

# Ameliorative Effect of Vitamin C On Sodium Arsenate Induced Changes Of Blood Indices In Experimental Albino Mice

<sup>1</sup>Vijaya G, <sup>2</sup>Yamini T, <sup>3</sup>Jameela V, <sup>4</sup>Meena Kumari G, <sup>5</sup>Nagaraju K.

Dept. of Zoology  
S.V. University  
Tirupati AP, India.

**Abstract-** It is well known that Sodium arsenate is necessary for the bone health, but ingestion of Sodium arsenate (NaAs) beyond the permissible limits is toxic to the animals. In the present investigation the author studied the ameliorative effect of vitamin C (Vit-C), on selected haematological parameters in NaAs intoxicated mice.  $1/10^{\text{th}}$  of  $LD_{50}$  of NaAs (*i.e.* 4.2mg/kg/bw) was orally administered for 28 days to mice to induce toxicity. In the next group vitamin C (*i.e.* 200mg/kg/day) was administered prior to 30 min of NaAs to see the ameliorating effect. Under NaAs toxicity the total RBC, Hb, WBC, PCV, MCV, MCH and MCHC were reduced. When vitamin C was co-administered with NaAs the haematological parameters did not show any significant change as compared to control indicating that vitamin C acts as an ameliorant against NaAs induced toxicity. The results are discussed in the light of available literature.

**Key words:** Sodium Arsenate, Vitamin C, Albino Mice , Haematological Parameters.

## INTRODUCTION

Hematological studies helps to understand the cause, prognosis, treatment, and prevention of diseases related to blood. In order to treat diseases that are caused by toxins like pesticides and heavy metals the production of blood and its components, such as blood cells, hemoglobin, blood proteins, bone marrow, platelets, blood vessels, spleen, and the mechanism of coagulation are usually studied which helps the researchers to get an insight into diseases such as include hemophilia, blood clots, other bleeding disorders, and blood cancers such as leukemia, multiple myeloma, and lymphoma. Any alterations in the blood constituents can impede many functions of the body and cause a range of complications. For this reason, hematology implies the study and treatment of blood disorders is usually carried out whenever the animals are subjected to the exposure of toxic compounds.

The importance of hematological parameters in clinical biochemistry, population genetics and medical anthropology is well established and they provide important information about the internal environment of the organism (Masopust, 2000). (Hymavathi and Rao, 2000) reported that haematological studies are more rapid and any alterations during stress can be easily detected. In human medicine, investigation of hematological parameters is necessary for clinical diagnosis of a disease and pathological condition. Blood is the only available tissue in occupationally exposed workers and it is a patho physiological reflector of the whole body, hence blood parameters are routinely used for diagnosing and monitoring the disease conditions in humans (Rahman and Siddiquie, 2006).

It is well known that ingestion of excessive sodium arsenate results in anemia and eosinophilia of leukocytes. (Erdal Eren *et al.*, 2005) explored hematological effects in Albino mice induced by 100ppm of arsenate for four months. At the end of four months they measured the hematological indices (Hb, MCV, MCH, RBC, WBC, and platelet counts). They observed dysplastic changes on granulocytes in the bone marrow.

Arsenic (As) is considered to be one of the most important concerns for animal and human health as an environmental toxicant. As-contaminated environments result in severe health risks to fauna and their ecosystems (Susan *et al.*, 2019). Increased levels of As in the environment have become a global threat due to its harmful effects on human and animal health (Roshni *et al.*, 2015). Exposure to this metalloid results in severe health effects and pathology in livestock, wildlife, and humans including gastrointestinal damage, hematopoietic toxicity, and neurological dysfunction (Cárdenas-González *et al.*, 2016). As can also cause major reproductive dysfunction via endocrine disruption and reduced fertility (Iftikhar *et al.*, 2014). In terms of reproduction, studies have reported harmful effects of As in the mice testis (Souza *et al.*, 2016).

Arsenic, a naturally occurring toxicant that is present in food, soil and water. Exposure to higher level than the average level of arsenic occurs either in workplace, for example, in smelting industries, coal fired power plants, cosmetic industries, agriculture or through arsenic-contaminated food and drinking water. The most common forms of arsenic are water-soluble arsenite (the trivalent form, As III) and arsenate (the pentavalent form, As V); trivalent arsenic is more toxic than the pentavalent form and its inorganic forms are more toxic than the organic forms [1]. Moreover, it is reported that the inorganic As (III) form as  $H_2AsO_3$  is 40–60 times more toxic than the As (V) form as  $H_2AsO_4$  [2]. Arsenic has been reported to be associated with multi-site cancers, cardiovascular diseases, diabetes mellitus, dermatitis, immuno toxicity, lymphoproliferative disorders, peripheral neuropathy and many other complications Vahidnia, A, Romijn, F, van der Voet, G.B, *et al.*, 2008; Chen, C.J, Wang, S.L, Chiou, J.M, *et al.*, 2007 ;

Toxicity due to metals such as cadmium, chromium, lead, and arsenic has generated interest to understand their interactions with biomolecules. Concentration of arsenic in various sources ranges from 2 ppb to 5000 ppb .The WHO expert committee on food additives has evaluated the effects of arsenic on human health and also concluded that for certain regions of the world, the concentrations of inorganic arsenic in drinking-water exceed 50–100 mg L<sup>-1</sup> (Quansah *et al.*, 2015). Higher concentrations of arsenic have been observed during acute promyelocytic leukemia, a condition wherein arsenic trioxide has been clinically administered (Ahmada *et al.*, 2013). It is known that As(III) species are more toxic than those of As(V) and biological methylation process can increase or decrease the toxicity of different forms. Cellular metabolism of arsenic is characterized by the reduction of As(V) to As(III) through a redox cycle and methylation of As(III) by S-adenosyl-methionine to yield mono- and di-methyl derivatives is a sequential process.

## MATERIAL AND METHODS

**Materials:** Sodium Arsenate (99%) was used as a toxicant supplied by BDH Chemical Division, Bombay.

**Animal model:** Male Albino Mice

**Animal Selected:** Healthy Wister strain Albino mice of the same age group  $60 \pm 05$  days and weight  $30 \pm 05$  g were procured as experimental animals for the present study. The mice were procured from Indian Institute of Science (I.I.Sc.), Bangalore. Prior to experimentation the animals were acclimatized and maintained at laboratory conditions in the animal house at  $25 \pm 2^\circ C$  with a photoperiod of 12h light and 12h darkness throughout the course of the present study. The mice were fed with standard pellet diet supplied by Sai Durga feeds and foods, Bangalore and water ad libitum.

**Concentration of NaAs selected:** The LD<sub>50</sub> as per the latest reports is 42mg/Kg body weight (Finney *et al.*, 1971). In the present investigation 1/10th LD<sub>50</sub> (i.e. 4.2 mg/kg body weight) was selected for sub-lethal treatment to the experimental mice to induce the toxic effects of arsenate in Albino mice.

**Sodium arsenate stock solution:** Stock solution of NaAs was prepared in distilled water and it was diluted in such a manner that the experimental mice received 1/10th dose of LD<sub>50</sub> (i.e. 4.2 mg/ kg bw/ day.)

**Vitamin C stock solution:** Stock solution of NaAs was prepared in distilled water and it was diluted in such a manner that the experimental mice received 200mg/ kg bw/day (Devendra *et al.*, 2009).

**Course of study:** 28 days

**Route of administration:** Oral through gavage.

### Experimental Design:

Four groups of Albino mice were selected and each group consists of six mice. Group I: Control mice Group II: NaAs (exposed Albino mice for 28 days) Group III: Vitamin C treated Albino mice Group IV: NaAs + Vitamin C (exposed Albino mice for 28 days)

### Hematological Parameters

RBC count was made with a Neubauer crystalline counting chamber as described by Davidson and Henry (1969). The hemoglobin was estimated by Acid - Haematin method (Sahli, 1962). PCV estimated by micro hematocrit method (Schalm *et al.*, 1975). MCV expresses the average volume of the red blood cells. For obtaining the mean corpuscular volume, the packed cell volume is divided by red blood cell count and the result is multiplied by 10. MCV is expressed in cubic microns ( $\mu m^3$ ). MCH represents the average weight of hemoglobin contained in each cell. MCH is influenced

by the size of the cell and concentration of hemoglobin. For getting MCH the Hb concentration is usually divided by red blood cell count and the result is multiplied by 10 and is expressed as pictograms (Pg). MCHC refers to the average concentration of the Hb in the red blood cells. In contrast to MCH, MCHC is not influenced by the size of the cell. For getting MCHC the hemoglobin is divided by packed cell volume and the result is multiplied by 100. The MCHC value is expressed in terms of percentage. The rest of the procedure is the same as described by Davidson and Henry (1969) for RBC count. Counting was started under high power oil immersion objective from the edge of the smear moving the smear towards center. Leucocytes were identified and the movement was repeated till a total 100 cells were counted. The values of different morphological types were expressed as the percentage.

## RESULTS AND DISCUSSION

In the present investigation severe alterations in hematological parameters like RBC, Hb, WBC, PCV, MCV, MCH and MCHC were observed in NaAs exposed mice. Oral administration of NaAs caused a gradual decline in R.B.C, Hb, PCV, WBC and fluctuations in MCV, MCH and MCHC were observed in 28 days exposed groups and all the values are statistically significant (Table 1). When vitamin C was co-administered with NaAs the haematological parameters did not show any significant change as compared to control indicating that vitamin C acts as an ameliorant against NaAs induced toxicity. The results are discussed in the light of available literature.

Sodium arsenate administered mice showed decrease in RBC count over control which was found to be statistically significant. When Vitamin C was administered along with sodium arsenate the RBC count did not decline indicating that vitamin C shows an ameliorating the effect in nullifying sodium arsenate induced toxicity.

The Hb content in sodium arsenate exposed mice showed a decline when compared to controls. Vitamin C mitigated the toxic effect of sodium arsenate.

WBC count decreased by in sodium arsenate intoxicated mice. When an ameliorating agent Vitamin C was co-administered with sodium arsenate WBC values were not altered indicating that Vitamin C shows a mitigating effect induced by sodium arsenate. All the values were found to be statistically significant.

The PCV in the present investigation showed decrease in sodium arsenate intoxicated mice. When with Vitamin C was given along with sodium arsenate, the toxic effects of sodium arsenate were nullified.

The MCV, MCH and MCHC also showed a decline under sodium arsenate toxicity. Vitamin C mitigated the toxic effects of sodium arsenate and the results were found to be statistically significant.

Arsenicals are the potent environmental pollutants and well-established human carcinogen. The main cause of arsenic toxicity is the drinking of ground water contaminated by arsenic.

In the present investigation, the toxic effect of Sodium Arsenate on the Hematological parameters was studied in Albino mice. Mice treated with Sodium Arsenate became anaemic. Sodium Arsenate has an adverse effect on haemopoietic system. Sodium Arsenate administration might have caused destruction of erythrocytes directly or the decreased RBC count may be due to the effect of Sodium Arsenate on erythropoietic tissue viz., the bone marrow. In general, the manifestation of anaemia is due to reduction in the number of red blood cells or hemoglobin or impaired production of erythrocytes.

Although many aspects of how Sodium Arsenate affects the body are still unclear, it is known that Sodium Arsenate can block or induce various changes in blood. The Sodium Arsenate -induced anemia observed in this study might have resulted from inhibition of globulin synthesis, depression of erythropoiesis, or a decrease in the level of blood folic acid. There are evidences which indicate that Sodium Arsenate can affect the bone marrow microenvironment and the *ex vivo* studies of bone marrow cells indicate that Strain-dependent effects on hematopoietic colony-forming cell unit (CFU).

Blood tissue is a pathological reflector of the health status of animals exposed to toxicants and other conditions (Olafedehan, C.O, Obun, A.M, *et al.*, 2010). The interaction of a toxin or its metabolites with cellular constituents may eliminate in hematological parameters (Arika, W.M, Nyamai, D.W *et al.*, 2016) that indicate hematological disorders such as anemia (low hemoglobin content), leukopenia (reduced white blood cells), thrombocytopenia (low blood platelet level) (Price, E.A, Schrier *et al.*, 2008 ; Bradbury, C; Murray, J *et al.*, 2013; Izak, M, Bussel, J.B *et al.*, 2014). Thus, hematological parameters are very useful in assessing toxicity and health and physiological status of animals (Togun, V.A, Oseni, B.S.A, *et al.*, 2007; Pankaj, P.P, Varma, M.C *et al.*, 2013).

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 (Wintrobe, M.M, *et al.*, 2009). 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 (Kolanjiappan, K, *et al.*, 2002;

Junqueira, LC, *et al.*, 2006). The mice of hemoglobin destruction, or a decrease in the mice of hemoglobin synthesis may have influenced the significant decrease in Hb concentration observed in this study. This may have also contributed to the significant decreases in PCV. (Wintrobe MM *et al.*, 2009).

In addition, administration of sodium arsenite alone caused significant decrease 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 decrease in WBC may be as a result of the necrotic activities of sodium arsenite in the cells. Decreased WBCs in mice following treatments with arsenic are reported. The immune system to be a major target of arsenic, and suppressive effects on the immune system are noted in different animal models are studied.

Alteration in MCV, MCH, and MCHC values imply the macrocytic anemia which can lead to very slow production of erythroblasts in bone marrow. As a result they grow over in size with shape and have fragile membranes called megaloblast which is characteristic of pernicious anemia which can lead to megaloblast anemia. MCV, MCH, and MCHC observed in this study and may connote the presence of hypochromic and microcytic anemia. Decrease in MCV, MCH and MCHC were reported in mice.

The reduction in Hb, RBC, WBC, MCV, MCH, and MCHC indicate that sufficient damage was caused to haemopoietic tissue under Sodium arsenate toxicity. The decreased level of RBC count, Hb concentration and PCV values supports the increased damage of red cells due to ribosomal abnormality and decreased protein synthesis. Sodium arsenate when accumulated in the blood causes reduction in the blood components and disturbed the protein level in the body. The hematological parameters on the whole showed a significant change under the impact of sodium arsenate exposure after 28 days. From the results of this study and others, we suggest that vitamin C may stimulate the activity of the bone marrow stem cells and that nutritional supplementation which it may help to overcome anemia in animals. However, further research is required to investigate its effects on the regulation of the immune system and erythrocyte production in bone marrow (Meena kumari *et al.*, 2022)

Sodium arsenate treatment has the adverse effects on kidney and liver and vitamin C potentially inhibited the sodium arsenate action. It has been well-documented that ROS produced by arsenic acts as a second messenger for transducing intracellular signal. (Hossain K, Akhand AA, Kato M, *et al.*, 2000; Hossain K, Akhand AA, Kawamoto Y, *et al.*, 2003). The hematopoietic and immune system both are clear targets for As-induced toxicosis in albino mice. Feeding of antioxidants like vitamin C neutralized the toxic effects of sodium arsenate in terms of RBC count, hemoglobin content, and PCV. In other heavy metals like cadmium toxicity enhanced the serum levels of ALT, AST, ALP, Urea, and Creatinine decline in body weight and relative organ weights under toxic insult are altered under zinc and vitamin C supplementation. (Keshavulu *et al.*, 2021).

Vitamin C can act to reduce the accumulation and binding of metals in the small intestine. This vitamin C is equipped with high numbers of sulfhydryl groups. These groups bind with metals and reduce their bioavailability to tissues (Rana *et al.*, 2010). Supplementation of vitamin C greatly improved the blood parameters measured in albino mice. Similar improvements from vitamin C have been reported in mice (Amer Sayed *et al.*, 2019).

The immune system is a major target of arsenic, and suppressive effects on the immune system are reported in mammals (Sakurai *et al.*, 2006). As pointed out, vitamin C also has unique antioxidant capacity. These beneficial influences of vitamin C may be due to efficient antioxidant and scavenger of reactive oxygen species (Kojo 2004; Catani *et al.*, 2005). Feeding vitamin C along with sodium arsenite significantly ameliorates the effect of arsenic toxicosis in Albino mice. Protective effects against arsenic-induced toxicity have been reported in mice (Hasan *et al.*, 2015). Here we also demonstrates that vitamin C can be an excellent protective mechanism against arsenic toxicosis in Albino mice. Hematologic and biochemical analyses are considered to be reliable parameters for the evaluation of health status of humans, as well as animals, resulting from arsenic poisoning (Sexena *et al.*, 2011; Ohaeri and Eluwa *et al.*, 2011).

## CONCLUSION

Blood is promptly affected by environmental pollutants and toxicants that can cause many metabolic disorders. The high level of sodium arsenate acts as a potential pollutant, with very high toxicity, associated with the hematological damage. This study aimed to determine the toxic effect of sodium arsenate on the hematological parameters like RBC, Hb, WBC, PCV, MCV, MCH and MCHC are commonly examined to assess the toxic stress in sodium arsenate exposed mice. Its toxicity produces hematological alterations. In the present investigation sodium arsenate administration might



have caused destruction of erythrocytes directly may be due to the effect of sodium arsenate on erythropoietic tissue. WBC count was decreased, suggesting that defense mechanism will be affected under sodium arsenate toxicity. Many investigators reported decreased RBC count, Hb concentration and PCV values under various stress conditions in mice. This might be due to metabolic alterations in erythrocyte and Hb carrying capacity of the blood, consequently resulting in lowering Hb concentration. The outcomes of the current investigation indicated the reduction in Hb, RBC, WBC, MCV, MCH, and MCHC on sodium arsenate intoxication. Mice treated with sodium arsenate became anaemic. Anemia caused by sodium arsenate can be reversed by the antioxidant properties of vitamin C. Sodium arsenate has an adverse effect on haemopoietic system. Vitamin C is useful for amelioration of sodium arsenate toxicity which is used as dietary supplement, it has antioxidant potential and also to protect the hematotoxic to the Sodium arsenate. The hematological parameters found within normal range after ingestion of vitamin C.

**Table 1: NaAs Induced Toxicity and Ameliorative Effect of Vitamin C Related to Haematological Parameters in Albino mice.**

	Control	NaAs (28 D)	Vit. C (28 D)	NaAs+ Vit. C (28 D)	F ratio
<b>RBC (Mean)</b> ± SD (% Change)	8.561 0.013	5.874 0.013 (31.380)	8.333 0.040 (2.659)	8.241 0.006 (3.736)	197.154*
<b>HB (Mean)</b> ± SD (% Change)	10.117 0.003	7.850 0.130 (22.405)	9.997 0.002 (1.188)	9.557 0.012 (5.532)	155.002*
<b>WBC</b> ± SD (% Change)	11.359 0.014	10.942 0.036 (3.670)	11.120 0.005 (2.103)	11.082 0.018 (2.434)	388.973*
<b>PCV</b> ± SD (% Change)	49.731 0.023	36.453 0.010 (26.701)	49.523 0.013 (0.419)	49.223 0.012 (1.022)	104.721*
<b>MCV</b> ± SD (% Change)	47.379 0.008	40.599 0.003 (14.310)	47.256 0.010 (0.259)	46.985 0.013 (0.830)	764.985*
<b>MCH</b> ± SD (% Change)	12.892 0.005	9.931 0.060 (22.972)	12.761 0.006 (1.017)	12.107 0.006 (6.088)	122.137*
<b>MCHC</b> ± SD (% Change)	22.158 0.006	17.545 0.008 (20.817)	22.118 0.003 (0.179)	21.995 0.003 (0.736)	113.379*

Values are Mean ± SD of six individual animals. Mean values with the same superscript do not significantly differ among themselves through S-N-K test.

\*p < 0.01

#### Units:

RBC - Cu.mm; HB - g / 100ml; WBC - Cu.mm; PCV - percent ; MCV - µg ; MCH - µ.µg ; MCHC - percent ;

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