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Research Article

Lycopene Reverses Haematological, Oxidative, Hepatic and Renal Damage in Arsenic - Toxic Male Wistar Rats

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Abstract

Arsenic is a toxic heavy metal reported to induce haemotoxicity, nephrotoxicity and hepatotoxicity among other toxic conditions. Antioxidants are known to ameliorate toxicity and damage by heavy metals. Lycopene is a reported antioxidant but there is dearth of information on its effect in arsenic toxicity. This study investigated the effect of lycopene on oxidative, haematological, hepatic and renal damage in arsenic-toxic rats.

Thirty-five male Wistar rats (150 - 200g) were grouped into five of seven animals each: Groups 1 (Normal - 0.1 mL distilled water), 2 (Corn oil - 0.5 mL/kg), 3 (Lycopene - 20 mg/kg), 4 (Sodium arsenite - 2.5 mg/kg), and 5 (Sodium arsenite - 2.5 mg/kg and Lycopene - 20 mg/kg). Treatments were given orally for 28 days. Blood was collected through retro-orbital puncture for haematological studies, liver and kidney function tests. Liver and kidneys were excised for malondialdehyde (MDA) level, superoxide dismutase (SOD), catalase (CAT) activities evaluation and histological examination. Level of significance was taken at $\alpha_{0.05}$ using one-way ANOVA.

Treatment with lycopene caused a significant ($\alpha_{0.05}$) increase in packed cell volume, haemoglobin concentration, red blood cell count, hepatic and renal SOD and CAT activities compared to arsenic alone treated group. However, a significant ($\alpha_{0.05}$) decrease in blood urea nitrogen, creatinine, activities of aspartate aminotransferase, alanine aminotransferase, hepatic and renal MDA was observed with lycopene treatment. Histological examination showed reversal of hepatocellular damage in lycopene-treated rats but no apparent alteration was observed in the kidney. Lycopene reversed haematological alterations, oxidative, hepatic and renal damage in arsenic-toxic rats.

Keywords: *Arsenic; Lycopene; Haematological profile; kidney and Liver Function Tests*

Introduction

Arsenic is a known environmental toxicant which exist both in organic and inorganic forms [1]. Its prevalence and exposure depends on natural processes and anthropogenic activities such as mining and pesticide use in agricultural practices [2,3]. Humans are exposed to arsenic from contaminated air, food and water [4,5]. Drinking arsenic-contaminated water is the major route of human exposure to arsenic [6]. Toxicity in humans and experimental animals has been recorded from acute or chronic exposure to inorganic form of arsenic compared to the organic form such as arsenobetaine [7,8]. Ingestion of arsenic has been shown to cause haematological, neuronal, renal and lung damage among other conditions [9]. Reports have shown arsenic to cause excess generation of reactive oxygen species which results in oxidative stress [9]. However, antioxidants have been reported to protect against arsenic-induced tissue damage [10].

Lycopene is a naturally occurring carotenoid contained in red tomatoes (*Solanum lycopersicum)* and other fruits such as watermelon, pink guava and pink grape [11] which has been shown to exhibit powerful antioxidant potentials [12]. Studies have reported lycopene to possess an anti-diabetic activity, protect against methylmercury-induced neurotoxicity in cultured rat cerebellar granule neurons and cadmium-induced renal toxicity in mice [13]. These activities were linked with its antioxidant potentials [14,15]. This study investigated the effect of lycopene on arsenic-induced oxidative, haematological, hepatic and renal damage in male Wistar rats.

Materials and Methods

Chemicals

Lycopene (Vitabiotics Company, London, England), Sodium arsenite (Sigma-Aldrich, USA), Corn oil, and other reagents used were of analytical grade.

Animal and experimental protocols

Thirty-five male Wistar rats weighing 150 - 200g were obtained from the Central Animal House, University of Ibadan. The rats were housed in well-ventilated cages and acclimatized to standard laboratory conditions for two weeks. They were maintained on standard rat chow and watered *ad libitum*. They were randomly allocated into five groups of seven animals each: Group 1 (normal) received 0.1 mL distilled water; Group 2 received 0.5 mL/kg corn oil [16]; Group 3 was treated with 20 mg/kg lycopene [11,15]; Group 4 received 2.5 mg/kg sodium arsenite [3,17] daily for 28 days; Group 5 received 2.5 mg/kg sodium arsenite with 20 mg/kg lycopene treatment daily for 28 days. All treatments were administered orally. Lycopene was reconstituted in corn oil while arsenic was dissolved in distilled water.

Blood collection

Blood samples were collected after 28 days of treatment from the retro-orbital sinus under mild ether anesthesia into heparinized and plain bottles. Heparinized blood samples were immediately subjected to haematological assessment using an auto analyzer (Sysmex Hematology Analyzer, K4500 model). Plain blood samples were allowed to clot at room temperature and then centrifuged at 3500 rpm for 10 minutes to separate out serum. Serum was carefully aspirated into new sterile plain bottles for alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, serum creatinine and blood urea nitrogen analysis.

Organ sampling

Liver and kidney samples were harvested from each animal after euthanasia for both biochemical analysis and histological studies. Excised liver and kidney were weighed, homogenized in 5 times ice cold 10% weight/volume phosphate buffer (0.1M, pH 7.4). Homogenates were centrifuged at 10,000 rpm for 15 minute at 4ºC and supernatant was collected for assessment of malondialdehyde level, superoxide dismutase and catalase activities.

Biochemical assays

Serum alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, creatinine and blood urea nitrogen were estimated using commercially available kit (Fortress Diagnostics, UK). Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) using the method of Varshney and Kale [18]. Superoxide dismutase and catalase activities were determined by the method described by Misra and Fridovich [19] and Sinha [20] respectively.

Relative organ weight

This was calculated using the formula:

Relative organ weight = (absolute organ weight / body weight at sacrifice) × 100 [21].

Histological examination

Liver and kidney were fixed in 10% formaldehyde for 24h and processed for histological observation using Hematoxylin and Eosin staining method.

Statistical analysis

Data were presented as Mean ± standard error of mean and analyzed using Graph pad Prism statistical package (version 5.01, USA). Statistical significance at $\alpha_{0.05}$ was established using one-way Analysis of Variance (ANOVA) and Newman Keuls' post-hoc test.

Results and Discussion

Effect of lycopene on relative liver and kidney weights in arsenic - toxic male Wistar rats

Arsenic exposure significantly reduced ($\alpha_{0.05}$) relative liver weight (2.59 ± 0.11%) compared to control (3.31 ± 0.18%). Treatment with lycopene significantly increased relative liver weight (3.52 ± 0.09%) compared to arsenic alone group (Figure 1).

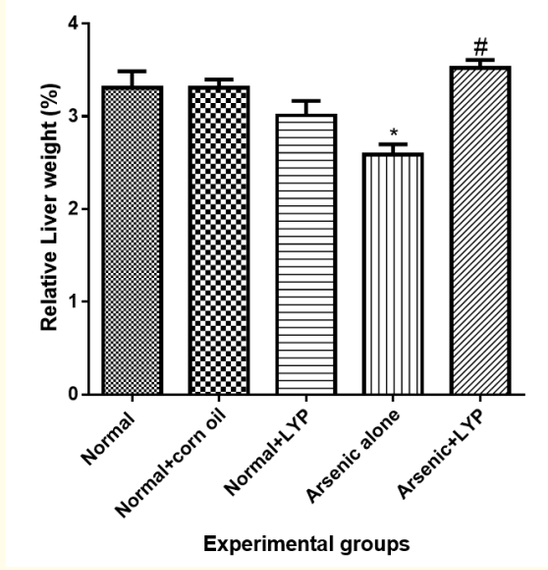


Figure 1: *Effect of lycopene on relative weights of the liver in arsenic-toxic male Wistar rats.*
Values were expressed as mean ± SEM; n = 7, α0.05.
**: Indicates values significantly different from control.*
#: Indicates values significantly different from arsenic alone.
Note: LYP: Lycopene.

Relative kidney weight (%) in arsenic alone (0.28 ± 0.022), lycopene alone (0.27 ± 0.002) and arsenic + lycopene (0.27 ± 0.002) were not significantly different ($\alpha_{0.05}$) when compared to control (0.25 ± 0.009) (Figure 2).

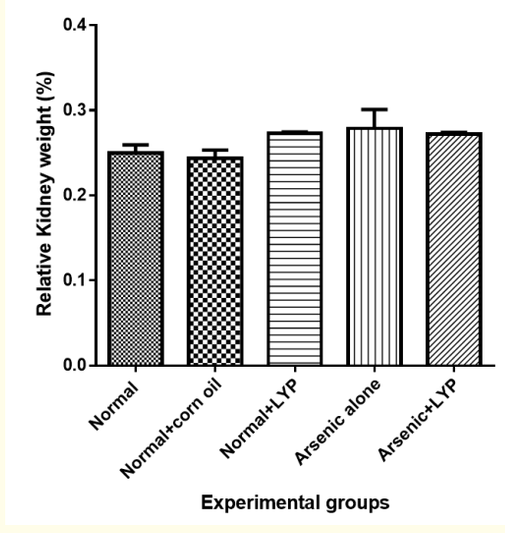


Figure 2: *Effect of lycopene on relative weights of the kidney in arsenic- toxic male Wistar rats.*
Values were expressed as mean ± SEM; n = 7, α0.05.
Note: LYP: Lycopene.

Effect of lycopene on packed cell volume, haemoglobin concentration, red blood cell count and white blood cell count in arsenic - toxic male Wistar rats

Packed cell volume, haemoglobin concentration and red blood cell count in group 2 (arsenic alone) were significantly decreased ($\alpha_{0.05}$) when compared to control. Treatment with lycopene significantly increased ($\alpha_{0.05}$) packed cell volume, haemoglobin concentration and red blood cell count compared to arsenic alone (group 2) (Table 1).

	Packed cell volume (%)	Haemoglobin conc. (g/dl)	Red blood cell count ($\times 10^6$ mm^{-3})	White blood cell count (mm^{-3})
Normal	36.40 ± 1.66	11.82 ± 0.39	6.250 ± 0.296	7050 ± 1021
Normal + corn oil	36.60 ± 1.50	11.80 ± 0.35	6.012 ± 0.347	7170 ± 798.8
Normal + LYP	38.40 ± 1.12	12.76 ± 0.36	6.446 ± 0.083	7030 ± 1094
Arsenic alone	30.00 ± 1.41*	9.70 ± 0.37*	5.044 ± 0.170*	6820 ± 489.0
Arsenic+ LYP	38.00 ± 1.55#	12.82 ± 0.63#	6.358 ± 0.280#	7270 ± 551.7

Table 1: *Effect of lycopene on packed cell volume, haemoglobin concentration, red blood cell count and white blood cell count in arsenic- toxic male Wistar rats.*

Values were expressed as mean ± SEM; $\alpha_{0.05}$; n = 7.

**: Indicates values significantly different from Control, #: Indicates values significantly different from Arsenic alone. Note: LYP: Lycopene.*

White blood cell count in arsenic alone group (6820 ± 489.0), lycopene alone (7030 ± 1094) and arsenic + lycopene group (7270 ± 551.7) were not significantly different ($\alpha_{0.05}$) when compared to control (7050 ± 1021) (Table 1).

Effect of lycopene on alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in arsenic - toxic male Wistar rats

Alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities of arsenic alone group were significantly increased by 43.7, 64.4 and 76.8 % respectively when compared to control. There was no significant difference between lycopene and corn oil treated groups. However, arsenic +lycopene group showed 52.3, 35.4 and 73.6 % significant increase in ALP, AST and ALT activities compared to arsenic alone (Figure 3-5 respectively).

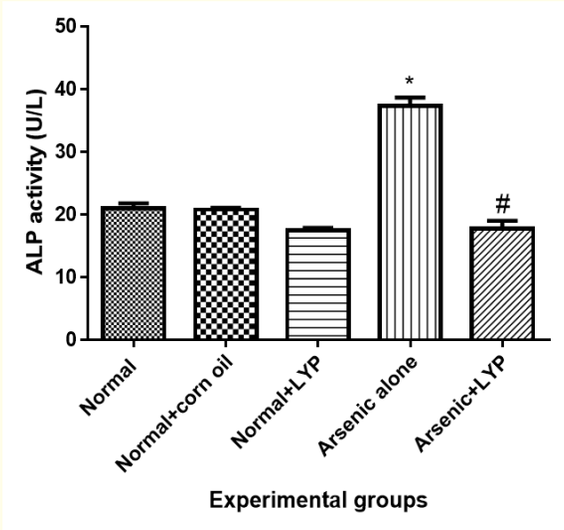


Figure 3: *Effect of lycopene on alkaline phosphatase activity in arsenic-toxic male Wistar rats.*
Values were expressed as mean ± SEM; n = 7, α0.05.
**: Indicates values significantly different from control.*
#: Indicates values significantly different from arsenic alone.
Note: LYP: Lycopene.

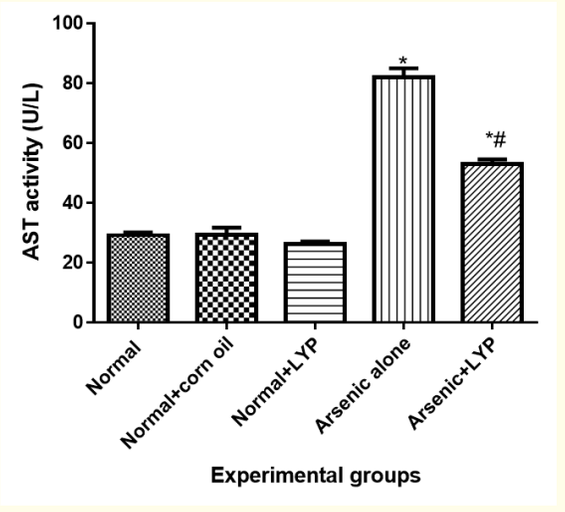


Figure 4: *Effect of lycopene on aspartate aminotransferase activity in arsenic-toxic male Wistar rats.*
Values were expressed as mean ± SEM; n = 7, α0.05.
**: Indicates values significantly different from control.*
#: Indicates values significantly different from arsenic alone.
Note: LYP: Lycopene.

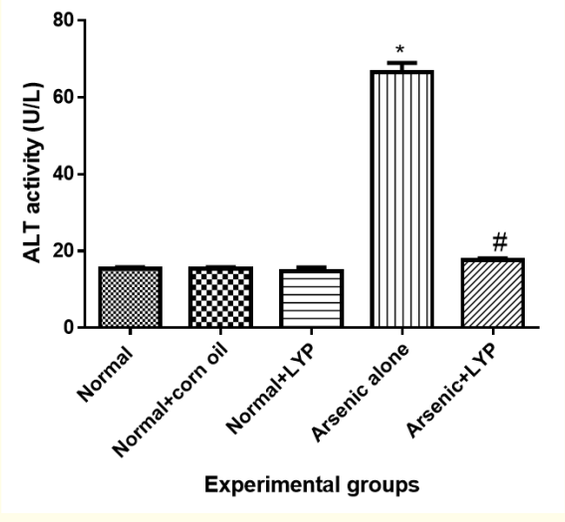


Figure 5: *Effect of lycopene on alanine aminotransferase activity in arsenic-toxic male Wistar rats.*
Values were expressed as mean ± SEM; n = 7, α0.05.
**: Indicates values significantly different from control.*
#: Indicates values significantly different from arsenic alone.
Note: LYP: Lycopene.

Effect of lycopene on serum blood urea nitrogen and creatinine level in arsenic - toxic male Wistar rats

Arsenic exposure in group 4 (arsenic alone) significantly increased serum blood urea nitrogen and creatinine by 39.3% and 48.9% respectively when compared with control. Treatment with lycopene significantly reduced blood urea nitrogen and creatinine by 40.5% and 47.8% respectively in group 5 (arsenic + lycopene) when compared with arsenic alone (Figure 6 and 7).

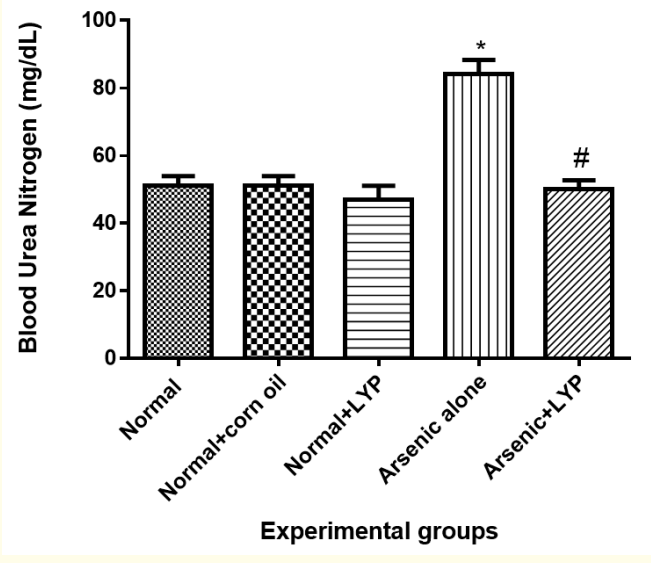


Figure 6: *Effect of lycopene on serum blood urea nitrogen level in arsenic-toxic male Wistar rats.*
Values were expressed as mean ± SEM; n = 7, α0.05.
**: Indicates values significantly different from control.*
#: Indicates values significantly different from arsenic alone.
Note: LYP: Lycopene.

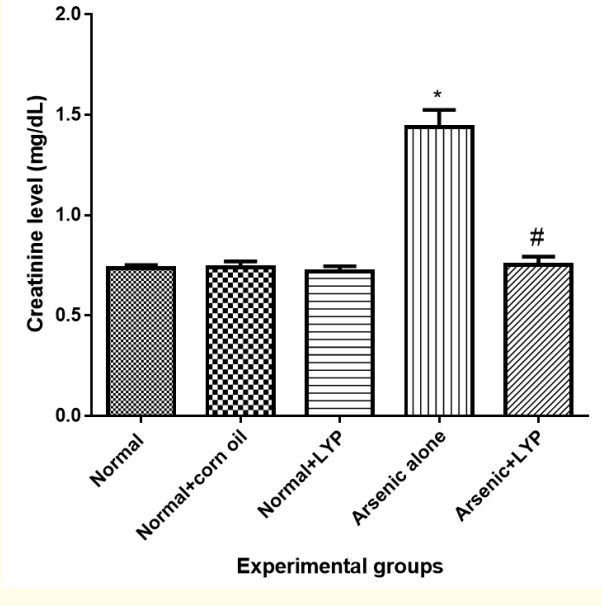


Figure 7: *Effect of lycopene on serum creatinine level in arsenic-toxic male Wistar rats.*
Values were expressed as mean ± SEM; n = 7, α0.05.
**: Indicates values significantly different from control.*
#: Indicates values significantly different from arsenic alone.
Note: LYP: Lycopene.

Effect of lycopene on malondialdehyde level, superoxide dismutase and catalase activities in arsenic - toxic male Wistar rats

Liver malondialdehyde level of group that received arsenic alone significantly increased ($\alpha_{0.05}$) by 77.8% when compared to control. This was significantly reduced by 55.6% with lycopene treatment in group 5 (arsenic + lycopene) when compared to arsenic alone group.

Liver superoxide dismutase and catalase activities of arsenic alone treated rats (Group 4) were significantly lowered ($\alpha_{0.05}$) by 67% and 0.97% respectively when compared to control. The activities of these enzymes were significantly increased 68.4% and 0.39% with lycopene treatment in group 5 (arsenic+lycopene) compared to arsenic alone (Table 2).

Kidney malondialdehyde level in arsenic alone (Group 4) was significantly increased ($\alpha_{0.05}$) by 50% when compared to control. Treatment with lycopene (arsenic+lycopene) significantly reduced kidney MDA level by 50% compared to arsenic alone.

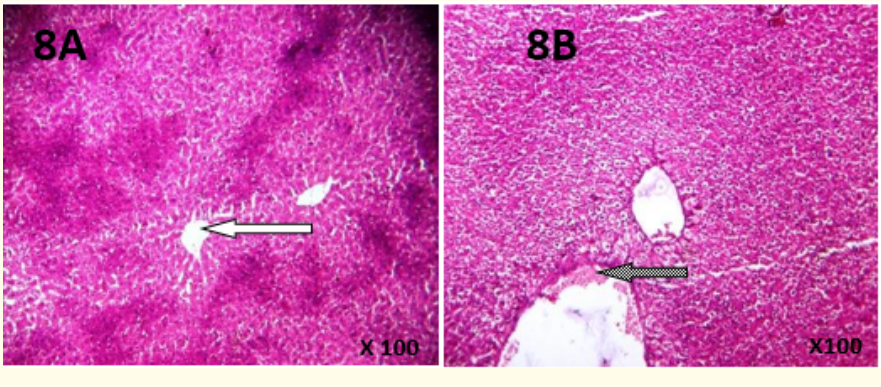
Kidney superoxide dismutase and catalase activities in arsenic alone group were significantly decreased ($\alpha_{0.05}$) by 74.1% and 0.87% respectively when compared to control. The activities of these enzymes were significantly increased by 85.4% and 1.1% respectively with lycopene treatment in Group 5 (arsenic+lycopene) compared to Group 4 (arsenic alone). Kidney superoxide dismutase activity was also significantly increased in Group 5 compared to control (Table 2).

	Liver			Kidney		
	MDA (U/mg of protein)	**SOD (U/mg of protein)**	**CAT (U/mg of protein)**	**MDA (U/mg of protein)**	**SOD (U/mg of protein)**	**CAT (U/mg of protein)**
Normal	0.0022 ± 0.0003	0.6682 ± 0.0549	7.500 ± 0.0029	0.0053 ± 0.0002	0.3083 ± 0.0255	7.508 ± 0.0017
Normal + corn oil	0.0024 ± 0.0003	0.5358 ± 0.0947	7.502 ± 0.0043	0.0053 ± 0.0001	0.3375 ± 0.0392	7.497 ± 0.0070
Normal + lycopene	0.0021 ± 0.0005	1.427 ± 0.0774^{*+}	7.513 ± 0.0043	0.0055 ± 0.0002	0.5958 ± 0.0893^{*+}	7.495 ± 0.0042
Arsenic	0.0085 ± 0.0004*	0.2206 ± 0.0059*	7.427 ± 0.0148*	0.0114 ± 0.0004*	0.0803 ± 0.0050*	7.443 ± 0.0167*
Arsenic + lycopene	0.0038 ± 0.0008$^{\#}$	0.6974 ± 0.0993$^{\#}$	7.529 ± 0.0180$^{\#}$	0.0053 ± 0.0011$^{\#}$	0.5465 ± 0.0636$^{*\#}$	7.522 ± 0.0075$^{\#}$

Table 2: *Liver and kidney malondialdehyde level (MDA), superoxide dismutase (SOD) and catalase (CAT) activities in arsenic-toxic male Wistar rats.*

Values were expressed as mean ± SEM; $\alpha_{0.05}$; n=7.

* indicates values significantly different from Control, $^{\#}$ indicates values significantly different from Arsenic alone, $^{+}$indicates values significantly different from corn oil.



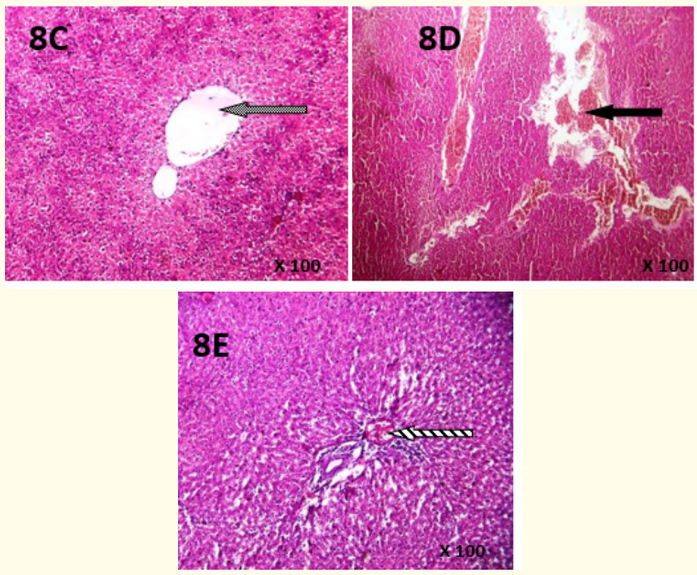


Figure 8: *Photomicrographs showing liver sections of normal, normal+cornoil, normal+lycopene, arsenic-toxic and arsenic+lycopene rats using Haematoxylin and Eosin (H&E) stain.*

Liver section in normal rats shows normal architecture, central venules and portal tracts that are not congested (white arrows) (8A). Figure 8B and 8C show tissue sections of normal+corn oil and normal+lycopene rats respectively with normal sinusoids without infiltration of inflammatory cells but fat infiltration of cytoplasm (dotted arrow). The central venules in arsenic-toxic rats appear congested with haemorrhage (black arrow) (8D). Figure 8E shows liver sections of arsenic+lycopene rats with moderate haemorrhage (striped arrow).

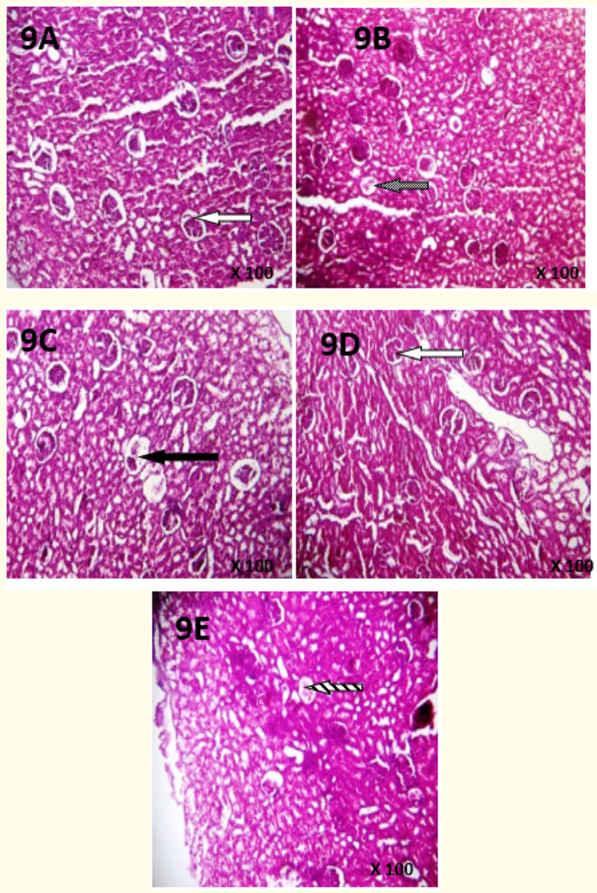


Figure 9: *Photomicrographs showing kidney sections of normal, normal+cornoil, normal+lycopene, arsenic-toxic and arsenic+lycopene rats using Haematoxylin and Eosin (H&E) stain.*

Kidney sections in normal and arsenic-toxic rats (9A and 9D) respectively show normal architecture and renal cortex with normal glomeruli (white arrow). Figure 9B shows tissue section of normal+corn oil rats with the glomeruli showing thick and condensed basement membrane (dotted arrow). Tissue section of normal+lycopene rats in figure 9C shows moderate architecture and renal cortex with widened capsular spaces (black arrow). Figure 9E shows kidney sections of arsenic+lycopene rats with mild glomerulosclerosis in the renal cortex (striped arrow).

Discussion

Increase or decrease in either absolute or relative weight of organs after administration of a chemical or drug has been reported to be an indication of toxicity [21]. A decrease in relative liver weight was observed in the present study. This finding is consistent with earlier report of Manish., *et al.* [22] who reported a decrease in relative liver weight. The significant reduction in relative liver weight as observed in the present study may be as a result of degeneration of hepatic cells which was observed in the histological evaluation of the liver.

Haematological assessment remains the frontline indicator of exposure to toxicants before other major injuries become obvious [23]. The present study recorded anaemia as indicated by a reduction in packed cell volume, hemoglobin concentration and red blood cell count in sodium arsenite alone exposed rats. These findings are in agreement with the reports of Al-Forka., *et al.* who reported occurrence of anemia after exposure of Wistar rats to sodium arsenite [24]. Alterations in haematological variables in the arsenic exposed rats could be attributed to either an altered erythropoietic system or hindered maturation of blood cells [24]. Lycopene treatment restored arsenic-induced haematological alterations observed in this study.

Akin-Idowu., *et al.* reported an elevation in the serum activities of liver enzymes in animals that were exposed to arsenic toxicity. The observed alterations in the serum liver enzyme activities were attributed to the occurrence of hepatocellular damage [3]. In the present study, an increase in the serum activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) was also observed in sodium arsenite alone toxic rats which agrees with previous reports. Histological examinations of liver sections of group treated with arsenic alone showed occurrence of hepatotoxicity as reflected by moderate haemorrhage, congested central venules and sinusoids. The use of exogenous antioxidants and food rich in antioxidants has been shown to protect against liver damage and oxidative stress [17]. Lycopene treatment caused an obvious decrease in the serum activities of the liver enzymes of arsenic toxic rats. The ability of lycopene to cause the observed decrease in serum activities of liver enzyme of rats exposed to arsenic toxicity could be attributed to its reported antioxidant properties. However, further studies are needed to understand the mechanism by which lycopene causes the observed decrease in the serum liver enzymes activity.

Renal function was assessed using serum levels of blood urea nitrogen (BUN) and creatinine. There was a significant increase in the levels of BUN and creatinine in the group that received sodium arsenite alone which is in agreement with the findings of Wang., *et al*. [4]. The increase in the levels of BUN and creatinine is an index of arsenic-induced toxicity and could be due to alteration in the normal kidney function [25]. Administration of lycopene caused significantly reduced levels of blood urea nitrogen and creatinine in the group that received both sodium arsenite and lycopene. This suggests that lycopene improved renal clearance of these metabolic wastes and as well prevented renal dysfunction from arsenic exposure.

Most toxic substances have been reported to induce toxicity by causing oxidative damage of biomolecules [13]. Superoxide dismutase (SOD) and catalase (CAT) are both endogenous antioxidant enzymes that protect the body against superoxide ions and hydrogen peroxides [26]. Results of this study showed a decrease in the activities of superoxide dismutase and catalase and a considerable increase in malondialdehyde (MDA) level in the liver and kidney of arsenic alone treated group. Akin-Idowu., *et al*. [3] had earlier shown arsenic to induce generation of reactive oxygen species (ROS) and decrease antioxidant enzyme activities as observed in this study. Administration of lycopene reduced ROS generation and prevented down-regulation of SOD and CAT activities. Lycopene may have exerted these effects by either preventing depletion of endogenous antioxidant co-factor or arsenic-induced generation of reactive oxygen species which may have helped in scavenging generated ROS [21]. The antioxidant activities exerted by lycopene in this study are consistent with earlier reports that lycopene is a potent free radical scavenger [27]. Lycopene caused a notable reduction in liver and kidney levels of MDA possibly by mopping up the generated free radicals.

Conclusion

Lycopene reversed arsenic-induced haematological alterations, hepatic injury and renal dysfunction by reducing reactive oxidative species level and up-regulating the activities of anti-oxidative enzymes.

Conflict of Interest

None.

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