ORIGINAL ARTICLE

Hepato-Protective Effect of Vit. E on Arsenic Induced Microscopic Hepatic Changes among Albino Rats: Cross Sectional Study

AMRAT IJAZ[1], IMTIAZ ASLAM[2], AMMARA RASHEED[3], SUMMAYAH NIAZI[4], RAAFEA TAFWEEZ KURAISHI[5], TALHA LAIQUE[6]

[1]*Department of Anatomy, Avicenna Medical and Dental college, Lahore-Pakistan*
[2]*Department of Anatomy, Fatima Jinnah Medical University, Lahore-Pakistan*
[3]*Department of Anatomy, Nishtar Medical University, Multan-Pakistan*
[4]*Department of Physiology, Quetta Institute of Medical Sciences, Quetta-Pakistan*
[5]*Department of Anatomy, King Edward Medical University, Lahore-Pakistan*
[6]*Department of Pharmacology, Allama Iqbal Medical College, Lahore-Pakistan*
Correspondence to Dr. Talha Laique, Email: talhalaique51@gmail.com Tel:+92-331-0346682

ABSTRACT

Background: Arsenic contamination among heavy metals in ground water is a health hazard due to its toxicological concern, affecting millions of humans globally.
Aim: To observe hepato-protective effect of vitamin-E on sodium arsenite induced microscopic hepatic changes among albino rats.
Study Design: Cross Sectional study.
Methodology: 90 adult albino male rats (30 in each group), weighing 140 to 180 grams were taken. They were kept under controlled temperature of 23+2 and humidity 50+5% in the experimental research lab of postgraduate medical institute Lahore. The night and day cycle was maintained for 12 hours each. Each animal of every group was labeled from 1-30 with different color for every group. Standard diet and tap water and libitum was given
Results: The initial and final body weights of animals in all the three study groups were shown in table-1 as mean ± SD. There was significant difference among three groups as regard to fatty degeneration of hepatocytes with p <0.001. Significant difference was noted among each group as regard to pyknosis. The difference among groups for number of inflammatory cells was significant with p-value <0.001.
Conclusion: Vitamin-E can be employed as anti-oxidant agent for treatment against arsenic induced hepatoxicity. It improves microscopic changes in lever hence its can be used as hepato-protective agent.
Keywords: Vitamin-E, Hepato-protection, Relative Tissue Weight Index and Albino Rats.

INTRODUCTION

Arsenic contamination among heavy metals in ground water is a health hazard due to its toxicological concern, affecting millions of humans globally[1]. Arsenic is a ubiquitous naturally occurring metalloid formerly known as 'King of poisons'[2]. It was commonly used in political assassination due to its tasteless and odorless characteristic in water[3].

Sources of arsenic intoxication for general population include drinking water, agricultural products such as food crops and seafood like prawns and fishes[2,3]. It is commonly employed in many industrial processes like purification of industrial gases, electronics manufacturing products and clarifying glass and ceramics. It is an important ingredient of air nowadays due to processes like fossil fuels as well as wood burning treated with arsenic preservatives[4].

In addition to ground water, it is found in cigarettes, foods and in air[5]. As its compounds are used extensively as components of herbicides, insecticides, rodenticides, food preservatives, and drugs[4,6]. According to an estimate, 50 to 60 million people in Pakistan are using water that contains more than 50 µg/L of arsenic, the values being five times more than WHO's safety limit[7].

Oxidative stress is the main mechanisms of its toxicity. Oxidative stress damages DNA which causes disturbances in cellular biology[8]. Among humans it is mainly metabolized by liver. Ingested inorganic arsenic is mostly absorbed in the gastrointestinal tract. After absorption, its main storage sites include hepatic, renal, cardiac, and pulmonary tissues[9]. In humans acute arsenic poisoning causes vomiting, abdominal pain, and watery stools[10].

With this background, any agent having powerful antioxidant property can prove beneficial in chelating arsenic and excretion of heavy metals[11]. Vitamin E, most potent antioxidants, occurs in edible vegetable oils such as corn, peanut, olive and soybean etc[12,13]. It is now accepted as a major free radical scavenging antioxidant in humans and protects biological molecules from detrimental oxidative modifications[14].

In Pakistan poor nutrition and cooking practices have contributed to its deficiency. One study by Casal et al have demonstrated that foods when subjected to high temperatures would lose a significant amount of vitamin E[15]. Due to increasing arsenic poisoning among humans, we carried-out present study to find vitamin E as protective agent against arsenic poisoning.

The objective of the study was to observe hepato-protective effect of vitamin-E on sodium arsenite induced microscopic hepatic changes among albino rats.

METHODOLOGY

Total 90 adult albino male rats (30 in each group), weighing 140 to 180 grams were taken. They were kept under controlled temperature of 23+2 and humidity 50+5% in the

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experimental research lab of postgraduate medical institute Lahore. The night and day cycle was maintained for 12 hours each. The cages were labeled according to the group and number of animals. Each animal of every group was labeled from 1-30 with different color for every group. Standard diet and tap water ad libitum was given to animals. Before starting the experiment animals were acclimatized for 10 days.

Grouping of animals: Animals were divided into 3 groups:

Group A: Control group: were given only distilled water according to weight of the animal by oral gavage method for 30 days.

Group B: Arsenic group: were given sodium arsenite 0.5 mg/100gm/day dissolved in distilled water by oral gavage method for 30 days.

Group C: Arsenic +Vitamin E**:** Sodium arsenite was given as 0.5 mg/100gm/day and Vitamin E were given to each animal as 5 mg/100gm/day by oral gavage method for 30 days.

Statistical analysis: Data analyzed by SPSS 22.0v. For initial and final weight of rats as well as number of inflammatory cells were described as mean +/- SD. Tukey's test was used for post hoc analysis. Parameters like fatty changes in hepatocytes and pyknosis were described in frequency and percentages. Comparison among groups was performed by using Chi-square test. P-value ≤ 0.05 was considered significant.

RESULTS

The initial and final body weights of animals in all the three study groups were shown in table-1 as mean ± SD.

Microscopic changes in hepatocytes like fatty degeneration was analyzed and presented as frequency and percentages in table-2. There was significant difference among three groups as regard to fatty degeneration of hepatocytes with p <0.001 as shown in table-3. Microscopic analysis of changes were made during present study. Parameter like pyknosis of hepatocytes was presented as frequency and percentages in table-4. Significant difference was noted among each group as regard to pyknosis with p-value <0.001 as shown in table-5. Number of Inflammatory cells in all the three study groups were presented as mean ± SD. The difference among groups this parameter was significant with p-value <0.001 shown in table-6.

Table-1: The initial and final body weight of animals in three study groups

	Initial Weight			Final Weight		
Groups	N	Mean	SD	N	Mean	SD
Group A (Control group)	30	153.03	8.01	30	201.43	9.90
Group B (Arsenic group)	30	154.60	8.27	30	179.57	8.50
Group C (Arsenic+ Vit E)	30	155.47	11.82	30	199.17	14.09

Table-2: Status and comparison of Fatty change among 3 groups

	Control group (A)	Arsenic group (B)	Arsenic + Vit E (C)	Total
Absent	30 (100%)	0 (0%)	17 (57%)	47(52.2%)
Mild	0 (0%)	0 (0%)	13(43.3%)	13(14.4%)
Moderate	0 (0%)	6 (20%)	0 (0%)	6 (6.7%)
Marked	0 (0%)	24 (80%)	0 (0%)	24(26.7%)

P value <.001* *Statistically Significant

Table-3: Group wise comparison for fatty change among 3 groups

Group	(J) Group	Chi-square	Df	P-value
Group A	Group B	60.000	2	<0.001*
Group A	Group C	16.596	1	<0.001*
Group B	Group C	60.000	2	<0.001*

*Statistically Significant

Table-4: Status and comparison of Pyknosis among three groups

	Control group (A)	Arsenic group (B)	Arsenic+ Vit E(C)	Total
Absent	30(100%)	0 (0%)	30(100%)	60(67%)
Present	0 (0%)	30 (100%)	0 (0%)	30(33%)
Total	30(100%)	30 (100%)	30(100%)	90(100%)

P value <0.001* *Statistically Significant

Table-5: Group wise comparison for pyknosis among three groups

Group	(J) Group	Chi-square	Df	P-value
Group A	Group B	60.000	1	<0.0001*
Group A	Group C	-	-	N.A
Group B	Group C	60.000	1	<0.0001*

*Statistically Significant

Table-6: Number of Inflammatory cells in three study groups

	Mean±SD	Between Groups		P-value
		Mean Square	F	
Control group	0.00±0.00	3991.811	547.628	<0.001*
Arsenic group	21.70±3.96			
Arsenic+ Vit E	4.07±2.49			

*Statistically Significant

DISCUSSION

Polluted water with toxic elements is a great threat to the living species in the whole world and among toxic elements arsenic is gaining more attention because of its deleterious effects on health of humans[4,5]. It has been proved through various studies that prolonged exposure to AS and its compounds leads to many skin diseases, cardiovascular disorders, reproductive problems, neurovascular, gastrointestinal and renal dysfunction[16,10].

One of the parameters of the present study was the weight of the animals. Weight gain may be taken as an indicator of general well being of animal. In the present study, there was weight gain in all animals but the final weight at the end of experiment was found significantly different among three groups. There was more increase in weight of animals of group A (control) and C (Arsenic + vitamin E) as compared to group B (Arsenic only). Although there was weight gain in animals of arsenic treated group as well, but the extent of weight gain was less in comparison to control. This finding was comparable to the study of Sayed S et al. who studied on mice to find the Protective effects of phyllanthus emblica leaf extract (PLE) on sodium arsenite-mediated adverse effects in the duration of 4 weeks. In that study sodium arsenite treated group animals gained less weight as compared to other groups (control and PLE treated group).[14] This study supports findings of present study that animals treated with sodium arsenite gained less weight.

On the basis of histopathology, fatty degeneration of hepatocytes, was evaluated. Although it is apoptosis. In the present study fatty change was present in 100% animals of group B while absolutely absent in control group. Similar findings were observed by Saima Qureshi et al when rats

were treated with SA experimental group showed excessive fatty degeneration of hepatocyes[17].

Ballooned cells have (small) pyknotic nuclei or nuclei that are undergoing karyorrhexis, i.e. in the process of disintegration. In current study pyknosis was present in 100% animals of group B while 100% absent in Groups A and C. Akanda MR et.al conducted a study on Sodium Arsenite-Induced toxicity among rats and concluded similar findings of pyknotic nuclei and blurred cytoplasm in hepatic tissue in all SA treated rats[18]. They concluded these findings due to excessive accumulation of free radicals in hepatic tissue by SA. Similar mechanism might have operated in present study to give similar results. Absence of pyknosis documented by Abddel Daim in 2018 by treatment with Vitamin E against fipronil mediated oxidative damage in rat liver[19]. These findings are similar to group C of our study as pyknosis was absolutely absent in these animals given vitamin E.

In present study, the highest number of inflammatory cells was found in arsenic treated group which is comparable with findings of Al-Forkan M and Fatima N[20,21]. These leucocytic infiltrations were considered as a prominent response of the body tissue facing injurious impacts. As in present study among animals of Group C only few showed inflammatory cells. Similar findings were noticed by Zubairi MB in a research to determine the hepatoprotective role of vitamin E on metoprolol induced liver injury on rabbits. Vitamin E treated group showed mild inflammatory infiltration and mild congestion as compared to metoprolol group.[22] This result substantiates that the molecules of the Vitamin E exerts anti-inflammatory activities[23]. Therefore, liver inflammation either changed to mild following Vitamin E treatment or disappeared entirely.

Limitations: Present study had small sample size, financial contrains and limited resources.

CONCLUSION

This study concluded that vitamin-E can be employed as anti-oxidant agent against arsenic induced hepatoxicity as a treatment option. It improves microscopic changes in lever hence its can be used as hepato-protective agent.

Author's contribution: AI & IA: Overall supervision, write up and literature review. AR & SN: Statistics application analysis literature review, help in write up. RTK & TL: Literature review help in write-up.

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