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Effects of combined leaf extracts of *Vernonia amygdalina* and *Ocimum gratissimum* on biochemical parameters of sodium arsenite induced toxicity in albino Wister rat

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Abstract

This study was carried out to examine the effect of different leaf extract treatment on Sodium Arsenite toxicity in albino Wister rat model. Twenty-five (25) rats weighing between 120-270 g were used in this experiment and randomly divided into five (5) groups containing five rats each. Group 1 animals served as control and was administered placebo, while group 2 was induced with only sodium arsenite, 3 and 4 were both induced with sodium arsenite sand treated with *Ocimum gratissimum* ethanoic extract and *Vernonia amygalina* ethanoic extract respectively. Group 5 received concomitant administration of both extract after induction for 14 days. All drugs and extract administration were dose dependent on kilogram body weight using a cannula attached to a syringe. The result showed a significant elevation of sodium arsenite in group 2 serving as a biochemical marker defining sodium arsenide toxicity to living rat model tissue when compared to group 1. Group 5 showed no significant ($p<0.05$) difference when compared to group 1. Thus, showing an overall improvement in the effect of combined administration of the extract in the management of sodium arsenite level in sodium asenite induced toxicity when compared to groups 3 and 4 which may be associated with the phytochemicals present in both herbs.

Keywords: Sodium Arsenite; *Ocimum gratissimum; Vernonia amygalina,* Wistar Albino Rat; Extract

1. Introduction

Indigenous pharmacopoeias have shown medicinal plants with significant and potent curative properties [1]. Both industrialized and developing nations now accept the widespread use of medicinal plants to treat a variety of illnesses [2].

A perennial herb with the common name "alfavaca," *Ocimum gratissimum* is commonly cultivated in tropical and warm-temperate regions of various African nations, including Nigeria. It is used in folk medicine to cure a variety of conditions, including pneumonia, diarrhea, upper respiratory tract infections, headaches, and fever [3].

As the name "bitter leaf" suggests, *Vernonia amygdalina* is a tiny shrub that grows in tropical Africa. It is a member of the daisy family. It can reach heights of 2 to 5 meters, and has up to 20 cm long, elliptical leaves [4].

According to studies, hepatic and oxidative damage-related disorders are historically treated with Vernonia amygdalina and *Ocimum gratissimum.* Although these leaves are widely used in traditional medicine, there has been remarkably little research on the pharmacology of this plant, and only a preliminary report has suggested that its extract may have nociceptive properties in Nigeria [5]. Therefore, the present studies were carried out to assess the possible roles of the ethanolic extract of *Vernonia amygdalina* and *Ocimum gratissimum* in rat induced with sodium arsenite.

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Sodium arsenite has some carcinogenic and teratogenic effects meaning it can lead to the onset or formation of cancer, or it can lead to abnormalities of physiological development. Other than these, symptoms brought on by substance contact include burns, itching, irritation, thickened skin, rash, loss of pigment, stomach pain, nausea, vomiting, diarrhoea, convulsion, low blood pressure, and headaches. When the neurological system is severely damaged, symptoms like weakness, poor coordination, or "pins and needles" may develop, finally leading to paralysis and death [6].

Sodium arsenite toxicity has a great influence in altering some biochemical indices of a physiological being, this includes its effect in some haematological parameters, its teratogenic effects and its carcinogenic effects. the extracts on the other hand have been proved to be efficient in restoring back homeostasis. This study helps to elucidate the potency of these extracts which can then be used in drug formulation and dieting for people who are exposed to sodium arsenite.

2. Material and methods

2.1. Chemicals

The study's source of Sodium Arsenite was Qualikem Fine Chemistry Pvt Ltd in Vadodara, India.

2.2. Experimental animals

At the University of Uyo Akwaibom State Faculty of Basic Medicine animal house, 25 male albino rats weighing 120–180g were purchased. Before the studies, the animals had a week acclimatization period. The animals were housed in small, well-constructed cages to prevent feed and rat waste from mixing. The institution's Animal Ethics Committee's ethical principles [7] were followed in the handling and care of the animals.

2.3. Sample collection and preparation

The plant materials which are *Vernonia amygdalina* and *Ocimum gratissimum* (Bitter leaf and Scent leaf respectively) were obtained from Itam market, in Itam, Itu Local government area Uyo, Akwaibom State Nigeria. The plant sample was identified by the lab technologist of the Department of plant Science and Biotechnology, University of Uyo. The leaves were first washed free of sand and debris, air dried and then shredded manually with hands in preparation for extraction

2.4. Ethanol Extraction

5L of 80% ethanol were used for extraction of both leave samples. The leave samples were macerated in the 80% ethanol, it was then stirred using a stirring glass rod and then allowed for five Hours. The mixture was then filtered into a stainless container. The silt was discarded while the filtrate was slowly evaporated to dryness at 50^{o} C using water bath for 48 hours. The dried extract was transferred to a plastic container and preserved in a refrigerator.

2.5. Experimental induction of Sodium Arsenite

The chemical sodium arsenite was administered intraperitoneally based on the body weight of the rat. Due to the hazardous nature of the chemical, laboratory safety measures were observed strictly throughout the period of administration; face masks and clean lab coat with gloves was used. The dose of administration for the chemical compound is 10 mg/kg body weight which is 4/10 of its lethal dose [8], and the stock concentration is 1 mg/ml.

The chemical was administered based on the body weight using the formula:

$$\text{Dose (mg)} = \frac{\text{Weight (g)}}{1000} \times \frac{\text{Dose(mg/kg)}}{\text{Stock Conc. (mg/ml)}}$$

2.6. Experimental design

Twenty-five albino rats were divided into 5 groups of five rats each

Table 1 Experimental Design

Groups	No of animals	Treatment
I (NC)	5	Placebo
II (SA)	5	10 mg/kg weight SA only
III (SAOG)	5	10 mg/kg SA + 100 mg/kg OG
IV (SAVA)	5	10 mg/kg SA + 100 mg/kg VA
V (SAOGVA)	5	10 mg/kg SA + Combined (50 mg/kg OG + 50 mg/kg VA)

NC = Normal Control group; SA = Sodium arsenite induced group; SAOG = Sodium arsenite induced group treated with *Ocimum gratissimum* extract; SAVA = Sodium arsenite induced group treated with *Vernonia amygalina* extract; SAOGVA = Sodium arsenite induced group treated with combined extract of *Ocimum gratissimum* (50 mg/kg) and *Vernonia amygdalina* (50 mg/kg)

2.7. Preparation of Stock Solution

900 mg of *Vernonia amygdalina* and *Ocimum gratissimum* was dissolved in 18 ml of distilled water each, to make a stock solution of 50 mg/ml. 180.8 mg of sodium arsenite was dissolved in 180.8 ml of distilled water to make a stock solution of 1mg/ml. the required dose for each animal was measured from the stock solution and administered. The stock solution was prepared every three days throughout the study.

2.8. Administration of Plant Extract

Plant Extract of both *Ocimum gratissimum* and *Vernonia amygdalina* were administered to the experimental animal based on their body weight. The extracts were administered through the oral route using a sterile syringe.

2.9. Haematological Analysis

The blood sample was used to carry out full blood count which included the white blood cell count, differential count, red blood cell count, packed cell volume and other haematological parameters like the Mean Corpuscular Haemoglobin concentration.

2.10. Statistical Analysis

The results were expressed as ± standard error of means (S.E.M) with five rats in each group except group one which has four rats. The result was analysed using one-way analysis of variance (ANOVA) followed by LSD post hoc test for comparison of different means using SPSS (Standard Package for Social Sciences) statistical software. Statistical significance was set at $P \leq 0.05$.

3. Results

Table 2 showed the effect of extracts treatment (singly and in combination) of *Ocimum gratissimum* (OG) and *Vernonia amygdalina* (VA) on haematological indices of sodium arsenite (SA) induced toxicity in albino Wister rats.

The result disclosed a non-significant decrease in WBC of the groups treated with single extract (11.66 ± 0.69 for SAOG) and (11.66 ± 2.09 for SAVA) when compared with the normal control (12.25 ± 2.73 for NC), while showing a non-significant increase in WBC of in the group administered with SA only (13.32 ±1.10) and combined extract (11.66 ± 0.69 for SAOGVA) when compared to the normal control (12.25 ± 2.73 for NC).

The result also revealed a significant decrease ($P \leq 0.05$) in LYM on the group administered with SAVA (81.1± 2.94) when compared with the normal control (88.7 ± 1.26). a non-significant decrease was evidently shown in the group treated with SA (84.3 ± 1.82) only and SAOG (85.4± 1.97) when compared with the normal control (88.7 ± 1.26). the result also disclosed a significant decrease ($P \leq 0.05$) in LYM on the group administered with SAVA (81.1± 2.94) when compared with the combined extract treated group SAOGVA (88.82± 2.17).

The result disclosed a significant increase ($P \leq 0.05$) in MON in the single extract treated group SAVA (5.08 ± 1.1) and a non-significant increase in SA (3.04 ± 0.69), SAOG (3.02 ± 0.35) and SAOGVA (4.42 ± 1.24) when compared to the normal control (2.00 ± 0.37). there was a non-significant decrease in SAOG (3.02 ± 0.35) when compared to the combined extract administered group. There was also a non-significant increase in SAVA (5.08 ± 1.1) when compared to SAOGVA (4.42 ± 1.24).

The result also showed a non-significant increase in NEU in the group administered with SA only (8.36 ± 1.04), SAOG (7.02 ± 1.05) and SAVA (8.48 ± 1.3) when compared to group one which is the normal control. There was a significant increase (P ≤ 0.05) in the combined extract treated group SAOGVA (4.18 ± 0.6) when compared with SA treated group SA (8.36 ± 1.04). there was an evident significant increase (P ≤ 0.05) in SAVA group (8.43 ± 1.23) when compared to the double extract treated group SAOGVA (4.18 ± 0.6).

There was an evident increase in EOS value for SA (0.16 ± 0.05), SAVA (0.18 ± 0.08) and SAVAOG (0.14 ± 0.40) when compared to the normal control (0.12 ± 0.04). the result also showed a non-significant decrease in SOOG (0.12 ± 0.04) and SAOGVA (0.14 ± 0.40) when compared to SA (0.16 ± 0.05).

From the result, BAS values significantly increased (P ≤ 0.05) in SAVA (5.02 ± 0.9) when compared to SAVAOG (2.44 ± 0.66). there was also a non-significant increase in SA (4.08 ± 0.62) and SAOG (4.40 ± 0.87) when compared to NC (2.75 ± 0.58).

From the result, there was a non-significant decrease in RBC values in SA (8.25 ± 0.38), SAOG (8.67 ± 0.21), SAVA (8.64 ± 0.44) and SAOGVA (9.05 ± 0.38) when compared to the normal control group (9.74 ± 1.22). The result also disclosed a non-significant increase of RBC in SAOGVA (9.05 ± 0.38) when compared to the SA (8.25 ± 0.38) treatment group

The result showed a significant increase (P ≤ 0.05) in HGB in SA (13.66 ± 0.7), SAOG (14.28 ± 0.5), SAVA (13.92 ± 1.2), and SAOGVA (13.98 ± 0.6) when compared to the normal control (12.32 ± 0.66). there was also a non-significant decrease in PCV value in all groups when compared to the normal control group.

Table 2 Effect of extract treatment on haematological indices of sodium arsenite induced toxicity

Groups/ treatment	WBC (10^3 µl)	LYM (%)	MON (%)	NEU (%)	EOS (%)	BAS (%)	RBC (10^6 µl)	HGB (g/dl)	PCV (%)	MCV (µm^3)	MCH (pg)	MCHC (g/dl)
I (NC)	12.25 ± 2.73	88.75 ± 1.26	2.00 ± 0.37	6.37 ± 0.86	0.12 ± 0.04	2.75 ± 0.58	9.74 ± 1.22	12.32 ± 0.66	53.35 ± 7.13	54.57 ± 0.52	13.2 ± 1.45	24.27 ± 2.92
II (SA only)	13.32 ± 1.10	84.36 ± 1.82	3.04 ± 0.69	8.36 ± 1.04	0.16 ± 0.05	4.08 ± 0.62	8.25 ± 0.38	13.66 ± 0.7*	45.82 ± 1.99	55.60 ± 0.90	16.66 ± 0.52*	29.94 ± 0.85*
III (SAOG)	11.66 ± 0.69	85.40 ± 1.97	3.02 ± 0.35	7.02 ± 1.05	0.12 ± 0.04	4.40 ± 0.87	8.67 ± 0.21	14.28 ± 0.5*	47.62 ± 0.98	54.98 ± 0.89	16.50 ± 0.45*	30.04 ± 0.77*
IV (SAVA)	11.66 ± 2.04	81.1 ± 2.94*c	5.08 ± 1.1*	8.48 ± 1.3 c	0.18 ± 0.08	5.02 ± 0.9 c	8.64 ± 0.44	13.92 ± 1.2*	46.32 ± 2.44	53.60 ± 0.32	16.16 ± 0.34*	30.18 ± 0.83*
V (SAOGVA)	13.52 ± 0.23	88.82 ± 2.17	4.42 ± 1.24	4.18 ± 0.6 a	0.14 ± 0.40	2.44 ± 0.66	9.05 ± 0.38	13.98 ± 0.6*	48.80 ± 2.28	53.88 ± 0.54	15.54 ± 0.60*	28.84 ± 1.24*

Values are expressed as means ± SD, n = 5, WBC = White Blood Cell; HGB = Haemoglobin; MCH = Mean Cell Haemoglobin RBC = Red Blood Cell; HCT = Haematocrit; MCHC =Mean Cell Haemoglobin concentration MCV = Mean Cell Volume; PLT = Platelet count; LYM = Lymphocyte; MON = Monocyte; NEU = Neutrophils; EOS = Eosinophils; BAS = Basophiles. NC = Normal Control group; SA = Sodium arsenite induced group, SAOG = Sodium arsenite induced group treated with *Ocimum gratissimum* extract, SAVA = Sodium arsenite induced group treated with *Vernonia amygalina* extract, SAOGVA = Sodium arsenite induced group treated with combined extract of *Ocimum gratissimum* (50mg/kg) and *Vernonia amygdalina* (50 mg/kg)

The result disclosed a significant (P ≤ 0.05) increase in MCH in the all groups; SA (16.66 ± 0.52), SAOG (16.50 ± 0.45), SAVA (16.16 ± 0.34) and SAOGVA (15.54 ± 0.60) when compared to the normal control group (12.32 ± 0.66). there was a non-significant increase the single extract treatment groups SAOG (16.50 ± 0.45) and SAVA (16.16 ± 0.34) when compared to the combined extract treatment group SAOGVA (15.54 ± 0.60).

The values of MCHC were also significantly elevated (P ≤ 0.05) in all the groups SA (29.94 ± 0.85), SAOG (30.04 ± 0.77), SAVA (30.18 ± 0.83) and SAOGVA (28.84 ± 1.24) when compared to the normal control group. The single extract treatment groups evidently showed a non-significant increase SAOG (30.04 ± 0.77) and SAVA (30.18 ± 0.83) when compared to the combined extract treatment group SAOGVA (28.84 ± 1.24).

4. Discussion

Haematological determinations give the physiological information on the blood and its main function is to detect blood disorders such as anaemia or leukaemia. Other functions include investigations of coagulation defects and the control treatment in such diseases as coronary thrombosis and leukaemia.

The 14 days study showed that sodium arsenite had a high influence on haematological parameters and lead to an elevation in the level of HGB, MCH and MCHC. Increase in the level of HGB signifies that the bone marrow is producing more haemoglobin and this is associated with some disease conditions such as polycythaemia.

Haemoglobin is a protein in red blood cells that helps blood carry oxygen throughout the body. (Haemoglobin contains iron, which gives blood its red colour.) The haemoglobin count is an indirect measurement of the number of red blood cells in your body. When the haemoglobin count is higher than normal, it may be a sign of a health problem.

Packed Cell Volume, haemoglobin and mean corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals [9]. Furthermore, posited that high Packed Cell Volume (PCV) reading indicated either an increase in number of Red Blood Cells (RBCs) or reduction in circulating plasma volume. Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration indicate blood level conditions. A low level is an indication of anaemia

Red blood cells (erythrocytes) serve as a carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration. According to [10] red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs [10].

The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases [11] and enhance adaptability to local environmental and disease prevalent conditions [10].

Blood platelets are implicated in blood clotting. Low platelet concentration suggests that the process of clot-formation (blood clotting) will be prolonged resulting in excessive loss of blood in the case of injury. Packed Cell Volume (PCV) which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF), is the percentage (%) of red blood cells in blood [12]. According to [10] Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. Increased Packed Cell Volume shows a better transportation and thus results in an increased primary and secondary poly-cythemia. Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates with the exception of the fish family, channichthyldae as well as tissues of invertebrates. Haemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals.

5. Conclusion

The outcome of this study shows that the extract of the two leaves has the tendency to reduce the high level of sodium arsenite in the blood of the albino rats.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The ethical committee of the university approved the study protocol before the study commenced and was carried out accordance to the guidelines of the Animal welfare act.

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