	(p=0.1988)	(p=0.0155*)	(p=0.1678)	(p=0.5098)

End points are in mean \pm SD. Superscripts differ significantly (^ap<0.05) from corresponding control using Dunnett's multiple post hoc test. Dist.H₂O = Distilled water.

Cytogenotoxicity Analysis

When compared with the corresponding controls; aqueous (0 mg) and ethanol (0 mg), the 1000, 2000 and 5000 mg treated groups A and B rats did not significantly ($p < 0.05$) induced increase in the frequencies of micronucleated PCE and PCE/NCE ratio in bone marrow cells of rats (Table 4). Toluene (0 mg) marginally induced significant ($p = 0.0227$) increased in the frequencies of MNPCE and insignificantly ($p = 0.8100$) reduced PCE/NCE ratio in the treated rats. Toluene prepared camwood dye (1000, 2000 and 5000 mg) treated rats in group C presented frequencies of MNPCE and PCE/NCE ratio values that were close to those observed in group A and B (Table 4). Figure 3(a-c) presents cells observed as MNPCE, PCE and NCE in bone marrow of rats.

Skin Histopathology

Skin histology for the corresponding controls (0 mg) and treated (1000, 2000 and 5000 mg) rats in three Groups A, B and C is shown in Figures 4A - F. There is three layered stratum germinativum, with moderate keratin, few hair follicles and normal dermal layer in rats

treated with distilled water (0 mg) (control for Group A; figure 4A). Figure 4B presents discontinuous multilayered stratum germinativum, with marked keratin, moderate hair follicles and slightly thick dermal layer in rats treated with ethanol (0 mg) (control for Group B).

Figure 4C shows discontinuous stratum germinativum with marked necrosis of the epidermal cells. There were few hair follicles and densely thick dermal layer with widely dispersed areolar tissues in rats treated with toluene (0 mg) (Group C). Figure 4D shows three layers stratum granulosum and stratum germinativum with moderate keratin, numerous hair follicles and thin dermis in rats treated with aqueous prepared (1000 mg) *B. nitida* dye. Figure 4E shows relatively normal stratum germinativum with slight keratin, moderate hair follicle and thin dermis in rats treated with ethanol prepared (5000 mg) camwood dye, while figure 4F shows discontinuous and moderately normal epidermis and hair follicles in the dermis of rats treated with toluene prepared (5000 mg) camwood dye.

Table 4. PCE/NCE ratio and micronucleated polychromatic erythrocytes in bone marrow cells of rat treated with aqueous, ethanol and toluene prepared camwood dye.

	Conc (mg/kg)	MNPCE	p value	PCE/NCE	p value
Dist Water	Control	0.47±0.17		1.19±0.37	
	1000	0.33±0.88	0.5597*	1.25±0.28	0.4644*
	2000	0.33±0.03		1.26±0.74	
	5000	0.34±0.12		1.84±0.39	
Ethanol	Control	0.53±0.17		1.11±0.16	
	1000	0.57±0.38	0.3696*	1.36±0.89	0.6691*
	2000	0.69±0.17		1.75±0.81	
	5000	0.41±0.09		1.33±0.14	
Toluene	Control	2.02±0.10		0.83±0.23	
	1000	1.25±0.19 ^a	0.0227	1.10±0.47	0.8100*
	2000	1.02±0.06 ^a		1.09±0.15	
	5000	1.17±0.09 ^a		1.11±0.26	

End points are in mean ± SD. Superscripts differ significantly (^a $p < 0.05$) from corresponding control using Dunnett's multiple post hoc test. Dist.H₂O = Distilled water.

PCE – Polychromatic erythrocytes; NCE – Normochromatic erythrocytes

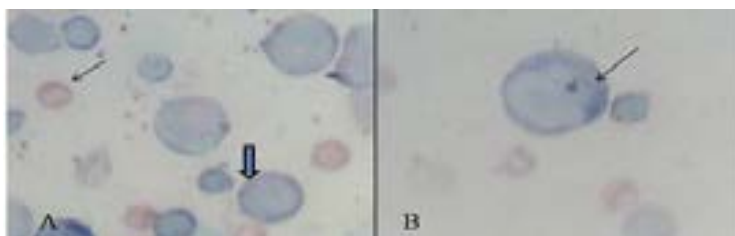


Figure 3. Shows bone marrow cells scored for cytogenotoxicity assessment.

A: Thin arrow shows normochromatic erythrocytes (NCE), they are somewhat smaller than PCE and stain light orange or orange-pink with Giemsa due to their acidophilic nature. Bold arrow shows polychromatic erythrocytes (PCE), they are basophilic and stains light blue or blue gray with Giemsa.

B: Arrow shows micronucleated polychromatic erythrocytes (MNPCE) observed in toluene (0 mg) treated rats.

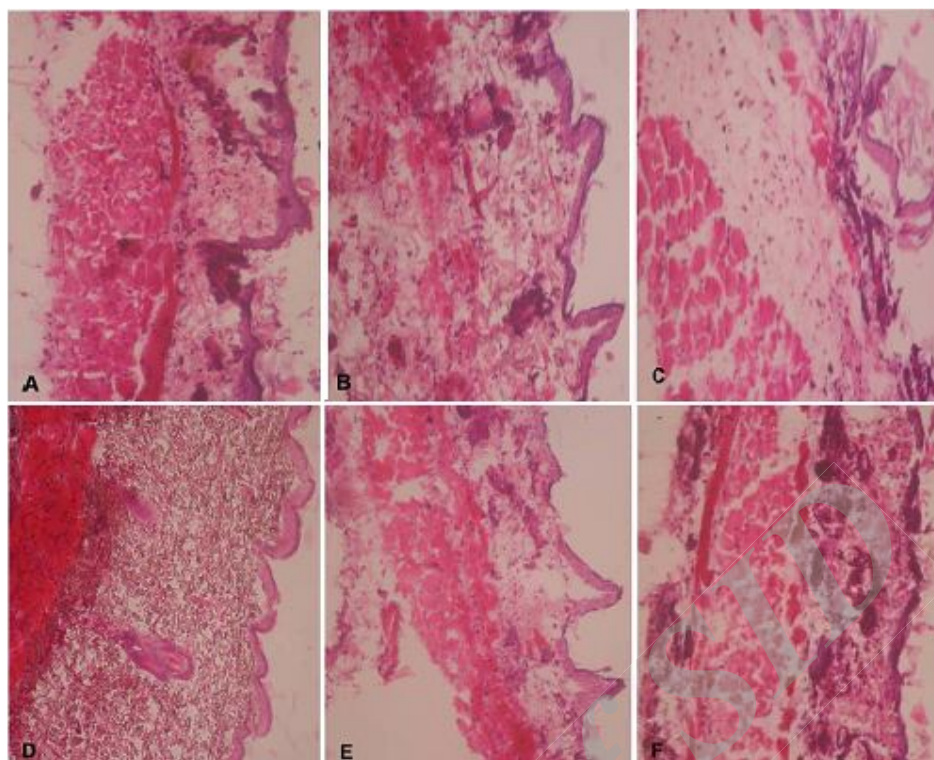


Figure 4. Skin histopathology from the site of exposure for the treated rats.

A: a three layered stratum germinativum, with moderate keratin, few hair follicles and normal dermal layer from rat treated with water sample (0 mg).

B: a discontinuous multilayered stratum germinativum, with marked keratin, moderate hair follicles and slightly thick dermal layer from rats treated with ethanol (0 mg).

C: a discontinuous stratum germinativum with marked necrosis of the epidermal cells, few hair follicles and densely thick dermal layer with widely dispersed areolar tissues from rats treated with toluene (0 mg).

D: a three layer stratum spinosum and stratum germinativum with moderate keratin, numerous hair follicles and thin dermis in rats treated with aqueous prepared camwood dye (1000 mg).

E: relatively normal stratum germinativum with slight keratin, moderate hair follicle and thin dermis in rats treated with ethanol prepared camwood dye (5000 mg).

F: very thin, discontinuous and fairly normal epidermis, with moderate hair follicles and thin dermis in rats treated with toluene prepared camwood dye (5000 mg).

DISCUSSION

Skin permeation of chemical xenobiotics is the penetration of molecules of these chemicals from one layer into another layer in the skin. The lipid matrix of stratum corneum layer plays an important role in determining the permeability of substances through the skin. Toluene is increasingly being used as solvent in most industries including paint, cosmetic and pharmaceutical industries [12, 13]. It has shown that toluene is readily absorbed into the body through the respiratory and gastrointestinal tracts during inhalation and accidental ingestion, respectively. It had also being reported to be absorbed through the skin [12], with the rate of absorption through forearm skin in the range of

14 – 23 $\text{mgcm}^{-2} \text{hr}^{-1}$ [29]. Moreover, when absorbed, toluene molecules are rapidly distributed throughout the body, mostly to the adipose (lipid) tissue, bone marrow, kidney, liver, brain and blood, where it induces deleterious effects [29, 30]. *B. nitida* (camwood) is increasingly being exploited as a potential source of drug, cosmetic and skincare product for human use. Its phytochemical compositions include flavonoid, alkanoids, tannin, phlobatanin, saponin, steroids, terpenoids and glycosides [10, 11, 31], which are known for their anti-inflammatory, anti-allergic, anti-viral, anti-bacterial and anti-tumoral activities [32]. Some of these phytochemicals are capable of preventing oxidative injury and cell death by several mechanisms, such as scavenging oxygen

radicals [33] and protecting against lipid peroxidation [34].

This study reports the 96 hr LD₅₀ toxicity, cytogenotoxicity, hematotoxicity and skin histopathology induced by toluene and the modulatory effects of *B. nitida* dye in rats after dermal exposure. The 96 hr LD₅₀ toxicity test predicts the safe dose of drugs or chemicals in biological systems [20]. The 96 hr LD₅₀ of aqueous, ethanol and toluene precipitated camwood dye in rats was indeterminate. This suggests that water, ethanol and toluene and the prepared *B. nitida* dye were unable to induced mortality (acute toxicity) in rats via dermal exposure. This may indicate that it's relatively safe to mammalian skin. Similar studies had shown that the methanol and acetone extracts of *B. nitida* leaves did not demonstrate any acute toxicity in orally exposed rats and mice [8]. The clinical signs of toxicity observed in toluene (0 mg) treated rats were attributed to its defatting action and extensive chemical burns due to its chemical nature. These observations had been previously reported in humans occupationally and environmentally exposed to liquid toluene from paints [30, 35, and 36]. Also in rabbits, 10 – 20 drops of repeated applications of undiluted liquid toluene on the skin for 2 - 4 weeks [30], and Guinea pigs treated with liquid toluene three times a day, for three days developed redness of the skin and increased epidermal thickness [37].

Toluene significantly reduce erythrocyte counts, hemoglobin concentrations and total leucocyte counts, with increased acanthocytes in the treated rats ($p < 0.05$). It is corroborated with previous report in male and female F344 rats exposed to 0, 30, 100, or 300 ppm toluene for 6 hr per day and 5 days per week through inhalation, showed altered hematological indices [38]. Alterations in the hematological parameters of the toluene treated rats suggest an adaptive response of the bone marrow cells and/or circulating erythrocytes to the physiological and immunological changes due to toluene induced stress or its direct contacts with the blood cells [25, 26]. *In vivo* micronucleus assay is the recommended test frequently used for investigating the cytotoxic, clastogenic and aneugenic profile of chemicals [28]. Toluene significantly ($p < 0.05$) increases micronucleus formation and insignificantly decreases PCE/NCE ratio in rats. It indicates its possible

genotoxic and cytotoxic effects in mammalian systems. These findings are in support of the report that workers occupationally exposed to 104 - 1170 ppm toluene expressed increased chromosome and chromatid breaks, and sister chromatid exchange in their peripheral lymphocytes compared to control group [37]. Histopathological examination of tissues is acknowledged as the most sensitive end point for detecting organ toxicity induced by xenobiotics that may not be readily detected by functional biomarkers [39, 40]. The marked necrosis of the epidermal layer and thickness of the dermal layer with few hair follicles observed in toluene (0 mg) treated rats suggest harmful effects of toluene on mammalian skin. Necrosis had been previously reported in the brain tissues of toluene treated rats [41], and it was attributed to the degreasing action of toluene and its ability to remove protective skin oils by free radical generations [15, 19, 42, 43]. These changes were not readily observed in water and ethanol (0 mg) exposed rats. It is plausible that the observed hematological, cytogenotoxic and histological alterations in toluene (0 mg) treated rats may have been induced by oxidative stress caused by toluene molecules after permeating through the skin in the 28 days exposure.

B. nitida dye attenuates the sub-lethal toxic effects of toluene in the treated rats. Generation of reactive oxygen species was implicated with toluene induced histopathology and biochemical alterations in rats [14, 15]. Therefore, *B. nitida* dye attenuates the hematotoxic, cytogenotoxic and histological effects of toluene in rats, may be attributed to its antioxidant activities. This assertion led credence to Akande et al. [31] who have reported that ethanolic extracts of *B. nitida* possess anti-oxidative phytochemicals which modulate the toxicological effects of diazepam in rats. Also, phytochemicals present in *B. nitida* are responsible for the anti-inflammatory and anti-bacterial properties of camwood against skin infections and gastrointestinal problems [6, 7, 10, 11, and 44]. The results presented herein are of significance in the cosmetic and skincare sectors as camwood dye is increasingly being use in the preparation of topical cosmetics and ointments, and pharmaceutical preparations in the treatment of bacterial and fungal diseases affecting skin, urinary tract, enteritis and gastrointestinal tract [7, 44].

CONCLUSION

96 hr dermal LD₅₀ for camwood dye preparations using water, ethanol and toluene was indeterminate. Camwood dye ameliorated the hematotoxicity, cytogenotoxicity and histopathological lesions induced by toluene in the treated rats. It suggests the protective effects of camwood dye on mammalian skin, and may be relatively safe for topical cosmetic and skincare preparations.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. John, Department of Veterinary Pathology, for the histological analysis and Ifeanyi, Adetunji Victor, and Stephnia, Department of Cell Biology and Genetics, University of Lagos, for their technical assistance.

REFERENCES

- Egharevba R, Ikhatua M. Ethno-medical uses of plants in the treatment of various skin diseases in Ovia North East, Edo State, Nigeria. *Res J Agric Biol Sci.* 2008;4(1):58-64.
- Ouedraogo M, Baudoux T, Stévigny C, Nortier J, Colet J-M, Efferth T, et al. Review of current and "omics" methods for assessing the toxicity (genotoxicity, teratogenicity and nephrotoxicity) of herbal medicines and mushrooms. *Journal of ethnopharmacology.* 2012;140(3):492-512.
- Kennedy J. Herb and supplement use in the US adult population. *Clinical therapeutics.* 2005;27(11):1847-58.
- Organization WH. The promotion and development of traditional medicine: report of a WHO meeting [held in Geneva from 28 November to 2 December 1977]. 1978.
- Neuwinger H. African Ethnobotany: Poisons and Drugs, In: Champman and Hall, ed. Chemistry, Pharmacology, Toxicology, Bundesrepublik Deutschland, 1996. P.942-3.
- Olowosulu A, Ibrahim Y. Studies on the antimicrobial screening of aqueous extracts of five plants used in Folk medicine in Nigeria. *West Afr J Boil Sci.* 2006;3(5):21-6.
- Gilles KNd, Djakalia O, Léopold AA, Dédéou S, Bamba K, Edouard NgK, et al. Anti-inflammatory and cicatrizing activity of Baphia nitida Lodd. exudates. *International Journal of Biosciences (IJB).* 2011;1(5):45-50.
- Onwukaeme D, Lot T. The effects of Baphia nitida Lodd (Leguminosae) extract on the gastrointestinal tract of rats and mice. *Phytotherapy Research.* 1992;6(3):129-32.
- Soladoye MO. A revision of Baphia (Leguminosae-Papilionoideae). *Kew bulletin.* 1985:291-386.
- Agwa O, Uzoigwe C, Mbaegbu A. Antimicrobial activity of camwood (Baphia nitida) dyes on common human pathogens. *African Journal of Biotechnology.* 2014;11(26):6884-90.
- Onwukaeme N. Anti-inflammatory activities of flavonoids of Baphia nitida Lodd.(Leguminosae) on mice and rats. *Journal of ethnopharmacology.* 1995;46(2):121-4.
- Agency for Toxic Substances and Disease Registry(ATSDR). *Case studies in environmental medicine: toluene toxicity.* [cited 2007 July 5]. Available from:<http://www.atsdr.cdc.gov/hec/csem/toluene/docs/toluene.pdf>.
- Faust RA. *Condensed Toxicity Summary for TOLUENE.* [cited 2011 July 5]. Available from: http://rais.ornl.gov/tox/profiles/toluene_c_V1.html.
- Ayan M, Tas U, Sogut E, Kuloglu T, Cayli S, Kocaman N, et al. The apoptotic effect of a high dose of toluene on liver tissue during the acute phase: an experimental study. *Toxicology and industrial health.* 2013;29(8):728-36.
- Kanter M. Protective effects of quercetine on the neuronal injury in frontal cortex after chronic toluene exposure. *Toxicology and industrial health.* 2012; 28:483-91.
- Evans EB, Balster RL. CNS depressant effects of volatile organic solvents. *Neuroscience & Biobehavioral Reviews.* 1991;15(2):233-41.
- Flanagan R, Ruprah M, Meredith T, Ramsey J. An introduction to the clinical toxicology of volatile substances. *Drug Safety.* 1990;5(5):359-83.
- Berenguer P, Soulage C, Perrin D, Pequignot J-M, Abraini JH. Behavioral and neurochemical effects induced by subchronic exposure to 40 ppm toluene in rats. *Pharmacology Biochemistry and Behavior.* 2003;74(4):997-1003.
- Mattia CJ, Ali SF, Bondy SC. Toluene-induced oxidative stress in several brain regions and other organs. *Molecular and chemical neuropathology.* 1993;18(3):313-28.
- Organization for Economic Cooperation and Development (OECD). OECD Guideline for testing of chemicals - Guideline 402: Acute Dermal Toxicity. Paris.1997.p.1-7.
- Organization for Economic Cooperation and Development (OECD). OECD Guideline for testing of chemicals - Guideline 410: Repeated Dose Dermal Toxicity. Paris.1981.p.1-8.
- Stone SH, editor. Method for obtaining venous blood from the orbital sinus of the rat or

- mouse 1954. American Association for the Advancement of Science. 1954; 119: 100-1.
23. Brown G. Haematology tests in toxicology: Time for a re-think? *Comparative haematology international*. 1992;2(4):231-5.
 24. Dacie JV, Lewis SM. *Practical Haematology*. 4th Ed. Churchill livingstone, Edinburgh, London, 1984.p. 420-1.
 25. Christopher MM. Interpretation of Red Blood Cell Morphology (Advanced Level), proceedings of 29th world congress of the world small animal veterinary Association. 2004.
 26. Cheesbrough M. *District laboratory practice in tropical countries*: Cambridge university press; 2005.p.263-47.
 27. Schmid W. The micronucleus test. *Mutation Research/Environmental Mutagenesis and Related Subjects*. 1975;31(1):9-15.
 28. Krishna G, Hayashi M. In vivo rodent micronucleus assay: protocol, conduct and data interpretation. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*. 2000;455(1):155-66.
 29. Defra and environment agency. Contaminants in soils: collation of toxicological data and intake values for humans. Toluene. R&D Publication TOX 14. Bristol: Environment Agency. 2004.
 30. International Programme on Chemical Safety (IPCS). *Chemical Environmental Health Criteria 52: Toluene*. WHO. Geneva, 1986.
 31. Akande I, Akande B, Gbenle G. Toxicological and antioxidant effects of ethanolic extract of *Baphia nitida* on diazepam induced oxidative stress in rats. *Af. J of Biochem Res*. 2011;5(8):255-63.
 32. Middleton Jr E. Effect of plant flavonoids on immune and inflammatory cell function. *Flavonoids in the Living System*: Springer; 1998. p. 175-82.
 33. Inal M, Akgün A, Kahraman A. Radioprotective effects of exogenous glutathione against whole-body g-ray irradiation: age- and gender-related changes in malondialdehyde levels, superoxide dismutase and catalase activities in rat liver. *Methods and findings in experimental and clinical pharmacology*. 2002;24(4):209-12.
 34. Laughton MJ, Evans PJ, Moroney MA, Houlst J, Halliwell B. Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives: relationship to antioxidant activity and to iron ion-reducing ability. *Biochemical pharmacology*. 1991;42(9):1673-81.
 35. Shibata K, Yoshita Y, Matsumoto H. Extensive chemical burns from toluene. *The American journal of emergency medicine*. 1994;12(3):353-5.
 36. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Toluene. [cited 2015 January 21]. Available from: <http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=161&tid=29>.
 37. Von Burg R. Toluene. *Journal of applied toxicology*. 1993;13(6):441-6.
 38. Gibson JE, Hardisty JF. Chronic toxicity and oncogenicity bioassay of inhaled toluene in Fischer-344 rats. *Toxicological Sciences*. 1983;3(4):315-9.
 39. Lanning LL, Creasy DM, Chapin RE, Mann PC, Barlow NJ, Regan KS, et al. Recommended approaches for the evaluation of testicular and epididymal toxicity. *Toxicologic pathology*. 2002;30(4):507-20.
 40. Travlos G, Morris R, Elwell M, Duke A, Rosenblum S, Thompson M. Frequency and relationships of clinical chemistry and liver and kidney histopathology findings in 13-week toxicity studies in rats. *Toxicology*. 1996;107(1):17-29.
 41. Program NT. NTP Toxicology and Carcinogenesis Studies of Toluene (CAS No. 108-88-3) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). National Toxicology Program technical report series. 1990;371:1-2.
 42. Agency for Toxic Substances and Disease Registry (ATSDR): Toxicological profile for toluene. Prepared by Life Systems. U.S. Department of Health and Human Services, Public Health Service, 1989.
 43. National Institute of Occupational Safety and Health (NIOSH). *Criteria for a Recommended Standard Occupational Exposure to Toluene*. [cited 1989 Jun 6]. Available from: <http://www.cdc.gov/niosh/docs/87-104>.
 44. Olukemi OA, Oluseyi JM, Olukemi IO, Olutoyin SM. The use of selected Nigerian natural products in management of environmentally induced free radical skin damage. *Pakistan Journal of Biological Sciences*. 2005;8(8):1074-7.