RESEARCH ARTICLE Indian Journal of Animal Research, Volume 58 Issue 12: 2198-2203 (December 2024)

Histopathological Grading of Induced Cardiotoxicity Due to Arsenic and its Alleviation by *Allium sativum* in Ducks

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10.18805/IJAR.B-4696

ABSTRACT

Background: Arsenic present in underground water affects the overall health. The therapeutic effects of *Allium sativum* was investigated for ameliorating the toxic effects of arsenic exposed ducks. Even though, heavy metals especially arsenic toxicity and ameliorative effects of *Allium sativum* has been largely studied, yet the histopathological cardiac lesion grading and its correlation with oxidative stress and biochemical parameters is scarce. This study is aimed at evaluating cardiotoxic lesions due to arsenic and ameliorative effects of *Allium sativum* in accordance with histopathological grading with respect to oxidative and biochemical parameters.

Methods: In the present study, 105 birds were randomly distributed in 5 groups. GRP II was given arsenic @ 30 ppm whereas GRP III, IV, V were given *Allium sativum* @ 0.5 gm/ kg feed, 1 gm/kg feed and 2 gm/kg feed respectively along with arsenic to study the ameliorative effects. At the end of 42 days, 6 birds from each group were slaughtered after blood collection and the extend of arsenic toxicity with *Allium sativum* effects were studied as biochemical and oxidative parameters with histopathological grading of lesions. The extent of lesions was graded as mild (+), moderate (++) and severe (+++).

Result: Grossly, heart revealed dilatation and thickening of ventricular wall in GRP II along with congestion in coronary vessels. In GRP V, grossly heart revealed no congestion and comparatively reduced enlargement. Whereas microscopically, myocardial and epicardial oedema, disruption of muscle fiber and hemorrhage was observed. Along with this, there was increase in cardiac biomarkers - Total serum creatinine kinase (CK), creatine kinase-MB (CK-MB) levels and tissue oxidative parameters (LPO), with decrease in GSH, CAT, total antioxidant and SOD levels in GRP II which was comparably restored in GRP V.

Key words: Arsenic, Ducks, Grading, Heart, Histopathology, Oxidative stress.

INTRODUCTION

Arsenic, is classified as a heavy metal and one of the most commonly found elements in the earth's crust (Hantson *et al.,* 2003). Trivalent form (Inorganic arsenic) is comparatively toxic than both the organic and the pentavalent form (Frost DV. 1967). Arsenic present in drinking water puts off the production of meat (Sharaf *et al.,* 2013).

Allium sativum (garlic) is used in food by people all over the world. Garlic extract has been reported to have beneficial effects in controlling hyperlipidemia in animals (Ali, 2000). Toxico-protective effects of *A. sativum* are attributed to the presence of various organosulfur compounds, mainly allicin (Augusti *et al.,* 1974) and having tremendous antioxidant property which exerts actions by scavenging ROS, enhancing cellular antioxidant enzymes and increasing glutathione in the cells (Borek *et al.,* 2001). Chan *et al.* (2013 and Ademiluyi *et al.* (2013) reported that *Allium sativum* dried powder improved antioxidant levels besides modulating oxidative stress.

Although extensive work has been carried out on arsenic and *Allium sativum* effects on birds, histological grading of cardiac tissue and its correlation with biomarkers and gross lesions is scarce. Looking at the paucity of information, the current study was aimed to grade the histological lesions and to correlate it with the gross appearance, extend of lesions, oxidative and biochemical parameters in ducks.

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How to cite this article: Das, D., Pamia, J., Kumar, D., Panda, S.K. and Jenna, G. (2024). Histopathological Grading of Induced Cardiotoxicity Due to Arsenic and its Alleviation by *Allium sativum* in Ducks. Indian Journal of Animal Research. 58(12): 2198-2203. doi: 10.18805/IJAR.B-4696.

Submitted: 26-06-2021 **Accepted:** 21-09-2021 **Online:** 14-10-2021

MATERIAL AND METHODS

Experimental design

The present experiment was carried out at ICAR-Regional Central Avian Research Institute, Bhubaneswar in collaboration with Department of Veterinary Pathology, Orissa University of Agriculture and Technology during the

period of February to April, 2020. White Pekin ducklings of 1 day old (n=105) were randomly selected and distributed into five groups of 21 ducks in each group, having 3 replicas of 7 each in deep litter system with ad libitum feed and water. The experimental trial was conducted for 6 weeks. *i.e.*, 42 days. A time of 1 week was given to ducklings for acclimatization before the start of the experimental trial.

The birds of therapeutic trial groups were supplemented with sodium arsenite (HIMEDIA^{R}) procured from commercial sources, was dissolved in water and given to birds of GRP II (Arsenic @30 ppm in water), GRP III (Arsenic @30 ppm in water+ 0.05% *Allium sativum* in feed, *i.e.*, 0.5 g/kg feed), GRP IV (Arsenic @30ppm in water+ 0.1% *Allium sativum* in feed, *i.e.*, 1 g/kg feed), GRP V (Arsenic @30 ppm + 0.2% *Allium sativum* In feed, *i.e.*, 2 g/ kg feed). All the experimental birds were observed daily for the development of clinical signs of toxicity including mortality (if any).

Blood collection and post mortem

At the end of 42 days, 6 birds from each group (2 birds from each replicate) were randomly selected and blood was successfully collected by detailed post mortem examination. 4 ml blood was collected during the cool hours of the day in clot activator vials for separation of serum after clot formation and centrifugation at 5000rpm for 10 mins. Serum was stored, for a maximum of one week, at -40°C for later use.

Representative cardiac tissue samples were collected for both oxidative parameters and histopathology study. For histopathological studies, tissues were preserved in 10% neutral buffered formalin for a period of 48 hours. The tissues were processed as per routine histopathological procedure. Based on the observed histological patterns, the lesions were graded depending on the extent of lesions due to arsenic toxicity and amelioration by *Allium sativum* as given in Table 1. The gross appearance of the heart was also recorded. Whereas for oxidative parameters, tissues were cleaned in ice-cold 1X phosphate buffer saline (PBS) of pH 7.4 and kept in separate boxes, labelled and instantly stored at -80°C awaiting homogenization.

Biochemical and oxidative assays

Serum was used for estimation of CK and CK-MB using CoralR Kit oxidative profiling and estimation comprised of LPO (lipid peroxidation), GSH (glutathione peroxidase), CAT (catalase), from tissue samples collected while performing post mortem. LPO was evaluated in terms of malondialdehyde (MDA) formed using thiobarbituric acid reactive substances (TBARS) defined by (Rehman 1984), GSH was assessed by free -SH groups using 5-5' dithiobis 2 - nitrobenzoic acid (DTNB) defined by (Sedlak and Lindsay, 1968). CAT was estimated as stated by (Aebi *et al.* 1984). The oxidative profiling was estimated using spleen and bursa tissue by tissue homogeniser. Ferric reducing antioxidant power (FRAP) assay has been done by ENZAssay antioxidant activity estimation kit (Product code: CCK072).

RESULTS AND DISCUSSION

Histopathological grading criteria

Ameliorating effect on arsenic by the therapeutic use of *A. sativum* on cardiac tissue in regards to serum biochemistry, oxidative stress and histopathology was studied and compared with control group besides individual groups in the present study. Overall, the results were more satisfactory in 2% *A. sativum* supplementation GRP-V among the different treatment groups (GRP-III, GRP-IV, GPR-V). The histopathological lesions along with grading are presented in Table 1. Arsenic cardiac toxicity was found to be severe (+++) with intense disrupted myocardial muscle fiber, myocardial and epicardial oedema, hemorrhage and congestion. Grossly, heart was dilated and coronary vessel congestion was evident. The lesions were consistently found in all 21 ducks of GRP II Fig 3, 6, 9, 12. In GRP III and GRP IV, the lesions were moderate (++) with microscopic lesions consisting of more or less similar disrupted myocardial fibers, epicardial and myocardial congestions with inconsistent hemorrhages amongst the 21 birds. Grossly, heart was dilated and coronary vessel congestion persisted Fig 2, 5, 8, 11. In GRP V, the microscopic and gross lesions were found to be mild (+). There was absence of coronary vessel congestion and dilation was reduced to near to normal. Microscopically, slight epicardial congestion was seen along with intact myocardial fibers Fig 1, 4, 7, 10. The present study was an attempt to grade histological lesions with reference to extent of gross lesions and serum biochemical and tissue oxidative parameters due to arsenic toxicity and reports the ameliorative effects of *Allium sativum* (garlic) towards the cardiac biomarkers, lipid profile and histopathological lesions. Heart is affected secondarily by arsenic and hence, was histopathologically analyzed for extent of lesion. Arsenic exposure leads to myocardial injury, cardiac arrhythmias and cardiomyopathy (Benowitz, 1992,

Table 1: Histopathological grading of cardiac lesions due to arsenic toxicity.

Degree of grading	Grading criteria
Mild(+)	Intact myocardial fiber, slight myocardial congestion
Moderate(++)	Disruption of myocardial muscle fiber, myocardial and epicardial congestion
Severe(+++)	Disrupted myocardial fiber, myocardial and epicardial oedema, hemorrhage and congestion

Goldsmith and From, 1980); while this cardiac toxicity is reported to be associated with the reduction of antioxidant capacity (Muthumani and Prabu, 2014).

Following the different therapeutic doses of dietary *A. sativum* in arsenic toxicity induced ducks, total serum creatinine kinase, creatine kinase-MB (CK-MB) levels decreased comparatively in different treatment groups. However, the most significant decreased ($P<0.01$) values of total serum creatinine kinase (CK), creatine kinase-MB (CK-MB) levels was recorded in GRP-V as compared with the rest *A. sativum* treatment groups at 42nd day of treatment. The administration of sodium arsenite lead to significant increase in cardiac biomarkers. Accordingly, total serum creatinine kinase (CK), creatine kinase-MB (CK-MB) levels elevated significantly ($P<0.01$) in the GRP-II group compared to control group GRP-I. Metabolically damaged myocardium due to disturbed metabolic functions possess increased concentration of biomarkers for diagnosis leading to extracellular fluid (Upaganlawar, *et al.,* 2009). Serum creatinine kinase (CK) and creatine kinase-MB (CK-MB) are crucial biomarkers which elevates following myocardial injury, CK-MB being more specific for heart muscle and myocardial damage (Jaffe *et al.,* 2000). Creatine kinase (CK) enzyme is found in in brain, colon, muscles apart from heart as its function is associated with balancing phosphorylated creatine in ATP level depletion maintainance during muscle contraction. The results are inconsistent with other literature, but other compounds are used instead of sodium arsenite (Gomaa *et al.,* 2018).

Statistical analysis revealed that significant ($P<0.01$) increased level of total serum creatinine kinase (CK), creatine kinase-MB (CK-MB) was 577.0±7.28 in GRP-II at 42nd day of arsenic treatment in comparison to the control groups. However, following the application of dietary *A. sativum* in arsenic toxicity induced ducks, the mean values of total serum creatinine kinase (CK), creatine kinase–MB (CK-MB) level were significantly decreased at 531.5±8.97, 489.0±6.02 and 369.33±8.07, respectively in GRP-III, GRP-IV and GRP-V. There was significant ($P<0.01$) decreased level of GSH, CAT, FRAP and SOD with increased LPO level as 7.04±0.58, 3.49±0.23, 196.17±12.24 and 22.75±1.02 in GRP-II at 42nd day of arsenic treatment in comparison to the control groups. However, following the application of dietary *A. sativum* in arsenic toxicity induced ducks, the mean values of GSH, CAT, FRAP and SOD level were significantly ($P<0.01$) increased at

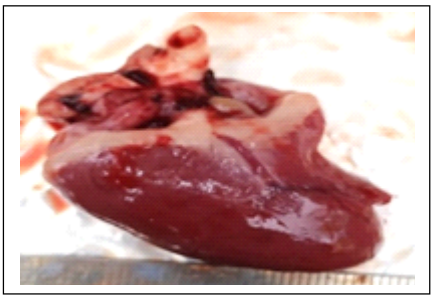


Fig 1: Heart from Grp V (mild).



Fig 2: *Allium sativum* treated heart (moderate) GRP III and IV.

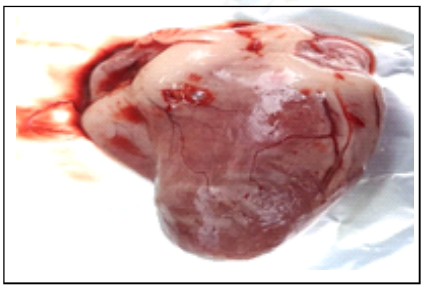


Fig 3: Arsenic induced thickening and congested hemorrhagic coronary arteries (GRP II) severe lesions.



Fig 4: Ventricular thickening from GRP V (mild).

Table 2: Cardiac biomarkers and oxidative parameters.

Parameter	G-I	G-II	G-III	G-IV	G-V
CK-MB	302.30±5.09^{a}	496.41±10.81^{b}	476.76±11.66^{b}	447.77±10.17^{b}	361.32±13.33^{ac}
TOTAL CK	263.5±15.37^{a}	577.0±7.28^{b}	531.5±8.97^{b}	489.0±6.02^{ab}	369.33±8.07^{a}
SOD	42.19±1.34^{a}	22.75±1.02^{b}	24.44±0.91^{b}	29.07±1.74^{bc}	35.47±1.42^{ac}
CAT	7.39±0.38^{a}	3.49±0.23^{b}	3.93±0.28 ^{b}	4.72±0.41^{ab}	6.32±0.32 ^{a}
LPO	246.84±14.58^{a}	474.89±15.98^{b}	450.53±16.32^{b}	450.51±15.75	359.05±12.02^{ac}
GSH	13.77±0.34^{a}	7.04±0.58^{b}	7.17±0.59^{b}	9.38±0.45^{ab}	10.79±0.42^{ac}
FRAP	314.34±11.27^{a}	196.17±12.24^{b}	212.79±13.60^{b}	244.59±17.39^{bc}	285.79±14.15^{ac}

10.79±0.42, 6.32±0.32, 285.79±14.15 and 35.47±1.42, respectively in GRP-V. All the parameters are presented in Table 2. The exact arsenic toxicity mechanisms are not fully understood, but arsenic increases reactive oxygen and nitrogen species (ROS/RNS), resulting in the lipid peroxidation, protein oxidation and DNA damage (Birben *et al.,* 2012). Arsenite inhibits antioxidant enzymes activity including catalase (CAT), superoxide dismutase (SOD) as well as the content of reduced glutathione (GSH) (Goudarzi *et al.,* 2018a). Hence, these indicates that oxidative stress is induced by arsenic due to increasing free radical generation and decreasing antioxidant capacity of cells, which contributes to the oxidative damage of tissues. Previous literature has shown protective effects of herbal



Fig 5: *Allium sativum* treated ventricular thickening (moderate) GRP III and IV.

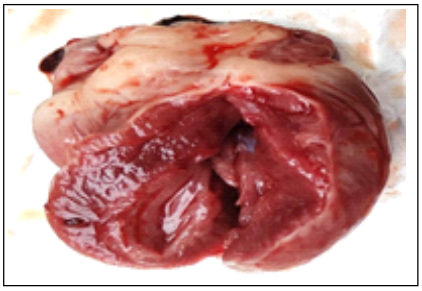


Fig 6: Arsenic induced thickening and congested ventricular muscle (GRP II) severe lesions.

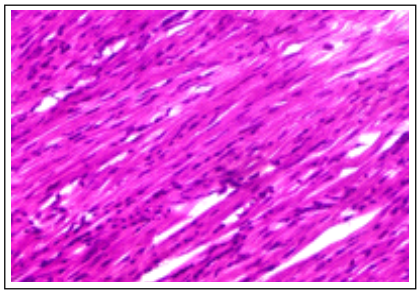


Fig 7: Reduced and slight myocardial congestion from GRP V (Mild).

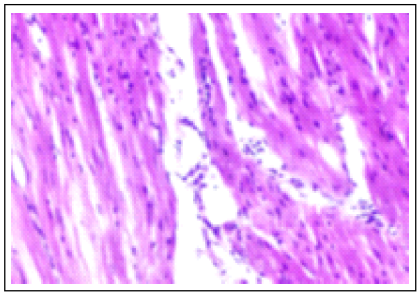


Fig 8: Moderate congestion with hemorrhage (GRP III and IV).

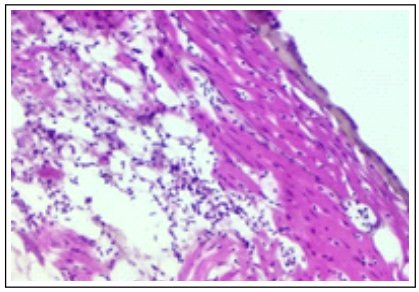


Fig 9: Severe myocardial hemorrhage and congestion (GRP II).

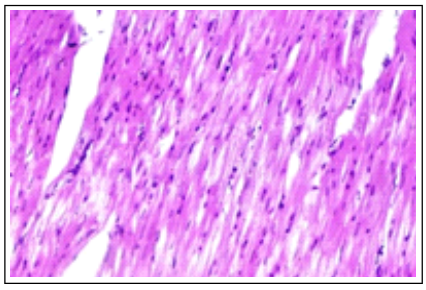


Fig 10: Mild lesion of intact myocardial muscle fibre (GRP V).

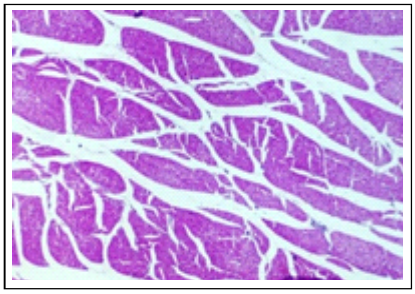


Fig 11: Moderately disrupted myocardial fibres from GRP III and IV

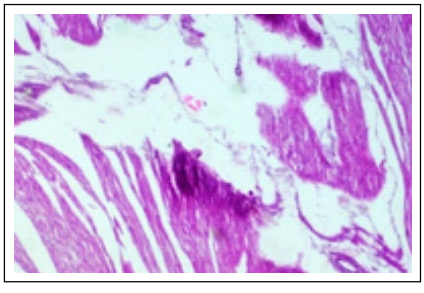


Fig 12: Severe myocardial disruption and oedema (GRP II).

medicine in the reduction of deleterious effect of arsenic on various organs and tissues (Goudarzi *et al.,* 2018c; Mehrzadi *et al.,* 2018a; Mehrzadi *et al.,* 2018b; Zhang *et al.,* 2014). Aged *Allium sativum* also exerted its antioxidant action by scavenging reactive oxygen species (Imai *et al.*, 1994) and enhancing the cellular antioxidants, like reduced glutathione superoxide dismutase, catalase and glutathione peroxidase of vascular endothelial cells (Geng *et al.*, 1997; Wei 1998).

Both the biochemical and oxidative parameters can be directly correlated with the histopathological changes and gross lesions. Hence, the grading is more prominently established. A correlation between gross and histological findings could be derived on detailed analysis along with biochemical findings. There are a few literatures available against microscopic changes of heart caused by arsenic toxicity. These findings may accordance with the previous findings of Kalavathi *et al.* (2011) in broilers. The histological changes in GRP II *viz*; disruption of cardiac muscle bundles can be because of oxidative stress within the tissues resulted peroxidative damage to the membrane lipids. (padmaja 2009) GRP III and GRP IV birds showed similar patterns of microscopic changes, with moderate improvement. GRP V was seen with maximum improvement histologically and grossly. It may be concluded that the anti-oxidant power of ginger have little improvement against oxidative damage to heart. There are a few literatures available against microscopic changes of heart caused by arsenic toxicity, hence this study was carried out.

CONCLUSION

Present study demonstrated that sodium arsenite causes cardiotoxicity and elevates cardiac biomarkers CK, CK-MB, along with LPO. *Allium sativum* has protective effects @ 2% towards gross and histologic cardiotoxic lesions due to arsenic. It also ameliorates the cardiac biomarkers and tissue oxidative parameters compared to control group. Arsenic administration results in the degenerative changes in cardiomyocytes, associated with the increased serum level of CK, CK-MB and LPO. In addition, arsenic also decreased levels of GSH, SOD activity and CAT in heart. Altogether, these effects accompanied histological changes in cardiac tissue which helped in grading of the toxic lesions in the ducks.

These obtained results are in agreement with previous studies indicating that arsenic compounds reduce antioxidant defenses and induces oxidative stress. The cardio-protective effect of *A. sativum* has been reported in previous studies indicating reduced functional and structural changes in cardiac tissue accompanied by the increased activity of antioxidant enzymes and the reduced level of lipid peroxidation in the heart tissue (Patel and Goyal, 2011). The treatment also reduced the serum level of cardiac bio-markers CK and CK-MB.

Conflict of interest

All authors declare that they have no conflict of interest.

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