Research Article

Protective Role of Tomato and Spinach on Toxic Effects Induced by Acute Arsenic Exposure on Serum Transaminases and Differential Leucocyte Count of Wistar Albino Rats

Suman Sharma, Saroj Rani*
Department of Zoology and Environmental Science, Punjabi University Patiala, Punjab, India.
***Corresponding author's E-mail:** saroj.rani1491@gmail.com

Received: 15-09-2022; **Revised:** 23-11-2022; **Accepted:** 30-11-2022; **Published on:** 15-12-2022.

ABSTRACT

The aim of the present study is to elucidate the toxicity induced by sodium arsenite on differential leucocyte count and serum transaminases and therapeutic role of tomato and spinach extract. Arsenic is a toxic element present worldwide and gaining attention due to its health hazards. Arsenic enters in living beings via ground water, anthropological activities, industrial products and use of herbicides and pesticides. From last few decades fruits and vegetables are being used to cure and prevent various body ailments. Antioxidants, vitamins and minerals present in them help to combat and reduce the oxidative stress generated by the free radical species produced as a result of various metabolic activities occurring in the body. In the present study rats were divided into 8 different groups with 5 rats in each. The following dose pattern was used in different groups of rats. Group I was kept as a control. Group II received 10mg/kg b. wt. of As, Group III were administered 50mg/kg b. wt. of TE, Group IV had 50mg/kg b. wt. of SE, Group V was given 10mg/kg b. wt. of As + 50mg/kg b. wt. of TE, Group VI received 10mg/kg b. wt. of As + 50mg/kg b. wt. of SE, Group VII had 10mg/kg b. wt. of As + 50mg/kg b. wt. of TE + 50mg/kg b. wt. of SE and Group VIII received 50mg/kg b. wt. of TE + 50mg/kg b. wt. of SE. A marked significant ($p<0.001$, $p<0.05$) increase in the activities of serum ALT and AST were observed in sodium arsenite treated rats showing hepatic dysfunction. However, supplementation of tomato extract and spinach extract showed significant reduction in their activities. Upon arsenic administration WBC count, eosinophil count and neutrophil count were elevated significantly ($p<0.05$, $p<0.05$, $p<0.001$ respectively) and lymphocyte count dropped significantly ($p<0.001$). Thus, based on the results obtained from our study and considering the modulatory properties of extracts of tomato and spinach it is logical to think that phytoconstituents present in them showed antioxidant activity which maintained antioxidant/proxidant balance disturbed by arsenic toxicity. No alterations were observed in monocytes and basophils count.

Keywords: Sodium arsenite, Tomato extract, Spinach extract, WBC and Serum transaminases.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2022.v77i02.025

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v77i02.025

INTRODUCTION

Increasing pollution is one of the major concerns of climatic issue which is enhancing day by day because of excessive use of hazardous chemicals, xenobiotics and heavy metals containing synthetic products (Jagadeesan et al[1]). Heavy metals enter the environment via industrial effluents, agricultural products such as pesticides and herbicides. Their presence in environment can cause harm to all living creatures on earth i.e. birds and mammals including human beings and aquatic organisms alike (Akinyeye & Okorie [2], Miquel[3], Picot & Proust[4]). Out of numerous substances present in the environment, arsenic is found naturally and ubiquitously and belongs to group V of the periodic table (Sackett[5]). Arsenic is found profusely in earth's crust as well as in minerals, soil and water (Antman[6]). Arsenic enters in human body through dermal contact, contaminated water and food or by inhaling polluted air with arsenic dust particles (Jomova et al[7], Qiu et al[8]). Chronic exposure to inorganic arsenic can cause major health issues such as skin lesions on feet and palm, skin blackening, enlargement of spleen, neuro and cardiological disorders, hypertension, cancers of various organs and skin (Centeno et al[9], Saha et al[10], Sharma et al[11]). In the last two decades people have shown keen interest in having fruits and vegetables loaded with antioxidants which are essential to prevent and manage arsenic toxicity (Wang et al[12], Flora et al[13]). Antioxidants help to mitigate and cure oxidative stress caused by reactive oxygen species generated by arsenic and have potential to damage biomolecules like proteins, nucleic acids and lipids (Shi et al[14]). Antioxidants are necessary to maintain the redox potential or neutralize the oxidised conditions which could occur in the body due to various metabolic activities (Velioglu et al[15]). These play important role to protect the body from mutagenesis, carcinogenesis and other harmful effects arising due to oxidative stress (Cook & Samman[16], Huang et al[17]). Spinach is a vegetable consumed widely either raw or boiled. Fresh leaves of spinach contain approximately 1000mg/kg of flavonoids (Weatherby & Cheng[18]). Spinach leaves also contain many active phytochemicals with several properties such as antiproliferative, antioxidative, anticancerous, anti-inflammatory and antiaging (Lomnitski et al[19]). The most important flavonoids the spinach leaves exhibit are patuletin and spinacetin (Zane & Wender [20]). Flavonoids

possess anti-allergic, anti-oxidative and anti-viral properties (Verma et al[21], Deschner et al[22], Middleton et al[23], Ishisaka et al[24]). Further flavonoids are used in the chelation therapy due to their structural confirmation which has potential to trap and nullify reactive oxygen species (Bors et al[25]). Tomatoes contain various natural antioxidants in the form of flavonoids, carotenoids, phenolic compounds, vitamins and glutathione which have the potential to scavenge the free oxygen radicals (Maisuthisakul et al[26], Sarada et al[27]).

METHODOLOGY

Female wistar albino rats weighing 135±5g were brought from animal house of LUVAS Hisar. Rats were subjected to acclimatization for 2 weeks before the commencement of experiment. Rats were segregated into 8 groups on the basis of their body weight. Each group consisted of 5 rats. Rats had free access to standard rat feed and water *ad libitum*. Their body weight was monitored regularly. Rats were kept in polypropylene cages bedded with dried rice husk throughout the entire experimental period. Each cage was tagged using tag card. The cages were washed and cleaned frequently and bedding was replaced often. Water bottles were cleaned daily.

Experimental chemicals, spinach and tomato: Sodium arsenite was obtained from Himedia Pvt. Ltd. Spinach (*Spinacea oleracea*) and tomatoes (*Solanum lycopersicum*) were obtained from the organic agriculturist and brought to laboratory for the preparation of extract.

Extract preparation of spinach and tomato: Tomato extract was prepared by using the method of salawu[28]. Tomato puree prepared was incubated for 45 min at 80°C. Solution was filtered and stored as a stock solution

Spinach extract was prepared according to the method of Islam et al[29]. Leaves were washed properly to remove soil particles under the tap water, dried in shade and a fine powder was made using a blender. Stock solution was prepared by soaking the powder in the distilled water overnight.

Ethical approval: Experiment was conducted after getting the permission from ethical committee with an approval number 107/GO/ReBi/S/99/CPCSEA/2017-23. Animals were maintained and handled according to the guidelines provided by the ethical committee.

Collection and analysis of haematological parameters: Blood was collected in anticoagulant coated tubes from retro-orbital plexus of anesthetized rats. Differential leucocyte count was analysed using automated haematological analyzer. Serum transaminases SGPT and SGOT were estimated using the commercially available kits.

Experimental design and treatment:

Group I (cont): Control rats (untreated).

Group II (As): Arsenic (as sodium arsenite 10mg/kg b. wt. acute dose).

Group III (TE): Tomato extract (50mg/kg b. wt. for 30 days).

Group IV (SE): Spinach extract (50mg/kg b. wt. for 30 days).

Group V (As+TE): Arsenic (as sodium arsenite 10mg/kg b. wt. acute dose) + Tomato extract (50mg/kg b. wt. for 30 days).

Group VI (As+SE): Arsenic (as sodium arsenite 10mg/kg b. wt. acute dose) + Spinach extract (50mg/kg b. wt. for 30 days).

Group VII (As+TE+SE): Arsenic (as sodium arsenite 10mg/kg b. wt. acute dose) + Tomato extract (50mg/kg b. wt. for 30 days) + Spinach extract (50mg/kg b. wt. for 30 days).

Group VIII (TE+SE): Tomato extract (50mg/kg b. wt. for 30 days) + Spinach extract (50mg/kg b. wt. for 30 days).

Sodium arsenite, tomato extract as well as spinach extract were administered orally to experimental rats.

Table 1: Body weight of rats.

Interval Days	Experimental Days							
	Group I (Control)	Group II (As)	Group III (TE)	Group IV (SE)	Group V (As+TE)	Group VI (As+SE)	Group VII (As+TE+SE)	Group VIII (TE+SE)
1	133.3	133	132.8	135	137.7	139.0	134.5	132.1
5	142.2	136.5	141.6	144.1	142.6	147.5	143.2	147.6
10	148.3	144.4	150.1	150.9	148.1	153.1	150.8	155.8
15	151.8	148.2	158.3	157.3	153	158.9	159.1	164
20	164.7	155.1	165.2	162.9	159.9	163.4	166.8	170.0
25	170.5	162.8	173	174.2	166.8	169	172.5	178.1
30	182.2	169.3	180.9	185.1	173.4	177.4	179.1	182

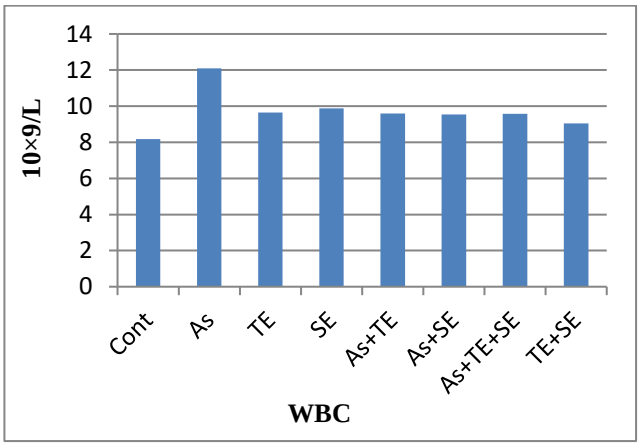

Figure 1: Total WBC count of rats.

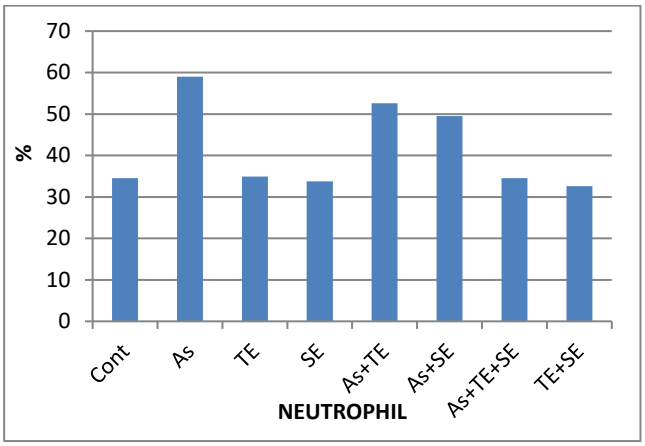

Figure 5: Neutrophil count of rats.

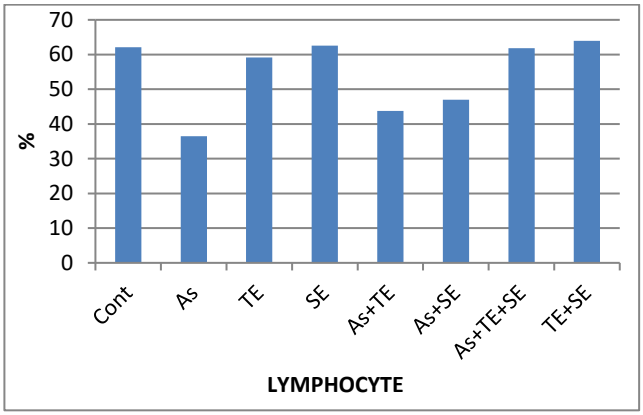

Figure 2: Lymphocyte count of rats.

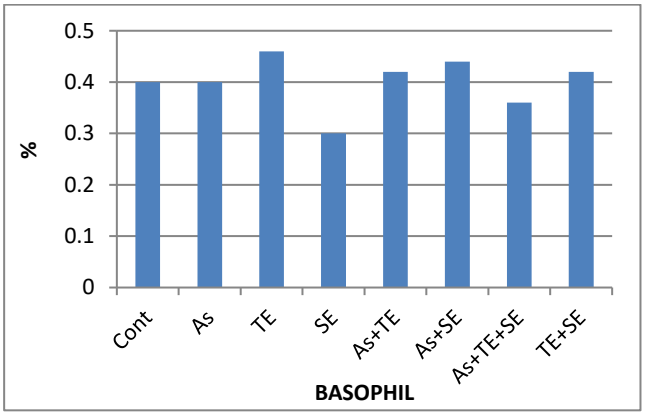

Figure 6: Basophil count of rats.

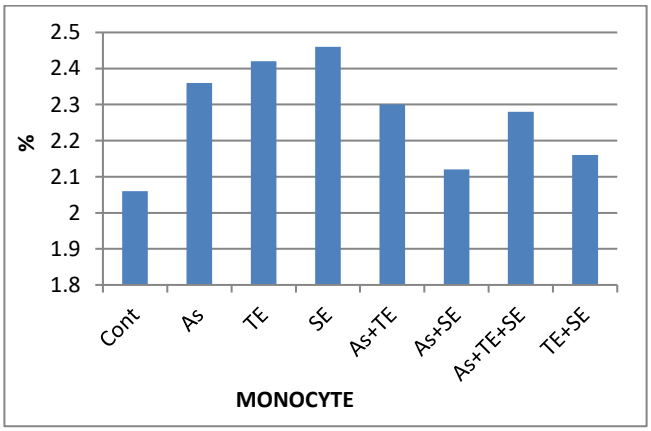

Figure 3: Monocyte count of rats.

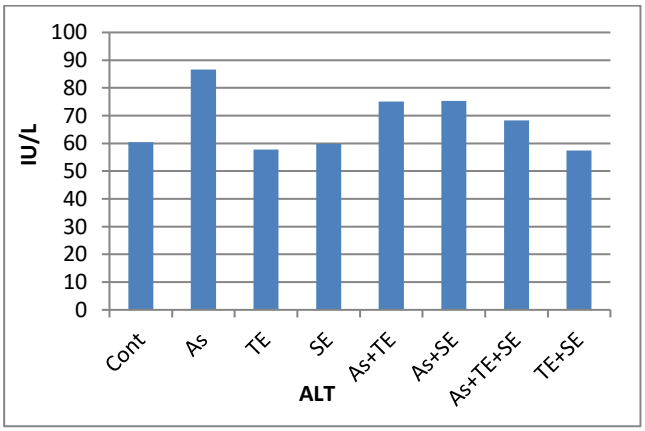

Figure 7: Serum ALT of rats.

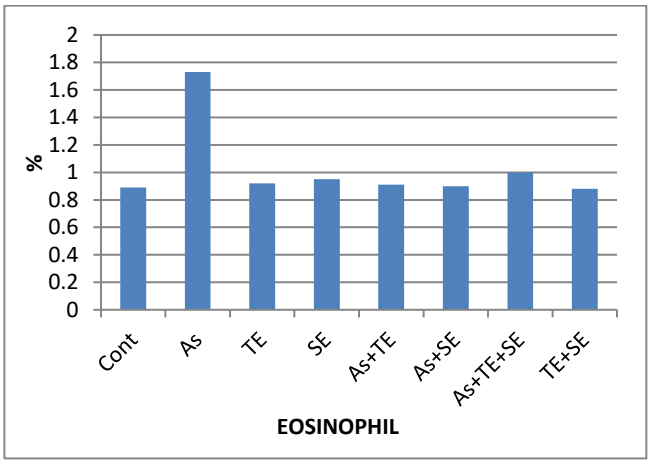

Figure 4: Eosinophil count of rats.

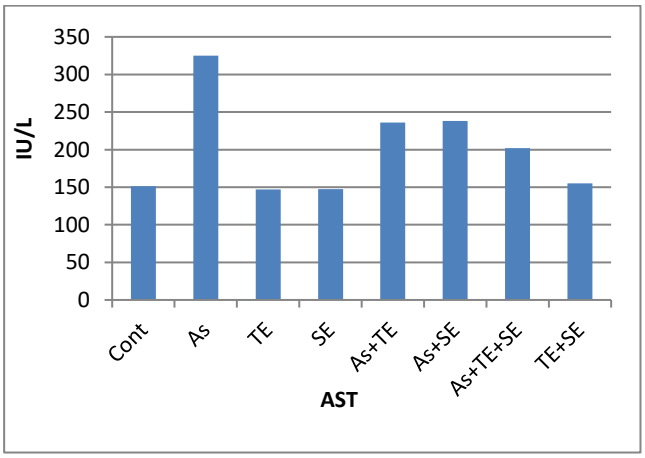

Figure 8: Serum AST of rats.

Table 2: Represents the correlation coefficient among differential WBC count of rats.

Parameters	WBC ($10^9 \times L$)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Neutrophils (%)	Basophils (%)
WBC ($10^9 \times L$)	1					
Lymphocytes (%)	-0.111	1				
Monocytes (%)	0.044	0.005	1			
Eosinophils (%)	0.228	-0.486**	0.110	1		
Neutrophils (%)	0.122	-0.988*	-0.070	0.460**	1	
Basophils (%)	-0.150	-0.063	0.077	-0.143	0.078	1

Table 3: Differential leucocyte count and serum transaminases (ALT and AST) of rats.

Parameters	Group 1 (CONT.) (n = 5)	Group 2 (As) (n = 5)	Group 3 (TE) (n = 5)	Group 4 (SE) (n = 5)	Group 5 (As+TE) (n = 5)	Group 6 (As+SE) (n = 5)	Group 7 (As+TE+SE) (n = 5)	Group 8 (TE+SE) (n = 5)
Total WBC ($10^9 \times L$)	8.180±1.443	12.10±0.374[a]	9.640±0.719[b]	9.88±0.409[b]	9.60±0.86[b]	9.54±0.88[b]	9.58±0.67[b]	9.04±1.05[b]
Lymphocytes (%)	62.09±2.10	36.50±2.79[a]	59.14±4.03[b]	62.55±1.87[b]	43.77±2.73[a]	47.03±4.88[a]	61.83±2.66[b]	63.93±2.65[b]
Monocytes (%)	2.06±0.15	2.36±0.30	2.42±0.27	2.46±0.28	2.30±0.23	2.12±0.30	2.28±0.42	2.16±0.29
Eosinophils (%)	0.89±0.06	1.73±0.27[a]	0.92±0.06[b]	0.95±0.08[b]	0.91±0.09[b]	0.90±0.06[b]	1.00±0.25	0.88±0.12[b]
Neutrophils (%)	34.55±1.97	59.00±2.71[a]	34.90±2.48[b]	33.73±1.95[b]	52.60±3.01[a]	49.50±4.75[a]	34.51±2.35[b]	32.59±2.77[b]
Basophils (%)	0.40±0.13	0.40±0.09	0.46±0.12	0.30±0.08	0.42±0.12	0.44±0.12	0.36±0.12	0.42±0.07
ALT (IU/L)	60.49±2.15	86.67±3.86[a]	57.78±4.73[b]	59.90±3.49b	75.13±1.93[ab]	75.33±2.28[ab]	68.23±2.83[b]	57.48±2.44[b]
AST (IU/L)	151.10±10.11	324.78±14.57[a]	146.95±11.23[b]	147.50±14.57[b]	236.24±32.69[ab]	238.14±32.76[ab]	202.14±17.01[ab]	155.45±15.32[b]

Data is presented as Mean±SEM. As = Sodium arsenite, TE = Tomato extract, SE = Spinach extract, WBC = White blood cell, ALT = Alanine aminotransaminase, AST = Aspartate aminotransaminase.

[a]p<0.05,0.01,0.001 significant changes in comparison to control group.

[b]p<0.05,0.01,0.001 significant changes among sodium arsenite treated group.

RESULTS AND DISCUSSION

The present study was conducted to evaluate the antioxidant efficacy of phytochemicals present in extract of tomato and spinach on differential leucocyte count and serum transaminases in sodium arsenite exposed rats. Due to paucity of literature on spinach and tomato extract we tried to explore the ameliorative potential of tomato and spinach on blood parameters as well as transaminases.

Exploding industrialization is a vital cause to create and spread pollutants which have became a threat to survival of animals and human beings (Akinboro et al[30]). Humans are exposed to the arsenic through well water, industrial effluents, different mining projects and agrochemicals (Klibet et al[31]). All rats seemed to be healthy with good morphology. The rats did not show any symptoms e.g. secretion from different parts such as nose, eyes, ears, genitalia and anal opening. The rats had normal diet and were not lethargic. Table 1 showed the body weight of rats. All rats in each group showed normal growth and weight gain but the gain was less pronounced in only sodium arsenite treated group as compared to control group.

Table 2 and fig1 depicts the total leucocyte count of control, only sodium arsenite, sodium arsenite + tomato, sodium arsenite + spinach, only tomato extract, spinach and tomato + spinach administered rats. Moreover, a significant ($p<0.05$) increase in total leucocyte count of Group II (As) was recorded as compared to Group I (Control); conversely, the changes in other treated groups demonstrated non significant differences with respect to control group. Upon administration of tomato extract along with spinach extract alone or in combination with sodium arsenite reduced the leucocyte count towards normal range. Group III (TE), V (As+TE), VI (As+SE), VII (As+TE+SE), VIII (TE+SE) ($P<0.05$) and Group IV ($p<0.01$) showed significant decrease in TLC in comparison to only sodium arsenite treated group. In the present work (Table 2) WBC count was positively correlated with monocytes (0.044), eosinophil (0.228) and neutrophil (0.122). However, negatively correlated with lymphocytes (-0.111) and basophil (-0.150). In this research we observed an

increase in total leucocyte count (TLC) upon sodium arsenite intoxication and the results are similar to the findings of Arhkuli et al[32], Amer et al[33], Witeska[34]. Leucocytes strengthen the immunological function of organisms and increase in WBC specified the immune responses to combat stress (Kotsanis et al[35]). A study conducted by suradkar *et al.* demonstrated that rats administered with lead acetate orally at different concentrations showed leucopenia as well as lymphopenia which might be due to the degradation of cells by toxic material or decreased production of these cells by lymphoid organs (Kumar et al[36]). Similarly rats treated with polluted water showed an increase in WBC count and neutrophils which have major phagocytic and biochemical function (Al-Terehi et al[37]). Opium treated rats also showed rise in the total leucocyte count. Various conditions such as alteration in the differentiation of WBC in bone marrow, infectious and inflammatory responses and changes in the endothelial cell's adhesion molecules lead to fluctuation in WBC count in blood (Asadikaram et al[38]). Administration of sodium arsenite generated significant increase in WBCs while pretreatment with *A. Conyzoides* restored the number of WBCs in animals. This increase was because of the action against the entry of foreign particles (Adebayo et al[39]). The increase in the WBC may be due to the release of the leucocytes from the immune organs into the blood upon triggering of the immune system by any foreign substances (Ola-Davies & Akinrinde [40]).

Table 2 fig 2 revealed the lymphocyte count in various groups of rats in the present study. Moreover, significant ($p<0.001$) decrease in lymphocyte count of (Group II and V) and Group VI ($p<0.05$) was noticed in comparison to control group. Whereas, a non significant change was observed in all other treated groups. After administration of only tomato extract or spinach extract and co-administration of both alleviated lymphocyte count significantly ($p<0.001$) in Group IV, VII and Group VIII, ($p<0.01$) and group III but non significant results were observed in groups V and VI ($p>0.05$). Lymphocyte count showed (Table 2) negative correlation with eosinophil (-0.486) and neutrophil (-0.988). A significant decline in lymphocyte count indicated the adverse effect on lymphopoiesis by arsenic administration.

Table 2 fig 3 represents the monocyte count. No alteration was observed in monocytes of control and all the treated groups. In this study monocyte count (Table 2) was positively correlated with eosinophils (0.110) and basophils (0.077) whereas, negatively correlated with neutrophils (-0.070).

Table 2 fig 4 show eosinophil count of sodium arsenite intoxicated rats increased significantly ($p<0.05$) in reference to control group. But non-significant changes were observed in all treated groups with respect to control group. A significant ($p<0.05$) reduction in eosinophil count of rats of groups III, IV, V, VI and group VIII was observed after supplementation of tomato extract and spinach extract. In Group VII eosinophil count dropped non-significantly ($p>0.05$) as compared to group II. Eosinophils showed (Table 2) positive correlation with neutrophils (0.460) and were negatively correlated with basophils (-0.143).

Table 2 fig 5 depict neutrophil count of control and other groups of rats. Neutrophil count of Group II ($p<0.001$) and Group VI ($P<0.05$) increased significantly as compared to control group. On the contrary, other treated groups showed non-significant ($p>0.05$) elevation. Rats administered the tomato extract and spinach extract showed significant ($p<0.001$) decrease in neutrophil count and almost matched the control values as compared to arsenic group. However, Group VI and Group VII ($P>0.05$) remained non significant. Neutrophils (Table 2) were positively correlated with basophils (0.078). In the present work, arsenic treatment increased the neutrophil count of rats. These results are supported by work of various workers (Fiati et al[41], Jalaludeen et al[42]).

Table 2 fig 6 show the basophil count in control and other experimental groups of rats. A non-significant ($p>0.05$) dissimilarities were witnessed in all treated group in comparison to Group I and Group II.

Evaluating serum and tissue enzymes helps in assessing hepatic health by pointing hepatocellular toxicity as well as related diseases (Awe et al[43]). AST and ALT are well known hepatic marker enzymes and leak into circulation during cellular destruction. Liver biotransforms arsenic to reduce its toxic properties (Lin et al[44]). It binds with the thiol group of enzymes and proteins present in the hepatic cells, thereby, disfunctioning the hepatocytic plasma membrane leading to increased AST and ALT activity (Goyer & Clarkson[45]).

Table 2 fig 7 depict significant ($p<0.001$, $p<0.01$) increase in ALT activity as compared to control group. When rats received antioxidant rich extracts activity of ALT was found almost near to control values showing statistically significant changes.

Table 2 fig 8 depict AST activity in serum of rats. A significant ($p<0.001$) elevation in activity of serum AST was observed in group II and ($p<0.05$) in groups V, VI and VII in comparison to group I. whereas, other groups showed non-significant ($p>0.05$) differences in AST activity. Supplementation of tomato and spinach extracts restore the AST activity significantly ($p<0.001$) in groups III, IV, VII and VIII but ($p<0.05$) in groups V and VI when compared to sodium arsenite exposed group. Thus, based on the results obtained from our study and considering the modulatory properties of spinach and tomato extracts it is logical to think that phytoconstituents present in spinach and tomato executed antioxidant activity which maintained antioxidant/proxidant balance disturbed by arsenic toxicity. The results of our study are consistent with the previous findings (Yasmin et al[46], Mehta & Hundal[47], Goudarzi et al[48]).

CONCLUSION

It can be concluded from this study that acute arsenic exposure can cause alterations in various parameters like differential leucocyte count and serum transaminases (ALT and AST). Biomethylation of the arsenicals is carried out in liver which in turn causes damage to hepatocytes leading to leakage of ALT and AST into the circulation. Arsenic exposure activates the immune responses which may elevate leucocyte count in blood. However, active phytochemicals present in extracts of tomato and spinach have antioxidant potential to prevent the arsenic induced toxicity.

Conflict of Interest: Authors declared no conflict of interest.

Acknowledgement: Authors are grateful to UGC for financial assistance and head of the department for providing laboratory facilities.

Abbreviations: DLC – Differential leucocyte count, WBC – White Blood Cell, TE – Tomato Extract, SE – Spinach Extract, As – Sodium Arsenite, ALT – Alanine Aminotransferases, AST – Aspartate Aminotransferases, b. wt. – body weight

REFERENCES

1. Jagadeesan G, Pillai SS. Hepatoprotective effects of taurine against mercury induced toxicity in rat. Journal of Environmental Biology. 2007 Oct 1;28(4):753. PMID: 18405108.
2. Akinyeye AJ, Okorie TG. Heavy metal studies of industrial effluent on Alaro stream sediment. Int Res J Biol Sci. 2012;1(6):1-9.
3. Miquel G., Les Effets des Métaux Lourds surl'Environnement et la Santé, Rapport d'Office Parlementaire d'évaluation des choix scientifiques et technologiques, Sénat (261), Assemblée Nationale (1979), Paris, France, 360p (2001).
4. Picot A, Proust N. Mercury and its compounds: from speciation to toxicity; Le mercure et ses composes. De la speciation a la toxicite. Actualite Chimique. 1998 Apr 1.
5. Sackett PD. Elemental cycles in the Anthropocene: Mining aboveground. Geological Society of America Special Papers. 2016 May 1;520:99-116. Doi: https://doi.org/10.1130/2016.2520(11).
6. Antman KH. Introduction: the history of arsenic trioxide in cancer therapy. The oncologist. 2001 Apr;6(S2):1-2. DOI: 10.1634/theoncologist.6-suppl_2-1; PMID: 11331433.
7. Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, Rhodes CJ, Valko M. Arsenic: toxicity, oxidative stress and human disease. Journal of Applied Toxicology. 2011 Mar;31(2):95-107. DOI: 10.1002/jat.1649; PMID: 21321970.
8. Qiu LQ, Abey S, Harris S, Shah R, Gerrish KE, Blackshear PJ. Global analysis of posttranscriptional gene expression in response to sodium arsenite. Environmental health perspectives. 2015 Apr;123(4):324-30. DOI: 10.1289/ehp.1408626; PMID: 25493608.
9. Centeno JA, Mullick FG, Martinez L, Page NP, Gibb H, Longfellow D, Thompson C, Ladich ER. Pathology related to chronic arsenic exposure. Environmental health perspectives. 2002 Oct;110(suppl 5):883-6. DOI: 10.1289/ehp.02110s5883; PMID: 12426152.
10. Saha JC, Dikshit AK, Bandyopadhyay M, Saha KC. A review of arsenic poisoning and its effects on human health. Critical reviews in environmental science and technology. 1999 Jul 1;29(3):281-313. DOI: https://doi.org/10.1080/10643389991259227.
11. Sharma AK, Tjell JC, Sloth JJ, Holm PE. Review of arsenic contamination, exposure through water and food and low cost mitigation options for rural areas. Applied Geochemistry. 2014 Feb 1;41:11-33. DOI: 10.1016/j.apgeochem.2013.11.012.
12. Wang TS, Kuo CF, Jan KY, Huang H. Arsenite induces apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species. Journal of cellular physiology. 1996 Nov;169(2):25668. DOI: https://doi.org/10.1002/(SICI)10974652(199611)169:2<256::AID-JCP5>3.0.CO;2-N.
13. Flora SJ, Flora G, Saxena G, Mishra M. Arsenic and lead induced free radical generation and their reversibility following chelation. Cellular and molecular biology. 2007 Apr 15;53(1):26-47. PMID: 17519110.
14. Shi HL, Noguchi N, Niki E. Introducing natural antioxidants. In J. Pokorny et al. Antioxidants in food: practical applications. 2001; Woodhead Publishing Ltd.
15. Velioglu Y, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of agricultural and food chemistry. 1998 Oct 19;46(10):4113-7. DOI: http://dx.doi.org/10.1021/jf9801973.
16. Cook NC, Samman S. Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. The Journal of nutritional biochemistry. 1996 Feb 1;7(2):66-76. DOI: https://doi.org/10.1016/S0955-2863(95)00168-9.
17. Huang MT, Ho CT, Lee CY. Phenolic compounds in food and their effects on health II. Washington, DC: American Chemical Society; 1992. DOI: **https://doi.org/10.1002/food.19930370225**.
18. Weatherby LS, Cheng AL. The determination of flavones or quercetin-like substances in certain naturally occurring products. Journal of Biological Chemistry. 1943;148:707-9. DOI: https://doi.org/10.1016/S0021-9258(18)72272-2.
19. Lomnitski L, Bergman M, Nyska A, Ben-Shaul V, Grossman S. Composition, efficacy, and safety of spinach extracts. Nutrition and cancer. 2003 Jul 1;46(2):222-31. DOI: 10.1207/S15327914NC4602_16. PMID: 14690799.
20. Zane A and Wender SH: Flavanols in spinach leaves. *J Org Chem.* 1961; 26**,** 4718. DOI: https://doi.org/10.1021/jo01069a531.
21. Verma AK, Johnson JA, Gould MN, Tanner MA. Inhibition of 7, 12-dimethylbenz (a) anthracene-and N-nitrosomethylurea-induced rat mammary cancer by dietary flavonol quercetin. Cancer research. 1988 Oct 15;48(20):5754-8. PMID: 3139283.
22. Deschner EE, Ruperto J, Wong G, Newmark HL. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. Carcinogenesis. 1991 Jul 1;12(7):1193-6. DOI: 10.1093/carcin/12.7.1193; PMID: 2070483.
23. Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological reviews. 2000 Dec 1;52(4):673-751. PMID: 11121513.
24. Ishisaka A, Ichikawa S, Sakakibara H, Piskula MK, Nakamura T, Kato Y, Ito M, Miyamoto KI, Tsuji A, Kawai Y, Terao J. Accumulation of orally administered quercetin in brain tissue and its antioxidative effects in rats. Free Radical Biology and Medicine. 2011 Oct 1;51(7):1329-36. DOI: 10.1016/j.freeradbiomed.2011.06.017; PMID: 21741473.
25. Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. In Methods in enzymology 1990 Jan 1 (Vol. 186, pp. 343-355). Academic Press. DOI: 10.1016/0076-6879(90)86128-i; PMID: 2172711.
26. Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food chemistry. 2007 Jan 1;100(4):1409-18. DOI:10.1016/j.foodchem.2005.11.032.
27. Sarada SK, Dipti P, Anju B, Pauline T, Kain AK, Sairam M, Sharma SK, Ilavazhagan G, Kumar D, Selvamurthy W. Antioxidant effect of beta-carotene on hypoxia induced oxidative stress in male albino rats. Journal of ethnopharmacology. 2002 Feb 1;79(2):149-53. DOI: 10.1016/s0378-8741(01)00360-9; PMID: 11801375.

28. Salawu EO, Adeeyo OA, Falokun OP, Yusuf UA, Oyerinde A, Adeleke AA. Tomato (Lycopersicon esculentum) prevents lead-induced testicular toxicity. Journal of human reproductive sciences. 2009 Jan 1;2(1):30. DOI: 10.4103/0974-1208.51346; PMID: 19562072.

29. Islam MZ, Awal MA, Mostofa M, Ghosh A, Khair A. Effect of spinach against arsenic toxicity in rats. Bangladesh Journal of Veterinary Medicine. 2009;7(2):358-63. DOI: https://doi.org/10.3329/bjvm.v7i2.6005.

30. Akinboro A, Adedosu OT, Badmus JA. Evaluation of redox status and organ protective effects of Aspalathus linearis extract in Arsenic exposed Rats. DOI:10.1016/j.phyplu.2021.100171.

31. Klibet F, Boumendjel A, Khiari M, El Feki A, Abdennour C, Messarah M. Oxidative stress-related liver dysfunction by sodium arsenite: Alleviation by Pistacia lentiscus oil. Pharmaceutical Biology. 2016 Feb 1;54(2):354-63. DOI: 10.3109/13880209.2015.1043562; PMID: 25946016.

32. Arhkuli KC, Malarvizhi, A, Kumaran SS, Ramesh M. Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp, Catla catla. *Food and Chemical Toxicology*. 2010; 48(10):2848-2854. DOI: 10.1016/j.fct.2010.07.017; PMID: 20654677.

33. Amer SA, AL-Harbi MS, AL-Zahrani YA. Protective role of some antioxidants on arsenic toxicity in male mice: physiological and histopathological perspectives. Biol. Med. 2016;8(1):18-24. DOI:10.4172/0974-8369.1000266.

34. Witeska M. The effect of toxic chemicals on blood cell morphology in fish. Fresenius Environmental Bulletin. 2004;13(12):1379-84.

35. Kotsanis N, Iliopoulou-Georgudaki J, Kapata-Zoumbos K. Changes in selected haematological parameters at early stages of the rainbow trout, Oncorhynchus mykiss, subjected to metal toxicants: arsenic, cadmium and mercury. Journal of Applied Ichthyology. 2000 Dec;16(6):276-8. DOI: **https://doi.org/10.1046/j.1439-0426.2000.00163.x**.

36. Kumar A, Chauhan RS, Singh NP. Immunopathological effect of lead on cell mediated immunity in chickens. Indian Journal of Veterinary Pathology. 1998;22:22-5.

37. Al-Terehi MN, Al-Ganimi YK, Al-Ameri QM, Al-Saadi AH, Zaidan HK, Ewadh MJ. Effect of Pollutant Water on Some Organs and Blood Parameters in Rats. Research in Pharmacy. 2015 Oct 29;2(5):35-39.

38. Asadikaram G, Sirati-Sabet M, Asiabanha M, Shahrokhi N, Jafarzadeh A, Khaksari M. Hematological changes in opium addicted diabetic rats. International journal of high risk behaviors & addiction. 2013;1(4):141-8. **DOI**: 10.5812/ijhrba.8777; PMID: 24971253.

39. Adebayo AH, Zeng GZ, Fan JT, Ji CJ, He WJ, Xu JJ, Zhang YM, Akindahunsi AA, Kela R, Tan NH. Biochemical, haematological and histopathological studies of extract of Ageratum conyzoides L. in Sprague Dawley rats. Journal of medicinal plants Research. 2010 Nov 4;4(21):2264-72. DOI:10.5897/JMPR10.470.

40. Ola-Davies OE, Akinrinde AS. Acute sodium Arsenite-induced hematological and biochemical changes in wistar rats: Protective effects of ethanol extract of Ageratum conyzoides. Pharmacognosy research. 2016 Mar;8(Suppl 1):S26. DOI: 10.4103/0974-8490.178645; PMID: 27114688.

41. Fiati KSS, Su H, Li Z, Kong L, Wang Y, Song X, Gu Y, Barber T, Aldinger J, Hua Q, Li Z, Ding M, Zhao J, Lin X. The systemic toxicity of heavy metal mixtures in rats. *Toxicology Research*. 2018;7(3):396-407. DOI: 10.1039/c7tx00260b; PMID: 30090589.

42. Jalaludeen AM, Ha WT, Lee R, Kim JH, Do JT, Park C, Heo YT, Lee WY, Song H. Biochanin A ameliorates arsenic-induced hepato-and hematotoxicity in rats. Molecules. 2016 Jan 9;21(1):69. DOI: 10.3390/molecules21010069; PMID: 26760991.

43. Awe EO, Banjoko SO. Biochemical and haematological assessment of toxic effects of the leaf ethanol extract of Petroselinum crispum (Mill) Nyman ex AW Hill (Parsley) in rats. BMC Complementary and Alternative Medicine. 2013 Dec;13(1):1-6. DOI: 10.1186/1472-6882-13-75; PMID: 23557241.

44. Lin SC, Chung TC, Lin CC, Ueng TH, Lin YH, Lin SY, Wang LY. Hepatoprotective effects of Arctium lappa on carbon tetrachloride-and acetaminophen-induced liver damage. The American journal of Chinese medicine. 2000;28(02):163-73. DOI: 10.1142/S0192415X00000210; PMID: 10999435

45. Goyer RA, Clarkson TW. Toxic effects of metals. In: Klaassen CD, editor. Casarett and Doull's Toxicology: The Basic Science of Poisons, 7th ed. USA: McGraw Hill, 2008.

46. Yasmin S, Das J, Stuti M, Rani M, D'Souza D. Sub chronic toxicity of arsenic trioxide on Swiss Albino mice. International journal of environmental sciences. 2011 Jan 1;1(7):1640.

47. Mehta M, Hundal SS. Induction of oxidative stress by sub-acute oral exposure of sodium arsenite in female rats. Indian J Appl Res. 2013;3(12):560-2.

48. Goudarzi M, Fatemi I, Siahpoosh A, Sezavar SH, Mansouri E, Mehrzadi S. Protective effect of ellagic acid against sodium arsenite-induced cardio-and hematotoxicity in rats. Cardiovascular toxicology. 2018 Aug;18(4):337-45. DOI: 10.1007/s12012-018-9446-2; PMID: 29383632.

Source of Support: The author(s) received financial support in the form of NFSC fellowship.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.