**Toxicology International,** *30(4) : 489-501; 2023. DOI: 10.18311/ti/2023/v30i4/34093*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi




# Ameliorative Role of Allicin in Arsenic-Induced Liver and Gonad Apoptosis in Male *Swiss* Albino Mice (*Mus musculus*)

**Payel Sarkar* and Jayanta Kr. Kundu**

*Genetics and Molecular Biology Research Unit, Department of Zoology, Vidyasagar University, Midnapore - 721102, West Bengal, India; payel_sarkar@yahoo.com*

## Abstract

Arsenic toxicity is a matter of concern in the present-day world. Arsenic, a potentially hazardous metalloid easily gets bio-magnified through the food chain and also affects not only the vital organs such as the kidney, and liver of human beings but also the reproductive organs of males. This study was designed to propose allicin, the main bio-active component of garlic to address arsenic toxicity more efficiently and without any side effects apart from the costly conventional chelation therapy which is not free from various side effects. To conduct this study, allicin has been quantified and collected from ethanolic extract of garlic by High-Performance Liquid Chromatography (HPLC) using C18 column at 254nm wave-lengths against standard allicin at the retention time of 18.775min. The calculated concentration of allicin is 77.80%. Next, arsenic trioxide ($As_2O_3$) (20mg/kg) and allicin (100mg/kg) were administered orally for toxicity and treatment respectively in mice (*Mus musculus*) for 30 days. Compared to the control group, liver marker enzymes i.e., Serum Glutamic Pyruvate Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), and Alkaline Phosphatase (ALP) levels were significantly increased in the arsenic-induced group. On the other hand, co-treatment with allicin significantly recovered liver enzyme parameters to normal levels ($p<0.05$). Decreased weight of testis, sperm count, and increased numbers of Sperm Head Anomalies (SHA) indicate reduced reproductive potential in the arsenic-induced group of male albino mice. On the contrary, co-treatment with allicin significantly increased testis weight, sperm count and decreased SHA count ($p<0.05$). On examining histological slides of the liver and testis, normal histo-architecture was observed in both control and arsenic-induced allicin co-treated groups; whereas damage was observed in the arsenic-induced group. Generated data pointed out that allicin offers significant protection to the mammalian liver and male gonad (testis) against arsenic-induced toxicity.

**Keywords:** Allicin, Arsenic Toxicity, HPLC, Hepatoprotective, Liver histology, Testis Histology

**Author for correspondence*

Article Received on: 16.06.2023 Revised on: 01.09.2023 Accepted on: 03.09.2023

# 1. Introduction

With its wide distribution and biomagnification power, Arsenic (As), a non-essential trace element, intimated itself as the most dangerous and hazardous to human health. Continuous infiltration of arsenic from different sources viz. mining, factory effluents, bedrock leaching, etc. in water bodies causes severe damage to the biological system. Nevertheless, accidental acute or chronic exposures to moderate or high levels of arsenic can cause liver and testicular injuries, sometimes persisting long after the exposure. Arsenic pollution in drinking water has been reported in different regions of the world, with the majority of reports coming from China (WHO, 1996). In India, arsenic-contaminated drinking water has been widely found in different regions of West Bengal due to the dissolution of arsenic from arsenic-containing bedrock. The prescribed provisional guideline value of the WHO is 10µg/l for arsenic in drinking water. According to the Indian Standard Drinking Water Specification Act of 1991, the desirable limit is 50µg/l without any relaxation. Early clinical symptoms of acute intoxication include abdominal pain, vomiting, diarrhea, muscular pain, and flushing of the skin. Whereas, chronic exposure due to arsenic-contaminated drinking water includes dermal lesions, peripheral neuropathy, skin cancer, and peripheral vascular disease[1]. Noman A. *et al*., experimentally proved that arsenic showed marked degenerative changes in the histopathology of the liver, kidney, brain, etc. of mice[2]. Oxidative stress plays a vital role in arsenic-induced adverse effects on the liver of mammal's body[3,4]. Machado-Neves M., reported that in males, arsenic causes a reduction in testicular weight and alterations in spermatogenesis[5]. To combat arsenic-induced toxicity, conventional chelation therapy using Meso-2,3-Dimercaptosuccinic Acid (DMSA), British Anti Lewisite (BAL), Calcium Disodium Ethylenediamine ($CaNa_2EDTA$), etc., are causing various side effects such as vomiting, diarrhoea, weakness, nausea, stomach pain, dehydration, anaemia, etc[6]. In Bangladesh, the clinical trial of chelation therapy with DMSA was conducted on patients chronically exposed to arsenic. This study was found to be ineffective[7]. The main drawbacks of chelation therapy are a short active period, various side effects, high cost, and a dose that must be cautiously calculated by trained medical practitioners based on pathological reports, etc. Considering the intensity of arsenic toxicity and limitations of conventional chelation therapy, it is high time to find a less expensive, easily available natural remedy without any side effects to combat arsenic-induced hepatotoxicity and male infertility-related problems. In various research studies, natural herbs have been proven effective in remediating heavy metal toxicity[8,9].

Garlic (*Allium sativum)*, a well-known spice, is commonly used in the preparation of traditional medicine[10]. Allicin is the main bioactive compound present in the garlic. The medicinal value of allicin is immense due to its antioxidative potential[11]. In the research article by Lawson, it has been reported that Allicin Bioavailability or Bioequivalence (ABB) is unknown and in question at the time of consumption of raw garlic[12]. Allicin is formed from alliin in the presence of garlic alliinase enzyme. This transformation usually occurs after consumption under enzyme-inhibited gastrointestinal conditions. So, it can be stated that treatment with allicin rather than using raw garlic assures the quantity of allicin taken. This article focuses on the isolation and quantification of allicin using Thin-Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) methods. Simultaneously an *in vivo* study with *Swiss* albino mice (*Mus musculus*) has been planned to investigate the protective role of allicin on liver and male reproductive organs (testis) over arsenic trioxide-induced oxidative stress. This study also paved a path for the natural way to treat arsenic toxicity against conventional chelation therapy with various side effects[13].

# 2. Materials and Method

## 2.1 Chemical

SGOT, SGPT, ALP kit Reckon HIMEDIA, Arsenic Trioxide ($As_2O_3$), Bovine Serum Albumin (BSA), Giemsa, Allicin standard, Syringe filter (0.45µm), TLC plate.

## 2.2 Garlic Extract Preparation

The outer skin of garlic cloves was peeled measuring 20gm in weight. Garlic cloves were added with 20ml ethanol and were ground in a grinder to avoid heat transfer. The mixture was initially filtered with the Whatman Filter Paper. Again, the sample was filtered through a 0.45µm filter membrane and stored in a falcon tube for HPLC analysis.

## 2.3 Allicin Detection by TLC

The Thin Layer Chromatography Method (TLC) is a very sensitive, effective, and quick analytical method. The procedure for the preliminary detection of allicin in an ethanolic solution of garlic was performed according to Kanaki *et al*[14].

## 2.4 Allicin Quantification and Isolation by HPLC

Allicin standard was bought from the registered vendor. 0.5% standard ethanolic allicin solution was prepared to use as the standard for HPLC. For HPLC, $C_{18}$ Column was used. In the ratio of (50:50), methanol and water were used as the mobile phase, the injection volume was kept at 10µl, the column temperature was maintained at 40°C, and the total running time was 25 min. A single injection of ethanol was used as a blank. Using a peak versus concentration graph, a linearity curve for allicin was prepared. Linear regression analysis was performed ($r^2 \geq 0.99$).

## 2.5 Experimental Design

The experiment was conducted on 12 *Swiss* albino male mice (*Mus musculus*) three weeks old and 20-22 gm each. Animals were bought from the registered vendor of Vidyasagar University (Saha Enterprise, 386/2, Nilachal, Birati, Kolkata-700051, CPCSEA REGD. NO.1328/PO/Bt/S/15/CPCSEA). The mice were kept in a 10-day acclimatisation period before the experiment was conducted. Those mice were randomized into four following groups (n = 3).

**SET I:** Normal (Normal food + Water *ad libitum*)

**SET II:** Allicin-treated group (Normal food + allicin + Water *ad libitum*)

**SET III:** Arsenic induced group (Normal food + Water *ad libitum* + $As_2O_3$)

**SET IV:** Arsenic induced allicin treated group (Normal food + Water *ad libitum* + $As_2O_3$ + allicin)

Arsenic trioxide ($As_2O_3$) was given at 20mg/kg and allicin was given at 100 mg/kg of body weight to those mice for a treatment period of 30 days. All animals were kept in the animal house facility of Vidyasagar University (Regn. No.: 2013/GO/Re/S/18/CPCSEA) following the protocol approved by the Intuitional Animal Ethics Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Project proposal no. VU/IAEC/CPCSEA/11/6/2022, Dated: 26.04.2022). After the completion of 30 days of the experimental phase, all those mice were fasted overnight and sacrificed using Ketamine anesthesia. Blood samples were collected directly from their hearts by puncturing with a hypodermic needle. To collect blood serum, blood samples were centrifuged at 10,000 RPM for 10min. at 4°C and the supernatant was collected for serum assay. Mature sperms were collected by squeezing out the epididymis and were collected in a watch glass in 1ml double distilled water. After that, the sperm samples were kept in different micro centrifuge tubes and were stored at 4°C.

## 2.6 Liver Function Test

### *2.6.1 Liver Protein Estimation*

A part of liver tissue of all experimental groups were washed with ice-cold saline and homogenized using the phosphate buffer (pH 7.4). Total protein of different sets of mice liver were estimated form liver homogenates by Lowry's Method using BSA standard curve.

### *2.6.2 Liver Marker Enzymes Assay*

The marker enzymes which are used for testing the functioning of liver are Serum Glutamic Pyruvate Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT) and Alkaline Phosphatase (ALP). These parameters were measured spectrophotometrically by conventionally used experimental kits of Reckon purchased from HIMEDIA.

## 2.7 Reproductive Potential Test

### *2.7.1 Sperm Count*

Sperm count is a technique for detecting fertility. When sperm count becomes less, the animal is called infertile i.e., unable to produce offspring. The sperm suspension was taken in Neubaur's chamber and counted under the microscope (400x or 100x magnification). The equation which is followed to convert the counts in 5 squares to concentration/ml is:

$$\text{Concentration/ml} = (\text{Dilution factor}) \times (\text{Count in 5 squares}) \times (0.05 \times 10^6)$$

### *2.7.2 Sperm Head Anomalies (SHA)*

SHA is a special kind of test to observe sperms and to analyze their head structure. It was done to compare

normal and abnormal head-shaped sperms and to know the effects of arsenic as well as allicin on sperm cells. 50µl sperm suspension was taken in a grease-free slide and a smear was prepared. After air drying, the slides were stored for later staining and SHA counting. Slides for SHA were stained with crude Giemsa (Triple filtered) and after keeping it undisturbed for 40min., the slides were observed under a microscope (40x or 10x magnification).

### 2.8 Histopathological Study

For histology, tissues of the liver and testis were collected from different sets and were subjected to paraffin wax block. With the help of microtome these blocks were sectioned and collected on grease free slide. After that the slides were double stained with Haematoxylin and Eosin (H and E) and were mounted. Thereafter, they were examined under a microscope to assess the damage done to the liver and testis.

### 2.9 Statistical Analysis

To draw statistical significance between the collected data; one-way ANOVA was performed in combination with descriptive statistics by using SPSS software. These data were analyzed at a significance level of 0.05.

## 3. Results

### 3.1 Allicin Detection by TLC

The TLC silica gel plate when observed under a UV light chamber, showed that the standard i.e., Alanin, an amino acid with a similar chemical formula to that of allicin, produced a blue patch at 5.1cm from the bottom of the gel plate ($R_f$ value 0.927). On the contrary, the ethanolic extract of fresh garlic showed a blue patch almost at the same level covering a distance of 5cm ($R_f$ value of 0.909). The results demonstrate that the active component of the ethanolic extract of garlic is nothing but allicin (Figure 1).

$R_f$ value of standard alanine: $R_f = 5.1/5.5 = 0.927$

$R_f$ value of ethanolic extract of fresh garlic: $R_f = 5/5.5 = 0.909$

### 3.2 Allicin Quantification and Isolation by HPLC

HPLC technique is not only useful in isolation but also helps in the quantification of allicin concentration in ethanolic extract of garlic. At 18.787min retention time, standard allicin showed a single main peak with an 18160126 area and 1460749 heights (Figure 2) whereas the ethanolic solution of garlic showed two major peaks at 18.775min and 19.212min retention time (Figure 3). As the component in sample solution showed a retention time of 18.775min (area 28259243) which can be considered as perfectly compatible with the retention time of standard allicin i.e., 18.787min. Hence, the unknown component at the retention time of 18.775 min in the garlic sample is nothing but allicin. The concentration of allicin in the sample can be calculated by comparing the known

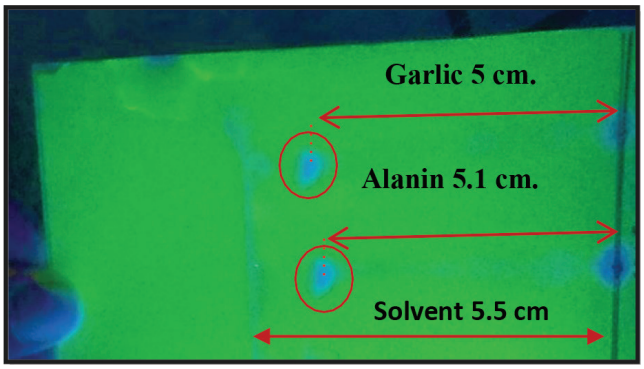



**Figure 1.** TLC plate under UV light.

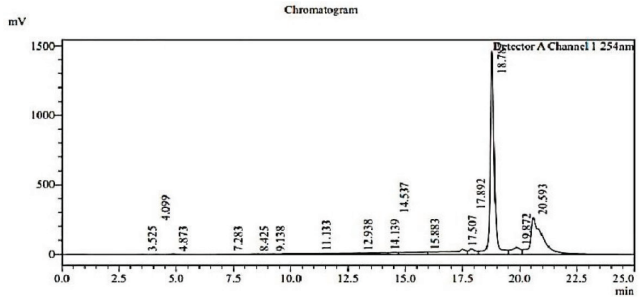



**Figure 2.** HPLC graph of standard allicin showing a peak at Ret. Time 18.787min.

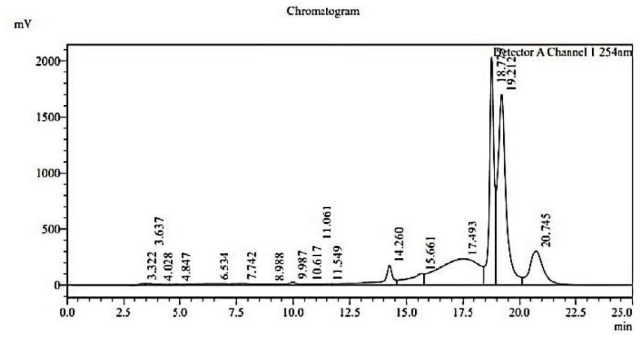



**Figure 3.** HPLC graph of ethanolic extract of garlic showing a peak at Ret. Time 18.775 min.

concentration and area covered by the peak of the known allicin standard with that of the area covered by the peak of the unknown concentration of allicin in the garlic sample. In this way, the concentration of allicin in the sample is calculated and found to be 77.80%.

## 3.3 Measurement of the Liver Weight of the Experimental Animal

The mean liver weight of the arsenic-induced mice group ($p<0.001$, one-way ANOVA) was significantly decreased up to 26.43% as compared to the normal control group. In the arsenic-induced allicin co-treated group, liver weight increased to the extent of 28.78% compared to the arsenic-treated group (Figure 4).

## 3.4 Measurement of Total Protein of Liver of Experimental Animal

Result of the total protein estimation of the arsenic-treated group indicates a 52.34% reduction as compared to the normal control group whereas an 80.25% increase in total protein is observed in the arsenic-induced allicin-treated group as compared to the arsenic-treated group which indicates ameliorative power of allicin ($p<0.000$, one-way ANOVA).

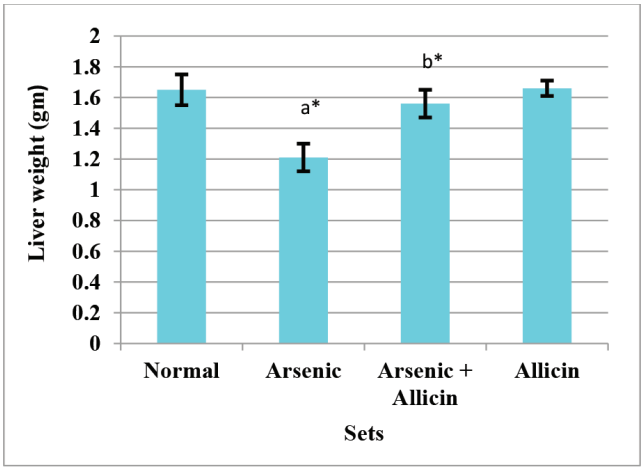



**Figure 4.** Result of the liver weight of different sets. 'a' significant with the control group, 'b' significant with the arsenic-treated group. Data are represented in mean ± SEM (* significant at 0.05 level).

## 3.5 Liver Enzymes Assay Result

In the arsenic-induced group; elevated activities of SGOT, SGOT and ALP enzymes to the extent of 29.60%, 43.64%, and 119.22% respectively are observed compared to normal control group whereas in the arsenic-induced allicin co-treated group; significant reduction in SGOT, SGPT and ALP level of 16.50%, 25.98% and 41.22% respectively are observed ($p < .000$, one-way ANOVA) (Table 1).

## 3.6 Testis Weight

Comparing the difference in weight of testis, 59.61% reduction in arsenic induced group is observed as compared to the normal group. 106.34% increase in the weight of testis is observed in arsenic-induced allicin co-treated group ($p < 0.000$, one-way ANOVA).

## 3.7 Sperm Count

In arsenic-exposed mice, sperm count decreased 64.27% as compared to normal control group whereas allicin co-treatment significantly recovered the sperm count by 144.9% compared to the arsenic-treated group ($p < 0.000$, one-way ANOVA).

## 3.8 SHA Results

In the case of SHA result, arsenic exposure increased sperm head abnormalities by 428.7% as compared to normal. Whereas in the arsenic + allicin group, sperm head abnormalities is found to increase by only 169.75% as compared to the normal control group ($p < 0.000$, one-way ANOVA).

**Table 1.** Showing liver SGPT, SGOT and ALP results. 'a' significant with control group, 'b' significant with the arsenic-treated group. Data are represented in mean ±SEM (*significant at 0.05 level)

| Experiments | Normal | Arsenic | Arsenic + Allicin | Allicin |
|---|---|---|---|---|
| SGOT (IU/L) | 58.49 ± 1.15 | 75.81 $^{a*}$ ± 1.53 | 63.29 $^{b*}$ ± 1.15 | 56.45 ± 2.01 |
| SGPT (IU/L) | 66.63 ± 0.90 | 95.726 $^{a*}$ ± 2.92 | 70.85 $^{b*}$ ± 1.65 | 66.64 ± 1.81 |
| ALP (IL/L) | 15.67 ± 1.04 | 34.36 $^{a*}$ ± 3.61 | 20.19 $^{b*}$ ± 1.38 | ± 1.38 |

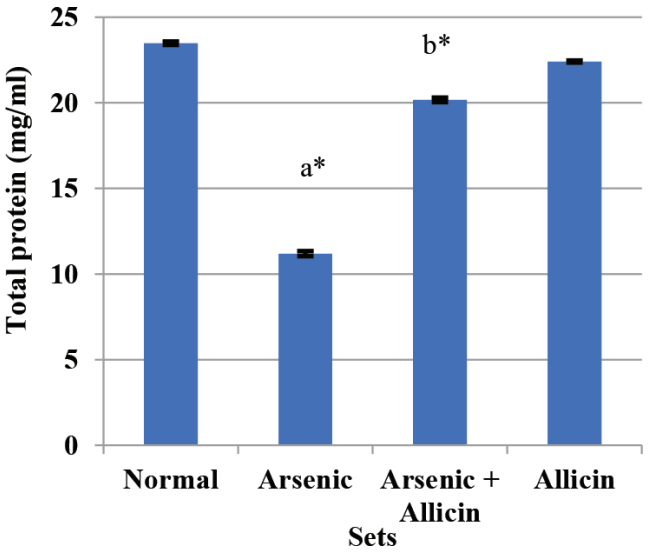



**Figure 5.** Result of total protein of liver of different sets. 'a' significant with control group, 'b' significant with arsenic-treated group. Data are represented in mean ± SEM (* significant at 0.05 level).

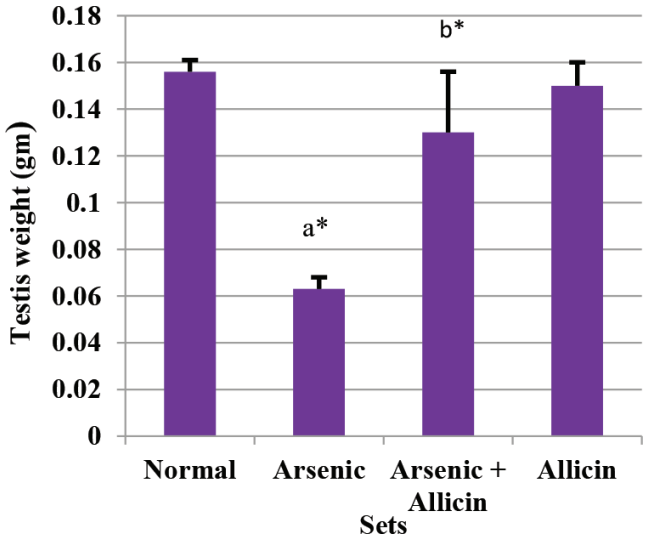



**Figure 6.** Result of testis weight (gm) of different sets. 'a' significant with the control group, 'b' significant with the arsenic-treated group. Data are represented in mean ± SEM (* significant at 0.05 level).

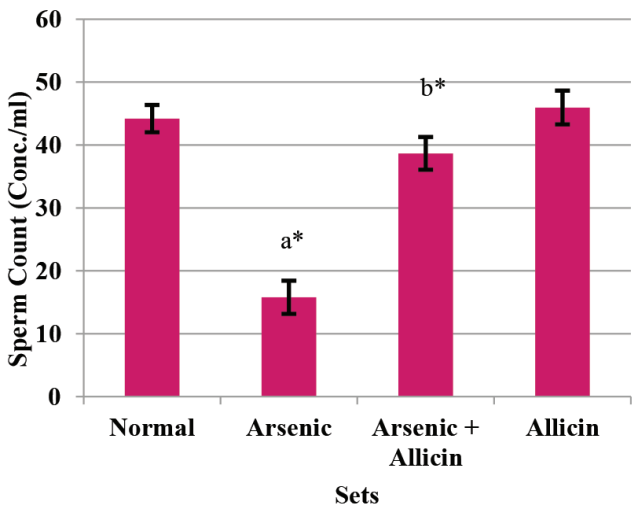



**Figure 7.** Result of sperm count (Concentration/ml) of different sets. 'a' significant with the control group, 'b' significant with the arsenic-treated group. Data are represented in mean ± SEM (* significant at 0.05 level).

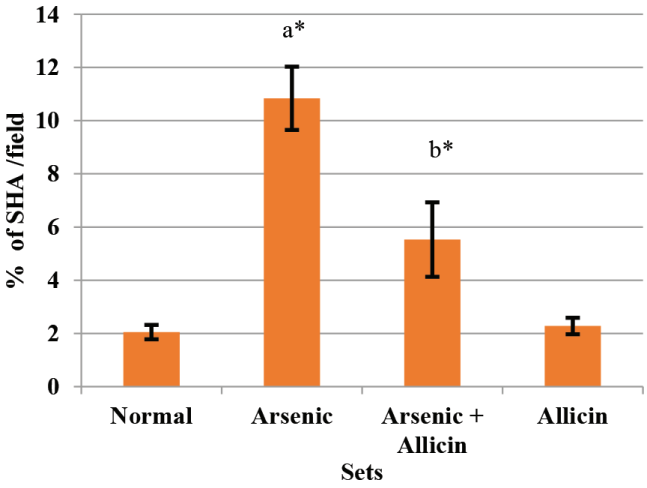



**Figure 8.** Showing % of SHA/field results of different mice sets. 'a' significant with the control group, 'b' significant with the arsenic-treated group. Data are represented in mean ± SEM (*significant at 0.05 level).

## 3.9 Histopathological Analysis of Liver

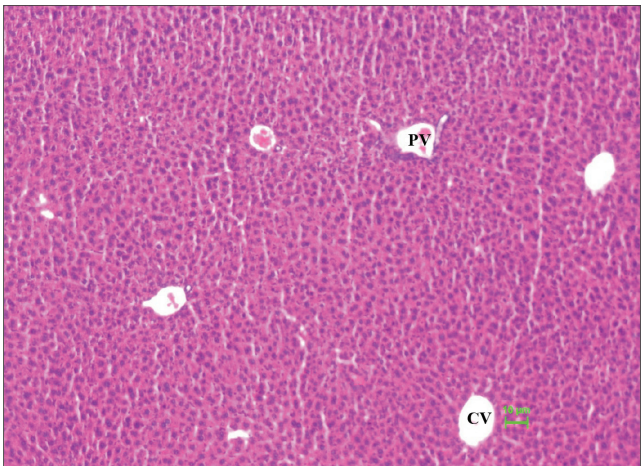



**Figure 9(a).** Photomicrographs of representative sections of Liver of *Swiss* albino mice from Normal group (100 magnification), here CV and PV represent central and portal vein respectively. Note: Normal cytoarchitecture.

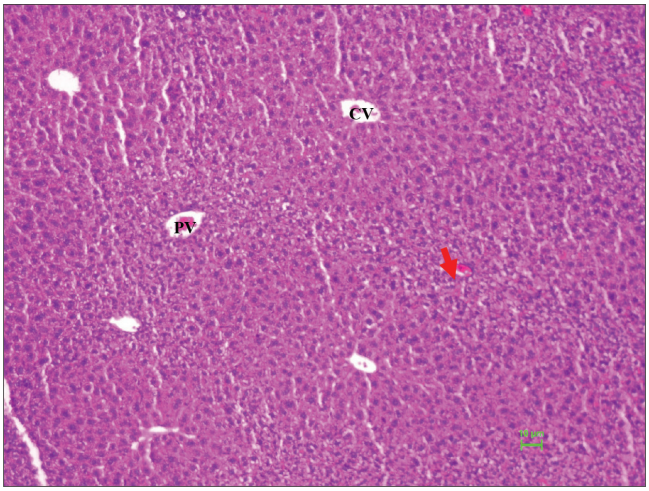



**Figure 9(b).** Photomicrographs of representative sections of the Liver of *Swiss* albino mice from Arsenic induced group (100 magnification), here red arrow represents haemorrhage, CV represents central vein, and PV represents portal vein. Note: Loss of cytoarchitecture.

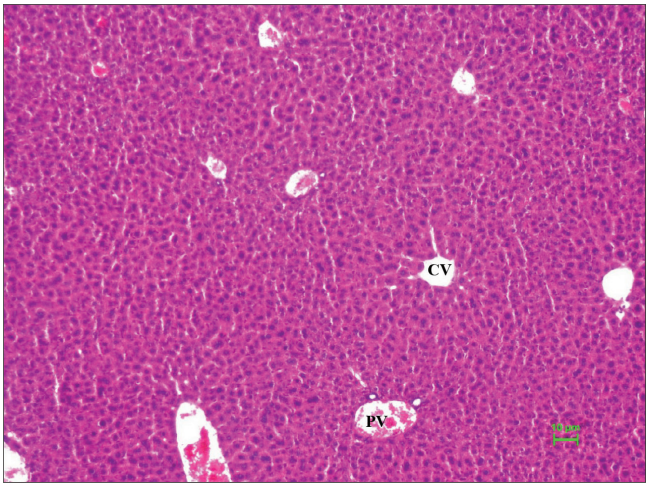



**Figure 9(c).** Photomicrographs of representative sections of Liver of *Swiss* albino mice from Arsenic induced allicin treated group (100 magnification), here CV and PV represent central and portal vein. Note: Normal cytoarchitecture.

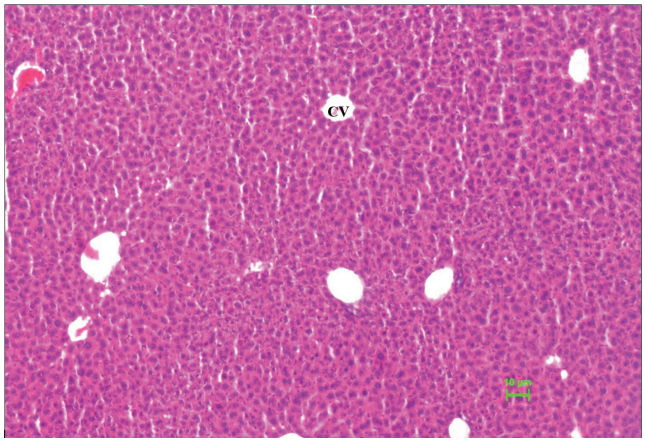



**Figure 9(d).** Photomicrographs of representative sections of the Liver of *Swiss* albino mice from only allicin treated group (100 magnification), here CV represents the central vein. Note: Normal cytoarchitecture.

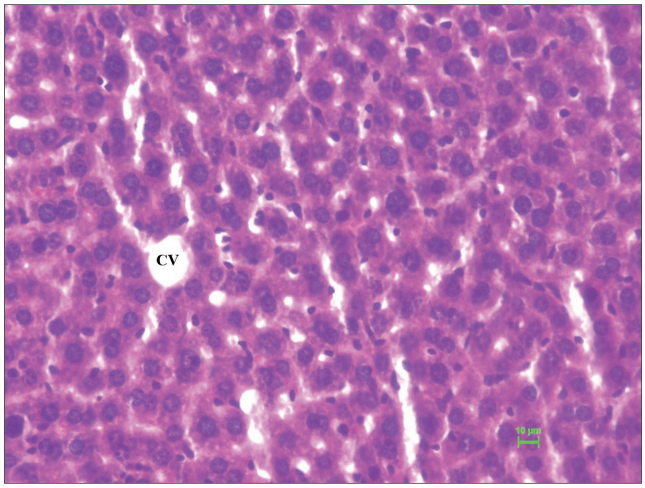



**Figure 9(e).** Photomicrographs of representative sections of the Liver of *Swiss* albino mice from Normal group (400 magnification), here CV represents central vein. Note: Normal cytoarchitecture.

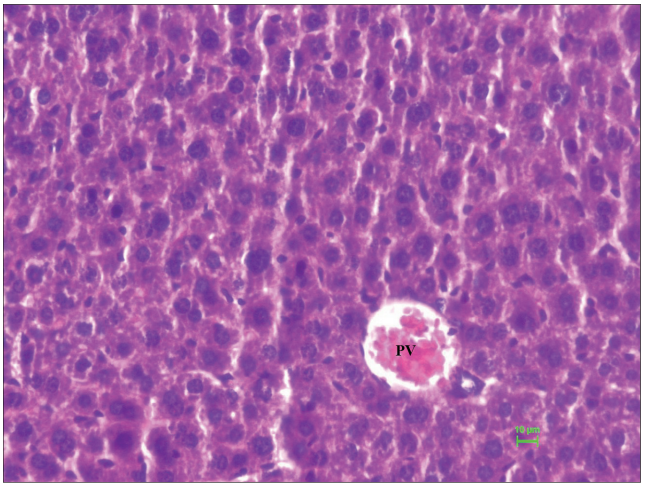



**Figure 9(g).** Photomicrographs of representative sections of the Liver of *Swiss* albino mice from Arsenic induced allicin-treated group (400 magnification), here PV represents portal vein. Note: Normal cytoarchitecture.

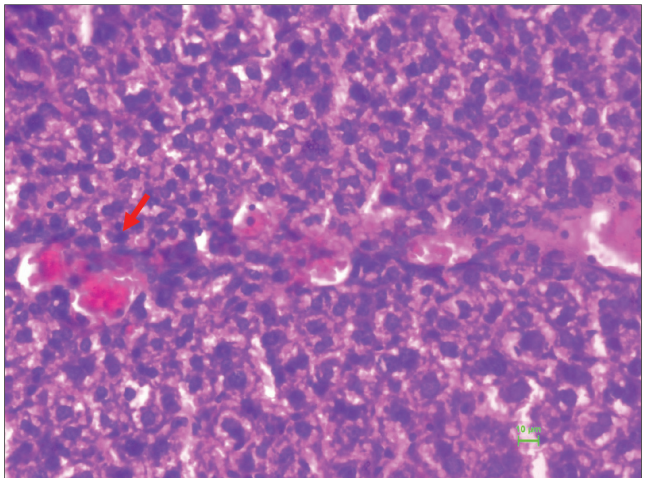


**Figure 9(f).** Photomicrographs of representative sections of the Liver of *Swiss* albino mice from Arsenic induced group (400 magnification), here red arrow represents haemorrhage. Note: Loss of cytoarchitecture.

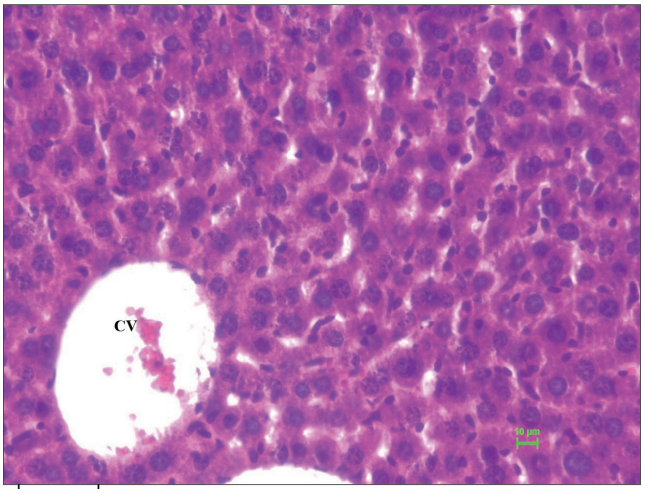



**Figure 9(h).** Photomicrographs of representative sections of the Liver of *Swiss* albino mice from Allicin treated group (400 magnification), here CV represents the central vein. Note: Normal cytoarchitecture.

## 3.10 Histopathological Analysis of Testis

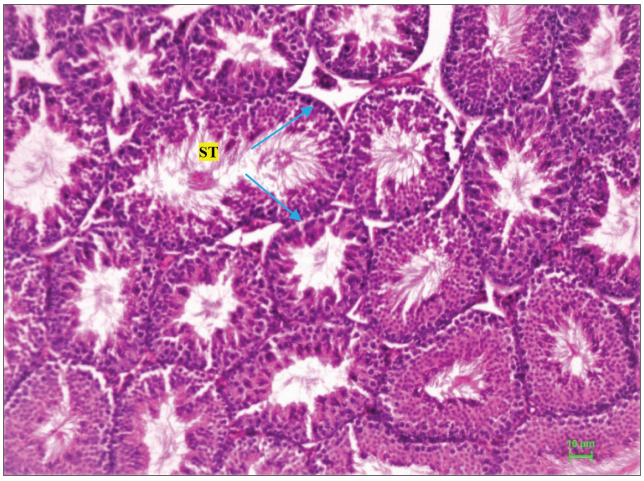



**Figure 10(a).** Photomicrographs of representative sections of Testis of *Swiss* albino mice from Normal group (100 magnification), here ST represents healthy seminiferous tubules. Note: Normal cytoarchitecture.

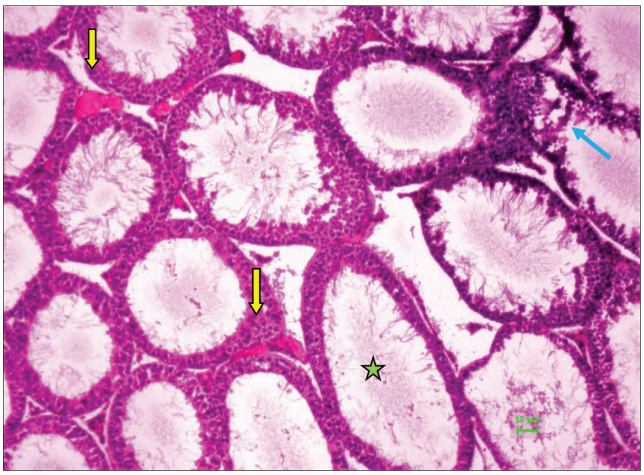


**Figure 10(b).** Photomicrographs of representative sections of Testis of *Swiss* albino mice from Arsenic induced group (100 magnification), here yellow and blue arrows indicate haemorrhage and membranous damage, green star indicates lumen less mature spermatozoa. Note: Loss of cytoarchitecture.

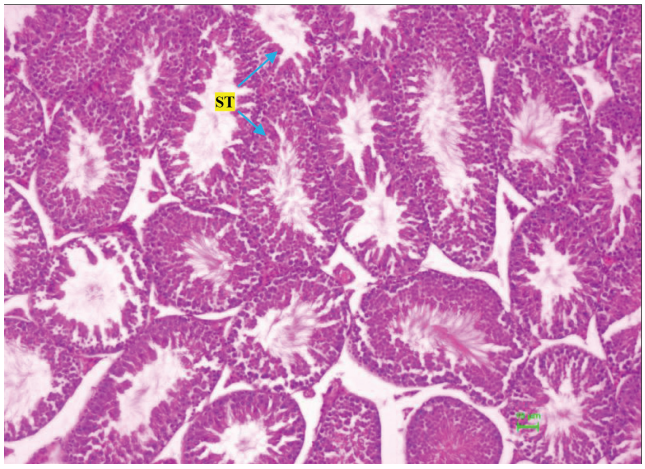



**Figure 10(c).** Photomicrographs of representative sections of Testis of *Swiss* albino mice from Arsenic induced Allicin treated group (100 magnification), here ST represents healthy seminiferous tubules. Note: Normal cytoarchitecture.

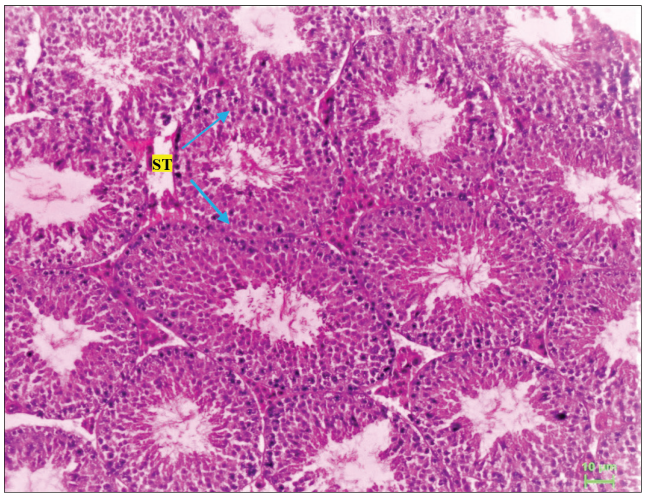



**Figure 10(d).** Photomicrographs of representative sections of Testis of *Swiss* albino mice from Allicin treated group (100 magnification), here ST represents healthy seminiferous tubules. Note: Normal cytoarchitecture.

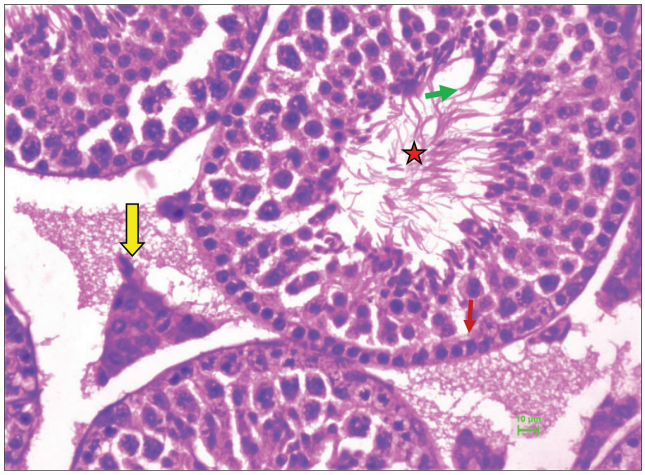


**Figure 10(e).** Photomicrographs of representative sections of Testis of *Swiss* albino mice from Normal group (400 magnification), here brown arrow indicates healthy spermatogonia, green arrow and red star indicate spermatozoa and healthy lumen, yellow arrow indicates Leydig cell. Note: Normal cytoarchitecture.

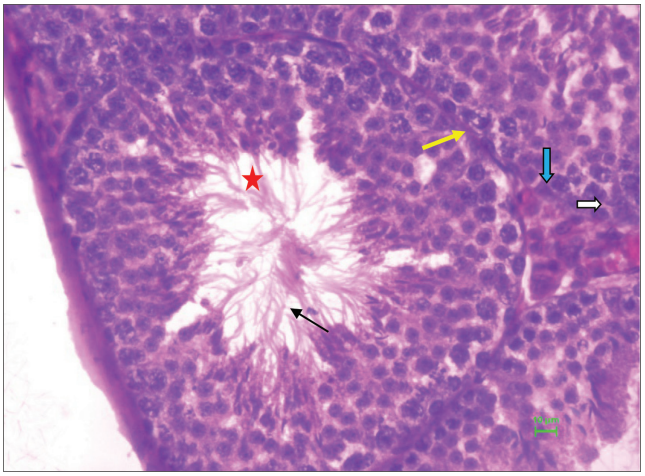


**Figure 10(g).** Photomicrographs of representative sections of Testis of *Swiss* albino mice from Arsenic induced Allicin treated group (400 magnification), here, white and blue arrow indicate hemorrhage and Leydig cell respectively. Black and yellow arrows indicate spermatozoa and spermatogonia respectively, red star indicates healthy lumen. Note: Normal cytoarchitecture.

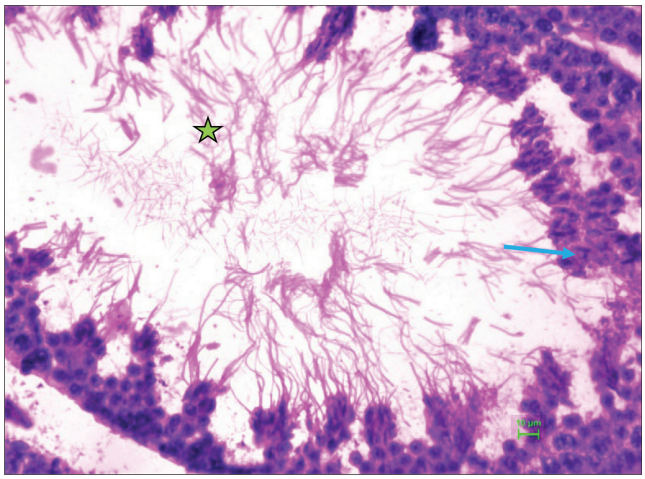
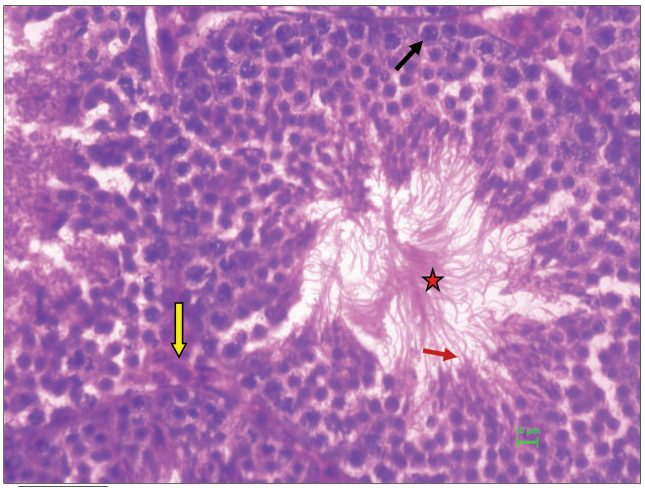


**Figure 10 (f).** Photomicrographs of representative sections of Testis of *Swiss* albino mice from Arsenic induced group (400 magnification), here green star indicates a lumen with very less spermatozoa, blue arrow indicates membranous damage. Note: Loss of cytoarchitecture.



**Figur 10(h).** Photomicrographs of representative sections of Testis of *Swiss* albino mice from Allicin treated group (400 magnification), here yellow, brown, and black arrows indicate Leydig cell, spermatozoa, and spermatogonia respectively, red star indicates healthy lumen. Note: Normal cytoarchitecture.

## 4. Discussion

Arsenic, a carcinogenic toxic metalloid to animals enters into the environment through anthropogenic activities. This study mainly focuses on the ameliorative potential of allicin fraction in an *in vivo* animal model against arsenic-induced toxicity. For this study, after the preliminary identification by the TLC method, the allicin fraction was collected by the HPLC method. Lee *et al.,* used the HPLC to identify allicin in garlic and reported its presence at 18.3 min which conforms with the present study[15]. By comparing the known concentration of standard and area with that of our unknown sample, the calculated concentration is 77.80%. The study of Liang *et al.*, using the HPLC method also reported that 70–80% allicin is present in garlic which supports the result of the present study[16].

In the case of arsenic toxicity, the liver acts as the primary organ that tries to detoxify the body. But during this process, increasing toxicity causes liver tissue degeneration[17]. Arsenic toxicity causes liver tissue damage which is established in the histological slides of the liver of arsenic-induced set shown in Figures 9(b), 9(f) above. Reactive Oxygen Species (ROS) generated by arsenic toxicity are the main cause of liver damage[18]. Moreover, arsenic toxicity-induced stress not only reduces the sperm count (Figure 7) but also increases sperms with a bent head, abnormal acrosomal process, etc. (Figure 8). Considering the histological slides of the testis of the arsenic-induced group, loss of cytoarchitecture indicates damage in the testis shown in Figures 10(b), 10(f). Chang SI *et al.*,[19] experimented on mouse testis induced with arsenic toxicity and Shangloo P. *et al.*,[20] experimented on albino rats induced with arsenic toxicity and reported marked distortion and degeneration of seminiferous tubules and germ cell lineage which also conforms to the findings of this study. Anti-microbial, anti-cancer, anti-inflammatory activities and cardiovascular protective roles of allicin are reported in many research papers[21-24]. From the present study, the hepatoprotective power of allicin against arsenic-induced toxicity can be proved by considering the significant recovery of liver marker enzymes to normal levels. Furthermore, considering the histological liver slides Figures 9(c), 9(g) of the arsenic-induced allicin co-treated group and the increase in total protein content (Figure 5) further supports the ameliorative power of allicin. Attia T *et al.*, 2006 in their research work reported that the carbon tetrachloride ($CCl_4$) induced hepatic injury in rats can be significantly treated with allicin[25]. The findings of their research work also support our findings regarding the hepatoprotective role of allicin. Accordingly, increasing sperm count (Figure 7), testis weight (Figure 6), a significant decrease in anomalies of sperm heads (Figure 8) and histological slides of the testis of the 'arsenic + allicin' group as shown in Figures 10(c), 10(g) which proves that allicin provides significant protection to male gonad (testis) from arsenic induced toxicity. To explain the reason behind the ameliorative power of allicin, three main reasons can be highlighted. The first reason is the chelation power of the sulphur content of allicin. J. Songsungkan *et al.*, reported the chelation capacity of allicin against copper, cobalt, and lead ($Cu^{2+}$, $Co^{2+}$, $Pb^{2+}$) heavy metals. He concluded that organosulfur plays the synergic role of both an antioxidant and a metal chelator[26]. On reacting with GSH and cysteine, allicin produces SAMG (S-Allylmercapto-Glutathione) and SAMC (S-Allylmercapto-Cysteine) respectively. SAMG is a powerful antioxidant derivative of allicin[27]. Being hydrophobic, allicin can efficiently cross the cellular membranes without inducing leakage, fusion, or aggregation of the membrane. This property of allicin greatly enhances its intracellular interaction capacity with internally entrapped thiol-containing compounds such as Glutathione (GSH) or 2-Nitro-5-Thiobenzoate (NTB)[28]. Liu H *et al.*, experimentally proved that allicin inhibits ROS generation, and reduces lipid peroxidation by preserving the endogenous antioxidant enzyme activities[29]. By suppressing mitochondrial dysfunction, decreasing Mitochondrial Membrane Protein (MMP) collapse, Cytochrome-c release, preserving mitochondrial ATP synthesis, and promoting mitochondrial $Ca^{2+}$ buffering capacity, allicin protects against oxidative stress.

## 5. Conclusion

From the current research work, it can be concluded that allicin provides significant protection over arsenic-induced severe liver and male gonadal degeneration. Although human trial with allicin is required further to evaluate the potential of allicin to ameliorate arsenic toxicity, still it can be said that treatment with allicin ameliorates arsenic-induced toxicity and can be used without prior pathological report.

## 6. Acknowledgements

The authors wish to express their heartfelt gratitude to all the faculty members of the Department of Zoology,

Vidyasagar University; fellow research scholars, and staff for their assistance in carrying out this research project smoothly. We also thank the Bose Institute (Sector-V, Salt Lake, West Bengal, India) for providing the facility of HPLC machines and their assistance in carrying out this research project smoothly. We also are thankful to Dr. Kalyani Naskar and Dr. M. C. Banerjee for their valuable evaluation of the histological slides.

## 7. References

1. 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; 29(3):281-313. https://doi.org/10.1080/10643389991259227
2. Noman AS, Dilruba S, Mohanto NC, Rahman L, Khatun Z, Riad W, Al Mamun A, Alam S, Aktar S, Chowdhury S, Saud ZA, Rahman Z, Hossain K, Haque A. Arsenic-induced histological alterations in various organs of mice. J Cytol Histol. 2015; 6(3):323. PMid: 26740907. PMCid:PMC4698904. https://doi.org/10.4172/2157-7099.1000323
3. Flora SJ, Kumar P, Kannan GM, Rai GP. Acute oral gallium arsenide exposure and changes in certain hematological, hepatic, renal and immunological indices at different time intervals in male Wistar rats. Toxicol Lett. 1998; 94(2):103-13. PMid: 9574807. https://doi.org/10.1016/S0378-4274(97)00110-0
4. Rahman MM, Chowdhury UK, *et al*. Chronic arsenic toxicity in Bangladesh and West Bengal, India - A review and commentary. J Toxicol Clin Toxicol. 2001; 39(7):683-700. PMid: 11778666. https://doi.org/10.1081/CLT-100108509
5. Machado-Neves M. Arsenic exposure and its implications in male fertility. Anim Reprod. 2023; 19(4):e20220119. PMid: 36819483. PMCid: PMC9924305. https://doi.org/10.1590/1984-3143-ar2022-0119
6. Flora SJ, Pachauri V. Chelation in metal intoxication. Int J Environ Res Public Health. 2010; 7(7):2745-88. PMid: 20717537. PMCid: PMC2922724. https://doi.org/10.3390/ijerph7072745
7. Mazumder DNG, Ghoshal UC, Saha J, Santra A, De BK, Chatterjee A, Dutta S, Angle CR, Centeno JA. Randomized placebo-controlled trial of 2,3-dimercaptosuccinic acid in therapy of chronic arsenicosis due to drinking arsenic-contaminated subsoil water. J Toxicol Clin Toxicol. 1998; 36(7):683-90. PMid: 9865236. https://doi.org/10.3109/15563659809162616
8. Tandan N, Roy M, Roy S. Ameliorative potential of *Psidium guajava* on hemato-biochemical alterations in arsenic-exposed wistar rats. Toxicol Int. 2012; 19(2):121-4. PMid: 22778508. PMCid: PMC3388754. https://doi.org/10.4103/0971-6580.97199
9. Salmataj SA, Bhat P, Kamath S. Rutin a citrus flavonoid attenuates sodium arsenate induced cytotoxicity in CHO cells. Toxicol Inter. 2020; 27(3):188-92. https://doi.org/10.18311/ti/2020/v27i3&4/26077
10. Rana SV, Pal R, Vaiphei K, Sharma SK, Ola RP. Garlic in health and disease. Nutr Res Rev. 2011; 24(1):60-71. PMid: 24725925. https://doi.org/10.1017/S0954422410000338
11. Salehi B, Zucca P, Orhan IE, Azzini E, Adetunji CO, Mohammed SA, *et al*. Allicin and health: A comprehensive review. Trends in Food Science and Technology. 2019; 86:502–16. https://doi.org/10.1016/j.tifs.2019.03.003
12. Lawson LD, Hunsaker SM. Allicin bioavailability and bioequivalence from garlic supplements and garlic foods. Nutrients. 2018; 10(7):812. PMid: 29937536. PMCid: PMC6073756. https://doi.org/10.3390/nu10070812
13. Flora SJ, Pachauri V. Chelation in metal intoxication. Int J Environ Res Public Health. 2010; 7(7):2745-88. PMid: 20717537. PMCid: PMC2922724. https://doi.org/10.3390/ijerph7072745
14. Kanaki NS, Rajani M. Development and validation of a thin-layer chromatography-densitometric method for the quantitation of alliin from garlic (*Allium sativum*) and its formulations. Journal of AOAC International. 2005; 88(5):1568-70. https://doi.org/10.1093/jaoac/88.5.1568
15. Lee J, Gupta S, Huang JS, Jayathilaka LP, Lee BS. HPLC-MTT assay: Anticancer activity of aqueous garlic extract is from allicin. Anal Biochem. 2013. https://doi.org/10.1016/j.ab.2013.01.033
16. Liang Y, Zhang JJ, *et al*. Release test of alliin/alliinase double-layer tablet by HPLC-Allicin determination. J Pharmaceut Analys. 2013. https://doi.org/10.1016/j.jpha.2012.08.001
17. Liu J, Waalkes MP. Liver is a target of arsenic carcinogenesis. Toxicol Sci. 2008; 105(1):24-32. PMid: 18566022. PMCid: PMC2734307. https://doi.org/10.1093/toxsci/kfn120
18. Muthusamy VR, Kannan S, Sadhaasivam K, Gounder SS, Davidson CJ, Boeheme C, Hoidal JR, Wang L, Rajasekaran NS. Acute exercise stress activates Nrf2/ARE signaling and promotes antioxidant mechanisms in the myocardium. Free Radic Biol Med. 2012; 52(2):366-76. PMid: 22051043. PMCid: PMC3800165. https://doi.org/10.1016/j.freeradbiomed.2011.10.440
19. Chang SI, Jin B, Youn P, Park C, Park JD, Ryu DY. Arsenic-induced toxicity and the protective role of ascorbic acid in mouse testis. Toxicology and Applied Pharmacology. 2007; 218(2):196–203. https://doi.org/10.1016/j.taap.2006.11.009

20. Shangloo P, Arban Kumar DM, Gupte B. Histopathological effects of arsenic on testes of adult male albino rats. an experimental study. Journal of Pharmaceutical Negative Results. 2022; 9340-4. https://doi.org/10.1016/j.tifs
21. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes Infect. 1999; 1(2):125-9. PMid: 10594976. https://doi.org/10.1016/S1286-4579(99)80003-3
22. Catanzaro E, Canistro D, Pellicioni V, Vivarelli F, Fimognari C. Anticancer potential of allicin: A review. Pharmacol Res. 2022; 177:106118. PMid: 35134476. https://doi.org/10.1016/j.phrs.2022.106118
23. Arreola R, Quintero-Fabián S, López-Roa RI, Flores-Gutiérrez EO, Reyes-Grajeda JP, Carrera-Quintanar L, Ortuño-Sahagún D. Immunomodulation and anti-inflammatory effects of garlic compounds. J Immunol Res. 2015; 2015:401630. PMid: 25961060. PMCid: PMC4417560. https://doi.org/10.1155/2015/401630
24. Liu C, Cao F, Tang QZ, Yan L, Dong YG, Zhu LH, Wang L, Bian ZY, Li H. Allicin protects against cardiac hypertrophy and fibrosis via attenuating reactive oxygen species-dependent signaling pathways. J Nutr Biochem. 2010; 21(12):1238-50. PMid: 20185286. https://doi.org/10.1016/j.jnutbio.2009.11.001
25. Attia MN, Ali MA. Hepatoprotective activity of allicin against carbon tetrachloride-induced hepatic injury in rats. Journal of Biological Sciences. 2006; 6(3):457-68. https://doi.org/10.3923/jbs.2006.457.468
26. Songsungkan J, Chanthai S. Study of allicin extract chelated with some heavy metals ($Cu^{2+}$, $Co^{2+}$ and $Pb^{2+}$) by fluorescence quenching method and its antioxidant activity. Asian J Chem. 2014; 26:132-6. https://doi.org/10.14233/ajchem.2014.15349
27. Trio PZ, You S, He X, He J, Sakao K, Hou DX. Chemopreventive functions and molecular mechanisms of garlic organosulfur compounds. Food Funct. 2014; 5(5):833-44. PMid: 24664286. https://doi.org/10.1039/c3fo60479a
28. Miron T, Rabinkov A, Mirelman D, Wilchek M, Weiner L. The mode of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. Biochim Biophys Acta. 2000; 1463(1):20-30. PMid: 10631291. https://doi.org/10.1016/S0005-2736(99)00174-1
29. Liu H, Mao P, Wang J, Wang T, Xie CH. Allicin protects $PC_{12}$ cells against 6-OHDA-induced oxidative stress and mitochondrial dysfunction via regulating mitochondrial dynamics. Cell Physiol Biochem. 2015; 36(3):966-79. PMid: 26087780. https://doi.org/10.1159/000430271