

Acute Sodium Arsenite-Induced Hematological and Biochemical Changes in Wistar Rats: Protective Effects of Ethanol Extract of *Ageratum conyzoides*

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ABSTRACT

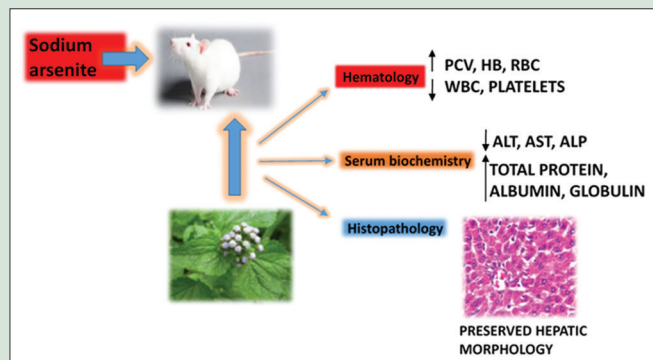
Background: *Ageratum conyzoides* L. (Asteraceae) is an annual herbaceous plant used in folklore medicine for the treatment of a wide range of diseases. **Objective:** To investigate the protective effect of the ethanol leaf extract of *A. conyzoides* (EEAC) against hematological, serum biochemical and histological alterations induced by Sodium arsenite administration to Wistar rats. **Materials and Methods:** Twenty male Wistar rats were randomly assigned into four groups of five rats each. Group I received propylene glycol and Group II rats were given the (EEAC, 100 mg/kg b.w.) orally for 7 days. Group III were given a single oral dose of sodium arsenite (NaAsO₂, 2.5 mg/kg b.w.). Animals in Group IV were pretreated with 100 mg/kg EEAC for 7 days followed by a single oral dose of sodium arsenite. **Results:** Arsenic exposure resulted in significant reductions ($P < 0.05$) in values of packed cell volume (PCV), hemoglobin concentration (Hb) and red blood cell (RBC) count, and elevation in total white blood cell (WBC) count with insignificant reductions in serum total protein, albumin, and globulin levels. Alterations in aspartate aminotransferase, alanine transferase, alkaline phosphatase, and gamma glutamyl transferase activities, as well as in serum levels of urea, creatinine, glucose, cholesterol, and triglyceride levels, were not statistically significant. EEAC significantly restored ($P < 0.05$) the PCV, Hb, RBC, and WBC as well as serum albumin, globulin, and total protein to normal values. **Conclusion:** The results of this study indicate that EEAC possess strong potentials to protect against toxicities induced by sodium arsenite.

Key words: *Ageratum conyzoides*, Hematology, Histopathology, Liver, Serum Biochemistry, Sodium Arsenite

SUMMARY

- *Ageratum conyzoides* produced significant reversal of the reduction in the erythrocytic indices (packed cell volume, red blood cell, and Hb) caused by sodium arsenite
- Sodium arsenite-induced slight elevations in serum aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), correlating with the histopathological lesions observed

- *Ageratum conyzoides* produced only slight reductions in AST, ALT, and ALP compared to the sodium arsenite group, but significantly reduced the severity of histopathological lesions.



Abbreviations Used: EEAC: Ethanol extract of *Ageratum conyzoides*; RBC: Red blood cell; WBC: White blood cell; Hb: Hemoglobin; ALT: Alanine transaminase; AST: Aspartate transaminase or Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transferase.

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INTRODUCTION

Arsenic (As) toxicity is known to globally affect humans and animals. The element was ranked second to lead (Pb) as a cause of heavy metal intoxication.^[1] It is found occurring ubiquitously in nature in the earth crust^[2] and major routes of exposure to humans may be via ingestion through drinking water^[3] with relatively minor routes being inhalation and skin absorption. Herbicides, insecticides, and other similar products also contain arsenic (Gupta and Flora, 2005). Domestic animals are also at risk of intoxication with this environmental toxicant and carcinogen.^[4,5]

A wide range of alterations has been investigated in arsenic poisoning. Arsenic has been reported to induce hematological and biochemical changes as well as oxidative stress in a chicken model of arsenic intoxication^[6] and other models.^[7] Epidemiological and mechanistic experimental evidence of arsenic carcinogenesis in animals and humans

are also available.^[8] The mechanisms of arsenic toxicity have been reported to include the induction of oxidative stress,^[9] inhibition of enzyme and mitochondrial function,^[10] and induction of stress-response genes.^[11]

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The search for specific, reliable, and safe treatment for arsenic toxicity still continues. Different approaches to treatment have included the use of chelating agents such as Meso-2, 3-dimercaptosuccinic acid, and 2,3-dimercaptopropane-1-sulfonate.^[4] These may be combined with few antioxidants, e.g. Vitamins C and E.^[12] Some of these metal-chelating agents, however, possess toxic side effects.^[13,14] Dimercaprol and dimercaptosuccinic acid are known to produce hypertension as an important side effect.

Ageratum conyzoides L. is an annual herbaceous plant belonging to the family Asteraceae with a long history of traditional medicinal uses.^[15] It is native to Central America, the Caribbean, Southeast Asia, South China, India, West Africa, Australia, and South America.^[16,17] It is utilized for medicinal purposes by various cultures worldwide, including as bactericide and antidiarrheal;^[18,19] treatment of fever, rheumatism, headache, and colic.^[20,21] Ethanolic leaf extracts of *A. conyzoides* (EEAC) are reported to have hematopoietic activities with increases in packed cell volume (PCV), hemoglobin (Hb) concentration, and red blood cell (RBC) counts.^[22] Its use in folk medicine against diabetes has also been investigated experimentally. It was found to possess blood glucose lowering effect in normoglycemic and in streptozocin-induced hyperglycemic rats. The diverse biological activities of *A. conyzoides* are thought to be due to its content of phytochemicals including flavonoids, tannins, saponins, triterpenoids, sesquiterpenes, chromenes, chromones.^[17,23]

As part of investigations into the protective roles performed by *A. conyzoides*, the present study sought to investigate the effects of the EEAC on sodium arsenite-induced hematological and biochemical alterations in Wistar rats.

MATERIALS AND METHODS

Chemicals

Sodium arsenite (BDH Chemicals Ltd., Poole, England) 2.5 mg/kg b.w. (corresponding to 1/10th of the oral LD₅₀) was administered to the experimental animals.^[24] All other reagents and chemicals were of analytical grade.

Collection and extraction of *Ageratum conyzoides* leaves

Leaves of *A. conyzoides* were harvested from the University of Ibadan, Ibadan, Nigeria Campus, and authenticated at the herbarium of the Department of Botany, University of Ibadan, Nigeria. The specimen voucher of the leaf (Voucher No. UIH-22423) was prepared and deposited in the herbarium. The leaves were cleaned, and air dried at room temperature and were thereafter blended with an electric blender. The powdered leaves were first defatted with n-hexane, after which it was soaked in ethanol for 24 h. The mixture was filtered, and the filtrate was concentrated using a rotary evaporator at 40°C. The yield of the extraction process was harvested and kept at 4°C for use.

Phytochemical screening

EEAC extract was subjected to the phytochemical test using Trease and Evans and Harbourne^[25,26] methods for alkaloids, saponins, tannins, anthraquinones, flavonoids, and cardenolides

Experimental animals

Twenty male Wistar albino rats weighing 140–150 g obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, were used in this study. They were housed in a well-ventilated animal house and were fed standard rat pellets (product of Ladokun feeds, Oyo state, Nigeria) and allowed access to drinking water *ad libitum*.

The animals were randomly assigned into four groups, comprising five rats per group. The rats were allowed to acclimatize for 10 days before extract administration commenced. The study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Ibadan.

Group I: Negative control, given propylene glycol (extract vehicle) in similar volume (0.2 ml) as other treatments for 7 days.

Group II: Administered a daily oral dose of (EEAC; 100 mg/kg b.w.) for 7 days.

Group III: Administered a single oral dose of sodium arsenite (2.5 mg/kg equivalent to 1/10th LD₅₀).

Group IV: Pretreated with *A. conyzoides* at 100 mg/kg for the first 7 days followed by a single oral dose of sodium arsenite (2.5 mg/kg b.w.).

Blood samples were collected from the animals after anesthetizing with diethyl ether. About 3 ml of the blood was allowed to clot at room temperature. The clotted blood samples were centrifuged at 3000 rpm for 10 min to obtain the serum, which was used for biochemical analyses. Another 2 ml of the blood samples were collected into heparinized tubes that were used for hematological assays. All the animals were then sacrificed by cervical dislocation 24 h after the last treatment.

Blood analysis

Blood samples were collected via the periorbital sinus into lithium heparinized bottles. The PCV was estimated by the microhematocrit method and the Hb concentration by the cyanmethemoglobin. RBC and white blood cell (WBC) counts were determined using the new improved Neubauer hemocytometer. Differential leukocyte counts were evaluated using standard method.^[27]

Biochemical evaluation

Commercially available kits were used according to the respective manufacturer's protocol for the measurement of serum liver enzyme activity. Serum alkaline phosphatase (ALP) activity was determined by a kit from BioSystems SA., Spain. Serum aspartate aminotransferase (AST), alanine transaminase (ALT) and gamma-glutamyl transferase (GGT) activities, urea, and creatinine levels were measured using RANDOX laboratory reagent kits obtained from RANDOX Laboratories Ltd., Ardmore, United Kingdom. Serum cholesterol and triglyceride levels were determined by Ecoline CHOD-PAP and Ecoline 25 GPO-PAP assay kits (1.14856.0001, Merck KGaA, Darmstadt, Germany), respectively.

Histological study

The liver samples were collected in 10% formalin for histopathological analysis. The organ tissues were processed and embedded in paraffin wax and sections were made of about 4–6 µm. After staining with hematoxylin and eosin, slides were examined under the microscope (Olympus, Japan) for histopathological changes and photographed.

Statistical analyses

All data were computed and presented as mean ± standard error of mean. Differences among experimental groups were determined using IBM SPSS Statistics software (Version 20) (USA). Group comparisons were done using the analysis of variance and significant differences were observed at $P < 0.05$.

RESULTS

Phytochemical screening of EEAC revealed the presence of alkaloids, saponins, anthraquinones, flavonoids, and cardenolides [Table 1].

Values of hematological parameters are presented in Table 2. Sodium arsenite caused significant reductions ($P < 0.05$) in PCV, Hb, and RBC values compared to control values, with increases in total WBC counts

and platelet counts. Pretreatment with EEAC produced a significant amelioration of the sodium arsenite-mediated reduction in erythrocyte indices, by increasing PCV, Hb, and RBC values significantly ($P < 0.05$) toward control values.

Table 3 presents serum biochemical parameters following exposures to sodium arsenite and EEAC. Total protein, albumin, and globulin values were reduced, though, insignificantly with exposure to sodium arsenite when compared with control whereas pretreatment with EEAC restored the values of these parameters to values similar to those of control rats. There were no statistically significant alterations ($P < 0.05$) in the values of ALT, AST, ALP, GGT with exposures to either sodium arsenite or EEAC. The serum creatinine levels of the rats increased nonsignificantly in the arsenite-treated group when compared with the values obtained for the rats in the control and EEAC pretreated groups [Table 3]. Also, the mean blood urea values insignificantly increased in rats from Group III when compared with that of rats in Groups I, II, and IV. However, the presence of EEAC in pretreated group (Group IV) reduced the values of blood creatinine and urea values.

Histopathology of liver from control group showed no visible lesions (i), EEAC-treated group (ii) was comparable to the control. SA treatment resulted in diffuse vacuolation of hepatocytes, congestion, and cellular

infiltration by mononuclear cells (iii). EEAC and SA group showed mild vacuolar degeneration of hepatocytes (IV) [Figure 1].

DISCUSSION

Exposure to environmental pollutants constitutes a major threat to animal and human survival in the ever increasing industrialized world. Arsenic contamination of drinking water from various sources has been reported in many parts of the world including developed and developing countries.^[28] The determination of hematological and serum biochemical parameters provides important information on the alterations that affect the physiology of the blood in disease states or exposures to toxic pollutants. Analysis of blood parameters is believed to be relevant in risk evaluation and response to therapy as changes in the hematological system have high predictive value.^[29]

In this study, pretreatment of rats with EEAC significantly increased the PCV, Hb concentration, and red cell counts all of which were significantly reduced upon sodium arsenite exposure, [Table 2]. This result is in accordance with reported erythropoietic activity of the plant.^[22] It has been previously suggested that bioactive components of *A. conyzoides* act to stimulate the kidney directly to secrete erythropoietin and stimulation of hematopoiesis.^[22] The erythropoietic effect is also thought to be due to the iron content of the plant, similar to that of other plants that reportedly contains a high level of iron.^[30]

The reduction in PCV, Hb, and RBC counts might be a result of inhibition of porphyrin or heme synthesis. Arsenic is known to cause inhibition of aminolevulinic acid dehydratase activity, thereby altering the heme synthesis pathway.^[4]

Exposure of rats in this study to sodium arsenite produced significant leukocytosis. The effect was significantly reversed in the animals pretreated with *A. conyzoides*. Increased number of WBCs is generally

Table 1: Phytochemical screening of ethanol extract of *Ageratum conyzoides*

Constituents	Inference
Alkaloids	Present
Saponins	Present
Tannins	Absent
Anthraquinone	Present
Flavonoids	Present
Cardenolides	Present

Table 2: Hematological parameters in Wistar rats exposed to sodium arsenite and *Ageratum conyzoides* extract

Parameters	Group I control	Group II (100 mg/kg EEAC alone)	Group III (2.5 mg/kg NaAsO ₂ alone)	Group IV (100 mg/kg EEAC + 2.5 mg/kg b.w sodium arsenite)
PCV (%)	46.60±1.36	48.50±3.14	39.17±1.28 ^{a,b}	46.17±1.74 ^c
Hb (g/dl)	14.40±0.4	15.00±1.90	11.83±1.17 ^{a,b}	14.67±2.07 ^c
RBC ×10 ⁶ (μl)	7.86±0.30	7.72±0.40	6.62±0.41 ^{a,b}	7.78±0.90 ^c
WBC (×10 ³ /mm ³)	427.00±56.29	452.50±28.45	500.83±52.72	331.67±55.94 ^c
Platelets	73,800.00±14,537.54	77,333.33±6955.41	89,833.33±7989.23	55,666.67±11,321.563 ^c
Lymphocytes (%)	70.80±4.40	73.67±2.74	72.00±1.86	65.33±4.13 ^b
Neutrophils (%)	26.40±4.12	22.33±2.43	23.83±2.06	30.50±2.36 ^b
Monocytes (%)	2.60±0.75	2.33±0.62	2.00±0.52	1.83±1.47
Eosinophils (%)	1.40±0.510	1.67±0.33	1.67±0.42	2.33±0.56

Values are presented as mean±SE of mean of five animals per group. ^aValues differ significantly from control; ^bValues differ significantly when compared with EEAC alone group; ^cValues differ significantly when compared with NaAsO₂ alone group. EEAC: Ethanol extract of *Ageratum conyzoides*; NaAsO₂: Sodium arsenite; WBC: White blood cell; RBC: Red blood cell; PCV: Packed cell volume; Hb: Hemoglobin

Table 3: Serum biochemical parameters in Wistar rats exposed to sodium arsenite and *Ageratum conyzoides*

Parameters	Group I control	Group II (100 mg/kg EEAC alone)	Group III (2.5 mg/kg NaAsO ₂ alone)	Group IV (100 mg/kg EEAC + 2.5 mg/kg NaAsO ₂)
Total protein	7.00±0.34	6.63±0.14	6.77±0.22	7.13±0.28
Albumin	4.28±0.22	4.12±0.13	3.98±0.45	4.27±0.19
Globulin	2.72±0.14	2.52±0.149	2.62±0.11	2.87±0.09
A: G ratio	1.56±0.07	1.67±0.15	1.48±0.08	1.47±0.02
AST	41.20±0.97	42.67±1.33	43.83±1.01	42.00±1.97
ALT	31.80±0.37	30.33±0.88	32.33±0.33	30.50±0.99
ALP	100.83±4.97	109.67±7.29	117.60±3.33	107.50±5.68
Urea	14.00±0.45	14.50±0.45	15.60±0.51	14.67±0.42
Creatinine	0.55±0.06	0.58±0.060	0.77±0.12	0.70±0.12
GGT	0.82±0.24	0.75±0.19	1.10±0.24	0.97±0.08

EEAC: Ethanol extract of *Ageratum conyzoides*; NaAsO₂: Sodium arsenite; GGT: Gamma glutamyl transferase; ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; A: Albumin; G: Globulin

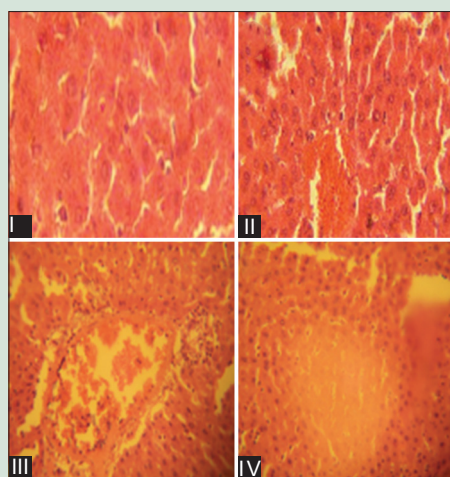


Figure 1: Photomicrograph of liver sections of rats treated with ethanol extract of *Ageratum conyzoides* and sodium arsenite for 7 days (Mag X400). (I) Control showing no visible lesion. (II) EEAC only (100mg/kg body weight) showing no visible lesion. (III) Sodium arsenite (2.5mg/kg body weight) showing diffused vacuolation of hepatocytes, congestion and cellular infiltration by mononuclear cells. (IV) EEAC + SA (100mg/Kg and 2.5mg/Kg body weight) showing very mild vacuolar degeneration of hepatocytes

known to be a normal reaction to foreign substances.^[31] Leukocytosis, mainly due to leukocyte mobilization into the circulation can be a result of stimulation of the immune system against infectious agents or chemicals. Thrombocytosis (increased platelet counts) observed in this study may indicate an immediate-type hypersensitivity to arsenic exposure.

Results from this study revealed that serum total protein levels was decreased nonsignificantly with exposure to sodium arsenite. Treatment with *A. conyzoides*, however, restored total protein levels to normal values. The same effects were observed for serum albumin and globulin. Decreased serum protein levels could be a result of damage, particularly protein oxidation by reactive oxygen species generated by arsenic toxicity.^[32] Increased breakdown (catabolism) of proteins due to possible oxidative stress may also contribute to decreased protein levels. From the results presented in Table 3, there is an increase in the activities of AST, ALP, ALT, and GGT in the serum of SA-treated rats when compared with the control. This might have resulted from sodium arsenite-induced oxidative stress-related damages to hepatocytes membrane and leakage of hepatic transaminases into extracellular spaces ultimately finding their way into the blood from the liver.^[33] However, there was a decrease ($P > 0.05$) in serum activities of AST, ALP, ALT, and GGT in the SA + EEAC pretreated group compared with SA only group. This is suggestive of hepatocytes protection from EEAC cotreatment against SA-induced damages. This can be attributed to the antioxidant present in EEAC. This is in accordance with the findings of Das and Sengupta,^[34] which recommend the use of antioxidant-rich foods and herbal medicinal plants for the management of arsenicosis. Increased concentration of serum urea and creatinine are considered for investigating drug-induced nephrotoxicity in animals and man.^[35] In this study, arsenite treatment interfered with kidney functions as seen by elevation of these values in rats. Urea, a waste product of protein catabolism, can rise when the kidney is defective. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of its clearance.^[36] Increased urea and creatinine levels observed in the positive control group (Group III) may be an indication of nephrotoxicity by sodium arsenite. This is in consonance with the findings of Anwar *et al.*^[37] and Nandi *et al.*^[38] that arsenite toxicity induces several metabolic disorders including urea and creatinine elevation following proximal tubule damage and glomerular

injury, respectively. Pretreatment with EEAC had a reversal effects on these parameters. This study, therefore, showed the nephroprotective effects of the leaves of *A. conyzoides* in arsenite-induced toxicity.

Our finding from liver histopathology suggests NaAsO₂-induced toxicity and ameliorative potentials of EEAC on SA-induced hepatocytes damage. Histological examinations of liver sections of treated animals showed that SA was potentially hepatotoxic as reflected by the congestion, vacuolation, and cellular infiltration by mononuclear cells observed. Liver sections from SA and EEAC-treated group exhibited very mild vacuolar degeneration of hepatocytes while those of the EEAC-treated group showed no visible lesions confirming a modulatory effect of EEAC on SA-induced hepatocytes damage. Findings from histopathology strengthen the claims for the potential of *A. conyzoides* to protect against liver damage at the dosage tested (100 mg/kg). In a previous study by Adebayo *et al.*,^[31] dosages (500–1500 mg/kg) of the EEAC were found to induce some hepatocellular necrosis (with corresponding increases in serum levels of ALT). It can be inferred that the EEAC appears safe at lower doses, such as that tested in our study (100 mg/kg). Therefore, the data obtained from this study further proved the usefulness of EEAC as a food supplement that may be recommended for the protection of humans and animals exposed to arsenic toxicity. Taken together, the results suggest that EEAC protects cells against damages generated from arsenic exposure.

CONCLUSION

The present study has demonstrated the mitigating effect of leaves of *A. conyzoides* on hematological, serum biochemical and histological parameters, altered by sodium arsenite exposure in Wistar rats. This amelioration may be partly due to the antioxidant effect of constituents of the plant extract. *A. conyzoides* possess remarkable potential to reverse the reduction in the erythrocytic indices (PCV, RBC, and Hb) caused by sodium arsenite. This finding is supported by previous reports of profound erythropoietic (hematinic) properties of *A. conyzoides*. (Ajayi *et al.*, 2000; Ita *et al.*, 2007). This hematinic property may be exploited in the treatment of anemia.

This study has raised no controversies with previous reports on the activities of extract of *A. conyzoides*. Because this present study has not assessed biomarkers of oxidative stress that may be involved in the mechanism of toxicity of sodium arsenite as well as possible protection against sodium arsenite-induced oxidative stress, we are currently seeking to understand the mechanisms underlying the protective potential exhibited by *A. conyzoides* by fractionation and purification of this extract to determine the active components responsible for the most profound biological activities of the plant. Also, evaluation of the involvement of oxidative stress in sodium arsenite-induced toxicity, the role of *A. conyzoides* in either mitigating or exacerbating oxidative stress and further assessment of the role of *A. conyzoides* and molecular evaluations of its biological activities would be studied.

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Conflicts of interest

There are no conflicts of interest.

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