# Mitigation of Arsenic-induced Increases in Pro-Inflammatory Cytokines and Haematological Derangements by Ethanol Leaf Extract of *Irvingia gabonensis*

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*Authors' contributions*

*This study was carried out in collaboration among all authors. Authors EGE and NPO designed the study. Author NPO supervised the study. Author EGE performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EGE and SAO managed the analyses of the study. Author EGE managed the literature searches and authors NPO and OEE edited the manuscript. Authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To investigate the effect of ethanol leaf extract of *Irvingia gabonensis* (ELEIG) on sodium arsenite (SA)-induced hepatic pro-inflammatory cytokines and haematological derangements in Wistar rats.
**Study Design:** Fifty five Wistar rats weighing between 100 g and 161 g were randomly distributed to eleven (11) groups (n=5). Group 1 (control) had feed and water only. Group 2 received SA at a dose of 4.1 mg/kg body weight (kgbw) for 14 days. Groups 3-11 received ELEIG with or without SA.
**Place and Duration of Study:** Biochemistry Department, University of Uyo and Biochemistry Department, University of Benin. The duration of the study was 14 days.

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**Methodology:** Treatment was done orally for 14 days. Immunological markers: tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-4 (IL-4), interleukin-10 (IL-10), and haematological indices: red blood cell (RBC) count, haemoglobin (HB) concentration, packed cell volume (PCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) count and its differentials and platelet (PLT) count were used to determine the immunomodulatory and haematological effects of the extract.

**Results:** Intoxication with SA caused significant ($p < 0.05$) increases in hepatic TNF-α, IL-1β, and IL-4 levels and a significant decrease in hepatic IL-10 level, relative to control. The SA treatment also caused significant ($p < 0.05$) decreases in RBC, HB, PCV MCH, MCHC, MCV, PLT and monocyte counts as well as significant ($p < 0.05$) increases in WBC, lymphocyte, basophil, eosinophil and neutrophil counts, relative to control. However, post-treatment and concomitant treatment with ELEIG ameliorated the noxious effect of SA. In addition, ELEIG alone at various doses produced results with most of the assayed parameters having values comparable with those control.

**Conclusion:** These results indicate that ELEIG mitigates SA-induced inflammation and haematological perturbations in Wistar rats.

## 1. INTRODUCTION

Arsenic is a human carcinogen that contaminates the environment via natural and anthropogenic activities. It contaminates groundwater in different parts of the World [1-7]. Arsenic exposure in many parts of the World occurs via the consumption of arsenic-contaminated drinking water and food and it causes noxious effects *in vivo*. There is a link between consumption of arsenic-contaminated drinking water and cancer development among residents of various countries [8-12]. Chronic or prolonged inflammation is associated with severe detrimental health effects such as cancer [13,14]. It predisposes the host to damaging consequences of the inflammatory response [13]. Chronic inflammation due to the consumption of arsenic-contaminated water and food materials plays a role in the development of respiratory, cardiovascular, and other metabolic diseases [15]. Cytokines are examples of soluble factors that mediate the inflammatory response [16]. One vital way of determining environment and nutrition-related stresses is derangements in haematological components of an animal [17]. A myriad of studies have reported arsenic-induced haematological derangements [15,18-19].

Medicinal plants have been used from time immemorial for the treatment of diseases. *Irvingia gabonensis* (bush mango) is one of such medicinal plants. The leaves are used as antidotes against poisons [20]. The decoction of the stem bark is employed in treating gonorrhea, liver and gastrointestinal illnesses [20]. Different studies have reported the hepatoprotective; anti-diabetic, haematological and prophylactic effects of the leaf and stem bark extracts of *Irvingia gabonensis* in animal models [21-24]. In addition, its leaf extracts have been reported to possess ameliorative effects under conditions of induced toxicities in rat models [25-28]. However, there is a scarcity of information on the immunomodulatory and haematological effects of *I. gabonensis* leaves against sodium arsenite intoxication in experimental animal models. This study was therefore based on this premise.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

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

### 2.2 Preparation of Leaf Extract

Fresh leaves of *I. gabonensis* were obtained from a locality in Akwa Ibom State, Nigeria. They were authenticated by a taxonomist in Department of Pharmacognosy and Herbal Medicine, University of Uyo. The leaf samples were rinsed with clean water to eliminate contaminants, before air-drying for 7 days in Biochemistry laboratory, University of Uyo, Nigeria. They were then pulverized using a clean manual grinder, and stored in an air-tight container prior to extraction.

About 850 g of the pulverized leaves was macerated in absolute ethanol (JHD, China) for 72 h with intermittent stirring to ensure that the bioactive ingredients were solubilized. The sample was filtered repeatedly through a clean muslin cloth and the filtrate was concentrated in a stainless steel bowl using a water bath at 45°C to obtain a paste-like gel extract and preserved in a refrigerator at 4°C before use.

## 2.3 Experimental Animals

Fifty-five (55) healthy and pregnancy-free female Wistar albino rats weighing between 100 g and 161 g were purchased at 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. The experimental animals had free access to feed and water.

## 2.4 Experimental Design

Sequel to the seven days acclimatization and prior to commencement of treatment, the experimental animals were randomly distributed into eleven (11) groups (n=5) in standard experimental animal cages. Their initial body weights were obtained using a digital weighing balance (Camry electronic scale EK5350, China) after overnight fast. The detailed experimental design is as shown in Table 1.

## 2.5 Sample Collection

Upon termination of treatment, the experimental animals were subjected to overnight fast, but still had free access to water, and their final body weights were recorded. About 24 hours after the last treatment, the animals were sacrificed under chloroform anesthesia using lower abdominal incision. Whole blood samples were obtained by cardiac puncture using sterile syringes and needles and transferred into sterile ethylenediamine tetraacetic acid (EDTA) bottles for haematological analyses. Liver tissues were excised and rinsed with 1.15% ice cold potassium chloride (KCl) solution to remove traces of blood and weighed. The liver tissues were placed in sterile universal containers and frozen prior to homogenization.

## 2.6 Homogenization of Liver Tissues

The tissue sample was cut into small pieces of about 50 mg in weight on a Teflon cutting board using a pair of scissors. The cut pieces were homogenized in HEPES–KOH Buffer (pH 7.6), at 10,000 revolutions per minute. Equal volume of the homogenate was taken and spun at 10,000 revolutions per minute using refrigerated centrifuge (TGL16000) for 15 minutes. The tissue homogenate supernatant was transferred into a fresh test tube and stored immediately on dry ice in a glass jar, covered with a Teflon-lined lid below-10$^{\circ}$C with lid loosely attached to allow sublimation of $CO_2$. Sequel to $CO_2$ sublimation, the lid on the homogenate container was tightened and stored in a freezer below -10$^{\circ}$C prior to use for cytokine analyses.

## 2.7 Assay of Pro-inflammatory and Anti-inflammatory Cytokine Levels

Enzyme-linked immunosorbent assay (ELISA) was used to determine rat tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-4 (IL-4) and interleukin-10 (IL-10) concentrations in liver homogenates using Sunlong Biotech Co. (Zhejiang, China) assay kits according to manufacturer's protocol.

## 2.8 Haematological Analyses

Blood haemoglobin (HB) concentration was determined according to the method of Tietz [29]. Red blood cell (RBC) count was done according to the method of Ochei and Kolhakaar [30]. Wintrobe method was used in estimation of packed cell volume (PCV). White blood cell (WBC) count and its differentials were done according to the method of Cheesbrough [31]. Platelet (PLT) count was estimated according to the method of Dacie and Lewis [32]. Mean cell haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) were determined from RBC, PCV and haemoglobin (Hb) according to the method of Jain [33].

## 2.9 Statistical Analysis

Data obtained are presented as mean ± standard deviation (SD) and were analysed using one-way analysis of variance (ANOVA) for differences between groups with the aid of SPSS Software (IBM, version 20). Values of $p < 0.05$ were considered statistically significant [28].

**Table 1. Experimental design**

| Groups | Treatment / Duration |
|---|---|
| 1. (Control) | Normal feed and water *ad libitum* |
| 2. | SA at a dose of 4.1 mg/kgbw for 14 days |
| 3. | SA at a dose of 4.1 mg/kgbw for 14 days then, 100 mg/kgbw ELEIG for another 14 days |
| 4. | SA at a dose of 4.1 mg/kgbw for 14 days, then, 200 mg/kgbw ELEIG for another 14 days |
| 5. | SA at a dose of 4.1 mg/kgbw for 14 days, then, 400 mg/kgbw ELEIG for another 14 days |
| 6. | SA at a dose of 4.1 mg/kgbw + 100 mg/Kgbw ELEIG concomitantly for 14 days |
| 7. | SA at a dose of 4.1 mg/kgbw + 200 mg/Kgbw ELEIG concomitantly for 14 days |
| 8. | SA at a dose of 4.1 mg/kgbw + 400 mg/Kgbw ELEIG concomitantly for 14 days |
| 9. | 100 mg/kgbw ELEIG only for 14 days |
| 10. | 200 mg/kgbw ELEIG only for 14 days |
| 11. | 400 mg/kgbw ELEIG only for 14 days |

*ELEIG = Ethanol leaf extract of Irvingia gabonensis; SA= Sodium arsenite; mg/kgbw = milligram per kilogram body weight*

**Table 2. Effect of ELEIG on pro-inflammatory and anti-inflammatory cytokine levels in liver homogenates of sodium arsenite-intoxicated and non-intoxicated experimental rats**

| Groups / Treatment | TNF-α (pg/mL) | IL-1β (pg/mL) | IL-4 (pg/mL) | IL-10 (pg/mL) |
|---|---|---|---|---|
| 1. Control | 18.48±2.49$^{bcdefghij}$ | 2.10±0.14$^{bcdefghijk}$ | 3.32±0.50$^{bcdefh}$ | 18.32±10.17$^{bcdefgh}$ |
| 2. SA only | 148.92±21.59$^{acdefgh}$ | 7.06±0.80$^{acdefgh}$ | 7.38±0.38$^{acdefgh}$ | 4.49±1.64$^{acdefgh}$ |
| 3. Post-Treatment (100 mg/kg) | 90.20±8.97$^{abde}$ | 6.14±0.64$^{abdef}$ | 5.58±0.95$^{abe}$ | 33.08±3.59$^{abdef}$ |
| 4. Post-Treatment (200 mg/kg) | 71.73±3.67$^{abceg}$ | 4.70±0.50$^{abceg}$ | 6.05±0.24$^{abeg}$ | 26.40±4.84$^{abc}$ |
| 5. Post-Treatment (400 mg/kg) | 59.36±4.89$^{abcd}$ | 3.36±0.38$^{abcd}$ | 4.36±0.25$^{abcd}$ | 26.76±3.19$^{abc}$ |
| 6. Concomitant (100 mg/kg) | 101.16±5.70$^{abh}$ | 4.36±0.23$^{abc}$ | 5.32±0.92$^{abgh}$ | 43.56±6.98$^{abcgh}$ |
| 7. Concomitant (200 mg/kg) | 93.25±4.01$^{abdh}$ | 4.02±0.17$^{abd}$ | 4.05±0.60$^{bdf}$ | 32.08±2.99$^{abf}$ |
| 8. Concomitant (400 mg/kg) | 63.80±4.21$^{abfg}$ | 3.58±0.39$^{abcf}$ | 4.35±0.44$^{abf}$ | 29.73±2.36$^{abf}$ |
| 9. ELEIG only (100 mg/kg) | 42.28±5.03$^{ak}$ | 3.48±0.41$^{ak}$ | 3.60±1.03 | 21.34±2.04 |
| 10. ELEIG only (200 mg/kg) | 37.86±8.04$^{ak}$ | 2.95±0.21$^{a}$ | 3.32±0.28 | 19.38±2.80 |
| 11. ELEIG only (400 mg/kg) | 23.86±5.75$^{jk}$ | 2.69±0.18$^{ai}$ | 2.80±0.64 | 22.02±2.83 |

*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; SA = Sodium arsenite; ELEIG = Ethanol leaf extract of Irvingia gabonensis*

**Table 3. Effect of ELEIG on hemogram of sodium arsenite-intoxicated or non-intoxicated experimental rats**

| GROUPS / Treatment | RBC (x 10$^6$/µL) | HB (g/dL) | PCV (%) | MCH (pg/cell) | MCHC (g/dL) | MCV (µm$^3$) |
|---|---|---|---|---|---|---|
| 1. Control | 7.62±0.51[bcdefgh] | 14.88±1.25[bcdefghijk] | 46.22±4.47[bcdefghijk] | 21.94±2.96[bcdefghijk] | 36.06±1.95[bcdefghi] | 49.92±3.43[bcdefgh] |
| 2. SA only | 4.44±0.48[acdefgh] | 9.97±2.04[acdefgh] | 15.32±1.45[acdefgh] | 11.10±0.62[acdefgh] | 19.35±4.96[acdefgh] | 19.86±3.69[acdefgh] |
| 3. Post-Treatment (100 mg/kg) | 5.40±0.54[abe] | 11.44±0.26[ab] | 25.40±6.70[abe] | 13.64±0.46[abef] | 25.44±1.96[abe] | 29.18±1.68[abe] |
| 4. Post-Treatment (200 mg/kg) | 5.73±0.51[ab] | 11.92±0.30[ab] | 27.85±2.14[ab] | 15.53±1.40[abeg] | 27.48±1.93[ab] | 31.38±5.94[abe] |
| 5. Post-Treatment (400 mg/kg) | 5.98±0.22[abch] | 12.11±0.37[ab] | 31.26±3.06[abc] | 17.54±1.36[abcd] | 29.50±1.39[abc] | 40.96±2.25[abcd] |
| 6. Concomitant (100 mg/kg) | 5.18±0.30[abh] | 11.52±0.24[ab] | 23.54±3.52[abgh] | 17.14±0.76[abcg] | 24.90±2.94[abh] | 28.50±1.94[abgh] |
| 7. Concomitant (200 mg/kg) | 5.73±0.30[abh] | 12.00±0.32[ab] | 31.53±3.06[abf] | 19.18±1.48[abdf] | 25.65±2.04[abh] | 35.20±2.65[abfh] |
| 8. Concomitant (400 mg/kg) | 6.55±0.48[abefg] | 12.37±0.43[ab] | 32.02±1.83[abf] | 18.85±0.99[ab] | 29.60±1.63[abfg] | 40.60±2.23[abfg] |
| 9. ELEIG only (100 mg/kg) | 7.34±0.36 | 12.74±0.30[a] | 35.50±3.12[a] | 19.06±0.72[a] | 31.16±1.89[ak] | 46.70±3.45 |
| 10. ELEIG only (200 mg/kg) | 7.32±0.41 | 12.83±0.63[a] | 37.22±1.70[a] | 19.20±1.67[a] | 33.64±1.87 | 47.92±2.51 |
| 11. ELEIG only (400 mg/kg) | 7.38±0.26 | 12.98±0.50[a] | 38.14±3.05[a] | 19.50±1.04[a] | 34.48±2.79[i] | 49.32±1.73 |

*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; SA = Sodium arsenite; ELEIG = Ethanol leaf extract of Irvingia gabonensis*

**Table 4. Effect of ELEIG on platelet count, white blood cell count and its differentials of sodium arsenite-intoxicated or non-intoxicated experimental rats**

| Groups / Treatment | WBC (x10$^3$/µL) | PLT (x 10$^3$/µL) | LYM (%) | MON (%) | BAS (%) | EOS (%) | NEU (%) |
|---|---|---|---|---|---|---|---|
| 1. Control | 5.14±2.09[bcdefghijk] | 659.60±16.27[bcdefgjk] | 38.00±4.30[bcdefghi] | 22.40±2.88[bcdefg] | 0.00±0.00[befhij] | 3.60±1.52[bcde] | 45.60±3.65[bcdefghijk] |
| 2. SA only | 13.28±2.82[acfgh] | 330.20±61.91[acdefgh] | 79.80±8.29[acdefgh] | 15.00±2.00[aefgh] | 1.00±0.00[acg] | 10.00±1.58[acdefgh] | 78.40±7.30[acdefgh] |
| 3. Post-Treatment (100 mg/kg) | 15.76±1.56[abef] | 473.00±2.94[abdef] | 65.80±5.89[abef] | 16.60±1.95[ae] | 0.40±0.55[b] | 8.40±1.82[abef] | 55.20±5.26[ab] |
| 4. Post-Treatment (200 mg/kg) | 14.03±1.58[aeg] | 554.25±39.09[abc] | 61.25±2.06[ab] | 16.75±2.99[ae] | 0.50±0.58 | 7.50±1.73[abeg] | 59.25±6.18[ab] |
| 5. Post-Treatment (400 mg/kg) | 11.89±1.09[acdh] | 580.00±40.60[abc] | 55.60±3.21[abc] | 19.60±2.30[abcd] | 0.60±0.55[a] | 5.20±0.84[abcd] | 53.80±3.19[ab] |
| 6. Concomitant (100 mg/kg) | 10.59±0.62[abc] | 569.80±22.54[abch] | 55.00±3.32[abc] | 18.60±1.67[ab] | 0.60±0.55[a] | 5.00±0.71[bc] | 53.60±5.64[ab] |
| 7. Concomitant (200 mg/kg) | 10.55±0.33[abd] | 598.25±42.22[abc] | 56.75±4.27[ab] | 18.50±2.08[ab] | 0.25±0.50[b] | 4.50±1.29[bd] | 55.50±2.65[ab] |
| 8. Concomitant (400 mg/kg) | 9.37±0.66[abe] | 626.75±13.07[bf] | 52.75±3.40[ab] | 20.25±0.50[bcd] | 0.75±0.50[a] | 4.50±0.58[b] | 56.00±1.41[ab] |
| 9. ELEIG only (100 mg/kg) | 9.53±0.86[ajk] | 637.40±48.49[ijk] | 44.60±6.77[ajk] | 23.20±1.92 | 0.80±0.45[ak] | 3.80±0.84[k] | 54.20±2.17[a] |
| 10. ELEIG only (200 mg/kg) | 7.73±0.59[ai] | 721.80±19.45[ai] | 33.20±5.07[i] | 24.00±1.22 | 0.60±0.55[a] | 2.40±0.55 | 54.20±2.95[a] |
| 11. ELEIG only (400 mg/kg) | 7.02±0.52[ai] | 760.80±72.99[ai] | 34.40±5.41[i] | 24.60±1.82 | 0.20±0.45[i] | 2.20±0.84[i] | 54.40±2.41[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; SA = Sodium arsenite; ELEIG = Ethanol leaf extract of Irvingia gabonensis*

## 3. RESULTS

### 3.1 Effect of ELEIG on Pro-inflammatory and Anti-inflammatory Cytokine Levels in Liver Homogenates of Sodium Arsenite-Intoxicated and Non-Intoxicated Experimental Rats

Exposure to SA (group 2) produced significant ($p < 0.05$) increases in TNF-α, IL-1β and IL-4 concentrations and a significant ($p < 0.05$) decrease in IL-10 concentration relative to control. Post-treatment with ELEIG at various doses led to significant decreases in TNF-α, IL-1β and IL-4 concentrations in dose-dependent manner and significant increases in IL-10 concentrations, compared with group 2. Concomitant treatment with ELEIG followed similar trend as the post-treatment. In addition, treatment with ELEIG only at various doses produced results with values comparable to control. The detailed results are shown in Table 2.

### 3.2 Effect of ELEIG on Hemogram of Sodium Arsenite-intoxicated and Non-intoxicated Experimental Rats

Exposure to sodium arsenite caused significant ($p < 0.05$) decreases in RBC, HB, PCV, MCH, MCHC and MCV (group 2), compared with control. Post-treatment with ELEIG at the various doses culminated in significant ($p < 0.05$) increases in all the aforementioned parameters in dose-dependent manner, compared with group 2. Similar trends were observed for the concomitantly-treated rats, relative to group 2. In addition, treatment with ELEIG alone at various doses produced similar results with the normal control. The detailed results are presented in Table 3.

### 3.3 Effect of ELEIG on Platelet Count, White Blood Cell Count and Its Differentials of Sodium Arsenite-Intoxicated and Non-intoxicated Experimental Rats

Results showed that exposure of the experimental rats to sodium arsenite (group 2) culminated in significant ($p<0.05$) increases in WBC, LYM, BAS, EOS and NEU counts and significant ($p < 0.05$) decreases in PLT and MON counts relative to control. Post-treatment with ELEIG at various doses produced significant

($p<0.05$) decreases in LYM, BAS, EOS and NEU in dose-dependent and independent manner, compared with group 2. Post-treatment with ELEIG at various doses also led to significant ($p < 0.05$) increases in PLT and MON (at the dose of 400 mg/kgbw) counts, compared with group 2. In addition, concomitant treatment with ELEIG culminated in significant ($p < 0.05$) decreases in WBC, LYM, BAS, EOS and NEU counts as well as significant ($p < 0.05$) increases in PLT and MON counts, relative to group 2. Furthermore, treatment with ELEIG alone at various doses produced results with values comparable to control except WBC, PLT and NEU counts. The detailed results are presented in Table 4.

## 4. DISCUSSION

Cytokines are small glycoproteins with a molecular weight of less than 30 kDa that are produced by different cell types such as white blood cells and control immunity, inflammatory process and haematopoiesis [34,35]. Tumor necrosis factor-$\alpha$ (TNF$\alpha$) and interleukin-1$\beta$ (IL-1$\beta$) which are pro-inflammatory cytokines are involved in the induction, amplification and perpetuation of inflammation. Continuous stimulated release of pro-inflammatory cytokines may therefore culminate in chronic inflammation [36,37]. Anti-inflammatory cytokines such as IL-10 and IL-4 on the other hand, inhibit the inflammatory process by regulating the actions of pro-inflammatory cytokines thereby halting their activities [36-38].

In this study, sodium arsenite alone caused significant increases in hepatic TNF-$\alpha$, IL-1$\beta$ and IL-4 concentrations as well as significant decreases in IL-10 concentration relative to control. Tumor necrosis factor-$\alpha$ (TNF-$\alpha$), a pro-inflammatory cytokine, has been implicated in carcinogenesis owing to its participation in chronic inflammatory diseases [39]. Interleukin-1$\beta$ (IL-1$\beta$), a pro-inflammatory cytokine, is primarily released by monocytes and macrophages as well as non-immune cells like fibroblasts and endothelial cells in conditions such as inflammation, cell injury, infection and invasion [36]. Interleukin-10 is regarded as the most effective anti-inflammatory cytokine with anti-inflammatory properties. It 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 [36,40]. The significant decrease in IL-10 induced by sodium arsenite in this study is indicative of the overwhelming continuous production of TNF-α and IL-1β stemming from the repeated administration of sodium arsenite. Interleukin-4 (IL-4) is an anti-inflammatory cytokine that can reduce the effects of IL-1, TNF-α, IL-8, and IL-6 by affecting activated macrophages and inhibits the generation of free radicals. IL-4 is reported to have therapeutic potential in several clinical conditions such as psoriasis, osteoarthritis, lymphoma, and asthma [40]. It is therefore confirmed that induction of inflammation is one of the mechanisms involved in sodium arsenite-induced toxicity in the experimental rats by orchestrating the release of pro-inflammatory cytokines. The observed increase in IL-4 concentration may be an adaptive response in an attempt to cub the activities of the pro-inflammatory cytokines. The findings from this study are consistent with previous studies on the effect of sodium arsenite on pro-inflammatory and anti-inflammatory cytokines [15,41-43]. Arsenic exposure via consumption of contaminated water and airway has also been reported to elevate pro-inflammatory mediators and decrease anti-inflammatory IL-10 level in circulation which may contribute to metabolic syndrome and cardiovascular diseases (CVD) [44,45].

Treatment with ELEIG both concomitantly and two weeks after (post-treatment), culminated in significant decreases in hepatic TNF-$\alpha$ and IL-1$\beta$ concentrations in dose-dependent manner, relative to group 2 administered sodium arsenite only. Similar trends were observed for IL-4 (however, in dose-independent manner). Treatment with ELEIG also produced significant increases in IL-10 concentration, compared with group 2, administered sodium arsenite only. Administration of ELEIG alone at various doses also produced comparable results in the assayed cytokines relative to control. This implies the reversal of sodium arsenite-induced inflammation by ELEIG which undoubtedly may be as a result of its constituent anti-inflammatory and antioxidant phytochemicals [26].

Evaluation of haematological indices is of immense help in the diagnosis of adverse effects of xenobiotics on the blood components of animals [46]. In this study, exposure of the experimental rats to sodium arsenite culminated in significant decreases in RBC, HB, PCV, MCH,

MCHC and MCV relative to control. Red blood cells (RBCs) are pivotal in the transport of dissolved oxygen due to their constituent haemoglobin [47]. The significant decrease in RBC count observed in this study, may be as a result of the cytotoxic effect of sodium arsenite, likely due its generation of free radicals that could have compromised the red cell membrane integrity via lipid peroxidation and can culminate in anaemia since a decreased concentration of RBCs is a sign of anaemia [48,49]. The significant decrease in haemoglobin concentration may be as a result of an increase in the rate of haemoglobin destruction or a decrease in the rate of haemoglobin synthesis [50]. This may have contributed to the significant decreases in PCV, MCHC, MCH and MCV observed in this study and may indicate the presence of hypochromic and microcytic anaemia. Ita and Udofia [51] had also reported decreases in these parameters in Wistar rats intoxicated with crude petroleum, petrol, kerosene and diesel.

Packed cell volume (PCV) represents the percentage of the erythrocyte volume of the whole blood volume [47]. Abnormally low PCV represents anaemia especially aplastic anaemia. This may either be as a result of a decrease in red blood cell count, a decrease in haemoglobin concentration in each erythrocyte or both. It may be caused by damage to myeloid tissue by chemicals / toxins which inhibit enzymes that are essential for haematopoiesis [52]. It is therefore suggestive that the observed significant decrease in PCV orchestrated by sodium arsenite in this study may be due to the inhibition of essential haematopoietic enzymes and destruction of RBCs by sodium arsenite.

Administration of sodium arsenite alone also culminated in significant decrease in platelet count relative to control. The significant decrease in platelet count may impede the formation of platelet plugs that are essential in preventing haemorrhage at the site of injuries as well as loss of integrity of the capillaries [53]. Administration of sodium arsenite alone also culminated in significant increases in WBC count, lymphocytes, monocytes, basophils, eosinophils and neutrophils compared to control. These are indications of the induction of leukocytosis, lymphocytosis, monocytosis, basophilia, eosinophilia and neutrophilia by sodium arsenite. White blood cells (leukocytes) help in providing immunity to the body against invasion by antigens. The increase in WBC observed in this

study may be as a result of the necrotic activities of sodium arsenite in the cells [54,55]. Perhaps, it could also be attributed to an increase in immune function in an attempt to protect the animals from damage by sodium arsenite [56]. It could also be due to a stimulated increase in lymphopoiesis [57]. The increase in total WBC count in this study supports findings from previous studies [55,58-60].

Peripheral blood leukocytes are made up of polymorphonuclear cells, which include monocytes, neutrophils, basophils, eosinophils and lymphocytes [52]. Oxidative stress and cytokines have been documented to facilitate the activation of polymorpho and mononuclear leukocytes [61,62]. Neutrophils are granular leukocytes that constitute 60-70% of circulating leukocytes in humans. An increase in blood neutrophils (neutrophilia or neutrophil 'leucocytosis') is usually caused by infection and tissue injury [52]. This may be due to the stimulation of activities of leptin and the leptin receptor that are parts of a pathway that stimulates haematopoiesis [63]. Inflammation or necrosis and other factors are other pathological causes of neutrophilia [52]. In addition, monocytosis and lymphocytosis have been reported to be caused by inflammation and hepatitis respectively [52]. Certain forms of cancers have also been documented to cause eosinophilia and basophilia [52]. Since oxidative stress is a major factor behind the etiologies of most cancers, it is therefore rational to say that sodium arsenite might have caused the observed leukocytosis in this study through the induction of oxidative stress and inflammation. Sodium arsenite and arsenic-induced haematotoxicity has also been reported by a myriad of researchers [15,18-19].

Treatment with ELEIG both concomitantly and two weeks after (post-treatment) culminated in significant increases in RBC, HB, PCV, MCH, MCHC and MCV in dose-dependent manner relative to group 2, administered sodium arsenite only. Concomitant and post-treatment with ELEIG also produced significant increases in platelets and monocytes in dose-dependent manner as well as significant decreases in WBC count (concomitant treatment), lymphocytes, eosinophils and neutrophils, compared with group 2, administered sodium arsenite only. Administration of ELEIG alone at various doses also produced comparable levels in the aforementioned parameters, compared with control. Therefore, ELEIG may have reversed the

haematotoxic effects of sodium arsenite by mitigating the oxidative stress and inflammation induced by same. This may not also be unconnected with the potent antioxidant and ant-inflammatory phytochemicals present in the extract [26]. The medicinal effect of ethanol leaf extract of *I. gabonensis* observed in this study corroborates the findings from previous studies [64,65].

## 5. CONCLUSION

The results obtained from this study imply that ethanol leaf extract of *I. gabonensis* possesses immunomodulatory and haematoprotective properties against sodium arsenite-induced inflammation and haematotoxicity in Wistar rats.

## 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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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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