

POSSIBLE PROTECTIVE ROLE OF AQUEOUS TOMATO EXTRACT ON HEMATO-BIOCHEMICAL PARAMETERS AGAINST SODIUM ARSENITE TOXICITY IN ALBINO RATS

ABSTRACT: Due to rapid industrialization and technological development, humans are constantly exposed to heavy metals at minimum to maximum tolerable levels. Arsenic is ubiquitously distributed and most concerned element now-a-days, inorganic arsenicals mainly enter into the body via the route of lungs and gastrointestinal tract and 55-95% of it gets absorbed. Sodium arsenite poisoning leads to disturbance in hematological and biochemical processes. In the present study, toxic effects of sodium arsenite on the hematological parameters, serum transaminases and possible modulatory role of aqueous tomato extract were studied. Female rats were allocated into four different groups: 1. Control (given normal diet), 2. Sodium arsenite treated (10mg/kg B.W.), 3. Combination of sodium arsenite (10 mg/kg B.W.) and aqueous tomato extract (50 mg/kg B.W.) treated rats 4. Only aqueous tomato extract treated (50mg/kg B.W.) rats. It was observed that arsenic significantly decreased the total erythrocytes count (RBC), hemoglobin concentration (Hb), hematocrit value (HCT) and platelets (PLT), whereas significantly elevated the total leucocytes count (WBC) and activities of serum transaminases such as ALT and AST. Supplementation of aqueous tomato extract to arsenic treated rats showed decrease in values of WBC, ALT and AST which almost matched to values of control rats. So, from this study it can be concluded that antioxidants and vitamins present in the aqueous tomato extract showed protective role against arsenic toxicity in albino rats.

KEYWORDS: Hematological parameters, Hemoglobin, Serum transaminases, Sodium arsenite.

ABBREVIATIONS:

ALT – Alanine Transaminase

AST – Aspartate Transaminase

RBC – Red Blood Cell

HB - Hemoglobin

HCT – Hematocrit

PLT – Platelets

WBC – White Blood Cell

INTRODUCTION:

Heavy metals are elements having high density, present in the environment naturally in a small quantity [1]. Across the 70 countries all over the world over 140 million people and equal number of animals are living in arsenic contaminated zone [2-4]. Inorganic arsenicals are released into the environment from earth's crust through natural processes like volcanoes, weathering of rock containing arsenic and by human activities such as mining and smelting. Arsenic is also used in pesticides [5]. Upon ingestion of inorganic arsenic, it is immediately absorbed via gastrointestinal tract and distributed in body's various tissues with the aid of circulatory system [6]. Exposure to arsenic for long periods of time results in vomiting, abdominal pain and diarrhea. Arsenic has various deleterious effects on hematopoietic system, leading to anemia, erythrocyte karyorexis, lowering of granulocytes and deficiency of platelets which further results in slow clotting of blood after injury [7]. Arsenic shows potential reactivity towards sulfhydryl groups of various proteins and enzymes. After binding to them it initiates intracellular signaling pathways that ultimately results in harmful effects [8-10].

Hematological indices like mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) are related to RBC. MCV reflects average weight of hemoglobin contained in a RBC. MCV tells about average size of RBC, whereas MCHC shows average concentration of hemoglobin present in the RBC [11]. From ancient times, fruits and vegetables concoctions were in use to cure minor illness like headache to severe heart attack [12]. Antioxidants play an important role to maintain endothelial functionality, prevent platelet aggregation and minimize the atherosclerotic plaque formation in animals [13]. All over the world, people of developing countries are not getting proper nutritious diet causing malnutrition [14].

Tomatoes possess major constitute like lycopene, glutathione, vitamin C, vitamin A, potassium and calcium [15]. Tomatoes and its processed products like sauce, juice, soup and spaghetti sauce constitute major source of lycopene which accounts almost for 85% of dietary source [16]. Tomatoes enhance the efficiency of hematopoietic system and lead to elevation in RBC, WBC

and HCT values.

MATERIALS AND METHODS:

PROCUREMENT OF CHEMICAL: Sodium Arsenite (NaAsO_2) was obtained from Himedia Laboratories Pvt. Ltd. MUMBAI. Tomatoes were obtained from organic agriculturist. All reagents and chemicals used in the experiment were of analytical grade.

PREPARATION OF AQUEOUS TOMATO EXTRACT:

Tomato extract was prepared by the method of Salawu, (2009).

The tomato extract was made by grinding tomatoes in a mixture grinder and the puree was incubated in a water bath for 45min at 80°C then the extract was filtered using muslin cloth and it was stored in a sterile vial.

EXPERIMENTAL RATS: Twenty wistar albino female rats (Eight weeks old) weighing $135\pm 5\text{g}$ were procured from LUVAS, Hisar (Haryana). All rats were maintained and housed in disease free animal house of Punjabi University, Patiala. Rats had free access to water and standard rodent feed. After acclimatization for a period of 2 weeks prior to the experiment, rats were segregated into 4 groups of 5 rats each and maintained in 4 different polypropylene cages tagged with identity cards. Rats of each group were marked with different colors on tail for identification. Experiment was conducted after the approval of Institutional Animal Ethical Committee (Approval No; 107/GO/ReBi/S/99/CPCSEA/2017-23).

GROUP DESIGN:

Group1 - Rats were kept as a control.

Group2 - Rats were administrated acute dose of sodium arsenite (10mg/kg B.W., As) with the help of oropharyngeal cannula.

Group3 - Rats received acute dose of sodium arsenite (10mg/kg B.W.) followed by tomato extract (50mg/kg B.W., As+TE) for 30 days.

Group4 - Rats were administrated only tomato extract (50mg/kg B.W., TE) for 30 days.

COLLECTION OF SAMPLE:

During experiment each rat was weighed after 5 days interval using digital weighing machine. After autopsies blood samples for the haematological and biochemical analysis, were collected by ocular puncture from each of the rats using capillary tube, and was dispensed into commercially available EDTA coated vials and mixed thoroughly for analyzing haematological parameters and in simple vials for biochemical parameters.

DETERMINATION OF HAEMATO-BIOCHEMICAL PARAMETERS: Haematological analysis included RBC, Hb, HCT, as well as blood indices MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) and were assessed on the day of autopsy by standard methods using automated haematological analyzer.

For biochemical analysis collected blood samples were centrifuged at 3000 rpm for 15 minutes to separate out the serum. Serum transaminases (ALT & AST) were analyzed using the commercial kits.

STATISTICAL ANALYSIS:

The control, arsenic and aqueous tomato extract treated rats were compared using the student's t test. The data obtained is presented as mean±S.E.M. Significance level was considered at p < 0.05.

RESULT:

Table 1. Shows activity of serum transaminases (ALT and AST) in control, arsenic treated (As), tomato extract (TE) and (As+TE) supplemented rats.

SERUM TRANSAMINASES	TREATED GROUPS			
	GROUP I (CONTROL)	GROUP II (As)	GROUP III (As+TE)	GROUP IV (TE)
ALT (IU/L)	60.49±2.15 ^a	86.672±3.86 ^b	75.138±1.93 ^c	57.788±4.73 ^a
AST (IU/L)	151.10±10.11 ^a	324.786±14.57 ^b	236.24±32.69 ^c	146.95±11.23 ^a

Results are expressed in MEAN ± SEM and values within the same row having different superscript are significantly different.

TABLE 2. Depicted the body weight of control, arsenic treated, (As+TE) and (TE) treated rats at the intervals of time.

INTERVAL DATS	EXPERIMENTAL GROUPS			
	GROUP I (CONTROL)	GROUP II (As)	GROUP III (As+TE)	GROUP IV (TE)
1	133.3	133	137.7	132.8
5	142.2	136.5	142.6	141.6
10	148.3	144.4	148.1	150.1
15	151.8	148.2	153	158.3
20	164.7	155.1	159.9	165.2
25	170.5	162.8	166.8	173
30	182.2	169.3	176.4	180.9

Results are expressed as mean value

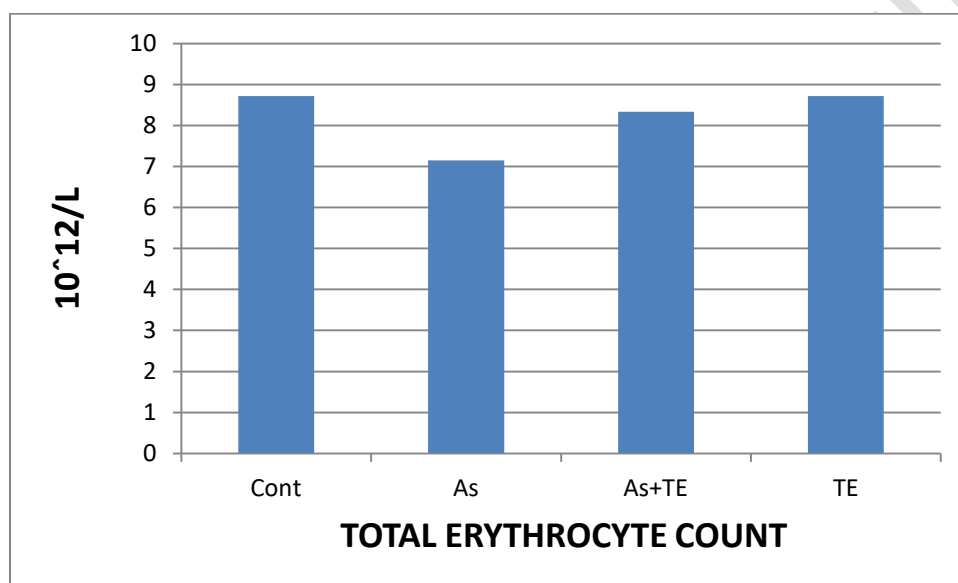


FIG. 1. Total number of erythrocytes of control, arsenic-exposed, (As+TE) and Tomato treated rats.

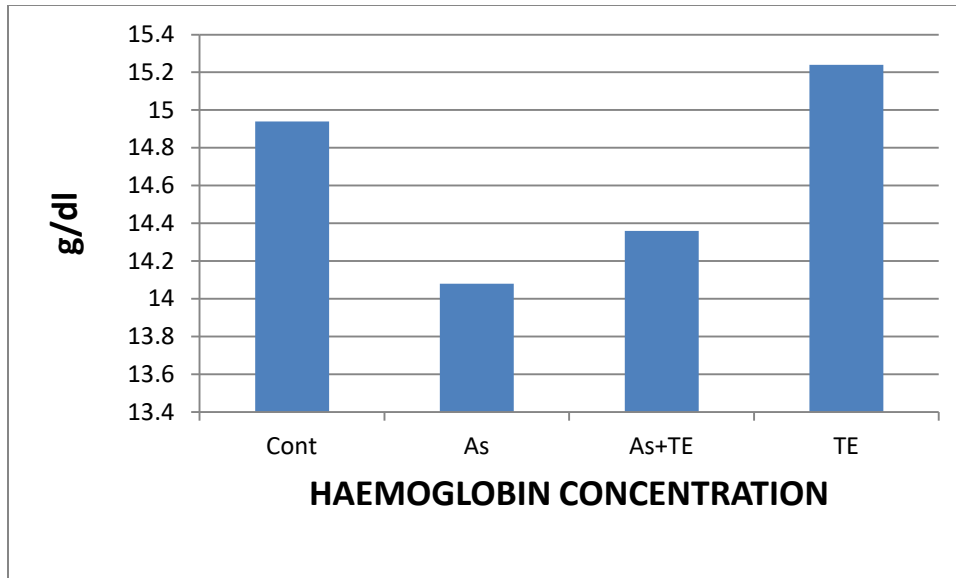


FIG. 2. Total haemoglobin concentration of control, arsenic-exposed, (As+TE) and Tomato treated rats.

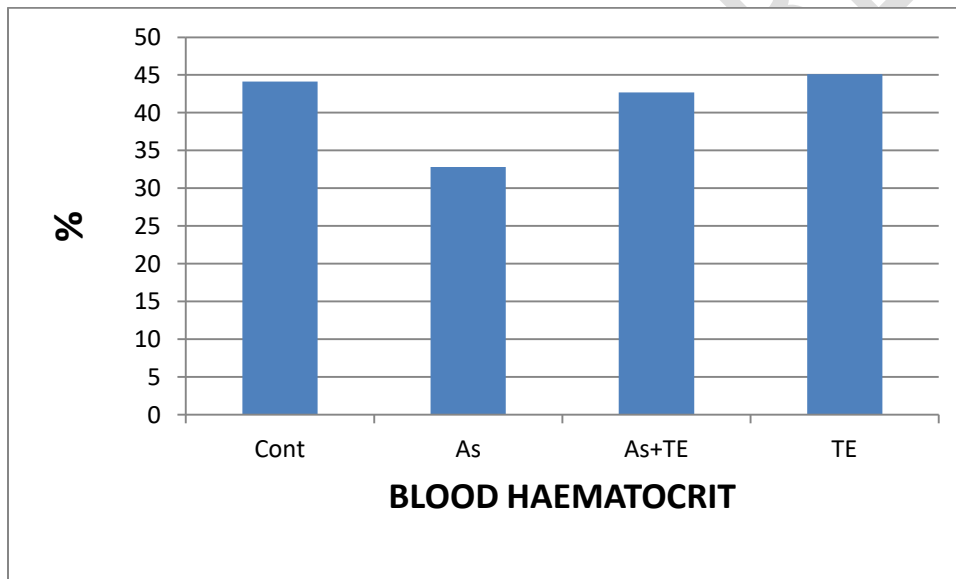


FIG. 3. Total haematocrit value of control, arsenic-exposed, (As+TE) and Tomato treated rats.

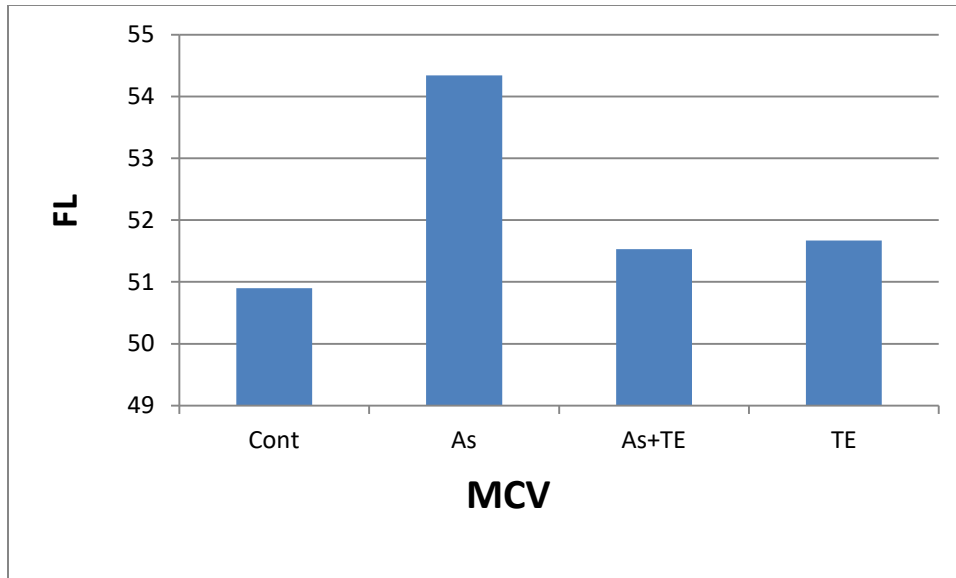


FIG. 4. Total MCV value of control, arsenic-exposed, (As+TE) and Tomato treated rats.

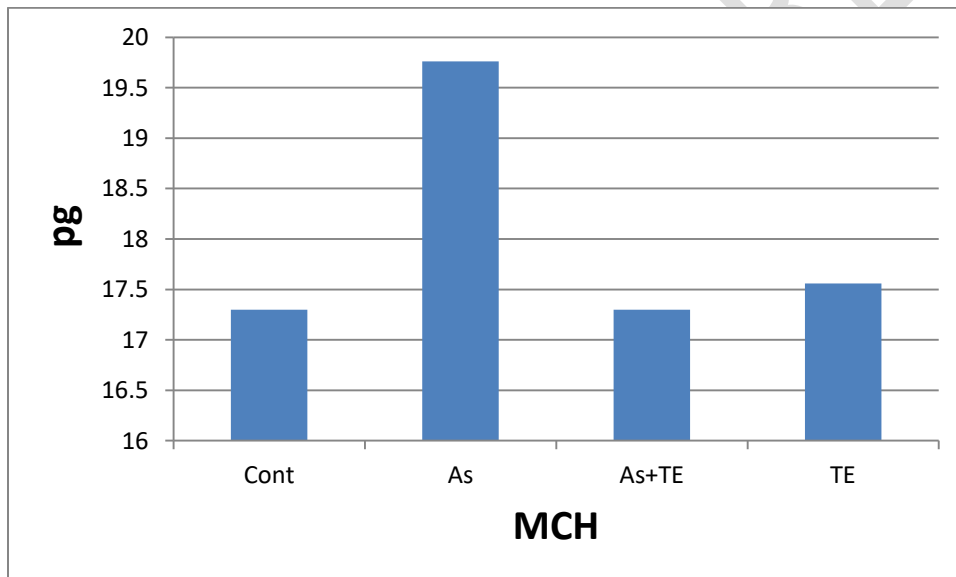


FIG. 5. Total MCH value of control, arsenic-exposed, (As+TE) and Tomato treated rats.

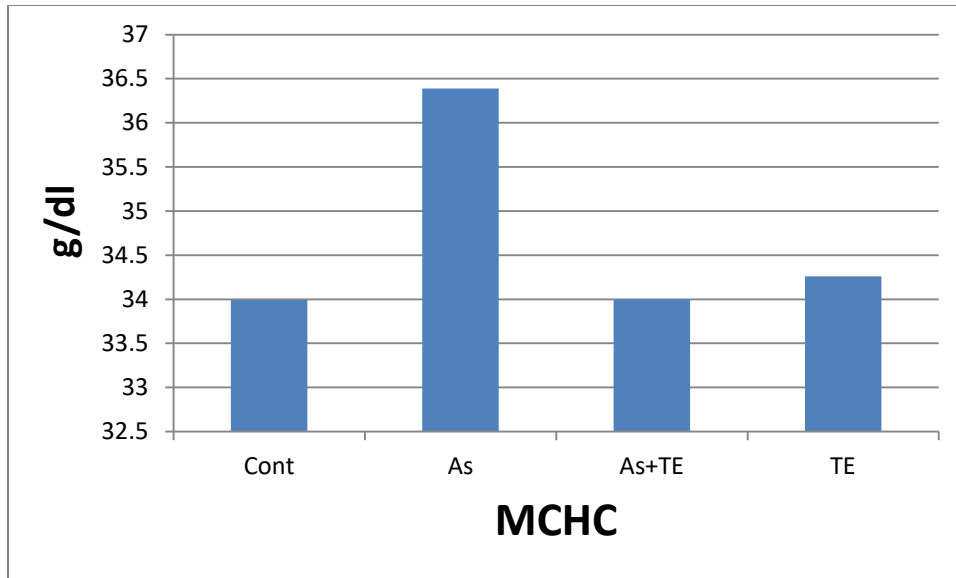


FIG. 6. Total MCHC value of control, arsenic-exposed, (As+TE) and Tomato treated rats.

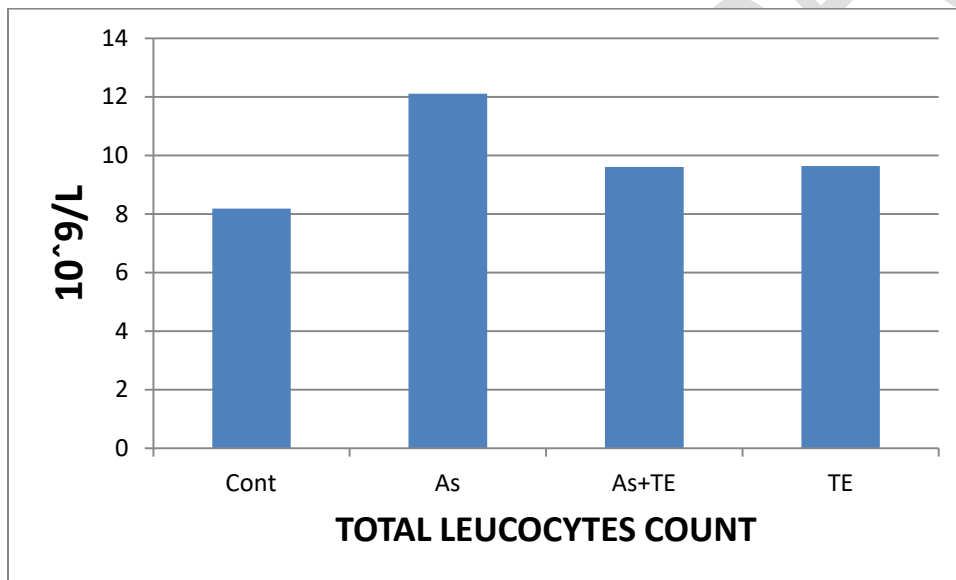


FIG. 7. Total MCHC value of control, arsenic-exposed, (As+TE) and Tomato treated rats.

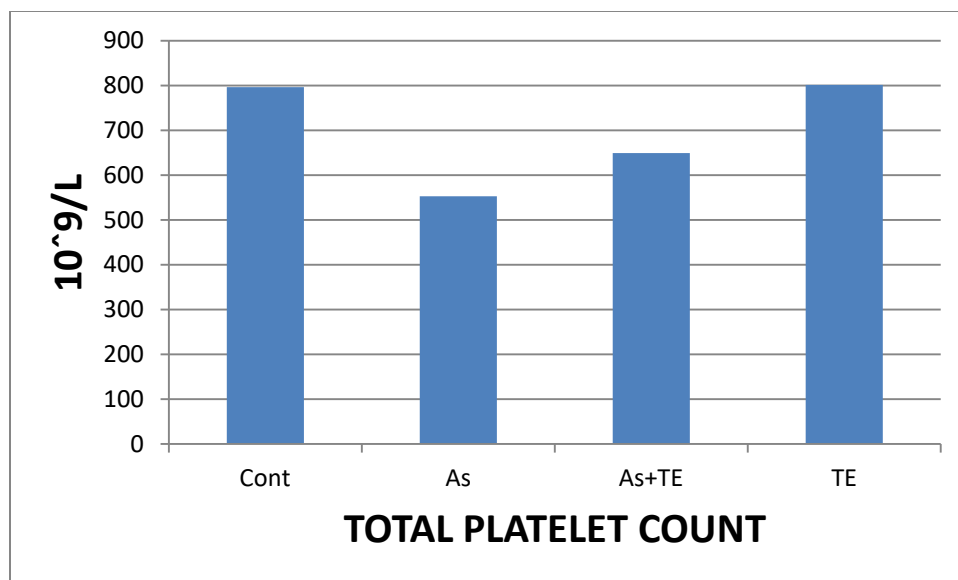


FIG. 8. Total platelet count of control, arsenic-exposed, (As+TE) and Tomato treated rats.

Animals were monitored throughout the experimental period of 4 weeks to notice any skin problem and tumor formation. The no mortality was observed throughout the experimental period. Rats showed no chronic toxicity as an acute dose of arsenic (10mg/kg B.W.).

Sodium arsenite treated rats showed slight increase in body weight shown in table 2 as compared to the other groups from their initial weight which might be due to reduced intake of water and food leading to dehydration.

Abnormalities in haematopoietic system of arsenic exposed rats are observed from the experimental data as shown in FIG. 1. A significant ($P = 0.01$) decrease in total erythrocyte count of sodium arsenite exposed rats was seen as compared to control rats. In the rats supplemented with tomato extract the erythrocyte count came almost equal to the control RBC count and thus a significant ($P = 0.01$) increase in erythrocyte count of As+TE was observed as compared to only arsenic group. This accounts for a partial reversal of the sodium arsenite caused toxic effects in As+TE treated rats and also maintained their erythrocyte count to the near normal range.

Similar pattern was observed in the hemoglobin concentration and hematocrit value. Results obtained from the experiment were depicted in FIG. 2. Hemoglobin concentration was decreased significantly ($P = 0.003$) in arsenic (As) treated rats as compared to control. After administration of tomato extract along with arsenic (As+TE), hemoglobin content increased significantly ($P = 0.03$) in (As+TE) as compared to arsenic (As) intoxicated rats. These results show that

antioxidants and vitamins enriched tomato extract has positive stimulatory effects on hematopoietic system. As it is well known that hematocrit value is largely dependent on total erythrocyte count, hematocrit value also declined significantly ($P = 0.00$) in sodium arsenite (As) administered rats as are quite visual in FIG. 3. as compared to control, whereas the detrimental effects were significantly reversed ($P = 0.04$) in rats treated with tomato extract (As+TE).

MCH showed no significant ($P = 0.06$) difference between all treated rats and control rats. Whereas MCH depicted significant decrease ($P = 0.01$) in (As+TE) group compared to only arsenic (As) given rats. Results are presented in the FIG. 5.

No significant ($P = 0.17$) difference was observed in the values of MCV of control, (As), (As+TE) and (TE) treated rats. Values are shown in FIG. 4.

Likewise, MCHC showed no significant ($P = 0.12$) difference among all treated groups. Results are shown in FIG. 6.

A significant ($P = 0.03$) increase was observed in total leucocyte count of sodium arsenite (As) treated rats compared to group I, group III and group IV. Rats administered with tomato extract (TE) after arsenic toxicity showed values almost equivalent to normal leucocytes and significantly decreased count was seen in group 3 in comparison to group 2. Data is presented in FIG. 7.

A Significant ($P = 0.00$) decline in the platelets count was observed in the arsenic exposed (As) rats as compared to group I, group III and group IV. When rats are supplemented with tomato extract significant ($P = 0.03$) increase was seen in (As+TE) group as compared to only arsenic treated (As) rats.

Liver is the major site for the xenobiotic and heavy metal metabolism. A significant ($P = 0.0004$) elevation in the activity of alanine transaminase (ALT) was observed in group II compared to group I, group III, and group IV. Supplementation of tomato extract to rats significantly ($P = 0.02$) lowered the value of ALT of group III rats in comparison to group II rats. Likewise, AST was significantly ($P = 0.0001$) increased in arsenic administered rats compared to control and other treated rats. In tomato extract supplemented rats the value of AST was significantly ($P = 0.03$) declined as compared to arsenic treated rats.

DISCUSSION: in the long run arsenic causes retarded growth, distortion in counts of different blood cells and marked elevation in various serum enzymes such as serum glutamic pyruvic transaminases (SGPT), alkaline phosphatase (ALP), lactate dehydrogenases (LDH) in rodents

and human [17-18]. To reduce arsenic burden in rats, supplementation of antioxidant, vitamins and micronutrient rich diet has been well documented [19]. Various studies are reported on sodium arsenite intoxication and supplementation of ascorbic acid, vitamin A, spirulina, zinc and iron which showed modulatory effects to reduce arsenic load of body [20]. In several studies, α - lipoic acid, ascorbic acid and α - tocopherol are shown to act synergistically more effective in comparison to their individual application. No significant alterations in Hb, RBC and WBC content were seen in sodium arsenite (4mg/kg B.W) treated rats, whereas SGPT and SGOT were significantly reduced. However, antioxidants loaded spirulina and thankuni partially reversed the arsenic toxicity [21]. Some studies reported alterations in hematological indices after arsenic intoxication which leads to anemia [22-24]. A study showed inhibitory effects of arsenic on hematopoiesis resulting in lower values of hematocrit and hemoglobin leading to anemia [25]. Mercuric chloride (0.3mg/kg B.W.) administration for 5 and 7 days in albino rats caused significant lowering in hemoglobin content. Whereas, supplementation of DL- α -Tocopherol acetate recovered the hemoglobin content almost near to control values [26]. Hemoglobin and hematocrit both are used as a marker to identify anemia. The ratio of RBC to total blood volume represents the hematocrit. Lower value of hematocrit represents anemia which may be due to the blood loss, leukemia and hyperthyroidism [27]. Hemoglobin is made up of haem group and globulin chain. Heavy metal toxicity interrupts hemoglobin synthesis pathway leading to decreased concentration of Hb in blood. It is observed that inorganic arsenic binds with the α -chain of hemoglobin and consequently accumulates in the erythrocytes [28]. The results of the present study are in agreement with the findings of similar work [29]. Similar hematological alterations were observed on administration of cadmium chloride (0.32mg/kg B.W.) for 15 days in albino mice but lycopene supplementation restored and maintained the normal function of haematopoietic system [30]. Rejigging in hemato-biochemical parameters were reported when cadmium chloride (5 mg/kg B.W.) was gavaged alone or in combination with vitamin E and β – carotene. Significant increase in activity of plasma ALT, AST and WBC, whereas significant reduction in RBC, PVC and tissue GST and ALP were observed. Increase in WBC might be due to the activation of immune system [31]. Leucocytes are considered soldiers of body originating from the myeloid cell that kill foreign particle by the phenomenon of phagocytosis. Major function of leucocytes is to provide immunity to body by producing and

distributing antibodies against antigens [32]. In our study the increase in the total WBC count was seen which reflects the fight against sodium arsenite caused contamination.

In the present study the significant increase in serum ALT, AST and WBC were observed. As it is well known that transaminases are found in low concentration in serum, their elevated concentration indicates hepatitis, hemolytic anemia and liver damage in animals as well as human beings [33-34]. Arsenic toxicity primarily targets the liver which leads to the leakage of transaminases into serum that casts hepatocellular destruction [35]. The activities of serum transaminases like ALT and AST were found to be increased in arsenite exposed rats; meanwhile, selenium supplementation protected the hepatocytes and prevented their leakage from liver [36]. In the present results of increased activities of ALT and AST were also supported by the studies conducted by above mentioned researchers. Tomatoes are packed of nutrients and antioxidants which protect the body from any kind of illness by detoxifying the body [37]. Tomatoes promote the function of immune system as well as enhance total erythrocyte count, total white blood cell count and HCT [38-39]. Amelioration of tomato paste against lead induced toxicity affecting hematological parameters like RBC, WBC and HCT is also reported. Decrease in the level of total serum protein, albumin and globulin were also observed in lead acetate treated rats. However tomato paste reversed the toxic effects exerted by lead acetate and maintained these values to normal range [40].

CONCLUSION: From the present study, it was observed that arsenic poisoning even at an acute dose of 10mg/kg B.W. can cause disturbances in hematological indices (RBC, Hb, HCT, MCV, MCH, MCHC, WBC and PLT). The supplementation of tomato extract 50mg/kg B.W. could reverse the ill effects of sodium arsenite by normalizing blood parameters which can be attributed to the antioxidants and vitamins present in the tomato extract.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation

but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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