(RESEARCH ARTICLE)

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Immunological and hematological effects of *Irvingia gabonensis* stem bark in sodium arsenite-exposed rats

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Abstract

This study investigated the effect of ethanol stem bark extract of *Irvingia gabonensis* (ESEIG) on sodium arsenite (SA)-induced pro-inflammatory cytokines and hematological perturbations in Wistar rats. Fifty-five Wistar rats weighing 100 g - 179 g were distributed into eleven groups (n=5). Group 1 had feed and water only. Group 2 received 4.1 mg/kg body weight (kgbw) of SA. Groups 3-11 received SA and/or ESEIG. Treatment was done orally for 14 days. Interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), interleukin-4 (IL-4) concentrations, hemoglobin (HB) concentration, red blood cell (RBC) count, packed cell volume (PCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), mean corpuscular volume (MCV), white blood cell (WBC) count and its differentials and platelet (PLT) count were used to investigate the immunological and hematological effects of ESEIG. Exposure to SA produced significant (p < 0.05) increases in hepatic IL-1β, TNF-α, IL-10 and IL-4 concentrations relative to control. Administration of SA also caused significant (p < 0.05) decreases in HB, RBC, PCV, MCHC, MCH, MCV and PLT and significant (p < 0.05) increases in WBC, lymphocytes, monocytes, eosinophils and neutrophils compared with control. Treatment with ESEIG concomitantly and 2 weeks after SA exposure, mitigated the deleterious effect of SA. However, ESEIG alone at various doses caused significant (p < 0.05) increases in some of the assayed parameters, compared with control. These results imply that ESEIG may be protective against SA-induced inflammation and hematological derangements in Wistar rats. Its exclusive administration on chronic basis may also be slightly toxic.

Keywords: Arsenic; Sodium arsenite; *Irvingia gabonensis*; Cytokines; Hematological derangements; Environmental pollutant

1. Introduction

Arsenic, an environmental carcinogen, contaminates groundwater in different parts of the World [1-3]. A myriad of people all over the World are exposed to arsenic via the consumption of arsenic-contaminated drinking water and food. This predisposes them to the several toxic effects of arsenic, thus compromising their health. Arsenic exposures stemming from the consumption of arsenic-contaminated drinking water have been linked to carcinogenesis among inhabitants of different countries [4,5]. Inflammation is an immunological body defense mechanism against injury, infection and allergy characterized by immigration of leukocytes and release of chemical toxins [6]. The inflammatory response is mediated by soluble factors such as cytokines [7]. However, prolonged inflammation is detrimental to health [8,9]. It makes the host susceptible to damaging consequences of the inflammatory response [8]. Arsenic-induced prolonged inflammation favors the development of several diseases [10]. Blood components are prone to change in relation to physiological health conditions [11]. Hematological derangements are used to determine stresses due to

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environmental and nutritional factors [12]. Arsenic and sodium arsenite have been reported to orchestrate hematological derangements [10,13,14].

Irvingia gabonensis O'Rorke Baill (IG), also called bush mango, is a plant that is used in traditional and modern medicine for treatment of several illnesses [15,16]. Its stem bark is used to treat hunch back and infections in Cameroon [17] and has been reported to possess antibacterial and antifungal activities [18]. In addition, the decoction of the stem bark is utilized for treating gonorrhea, gastrointestinal and hepatic disorders and the root bark is used in poultice form to treat wounds [19]. In French Equatorial Africa, the stem bark is also used as an antidote and for treating hernias, yellow fever and dysentery [20]. The hepatoprotective, anti-diabetic, hematological and prophylactic effects of the stem bark and leaf extracts of Irvingia gabonensis in animal models have also been documented [21-24]. However, there is a paucity of information on the effects of I. gabonensis stem bark against sodium arsenite-induced immunological and hematological disturbances in animal models and this was what informed this study.

2. Material and methods

2.1. Collection and Preparation of Plant Extract

Fresh and matured stem bark of I. gabonensis O'Rorke Baill was harvested from a village in Akwa Ibom State, Nigeria. The samples were identified and authenticated by a taxonomist of the Department of Pharmacognosy and Herbal Medicine, University of Uyo, Akwa Ibom State, Nigeria. They were washed using clean water to eliminate dust and other contaminants and air-dried for 7 days at room temperature. The dried sample was then pulverized using a clean mortar and pestle, and stored in an air-tight container.

About 2800 g of pulverized stem bark was macerated in absolute ethanol (JHD, China) and allowed to stand for 72 h with intermittent stirring to ensure that the active ingredients were dissolved. The sample was filtered thrice through a clean muslin cloth and the filtrate was concentrated in stainless steel bowl using a water bath at 45°C to obtain a paste-like gel extract and stored in the refrigerator at 4°C, prior to use.

2.2. Experimental Animals

Fifty-five (55) healthy and non-pregnant female Wistar albino rats of weighing between 100 g and 179 g were acquired from the animal house facility of Faculty of Basic Medical Sciences, University of Uyo, Nigeria. They were acclimatized for seven (7) days in the same facility under standard conditions, having free access to feed and water.

2.3. Experimental Design

Sequel to the seven-day acclimatization and just before treatment commenced, the experimental animals were randomly assigned to eleven (11) groups of five animals each in standard experimental animal cages and weighed using a digital weighing balance (Camry electronic scale EK5350, China) after overnight fast to obtain their initial body weights. The detailed experimental design and treatment is as represented in Table 1. The various doses of ESEIG administered, were based on the already established LD_{50} of the extract [25].

2.4. Collection of Sample

Upon completion of treatment, all experimental animals were denied feed overnight, but still had access to water ad libitum and their final body weights were taken. They were sacrificed under chloroform anesthesia using lower abdominal incision about 24 hours after the last treatment. Whole blood samples were obtained by cardiac puncture using sterile syringes and needles and collected in sterile ethylenediamine tetraacetic acid (EDTA) bottles for hematological analyses. Liver tissues were excised and rinsed with 1.15 % ice cold potassium chloride (KCl) solution to remove traces of blood before weighing. The liver tissues were placed in sterile universal container and frozen prior to homogenization.

Table 1 Experimental Design and Treatment Modalities

Groups / Treatment	Dose / Duration
1. (Normal control)	Normal feed and water ad libitum
2. (Negative control)	SA at a dose of 4.1 mg/kgbw for 14 days
3. (Post-treatment)	SA at a dose of 4.1 mg/kgbw for 14 days, followed with 100 mg/kgbw ESEIG for another 14 days
4. (Post-treatment)	SA at a dose of 4.1 mg/kgbw for 14 days, followed with 200 mg/kgbw ESEIG for another 14 days
5. (Post-treatment)	SA at a dose of 4.1 mg/kgbw for 14 days, followed with 400 mg/kgbw ESEIG for another 14 days
6. (Concomitant treatment)	SA at a dose of 4.1 mg/kgbw for 14 days + 100 mg/kgbw ESEIG concomitantly for 14 days
7. (Concomitant treatment)	SA at a dose of 4.1 mg/kgbw for 14 days + 200 mg/kgbw ESEIG concomitantly for 14 days
8. (Concomitant treatment)	SA at a dose of 4.1 mg/kgbw for 14 days + 400 mg/kgbw ESEIG concomitantly for 14 days
9. (ESEIG only)	100 mg/kgbw ESEIG only for 14 days
10. (ESEIG only)	200 mg/kgbw ESEIG only for 14 days
11. (ESEIG only)	400 mg/kgbw ESEIG only for 14 days

ESEIG = Ethanol stem bark extract of *Irvingia gabonensis*; SA= Sodium arsenite; mg/kgbw = milligram per kilogram body weight

2.5. Homogenization of Liver Tissues

The liver tissues of the experimental animals were homogenized as reported by Ewere et al. [26].

2.6. Reagents and Chemicals

Reagents / chemicals used in this study were of analytical grade and standard.

2.7. Determination of Hepatic Pro-inflammatory and Anti-inflammatory Cytokine Levels

Concentrations of pro-inflammatory cytokines [tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β)] as well as anti-inflammatory cytokines [interleukin-4 (IL-4) and interleukin-10 (IL-10)] in liver homogenates, were determined by Enzyme-linked immunosorbent assay (ELISA) using Sunlong Biotech Co. (Zhejiang, China) assay kits according to manufacturer's protocol.

2.8. Hematological Analyses

Red blood cell (RBC) count was carried out according to the method of Ochei and Kolhakaar [27]. Blood hemoglobin (HB) concentration was determined using the method of Tietz [28]. Wintrobe method was used in the determination of packed cell volume (PCV). Determination of White blood cell (WBC) count and its differentials was carried out according to the method of Cheesbrough [29]. Platelet (PLT) count was determined according to the method of Dacie and Lewis [30]. Red blood indices: mean cell hemoglobin (MCH), mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC) were determined from RBC, PCV and haemoglobin (Hb) using the method of Jain [31].

2.9. Statistical Analysis

Results obtained are presented as mean ± standard deviation (SD). They were analysed with one-way analysis of variance (ANOVA) for differences between groups using SPSS Software (IBM, version 20). Values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Effect of ESEIG on Pro-inflammatory and Anti-inflammatory Cytokine Levels in Experimental Rats

Administration of SA (group 2) caused significant ($p < 0.05$) increases in TNF-α, IL-1β, IL-4 and IL-10 relative to control. Post-treatment with ESEIG at various doses of 100, 200 and 400 mg/kgbw, produced significant decreases in all cytokines in a dose-dependent and independent manner, compared with group 2, except IL-1β. Concomitant treatment with ESEIG followed similar trend except IL-4 and IL-10. Treatment with ESEIG only at various doses of 100, 200 and 400 mg/kgbw, also produced comparable results as those of the control, except IL-4. The results of the effect of ESEIG on the immunological parameters in rats exposed to SA are presented in Table 2.

Table 2 Effect of ESEIG on Pro-Inflammatory and Anti-Inflammatory Cytokine Levels in Experimental Rats

Groups / Treatment	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-4 (pg/mL)	IL-10 (pg/mL)
1. Control	16.90±2.98[bf]	1.68±0.34[bcdefghijk]	2.72±0.37[bcefgi]	15.46±1.51[bf]
2. SA only	22.40±2.23[acdegh]	3.58±0.57[a]	5.32±0.76[acdegh]	18.08±1.29[ade]
3. Post-Treatment at 100 mg/kg	18.06±1.59[bd]	3.30±1.00[a]	3.85±0.47[abf]	16.04±1.74[f]
4. Post-Treatment at 200 mg/kg	14.92±2.24[bc]	2.80±0.48[a]	3.36±0.62[b]	15.16±1.08[b]
5. Post-Treatment at 400 mg/kg	14.87±1.42[b]	2.82±0.29[a]	3.63±0.54[ab]	14.19±2.87[b]
6. Concomitant at 100 mg/kg	20.50±2.93[agh]	3.60±0.26[a]	4.83±0.39[acgh]	20.50±0.85[acgh]
7. Concomitant at 200 mg/kg	15.88±0.68[bf]	3.10±0.48[a]	3.90±0.59[abf]	17.08±1.27[f]
8. Concomitant at 400 mg/kg	15.30±3.78[bf]	3.50±1.42[a]	3.13±0.85[bf]	16.10±1.35[f]
9. ESEIG only at 100 mg/kg	18.30±1.80[k]	3.55±0.75[a]	3.65±0.15[a]	14.75±0.65
10. ESEIG only at 200 mg/kg	15.60±0.98	4.03±0.80[a]	3.07±0.72	15.97±1.76
11. ESEIG only at 400 mg/kg	14.03±2.75[i]	3.37±0.64[a]	2.77±0.80	14.13±2.90

Data are expressed as mean ±SD (n=5); [a] $p < 0.05$ compared with group 1; [b] $p < 0.05$ compared with group 2 [c] $p < 0.05$ compared with group 3; [d] $p < 0.05$ compared with group 4; [e] $p < 0.05$ compared with group 5; [f] $p < 0.05$ compared with group 6; [g] $p < 0.05$ compared with group 7; [h] $p < 0.05$ compared with group 8; [i] $p < 0.05$ compared with group 9; [j] $p < 0.05$ compared with group 10; [k] $p < 0.05$ compared with group 11; ESEIG = Ethanol stem bark extract of *Irvingia gabonensis*; SA= Sodium arsenite

3.2. Effect of ESEIG on Red Blood Cell Indices of Experimental Rats

Results obtained showed that exposure of the experimental animals to SA (group 2) produced significant ($p < 0.05$) decreases in RBC, HB, PCV, MCH, MCHC and MCV, compared with the normal control. Post-treatment with the extract at various doses of 100, 200 and 400 mg/kgbw, led to significant ($p < 0.05$) increases in RBC, PCV and MCH in a dose-dependent manner as well as significant ($p < 0.05$) increases in HB and MCV in a dose-independent manner, relative to group 2.

In addition, post-treatment with the extract at a dose of 200 mg/kgbw produced a significant ($p < 0.05$) increase in MCHC, compared with group 2. Similarly, concomitant treatment with the extract at various doses of 100, 200 and 400 mg/kgbw, led to significant ($p < 0.05$) increases in all assayed red blood cell indices, compared with group 2.

Treatment with the extract alone, produced significant ($p < 0.05$) increases in RBC at doses of 200 mg/kgbw and 400 mg/kgbw, respectively and non-significant ($p > 0.05$) differences in MCH and MCHC, PCV (at doses of 200 mg/kgbw and 400 mg/kgbw, respectively), relative to normal control. Treatment with the extract alone at the various doses also led to significant ($p < 0.05$) decreases in HB and MCV (at doses of at doses of 100 mg/kgbw and 400 mg/kgbw, respectively) compared with the normal control. The detailed results are presented in Table 3.

Table 3 Effect of ESEIG on Red Blood Cell Indices of Experimental Rats

Groups / Treatment	RBC (x 10^6/μL)	HB (g/dL)	PCV (%)	MCH (pg/cell)	MCHC (g/dL)	MCV (μm³)
1. Control	7.50±0.52[bfhjk]	14.04±1.01[bcefghijk]	41.56±4.36[bcdefi]	18.46±0.54[bcdf]	33.74±1.86[bch]	50.28±1.79 [bcefghik]
2. SA only	3.95±1.32[acdefgh]	8.11±1.22[acdefgh]	22.15±5.29[acdegh]	8.94±1.71 [acdefgh]	23.03±4.54 [adefgh]	30.73±3.31 [acdefgh]
3. Post-Treatment at 100 mg/kg	7.53±0.45[bf]	12.57±0.63[ab]	27.96±5.18[abe]	15.56±1.82[abf]	20.26±4.78[adef]	42.02±1.52[ab]
4. Post-Treatment at 200 mg/kg	7.67±0.36[b]	13.37±0.73[beg]	30.60±6.25[abeg]	16.53±1.25[ab]	34.80±3.16[bc]	43.20±2.45[ab]
5. Post-Treatment at 400 mg/kg	8.13±0.68[bh]	12.10±1.14[abd]	36.78±3.63[bcd]	17.20±1.82[b]	32.90±5.88[bch]	42.90±1.94[ab]
6. Concomitant at 100 mg/kg	8.76±0.62[abc]	12.04±0.65[ab]	27.65±2.64[agh]	12.16±1.90[abcgh]	33.68±1.70[bc]	43.78±4.49[ab]
7. Concomitant at 200 mg/kg	8.24±0.97[b]	11.19±0.93[abd]	36.58±1.96[bdf]	16.90±1.52[bf]	38.95±1.96[bx]	45.10±4.17[ab]
8. Concomitant at 400 mg/kg	9.26±0.30[abe]	12.32±1.74[ab]	38.87±1.66[bf]	18.01±1.06[bf]	42.53±13.69[abef]	41.43±1.90[ab]
9. ESEIG only at 100 mg/kg	7.90±0.60	10.46±0.18[ajk]	27.90±1.30[ajk]	18.11±0.25	35.45±3.65	43.35±1.75[a]
10. ESEIG only at 200 mg/kg	8.57±0.56[a]	12.25±0.46[ai]	43.40±4.80[i]	18.12±0.20	37.87±4.46	47.13±0.49
11. ESEIG only at 400 mg/kg	9.06±1.01[a]	12.66±0.46[ai]	45.00±2.65[i]	18.42±0.78	38.00±1.44	44.90±4.18[a]

Data are expressed as mean ±SD (n=5); [a] $p < 0.05$ compared with group 1; [b] $p < 0.05$ compared with group 2 [c] $p < 0.05$ compared with group 3; [d] $p < 0.05$ compared with group 4; [e] $p < 0.05$ compared with group 5; [f] $p < 0.05$ compared with group 6; [g] $p < 0.05$ compared with group 7; [h] $p < 0.05$ compared with group 8; [i] $p < 0.05$ compared with group 9; [j] $p < 0.05$ compared with group 10; [k] $p < 0.05$ compared with group 11; ESEIG = Ethanol stem bark extract of *Irvingia gabonensis*; SA= Sodium arsenite

Table 4 Effect of Ethanol Stem Bark Extract of *Irvingia gabonensis* O'Rorke Baill on Platelets, White Blood Cell Count and Its Differentials of Experimental Rats

Groups / Treatment	WBC (x10³/µL)	Platelets (x 10³/µL)	Lymphocytes (%)	Monocytes (%)	Basophils (%)	Eosinophils (%)	Neutrophils (%)
Control	3.58±0.71[bcdefghijk]	700.60±28.52 [bcdefghijk]	37.40±4.51 [bcdefghijk]	24.80±4.09 [bcdefghijk]	0.00±0.00	0.80±0.84 [bcdefghijk]	47.20±3.90[bcdefgi]
SA only	8.04±1.46[acdegh]	334.75±87.79[acdefgh]	78.50±4.20 [acdefgh]	49.50±12.71[a]	0.50±0.58	12.75±0.96 [acdefgh]	80.75±4.03 [acdefgh]
Post-Treatment at 100 mg/kg	6.66±0.63[abf]	439.40±54.23[abf]	65.00±8.75[abdef]	49.00±5.24 [a]	0.40±0.55	10.00±0.71[ab]	69.00±4.58[abd]
Post-Treatment at 200 mg/kg	6.49±0.46[ab]	459.20±62.89[abg]	58.20±4.87[abc]	45.60±3.65 [a]	0.20±0.45	8.60±1.14 [ab]	63.00±6.75[abc]
Post-Treatment at 400 mg/kg	6.92±0.70[ab]	486.50±46.62[ab]	53.25±3.20[abc]	48.75±2.99 [a]	0.25±0.50	8.25±1.26 [ab]	63.25±1.71[abh]
Concomitant at 100 mg/kg	7.66±0.97[ach]	527.50±48.79[abc]	54.50±3.11[abc]	54.00±6.38 [a]	0.50±0.58	10.50±1.29[abh]	66.50±3.70[abh]
Concomitant at 200 mg/kg	6.84±0.60[ab]	541.75±26.29[abd]	55.00±6.68[ab]	47.50±3.70 [a]	0.00±0.00	8.75±0.96 [ab]	60.00±3.74[abh]
Concomitant at 400 mg/kg	5.96±0.19[abf]	544.33±52.99[ab]	52.33±4.04[ab]	45.33±4.93 [a]	0.00±0.00	8.00±1.00[abf]	52.00±2.65[befg]
ESEIG only at 100 mg/kg	5.31±0.05[a]	570.67±4.51[a]	51.00±2.00[a]	47.67±1.53 [a]	0.33±0.58	9.00±2.00[ak]	63.00±5.00[ajk]
ESEIG only at 200 mg/kg	5.78±0.34[a]	549.67±53.27[a]	54.33±4.73[a]	41.00±4.36 [a]	0.33±0.58	8.33±2.52[a]	53.00±7.00 [i]
ESEIG only at 400 mg/kg	5.48±0.44[a]	606.00±36.76[a]	49.00±5.29[a]	42.67±3.51 [a]	0.00±0.00	6.67±2.08[ai]	52.67±4.73[i]

Data are expressed as mean ±SD (n=5); [a] $p < 0.05$ compared with group 1; [b] $p < 0.05$ compared with group 2 [c] $p < 0.05$ compared with group 3; [d] $p < 0.05$ compared with group 4; [e] $p < 0.05$ compared with group 5; [f] $p < 0.05$ compared with group 6; [g] $p < 0.05$ compared with group 7; [h] $p < 0.05$ compared with group 8; [i] $p < 0.05$ compared with group 9; [j] $p < 0.05$ compared with group 10; [k] $p < 0.05$ compared with group 11; ESEIG = Ethanol stem bark extract of *Irvingia gabonensis*; SA= Sodium arsenite

3.3. Effect of ESEIG on Platelet Count, White Blood Cell Count and its Differentials of Experimental Rats

Administration of sodium arsenite to the experimental rats (group 2) produced significant ($p < 0.05$) increases in WBC, lymphocyte (LYM), monocyte (MON), eosinophil (EOS) and neutrophil (NEU) counts and a significant ($p < 0.05$) decrease in PLT count, compared with the normal control. Post-treatment with the extract at various doses of 100, 200 and 400 mg/kgbw, led to significant ($p < 0.05$) decreases in WBC, LYM, EOS and NEU in a dose-dependent and independent manner, compared with group 2. Post-treatment with extract at the various doses, also culminated in significant ($p < 0.05$) increases in PLT count in a dose-dependent manner, compared with group 2. Similar trends were observed for the concomitantly-treated rats, compared with group 2. Treatment with extract also produced insignificant differences ($p > 0.05$) in MON count when the treated groups were compared. There were no significant ($p > 0.05$) differences in BAS count across all experimental groups.

Treatment with extract alone at various doses of 100, 200 and 400 mg/kgbw, culminated in significant ($p < 0.05$) increases in WBC, LYM MON, EOS and NEU counts and significant ($p < 0.05$) decreases in PLT count, compared with the normal control. The results are presented in Table 4.

4. Discussion

Arsenic exposure via consumption of contaminated water has been reported to elevate pro-inflammatory mediators and lower anti-inflammatory IL-10 in circulation, which may cause various diseases [32,33].

In the present study, continuous administration of sodium arsenite alone caused significant increases in hepatic concentrations of the pro-inflammatory cytokines, TNF-α and IL-1β as well as significant increases in hepatic concentrations of the anti-inflammatory cytokines, IL-4 and IL-10, compared with the normal control. Pro-inflammatory cytokines such as TNF-α and IL-1β participate in the induction, amplification and perpetuation of inflammation. Continuous stimulated production of pro-inflammatory cytokines culminates in chronic inflammation [34,35], which has been implicated in the etiology of various disease conditions [8,9]. Conversely, anti-inflammatory cytokines, such as IL-10 and IL-4, mitigate the inflammatory process by regulating the activities of pro-inflammatory cytokines, thus putting a check to their activities [34-36]. This study therefore affirms that induction of inflammation is one of the various mechanisms involved in sodium arsenite-induced toxicity in experimental rats, by up-regulating the production of pro-inflammatory cytokines. The induction of IL-10 and IL-4 observed in this study may be an adaptive response in trying to curb the activities of TNF-α and IL-1β. This is because; IL-10 represses the expression of inflammatory cytokines like TNF-α, IL-6 and IL-1 by activated macrophages. It can also enhance the expression / production of endogenous anti-inflammatory cytokines and down-regulate pro-inflammatory cytokine receptors thereby counter-regulating the production and function of pro-inflammatory cytokines at several levels [34,37]. Interleukin-4 (IL-4) reduces the effects of IL-1 and TNF-α by affecting activated macrophages and inhibits the generation of free radicals [37]. The findings from this study agree with previous studies on the effect of sodium arsenite on pro-inflammatory and anti-inflammatory cytokines [10,38,39].

Post-treatment with ESEIG at various doses produced significant decreases in all assayed cytokines in dose-dependent and independent manner, compared with group 2, except IL-1β. Simultaneous treatment with ESEIG followed similar trend except IL-4 and IL-10. In addition, treatment with ESEIG only at various doses produced similar results as those of the control, except IL-4. This indicates the mitigation of sodium SA-induced inflammation by ESEIG which may be as a result of its constituent anti-inflammatory and antioxidant phytochemicals [25].

Blood tissue is a pathological reflector of the health status of animals exposed to toxicants and other conditions [40]. Changes in hematological indices are useful in determining stresses orchestrated by environmental, nutritional and pathological factors [12]. The interaction of a toxin or its metabolites with cellular constituents may culminate in perturbations in hematological parameters [41], that indicate hematological disorders such as anemia (low hemoglobin content), leukopenia (reduced white blood cells), thrombocytopenia (low blood platelet level) [42-44]. Thus, hematological parameters are very useful in assessing toxicity and health / physiological status of animals [11,45].

In the present study, administration of sodium arsenite alone caused significant decreases in RBC, HB, PCV, MCH, MCHC and MCV, compared with normal control. Red blood cells (RBCs) facilitate transport of dissolved oxygen due to their constituent hemoglobin [46]. The significant decrease in RBC may be as a result of the cytotoxic effect of sodium arsenite that could have disrupted the red cell membrane integrity by lipid peroxidation and may climax in anemia, since a decreased RBC count is a sign of anemia [47,48]. As reported by Reddy and co-workers, an increase in the rate of hemoglobin destruction, or a decrease in the rate of hemoglobin synthesis may have influenced the significant decrease in HB concentration observed in this study [49]. This may have also contributed to the significant decreases in PCV,

MCHC, MCH and MCV observed in this study and may connote the presence of hypochromic and microcytic anemia. Packed cell volume (PCV) is the percentage of the RBC volume of the whole blood volume [46]. Abnormally low PCV may be due to a decrease in RBC count, a decrease in HB concentration in each RBC or both. It can be caused by chemical-induced damage to myeloid tissue which inhibits enzymes that are vital for hematopoiesis [41]. A possible inhibition of essential hematopoietic enzymes and destruction of RBCs by sodium arsenite may be responsible for the significant decrease in PCV. Similar findings have been reported in cadmium-intoxicated rats [50].

Administration of sodium arsenite alone also produced a significant decrease in blood platelets level, compared with control. This could inhibit the formation of platelet plugs that are vital in the prevention of haemorrhage at the site of injuries as well as loss of capillaries integrity [51]. In addition, administration of sodium arsenite alone caused significant increases in WBC count, lymphocytes, monocytes, basophils, eosinophils and neutrophils, compared to control. These may be indicative of sodium arsenite-induced leukocytosis, lymphocytosis, monocytosis, basophilia, eosinophilia and neutrophilia. White blood cells (leukocytes) provide immunity against antigen invasion. The significant increase in WBC may be as a result of the necrotic activities of sodium arsenite in the cells, as reported by Rousselot *et al.* [52] and Kumar *et al.* [53]. It could also be due to an increase in immune function as an attempt to mitigate the damaging effect of sodium arsenite [54]. This corroborates findings from some previous studies [53,55].

Cytokines and oxidative stress have been reported to enhance the activation of polymorpho- and mononuclear leukocytes [56,57]. Neutrophils are granular leukocytes that make up the highest percentage of leukocytes in circulation. Tissue injury and infection are common causes of elevated blood neutrophils [41]. Elevated neutrophils may be as a result of enhanced activities of leptin and leptin receptor that stimulates hematopoiesis [58]. Other causes of neutrophilia include necrosis and inflammation. Furthermore, inflammation and hepatitis have been reported to cause monocytosis and lymphocytosis, respectively and some forms of cancers have also been reported to cause eosinophilia and basophilia [41]. Inorganic arsenic compounds have also been reported to induce oxidative stress (which promotes carcinogenesis) by inhibiting antioxidant enzymes [59]. Thus, sodium arsenite might have caused the observed leukocytosis in this study via the induction of oxidative stress. Sodium arsenite and arsenic-induced hematological perturbations have also been reported by various researchers [10,13,14].

Treatment with ESEIG simultaneously and two weeks after (post-treatment) caused significant increases in RBC, HB, PCV, MCH, MCHC and MCV, compared with group 2, that received sodium arsenite only. It also produced significant increases in platelets count in a dose-dependent manner as well as significant decreases in WBC count, lymphocytes, eosinophils and neutrophils, compared with group 2 (administered sodium arsenite only). Treatment with same extract led to no significant differences in monocyte and basophil counts, compared with group 2, administered sodium arsenite only. Thus, ESEIG may have ameliorated the hematotoxic effects of sodium arsenite by mitigating sodium arsenite-oxidative stress. This may be due to the actions of antioxidant phytochemicals inherent in the extract [25]. Administration of ESEIG alone at the various doses, also produced significant increases in WBC, platelets, lymphocytes, monocytes, eosinophils and neutrophils, compared with control. This is suggestive of its involvement in hematological perturbations when administered singly on chronic basis. Similar findings have been reported in a previous study [60].

5. Conclusion

The results from the present study suggest that ethanol stem bark extract of *I. gabonensis* has anti-inflammatory and hemomodulatory properties against sodium arsenite-orchestrated inflammation and hematological perturbations in Wistar rats. Caution in its use is however advised as its long-term administration may be slightly toxic.

Compliance with ethical standards

Disclosure of conflict of interest

Authors Efosa Godwin Ewere, Ngozi Paulinus Okolie, Jessie Idongesit Ndem and Samson Adewale Oyebadejo have declared that no competing interest exists.

Statement of ethical approval

All authors hereby declare that the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate Institutional Review Board.

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