ISSN 2581 – 4303

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2018; 9(4):231-236

RESEARCH ARTICLE

Protective Effect of Garlic Extract (*Allium sativum* L.) on the Liver Tissue of Arsenic-Induced *Channa punctatus*

Titikksha Das[1]* and Mamata Goswami[2]

[1]*Department of Zoology, Gauhati University, Guwahati, 781014, Assam* [2]*Department of Zoology, Cotton College, Guwahati, 781001 Assam*

Received: 25 August 2018; Revised: 15 September 2018; Accepted: 20 October 2018

ABSTRACT

Arsenic in its inorganic form is toxic and can cause severe health effects including cancers. In several *in vitro* experiments, arsenic exposure has shown multiple effects at the molecular level. However, the proper understanding of the role of arsenic in the cause of these diseases is still limited. In this work, we demonstrated the toxicity effect of sodium arsenite in the liver tissues of freshwater fish *Channa punctatus* and observed the histopathological as well as surface ultrastructural changes on it. A simultaneous study was performed to observe the protective effect of garlic extract (*Allium sativum* L.) on the liver tissue of arsenic-induced *C. punctatus*. The liver tissue of the control group showed a normal histoarchitecture. The arsenic-exposed liver tissue revealed hepatic lesions in the form of cloudy swelling of hepatocytes, vacuolar degeneration, karyolysis, dilation of sinusoids, and nuclear hypertrophy. Lesser hepatic alternation was observed in the liver tissue of arsenic-exposed *C. punctatus* concurrently treated with garlic extract. The protective effect of garlic was shown in the normalization of hepatocytes in the arsenic-induced liver tissue.

Keywords: *Channa punctatus*, garlic, histology, liver, sodium arsenite

INTRODUCTION

Arsenic occurs due to both geogenic and anthropogenic sources in water, soil, and air. Being a metalloid, it exists in both organic and inorganic forms and different oxidation states (−3,0,+3,+5).[1] The inorganic forms of arsenic, i.e., trivalent and pentavalent forms are considered to be more toxic than organoarsenic. The trivalent form binds to sulfhydryl groups of glycolysis and tricarboxylic acid cycle enzymes inhibiting their pathways, and the pentavalent arsenicals can interfere with the mitochondrial oxidative phosphorylation enzymes.[2,3]

On human population, arsenic exposure is confirmed to cause a wide variety of adverse health conditions. The earliest indication of arsenic toxicity is skin lesions including melanosis and hyperkeratosis which can later lead to skin cancer such as Bowen's disease, basal cell, and squamous cell carcinomas.[4-8] Ingested inorganic arsenic is very likely the causative agent of various other forms of internal cancers which has been confirmed from several evidences.[8,9-11] Therefore International Agency for Research on Cancer (IARC) of World Health Organisation (WHO) has classified arsenic as group 1 carcinoge. Arsenite is known to block the functioning of certain enzymes such as pyruvate dehydrogenase and glutathione reductase due to its high affinity for sulfur-containing ligands.[12] Both in laboratory and field studies histopathological investigations have been long recognized as reliable biomarkers of stress in fish[13] and the evaluation of the health of fish exposed to contaminants. Liver is the primary organ for detoxification of foreign compounds[14] and one of the most affected organs by contaminants in water.[15] Recent experiments are done to find an effective natural antioxidant which can be used for the prevention of heavy metal toxicity. Garlic (*Allium sativum* L.) is known to have many nutritive compounds and antioxidant substances as selenium, sulfur-containing compounds and vitamins (A, B, C, and E). Its ability to eliminate arsenic from blood and soft tissues has been explained in

***Corresponding Author:**
Titikksha Das
E-mail: titikkshadas.89@gmail.com

© 2018, IJPBA. All Rights Reserved

many researches.[2,8,16] The anti-arsenic activities of garlic are due to the presence of biologically active lipophilic sulfur-bearing compounds such as allicin, S-allylycysteine, diallyl-di-sulfide, and diallyl-sulfide.[8,17-19]

The present study carried on the toxicity effect of sodium arsenite in the liver tissue of freshwater fish *Channa punctatus* and observed the histopathological as well as surface ultrastructural changes on it. This work also aimed to study the ameliorative effect of aqueous garlic extract (AGE) in the liver tissue of arsenic-induced fishes. A critical value of the concentration of sodium arsenite has also been evaluated above which fishes are likely to be killed. A commonly useful measure of toxicity LC50 is used for this purpose. The aim of this study was, first, to observe the toxicity effect of arsenic on the histo-architecture of liver tissue and second, to evaluate the protective role of AEG on the ultra-structural changes of arsenic-induced liver tissue.

MATERIALS AND METHODS

Collection and Acclimatization of Fish

For the present study, healthy and disease free fishes *C. punctatus* (weight 22–50 g) were collected from local markets in Guwahati. After disinfection with a dip of 2% potassium permanganate ($KMnO_4$) solution, the fishes were acclimatized in aquaria for 2 weeks before initiation of the experiment. The water provided in the aquaria was from the tap water in the laboratory and was changed on the following day. The fishes were fed every day with fish food available in the market. Proper aeration was done during these periods.

Arsenic Treatment

Sodium arsenite ($NaAsO_2$), molecular weight - 129.91 Merck, India (Ltd.), was procured for performing the experiment. A stock solution was prepared with water from which the test concentration was prepared by dilution. The control group of fishes was kept in similar conditions without adding sodium arsenite. Fishes were exposed to five different concentration of sodium arsenite of 5, 15, 25, 35, and 45 ppm. The toxicity bioassay was performed in a semi-static system in triplicate with 10 specimens exposed for each concentration in each set in accordance with the standard methods of acute toxicity bioassay procedures given by American Public Health Association 2005.

Determination of 96 h - LC 50:

Fishes were transferred to each aquarium and exposed to five different concentrations such as 5, 15, 25, 35, and 45 ppm (parts per million) of sodium arsenite. In all cases, control groups of fishes were maintained. Each experimental trial was carried out for a period of 96 h. The mortality rate of the fish was recorded at logarithmic time intervals that is, after 6, 12, 24, 48, 72, and 96 h of exposure. The test media were renewed daily during the experimental period. The data obtained in the course of the investigation were analyzed statistically to see whether there is any influence of different treatment concentrations on the mortality of the fish.

The mortality rate of *C. punctatus* to different concentration of sodium arsenite can be seen in Table 1. In the present study, it was observed that 45 ppm sodium arsenite in water induced death of all the exposed fishes within 96 h. The 96 h LC50 of sodium arsenite for *C. punctatus* was found to be 25 ppm. Fishes treated with a concentration of 5, 10, and 12 ppm survived for >90 days with zero mortality rates. The sublethal concentration of sodium arsenite for the exposed group of fish was 10% of the LC50, i.e., 2.5 ppm. The method of arsenic treatment to the fishes, the method of determination of 96 h - LC 50 and its evaluation are in accordance with our earlier publication.[20]

Preparation of Garlic Extract

The outer layer of garlic (*A. sativum* L.) cloves was removed, and crushed mechanically in a mortar-

Table 1: Mortality rate of *Channa punctatus* in response to different concentration of sodium arsenite

Concentration of Arsenic (ppm: parts per million)	Mortality (%)	96 h LC 50 value (ppm)
5	0	25
15	20	
25	50	
35	80	
45	100	

pestle with 100 ml of autoclaved distilled water for 1 g of garlic. The homogenate was shaken for 20 min, filtered successively through gauze and 0.22 micron membrane filter to obtain the AGE.[8]

Experimental Design

Six fishes were used for each set and three sets of experiments were carried out for 15 days. First set was taken as control without any dose. Second set was given the sublethal dose 2.5 ppm of sodium arsenite. The third set received the same dose of sodium arsenite mixed with 10 ml of AGE. The water, arsenic and garlic treatments of each plastic pool were changed after 48 h.

Histological Studies in the Target Organs of *C. punctatus* Exposed to Sodium Arsenite

Fishes were randomly selected for histopathological examinations. Liver tissues were isolated from normal and experimental fish. Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissue. They were fixed in aqueous Bouin's solution for 24 h, processed through graded series of alcohols, cleared in xylene, and embedded in paraffin wax. Sections were cut at 4 micron thickness and stained with Hematoxylin and eosin stain. Histopathological lesions were examined and photographed with the help of computer attached Bright Field Microscope (LeicaDM3000).

Scanning Electron Microscopic (SEM) Study

Liver tissues of both the control and the treated groups were rapidly removed and processed routinely for SEM studies. Liver tissues were cut into small pieces of 1 mm thickness and fixed in 2.5% glutaraldehyde prepared in cacodylate (sodium phosphate) buffer adjusted to pH 7.4 for 24 h and afterward washed in phosphate buffer for 15 min. After dehydration in ascending series of acetone, samples were immersed in tetramethylsilane for 10 min at 4°C. Then, they were brought to room temperature to dry. The specimens were mounted on aluminum stubs coated with gold and observed through SEM in Sophisticated Analytical Instrument Facility, North-Eastern Hill University, Shillong – 793022.

RESULTS AND DISCUSSION

Morphological Changes

No morphological changes have been observed in the control group of fishes, and they were found in good condition. However, the sodium arsenite treated fish showed the rapid movement of fins and operculum. They produced a lot of slime around their body. The overall activities of the sodium arsenite treated fishes decreased with time.

Histological Studies

In the liver tissue of control group of *C. punctatus*, there was normal structure and systematic arrangement of hepatocytes. Hepatic cells (HC) were roundish, polygonal containing clear spherical nucleus which can be seen in Figure 1. The normal

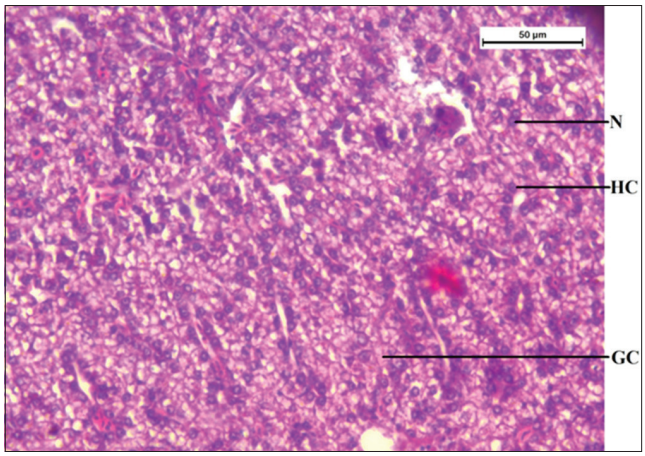

Figure 1: Histology of the liver tissue in the control group of *Channa punctatus* observed through optical microscope (Nucleus, Hepatic cell, and Granular cytoplasm)

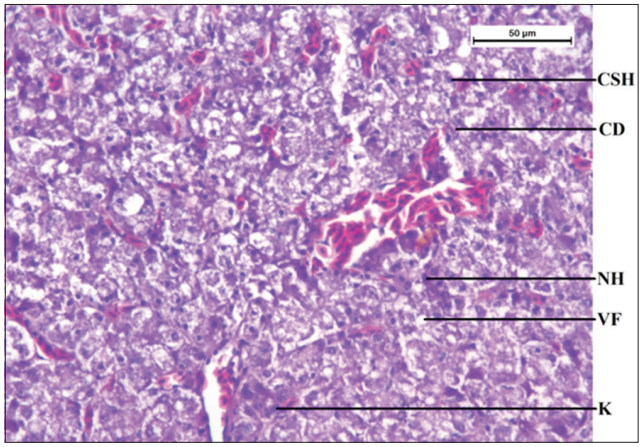

Figure 2: Histological changes observed through optical microscope in the liver tissue of sodium arsenite-treated *Channa punctatus* (vacuole formation, nuclear hypertrophy, cytoplasmic deformation, cloudy swelling of hepatocytes, and Karyolysis)

histological arrangement was not found in the liver tissue of sodium arsenite treated *C. punctatus*. A micrograph of liver tissue of sodium arsenite treated *C. punctatus* is shown in Figure 2. The micrograph shows a lot of rupture of blood vessels, necrotic tissue with marked loss of hepatocytes and extensive area of vacuolation in the liver tissue. These observations are in accordance with our earlier experiment described elsewhere.[20] Figure 3 shows the micrograph of liver tissue exposed to sodium arsenite and treated concurrently with garlic extract. It can be seen that there is the lesser extent of vacuolar degeneration and focal necrosis (FN). The hepatocytes are seen almost in normal structure.

SEM Study

A SEM of liver tissue in control *C. punctatus* is shown in Figure 4 which represents normal ultrastructural morphology of hepatocytes. Serous membranes with some connective tissue are seen on the surface of the liver tissue. HC are seen with clear spherical nucleus. In our study, it has been found that sodium arsenite caused several damages in the liver tissue which includes the destruction of normal arrangement of the cells, Vacuolar degeneration (VD) of cytoplasm, FN, and cloudy swelling of hepatocytes (CSH). These changes are represented in Figure 5. Similar effect has also been observed in our previous experiment.[20] Ultrastructural changes observed in the liver tissue of *C. punctatus* exposed to sodium arsenite and treated concurrently with garlic extract can be seen in Figure 6. The micrograph shows the lesser extent of damage to liver tissue with CSH and VD. Liver is the primary organ for detoxification of foreign compounds[14] and one of the most affected organs by contaminants in water.[15] In earlier studies[21,22] on sodium arsenite-treated

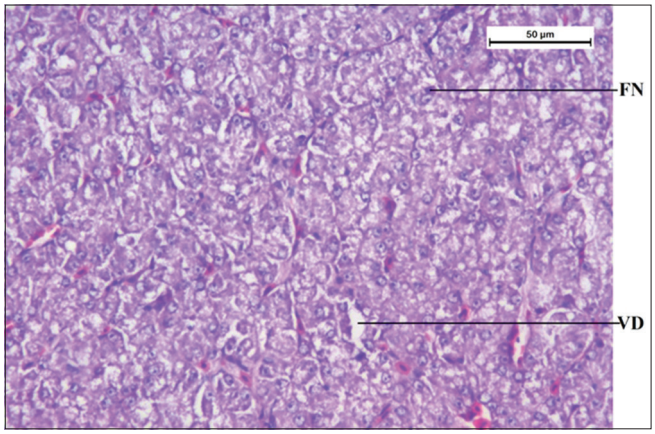

Figure 3: Optical micrograph of liver tissue of *Channa punctatus* exposed to sodium arsenite and treated concurrently with garlic extract (Vacuolar degeneration and focal necrosis)

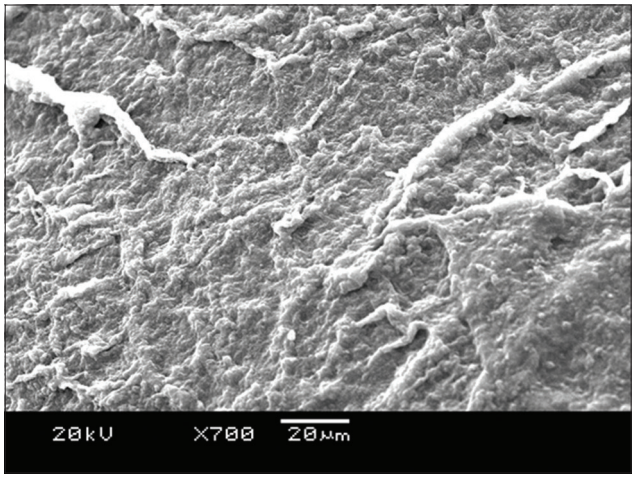

Figure 4: Surface ultrastructure of the liver tissue in the control group of *Channa punctatus* observed through scanning electron microscope

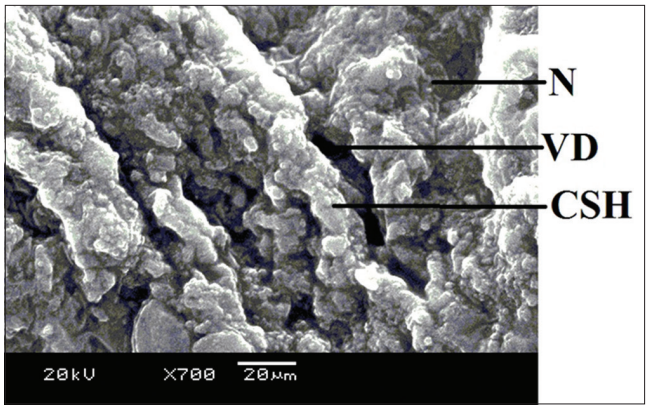

Figure 5: Surface ultrastructural changes observed through scanning electron microscope in the liver tissue of sodium arsenite treated group of *Channa punctatus* (cloudy swelling of hepatocytes, vacuolar degeneration, and necrosis)

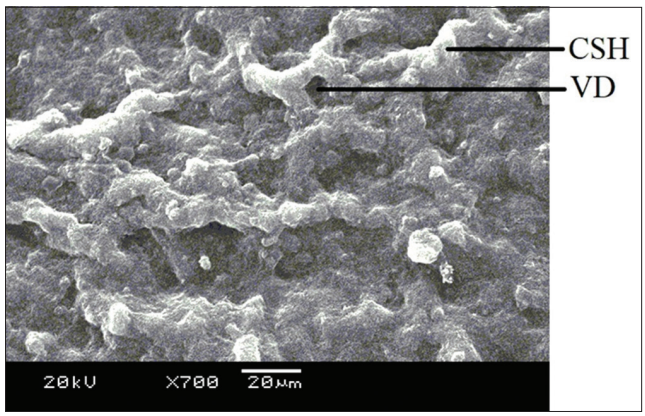

Figure 6: Scanning electron micrograph of liver tissue in the group of *Channa punctatus* exposed with sodium arsenite and treated concurrently with garlic extract (cloudy swelling of hepatocytes and vacuolar degeneration)

C. punctatus showed concentration-dependent reduced cell viability and chromosomal DNA fragmentation of liver cells. Findings of Ahmed *et al.* (2008)[21] revealed that lower concentration of sodium arsenite-induced apoptotic death of cells while higher concentration induced necrotic cell death. Ansari and Alam (2014)[23] revealed that acute toxicity of arsenic was significantly higher when compared to cadmium and copper, after 96 h exposure of *Danio rerio*. Sangeeta *et al.* (2012)[22] observed a decrease in the activities of alanine aminotransferases and aspartate aminotransferase in the arsenic-exposed *C. punctatus*. Studies on sodium arsenite-induced mice by Chowdhury *et al.*[24] revealed high frequencies of chromosomal aberrations and damaged cells. However, it was observed that garlic extract reduced the clastogenic effects of arsenic to a statistically significant level. Chowdhury *et al.*[8] observed in *in vitro* experiments that garlic reduced arsenic-induced cytotoxicity, reactive oxygen species production and repressed expression of p53 and hsp 90 genes in human skin malignant melanoma cells. The therapeutic efficacy of AGE over arsenic was also confirmed in $NaAsO_2$ intoxicated rats. Amer *et al.*[2] revealed that the histopathological studies in liver sections from arsenate treated mice showed venous congestion, sinusoidal dilation, mononuclear cell infiltration, and periportal fibrosis. However, the study revealed that the arsenic-exposed mice cotreated with different antioxidants including green tea, garlic, and vitamin C had reduced histopathological alterations.

CONCLUSION

This study highlights the histological alteration and the ultrastructural damages on liver tissue of freshwater teleost *C. punctatus* exposed to sublethal dose of sodium arsenite. High sensitivity and behavioral changes in the treated fish have also been observed. The ameliorative effect of garlic extract in sodium arsenite-treated liver tissue of *C. punctatus* has also been proved from the light and electron micrographs. The protective effect of garlic was shown in the normalization of hepatocytes in the arsenic-induced liver tissue. Hence, it can be concluded that garlic extract could be an effective natural agent to reduce the cellular damages in the arsenic-exposed liver tissue.

REFERENCES

1. Hughes MF. Arsenic toxicity and potential mechanisms of action. Toxicol Lett 2000;133:1-6.
2. Amer SA, AL-Harbi MS, AL-Zahrani YA. Protective role of some antioxidants on arsenic toxicity in male mice: Physiological and histopathological perspectives. Biol Med (Aligarh) 2006;8:266.
3. Flora SJ, Bhadauria S, Kannan GM, Singh N. Arsenic induced oxidative stress and the role of antioxidant supplementation during chelation: A review. J Environ Biol 2007;28:333-47.
4. Centeno JA, Mullick FG, Martinez L, Page NP, Gibb H, Longfellow D, *et al*. Pathology related to chronic arsenic exposure. Environ Health Perspect 2002;110 Suppl 5:883-6.
5. Khan MM, Sakauchi F, Sonoda T, Washio M, Mori M. Magnitude of arsenic toxicity in tube-well drinking water in Bangladesh and its adverse effects on human health including cancer: Evidence from a review of the literature. Asian Pac J Cancer Prev 2003;4:7-14.
6. Yoshida T, Yamauchi H, Fan Sun G. Chronic health effects in people exposed to arsenic via the drinking water: Dose-response relationships in review. Toxicol Appl Pharmacol 2004;198:243-52.
7. Yu HS, Liao WT, Chai CY. Arsenic carcinogenesis in the skin. J Biomed Sci 2006;13:657-66.
8. Chowdhury R, Dutta A, Chaudhuri SR, Sharma N, Giri AK, Chaudhuri K, *et al*. *In vitro* and *in vivo* reduction of sodium arsenite induced toxicity by aqueous garlic extract. Food Chem Toxicol 2008;46:740-51.
9. Bates MN, Smith AH, Hopenhayn-Rich C. Arsenic ingestion and internal cancers: A review. Am J Epidemiol 1992;135:462-76.
10. Morales KH, Ryan L, Kuo TL, Wu MM, Chen CJ. Risk of internal cancers from arsenic in drinking water. Environ Health Perspect 2000;108:655-61.
11. Smith AH, Marshall G, Yuan Y, Ferreccio C, Liaw J, von Ehrenstein O, *et al*. Increased mortality from lung cancer and bronchiectasis in young adults after exposure to arsenic in utero and in early childhood. Environ Health Perspect 2006;114:1293-6.
12. Nikaido M, Pi J, Kumagai Y, Yamauchi H, Taguchi K, Horiguchi S, *et al*. Decreased enzyme activity of hepatic thioredoxin reductase and glutathione reductase in rabbits by prolonged exposure to inorganic arsenate. Environ Toxicol 2003;18:306-11.
13. Paruruckumani PS, Maharajan A, Ganapiriya V, Narayanaswamy Y, Jeyasekar RR. Surface ultrastructural changes in the gill and liver tissue of Asian sea bass *Lates calcarifer* (Bloch) exposed to copper. Biol Trace Elem Res 2015;168:500-7.
14. Gernhofer M, Pawet M, Schramm M, Muller E, Triebskorn R. Utrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. Aqua Ecosyst Stress Recov 2011;8:241-60.
15. Camargo MM, Martinez C. Biological and physiological biomarkers in *Prochilodus lineatus* submitted to in situ tests in an urban stream in Southern Brazil. Environ Toxicol Pharm 2006;21:61-9.
16. Sumedha NC, Miltonprabu S. Antioxidant efficacy of

diallyl trisulfide on arsenic-induced toxicity in rats: An *in vivo* and *in vitro* study. Inter J Toxicol Appl Pharm 2013;2:33-8.
17. Kodera Y, Suzuki A, Imada O, Kasuga S, Sumioka I, Kanezawa A, *et al*. Physical, chemical, and biological properties of s-allylcysteine, an amino acid derived from garlic. J Agric Food Chem 2002;50:622-32.
18. Okada Y, Tanaka K, Fujita I, Sato E, Okajima H. Antioxidant activity of thiosulfinates derived from garlic. Redox Rep 2005;10:96-102.
19. Chung LY. The antioxidant properties of garlic compounds: Allyl cysteine, alliin, allicin, and allyl disulfide. J Med Food 2006;9:205-13.
20. Das T, Goswami M. Histopathological and ultrastructural changes in the gill and liver tissues of fresh water fish *Channa punctatus* exposed to sodium arsenite. Biosci Biotech Res Commun 2018;11:434-41.
21. Ahmed K, Akhand AA, Hasan M, Aslam M, Hasan A. Toxicity of arsenic (sodium arsenite) to fresh water spotted snakehead *Channa punctatus* (Bloch) on cellular death and DNA content. American-Eurasian J Agric Environ Sci 2008;4:18-22.
22. Sangeeta D, Unni B, Bhattacharjee M, Wann SB, Rao GP. Toxicological effects of arsenic exposure in a freshwater teleost fish *Channa punctatus*. Afr J Biotech 2012;11:4447-54.
23. Ansari S, Alam BA. Acute toxicity of copper, cadmium and arsenic to zebrafish, *Danio rerio (Cyprinidae)***.** Trends Biosci 2014;7:2357-60.
24. Chowdhury AR, Das T, Sharma A, Talukdar G. Inhibition of clastogenic effect of arsenic through continued oral administration of garlic extract in mice *in vivo*. Mutat Res 1997;392:237-42.