Assessing Health Consequences of Drinking Water from Tongi Industrial Area in Healthy Sprague Dawley Rats

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Dedicated to my parents who have always think about my happiness before thinking about their happiness and give me the inspiration to go forward and courage to face any difficulties and also to my beloved siblings.

Certification Statement

This is to certify that this project titled 'Assessing Health consequences of drinking water from Tongi Industrial Area in healthy Sprague Dawley Rats' submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Imon Rahman, Senior Lecturer, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed,

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Countersigned by the supervisor

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Abstract

Bangladesh has plentiful sources of drinking water from river and groundwater but due to the rapid industrialization and development processes the quality of drinking water is being deteriorated day by day. Therefore, the safety of drinking water has always been a major concern especially in global village. This study has been undertaken to evaluate the health consequences of tongi industrial areas drinking water in healthy Sprague Dawley rats and to measure the quality of drinking water by determining the presence of some heavy metals. The experimental study was continued for 8 weeks and 18 healthy Sprague Dawley rats were assessed by their hematological, biochemical and histopathological evaluation. The heavy metals that has taken accounted in this study includes Cr, Mn, Cu, Zn, Cd, Pb, As, Ni and Se. The concentration of Mn, Cd and As was found in higher amount than the maximum allowable limits of WHO. At the end of the 8 weeks of study, the hematological parameters of the experimental rat did not show any significant changes statistically, except the value of platelets which was found to decrease significantly. The serum enzymes ALT and AST found to decrease in the experimental group and showed significant decrease of ALT enzyme which was found to indicate the presence of liver dysfunction in several studies. The histopathological observation showed mild alteration of liver, kidney and spleen tissue as compared to the control. From the overall interpretation it has been observed that the decrease of platelets and serum ALT, AST level might have some relation with the high concentration of cadmium and arsenic in the sample water and it can be demonstrated as the after-effect getting exposure with those heavy metals. Because cadmium and arsenic is found to be very toxic even in a very trace amount and from several studies it has been noticed that these metals (Cd and As) exert oxidative damage of some organs especially in liver by instigating oxidative stress which in turn follows up with the reduction of serum enzymes and in the long term caused severe kidney dysfunction and chronic liver damage. However, the overall observation of this study does not show any conspicuous findings due to the very short term of study. Therefore, in order to have a clear outcome of this study long term study of some parameters is necessary.

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LIST OF ABBREVIATION

1. DoE: Department of Environment
2. ETP: Effluent treatment system
3. WHO: World Health Organization
4. DWASA: Dhaka water supply and sewerage authority
5. USEPA: United states Environmental Protection Agency
6. EPA: Environmental protection Agency
7. EU: European Commission
8. TDS: Total Dissolved solids
9. DO: Dissolved oxygen
10. COD: Chemical oxygen Demand
11. BOD: Biological oxygen Demand
12. TSH: Thyroid Stimulating Hormone
13. CIOMS: The Council for International Organizations of Medical Sciences
14. EDTA: Ethylene diamine tetra acetic acid
15. WBC: White blood cell
16. RBC: Red blood cell
17. PLT: Platelets
18. HCT: Hematocrit
19. MCH: Mean corpuscular hemoglobin
20. MCHC: Mean corpuscular hemoglobin concentration
21. WIC: WBC Impedance Count
22. WOC: WBC Optical Count
23. ALT: Alanine aminotransferase
24. AST: Aspartate aminotransferase
25. MP: Melting point
26. AAS: Atomic Absorption Spectrophotometer
27. H&E: Hematoxylin and Eosin
28. ROS: Reactive oxygen species
29. SOD: Superoxide dismutase

1. INTRODUCTION

Water is considered as the life preserving or life sustaining resources since it plays one of the most crucial roles for the survival of life on the earth. The existence of all types of living organism on the earth is intimately connected to the quality of water available to us. It is stated as an essential element for the survival of life because 75% of the human body consists water and only 25% constitutes the solid matter (Routledge and Stewart, 1998). Therefore, if the body does not get a continuous supply of water then the body cells become dehydrated and the vital organs remain no longer viable for human life (Routledge and Stewart, 1998). Another important function of water is acting as a purifier in our body. Therefore sufficient amount of water must be consumed to flush out the unhealthy toxins from the body (Global Healing Cente., 1998-2010). Not only the existence of human life or any other living organism is influenced by this vital element but also the environmental, economical growth and developments are also greatly influenced by the quality of surface and groundwater (Halder and Islam, 2015). An adequate supply of safe water and access to safe drinking water is the fundamental human need and prerequisite for a healthy life. Improving the access to safe water not only lessens the risk of several health diseases but also ensures a high standard of living. According to medical experts, an individual need to consume at least 2 liters of water per day for the basic survival (De Kok, Kok, Guidotti, Kjellstrom, and Yassi, 2001). Even though to lead a healthy life use of safe drinking water is very much crucial, but, unfortunately, the sources of fresh water is increasingly being threatened by the multitude forces that includes both natural activities and human activities. The percentage of fresh water available on earth is almost 3% which is very less to fulfill the social and economical demand (United Nations Environment Programme, 2008). This 3% of water not only supports the wide range human activities and nature but also maintains a balance in the global ecosystem. By several statistical analysis, it has been found that almost 70% of total fresh water is utilized for agricultural purpose and almost 22 % is used for industrial purpose and the percentage of fresh water used for domestic or household purpose is only 8% (Rosen, 2013). The use of domestic water greatly varies according to the standard of living of people living in urban or rural areas, and the use of water for the domestic purposes can be divided into several purposes such as drinking, cooking food, washing clothes and utensils, cleaning, gardening etc (Amin, Mahmud, Hosen, and Islam, 2011). In developed countries, high consumption of fresh water occurs in industrial sectors whereas in developing countries and least developing countries like Bangladesh, India, Africa higher consumption of water occurs in the agricultural sector (Environmental protection Agency, 2009). Even though the situation has turned quite a lot due to the rapid growth of population and development of modern technologies. In order to fulfill the demand of increased population rapid urbanization and industrialization are taking place and as a consequence, the higher percentage of water is consumed for the industrial and domestic purposes. Therefore, the risk of water pollution due to the population growth and development of technologies are also increasing to a great extent, thus ensuring the adequate supply of safe water and getting access to safe drinking water has become one of the most difficult tasks to do. Contamination of water sources can occur both by natural and human activities. Improper disposal of wastes, chemicals, naturally occurring substances such as several microorganism, heavy metals, use of agricultural products such as pesticides and fertilizers have the potential to contaminate the drinking water. Moreover drinking water if not properly treated or distributed through the poorly maintained distribution system then it also causes several health risks (Environmental protection Agency, 2009).

One of the most common types of water contamination occurs through the human and animal feces. In developed countries such as the United States has the proper means for the safe disposal of these types of pathogens, but in some developing countries like Bangladesh, India there is no proper way for the safe disposal of the feces. As a result, those feces enter into the water sources and spread through the population and cause several waterborne diseases such as Cholera, Diarrhea, Typhoid etc (National Resources Defense Council, 2008). Some populations are more susceptible to the diseases caused by pathogens than other population. For an example, children and infants are more vulnerable to several pathogen-related diseases due to the less strong and not fully developed immune system. But it has been proven experimentally that, the contamination of water through several microbial contaminants can be reduced to a safer extent by boiling water before using it (National Resources Defense Council, 2008). Another easiest way that pollutes water is several types of toxic chemicals and heavy metals that are produced by various types of natural and human activities such as disposal of industrial effluents into the water bodies, enormous use of fertilizers and pesticides (Amman, Michalke, and Schramel, 2002). In many developing countries rapid urbanization and industrialization has always been considered as the main source of water contamination because it plays a major role in the alleviation of poverty and also for the economic growth. Moreover, the rapid urbanization and industrialization have been always influenced by the higher growth of population in underdeveloped countries. Even though the implementation of modern technologies in the growth of industries is occurring but, unfortunately, environmental consideration is not properly integrated with the design of industrial processes mainly in the developing countries. As a result, though industries are providing basic necessities but, in the long run, it is making the survival more difficult (Jolly, Islam, and Akter, 2013).

Bangladesh has been popularly known as an agricultural country but since the early sixties, various types of industries has been started to grow sparingly and most of the industries are located near the rivers because of the suitability of transportation. Since the major percentage of the population of this country is in the poverty line, therefore, industrialization has started to play a vital role in the economic growth and alleviation of poverty in this country. The national pollution profile demonstrates that Bangladesh presently has more than 70,000 industrial units of which almost 50,000 are small and cottage like and the remaining 20,000 are large and medium industries (DoE, 2007). Every single industrial group has increased production substantially. As a result of the haphazard growth of the industries, the number of unregistered small-scale industries is also increasing in number and most of those industries do not have any treatment plan and facilities since the cost is beyond the capacity of small scale industries. Moreover, some industries do not have the provision of ETP (Effluent treatment system) and even though some of them have the provision of ETP but they are not operated due to the high operation cost and due to the lack of monitoring and effective policies discharge or dumping of that untreated wastage directly or indirectly into the river or nearby lakes has become very common phenomena. Therefore, those industrial pollutants very easily mix up with the water bodies and cause the pollution of water sources. Since Bangladesh is known as a riverine country and the capital Dhaka city is surrounded by a number of rivers and canals. Among them, Turag, Buriganga, Dhaleshwari, Balu, and Shitalakhya are considered as the most important one since these river ways make the transportation system suitable and easy for several industries and also work as the main source of fresh water for industrial, agricultural and household purposes.

Even though water is considered as one of the precious resources of Bangladesh but unfortunately due to the lack of plan and policies for the management of water resources, both the quality and quantity of water in these rivers is rendering to a high potential of risk that does not permit to use these water for instant use. The industries that are located near these riverside not only use the water of these rivers for their industrial purposes but they also play a major role in polluting these water as well. The water that has been discharged into the river without proper treatment by the industries has been found to be ten times more polluted than the water that has been consumed. The river Buriganga, Turag, Balu, Shitalakhya have been turned into the most polluted and poisonous river. The water of these rivers are continuously being polluted by untreated or partially treated sewage effluents, sewage polluted surface runoff, untreated industrial effluent from nearby residential and industrial area, various industrial wastage, domestic wastage and indiscriminate discharge of pathological and commercial wastage. As a consequence, the water of these rivers has become so noxious that it is not only killing the aquatic ecosystem but also giving rise to several health hazards to the dwellers of the city and the people having residents near the river side (Halder and Islam, 2015). According to Satter and Islam (2005), in present days, 10% of wastewater produced from distinctive industrial sources is being dealt with and the rest is released as into the closest water bodies. The contamination load from distinctive industrial and urban sources is increasing due to the fact that industries are increasing in number because most countries are becoming industrial. Since our country is linked with heaps of small and big streams, the contamination load is being mixed up with the fresh water bodies and demolishing the freshwater aquatic ecosystem of the country (Khan, Cao, Zheng, Huang, and Zhu, 2008). However, surface water being a real wellspring of water supply could be an extraordinary alternative for satisfying water emergency if the contamination level is under indicated limit. Pollution level has restricted surface water use as consumable water because surface water is considered as one of the major sources of industries for waste disposal. Improper discharge of untreated effluent from numerous industries contaminants the quality of surface water (Zakir and Shikazono, 2011; Mohiuddin, Ogawa, Zakir, Otomo, and Shikazono, 2011; Zakir, Rahman, Rahman, Ahmed, and Hossain, 2012; Zakir, Hasan, Quadir, Sharmin, and Ahmed, 2013; Bakali, Younus, and Zakir, 2014).

Groundwater is another source of fresh water that is used for domestic, agricultural and drinking purposes. Numerous parts of the industrial region are colonized and are in close vicinity of the industries and are utilizing groundwater for drinking, cleaning and washing purposes. In many countries like Bangladesh, groundwater is utilized as the major source of drinking water because it often has a low concentration of pathogens in light of the fact that the water is filtered during its travel through underground layers of sand, clay, or rocks. However, toxic chemicals, for example, arsenic and fluoride can be broken down from the dirt or rock layers into groundwater. But direct contamination can likewise happen from gravely composed unsafe waste sites or from industrial sites. The essential explanations behind groundwater pollution are industrial contamination and extensive cultivation that leads to the production of agrochemicals in groundwater. In the case of industries, the reason is same like before. The absence of treatment of effluents that are pumped into waterways and streams prompting groundwater contamination by several industrial toxic contents. Effluents from industries have a great impact on the contamination of water body; these effluents can modify the physical, chemical and biological nature of the accepting water body. The initial impact of waste is to debase the physical nature of the water. Later biological degradation becomes evident in terms of number, selection and organization of the living organisms in the water (Kaur, Sharma, and Sinha, 2010).

Industrial wastewater typically contains particular and promptly identifiable chemical compounds. It has been observed that 33% of the overall pollution comes in the form of effluent discharge, solid wastes, and other hazardous wastes and it has become a major environmental issue that is posing threats to the existence of human being. Industrial hazardous wastes may contain more than a single chemical or substance. Industrial pollution and wastes cause potential threats to human and ecological health if not properly managed. The consideration ranges from poisonous consequences for babies and youngsters to the health implications of low-level exposure to various toxins and the degradation environments and biological communities. These considerations do not stop at the borders; as a result, of some pollutants will travel a long distance and waste are transported to reusing and disposal sites across political boundaries. The chemical contaminants of surface water can produce several health risks because such waterways are frequently utilized specifically as drinking water sources or joined with shallow wells used for potable. In addition to that, flowing water have necessary roles for washing and cleaning and for diversion in towns. The danger of endless effect of surface water contamination is extremely frequently under assessed because of hidden durable action of pollutants. The poisonous risk of surface water contamination is decided from the result of analysis of venomous risk of the organic part and inorganic part of surface water pollution (Lokhande, Singare, and Pimple, 2011)

The uncontrolled dumping of immense industrial wastes of point and non-point sources is even to a great degree unsafe when the toxins are heavy metals and cannot be treated simply by conventional ways. The presence of toxic heavy metals has made the problem of water pollution worse to a great extent. Metals are considered as toxins and once they enter the body in more than the prescribed limit, they begin inflicting harm. Similarly, numerous physicochemical parameters assume a vital part in deciding the quality of water. Some of the Heavy metals like lead, cadmium, mercury, and arsenic are broadly studied and their consequences for human well-being are routinely evaluated by international bodies such as the W.H.O. Heavy metals are utilized by humans for thousands of years. Diverse studies demonstrate that the presence of a generative toxicant in drinking water is one possible rationalization of variations in spontaneous abortion rates between ladies who drink contaminated water and those who do not. The high values of heavy metals and physicochemical parameters have extreme health consequences. Metals contrast from other toxic substances in a way that they are neither created nor destroyed by humans. Traditionally, metal pharmacology or toxicology, for the most part, involved acute or over impacts, for example, damage to kidney, liver, and stomach colic from lead (Pb) toxicity, or growth retardation, muscle fatigue, sexual impotence from manganese (Mn) toxicity. These metals when to enter the body and their levels cross certain limits they begin delivering negative impact on health (Faisal, Majumder, Uddin, and Halim, 2014). The increasing trend in the concentration of heavy metals in nature has pulled in extensive consideration amongst ecologist globally throughout the last decades and has additionally started to bring significant concern in a large portion of the metropolitan urban areas. Untreated or purportedly treated industrial effluents and sewage water contain variable amounts of heavy metals, for example, arsenic, lead, nickel, cadmium, copper, mercury, zinc, and chromium (Singare, Lokhande, and Naik, 2010), that have the potential to contaminate crops growing below such irrigation. These heavy metals markedly affect the aquatic flora and fauna which through bio-amplification enter the evolved way of life and at last influence the individuals also (Lokhande et al. 2011). Most of the industrial effluent plants throughout the world are designed and controlled to get rid of nutrients from industrial effluents, but a giant amount of potentially toxic elements like heavy metals also remain in the industrial wastewater and released in the waterways in an untreated state thus polluting water and environment as well. In spite of the fact that the expansion of the industrial sector contributes fundamentally to the financial improvement of the nation, without a comprehensive environmental management set up, this expansion might not be sustained into the current millennium. Nowadays industrial wastewater contamination has turned into a major issue in Bangladesh. It rapidly degraded our water quality likewise as soil fertility and productivity. If this industrial wastewater treated and managed appropriately, it could be used as irrigation water to enhance the plant growth due to its nutrient content. It is an obvious fact that without enough good water our survival will be undermined but, unfortunately, ensuring the good quality of water has become an incredible challenge for the 21st century and is more essential than its quantity. Various researchers worked on quality of drinking water and found that industrial waste directly or indirectly affect on the quality of drinking water in appropriate location of wells with regard to storage tank, sewer pipe (industrial water seepage) and will build potential the draining of effluent in the well and groundwater system (Shaikh and Mandre, 2009). The effluents containing several toxic heavy metals and other chemical constituents is causing debasement of water quality since very few authorized company like DWASA (Dhaka water supply and sewerage authority) works for ensuring the supply of good quality water that is utilized for household and drinking purposes. An appreciable number of serious health concerns may happen as a consequence of the chemical contamination of drinking water (Begum and Ahmmed, 2010). Especially, the poisonous impact of several hazardous metals on health through the drinking water has become a major cause of concern in most of the metropolitan city like Dhaka because heavy metals are very harmful, dangerous and toxic even in ppb (Parts per billion) range (Trivedi, 2003).There are more than 50 components that can be named heavy metals, 17 of which are accounted to be very toxic and moderately accessible. The level of toxicity relies upon the type of metal, its biological role and, therefore, the kind of organism that are exposed to it. The heavy metals in drinking water connected most frequently to human poisoning are lead, iron, cadmium, copper, zinc, chromium and so on. They are needed by the body in little amounts, yet can likewise be harmful in large doses (Mohod and Dhote, 2013).

Heavy metals like copper are the vital trace elements yet show lethality due to overabundance amount in drinking water. Cadmium is dangerous to a great extent even in low concentration and can bio-accumulate in organism and ecosystem along with the biological half-life in the human body extending from 10 to 33 years. Long term exposure to cadmium actuates renal damage. Thus, cadmium is considered as one of the priority contaminants and observing in most of the countries and international organizations. The contamination of water is directly associated with the pollution thus, the persistent evaluation of the standard of ground and surface water sources is important (Ehi-Eromosele and Okiei, 2012). The known deadly impact due to the heavy metal toxicity in drinking water incorporates damaged or diminished mental and central nervous function and lower energy level. They likewise cause an anomaly in blood composition, severe effect on vital organs such as kidneys and liver (Mohod and Dhote, 2013). Most of the heavy metal affects the body simply by disrupting the body's immune response (Mahaffey and Fowler, 1977; Islam, Parvin, Pervin, Bari, and Khan, 2011). The long-term exposure of these metals results in physical, muscular, neurological degenerative procedure that causes Alzheimer disease (Brain disorder), Parkinson's disease (a degenerative disease of the brain), muscular dystrophy, multiple sclerosis (a nervous system disease that affects the brain and spinal cord). Lead is also the most widely recognized heavy metal in drinking water if occur more than its admissible limit it causes general metabolic toxicity and enzyme inhibition (Mebrahtu and Zerabruk, 2011). Zinc is also essential trace elements for human body but in higher doses it causes renal damage, muscle cramps etc. Arsenic is a heavy metal which is very poisonous even in a very little amount and in Bangladesh natural level of Arsenic in groundwater has been found to be causing harmful effects on the population (Anawara, Akaib, Mostofac, Safiullahd, and Tareq d, 2002). Upon several analysis it has been found that the metals- Lead, cadmium, chromium, Arsenic, Zinc may not have any significant purpose on the body but they do have a direct impact on liver and kidney and they are significantly nephrotoxic and hepatotoxic even at normal levels (Sabath and Robles-osorio, 2012). Moreover, cadmium is considered as one of the major pollutant of concern for many researchers, since it is found to be toxic in a very trace amount. Cadmium has found to induce the oxidative stress by reducing the antioxidant and thus follows up with several organ dysfunctions (Latinwo, Badisa, Ikediobi, Odewumi, Lambert, and Badisa, 2006; Kheradmand, Alirezaei, and Dezfoulian, 2013). Therefore, continuous assessment of the quality of groundwater and surface water sources is necessary and it should be taken as a mandatory action in those areas with the cluster growth of industries. Emphasis needs to be given for the analysis of toxic metals in drinking water so that the concentration does not get higher than the recommended limits set by different International Organizations such as USEPA, WHO, EPA and the European Union Commission.

Tongi is an administrative district in Gazipur that marks the northern border of Dhaka, the capital of Bangladesh. It is basically referred to and created as industrial zone according to the master plan of 1959. Different categories of large and small industries are present in Gazipur. Like other part of the country, these industries are not following the environmental rules and regulation set by the government and thus inflicting serious damage to health and ecology by means of polluting the environment. Gazipur is considered as an industrial hub in the country and thousands of industries are situated there that includes metal industries, garments, textile, paper and pulps, jute, pharmaceuticals, food manufacturing factories and so on, which do not have any effluent treatment plant (ETP) and thus contaminates land, water bodies, sediments and air in the encompassing zones. This mechanical toxic water flows down through drains and finally mix up with Turag river, Tongi khal and others and thus significantly polluting these waters sources (Bakali et al. 2014). On the other hand populations of around 350,000 living in Tongi, Gazipur district is greatly dependant on the water that comes from Turag River and Tongi Khal.

1.1 Rationale of the study:

Since water toxicity study has always been a critical issue of analysis for various researchers, therefore, since a very long time a large number of researches have been carried out, focusing on the issue of rapid industrialization and its impact on nearby areas and river water. Since Tongi is considered as the biggest industrial areas in Northern Bangladesh, it is an obvious fact that the condition of water sources is in a continuous deterioration stage. Moreover, being a densely populated area, it can be assumed that the deterioration condition of water might have some hazardous impact on the health of the inhabitants of those areas.

Even though, a great number of researches have been carried out to determine the presence of heavy metals in water, analyze the condition of Turag river water and the water of Tongi Khal by the physicochemical studies of water but Very few researches have been done so far to analyze the quality of water that the people living in slum areas and Tongi villages directly use for their drinking and other household purposes like bathing, washing clothes, cooking food etc (Zakir and Shikazono, 2011; Mohiuddin et al. 2011; Zakir et al. 2012; Zakir et al. 2013; Bakali et al. 2014). In order to study the present condition of the whole industrial area household water, samples were required to be collected anonymously from random villages of Tongi area. But due to time and economic constraints, only one study village had been focused. Since this study area is adjacent to many pharmaceutical industries, there is a high risk of water contamination which can be hazardous to human body. Therefore, the objective of this study is to observe the possible biological effects due to the use of water of industrial area for drinking and household purposes.

1.2 Aims and objective:

On the basis of existing tendency of rapid industrialization and urbanization along with its associated hazards and the work of various researchers on this issue, the preliminary study was undertaken to examine the quality of household water in Tongi industrial area. This concurrent study is therefore aimed to-

i. evaluate the water quality of Tongi Industrial area, Dhaka
ii. analyze the presence of heavy metals in drinking water of this area
iii. assess the health consequences of the dwellers who consumed this water in Tongi area by assaying hematological parameters and histopathology of liver, heart, kidney, Spleen, Brain and Lungs in rat models and,
iv. contribute to the existing knowledge of understanding relating to contamination of drinking water in the industrial area.

2. REVIEW OF LITERATURE

The purpose of this section is to review the past analysis works that are identified with the present study. Therefore, an endeavor has been made in this part to represent a short review of research information of previous studies that has been done to assess the drinking water quality and its biological effects. In Bangladesh the research related to this aspect is at initial stage, thus literature in this aspect is very few. Since, literature review forms a bridge between the past and present study, which helps to justify the research work and to draw a satisfactory conclusion. The most relevant studies that have been conducted within the recent past related to the current work are introduced in this part.

A considerable number of reports are available regarding water pollution and their reduction, but no detail report on the quality of drinking or household water of Tongi area in connection to Industrialization is available. Investigation of various Physico-chemical and microbiological parameters of different groundwater and surface water yield valuable information for comprehension the nature of water environment tosses a surge of light on the changes brought by the extraordinary human impedance.

Sarika and Chandramohanakumara (2008) stated that the urban aquatic biological systems are emphatically affected by the long term discharge of untreated domestic and industrial wastewater.

A study was conducted by Bakali et al. (2014) to assess physical parameters, major ionic constituents and trace metals content of surface and ground water of tongi area in Bangladesh. In the result of trace metals, the content of Pb in most of the surface water samples was found more than the permissible limit (< 0.05 mg L^{-1}) except all of the groundwater samples. The study result concluded that the quality of surface and ground water of Tongi area were in unsuitable condition for most of the parameters studied due to high industrial effluent contamination.

According to Rahman, Khan, Akib, and Biswas, (2013) Buriganga, Balu, Shitalakhya, Turag and Tongi canal around Dhaka city got huge amount of untreated sewage, industrial liquid and city waste which prompts serious surface water contamination. Their study concentrated on the presence of heavy metals in those rivers and canals. Five different parameters, Cd, Cr, Ni, Pb, and Zn were accounted for statistical analysis. The presence of these heavy metals mostly crossed the standard limit and recognizes water as defiled. The concentration of lead (Pb) was discovered higher than the permissive limit and may be harmful for all three cases.

According to Zoynab Banu, Chowdhury, Hossain, and Nakagami (2013) infringement, disposal of untreated domestic and industrial wastewater and dumping of solid wastes have corrupted the overall water and sediment quality of the river Turag, Bangladesh. The study found that Cr, Cu, Zn belong to moderately to highly polluted for Turag river.

A study was conducted by Zakir et al. (2006) to assess the metal pollution of Turag River in Bangladesh and found the concentration of Mn, Zn, Cr, Cu and Pb in higher amount. The author also reported that the higher amount of metals were found close to the industrial and municipal areas and were related to direct discharge of industrial sewage into the river.

Shaikh and Mandre (2009) conducted a seasonal study of Physico-chemical parameters of drinking water in Khed (Lote) Industrial area and found the different parameters like TDS, DO,COD, BOD and Hardness beyond the permissible limit in well water samples.

Mohod and Dhote (2013) conducted a research for determining the concentration of Heavy metals in drinking water and their effect on Human health. A similar study has also been performed by Mebrahtu and Zerabruk (2011) to assess the status of drinking water quality in the urban areas of Tigray region, northern Ethiopia and found the concentration of Arsenic, cadmium, chromium, nickel, iron, lead to cross the maximum desirable limit recommended by WHO (2008). Similarly, Salem, Eweida, and Farag (2000) focused on the presence of Heavy metals in drinking water and observed the occurrence of renal failure due to the contamination of drinking water with lead and cadmium.

Siglin, Mattie, Dodd, Hildebrandt, and Baker (2000) have been conducted a 90 days drinking water toxicity study in Rats to evaluate the subchronic toxicity of perchlorate when administered to rats as Ammonium perchlorate in their drinking water because perchlorate has been recognized as a persistent and pervasive contamination of drinking water supplies in a number of metropolitan areas. The result showed statistically significant changes in TSH and thyroid hormones at all Ammonium perchlorate dosage level.

Ray et al. (2000) has done a study on drinking water of Rohtas district, and found the presence of high amount of Iron, magnesium, fluoride and nitrate in water. Sajidu, Masumbu, Fabiano, and Ngongondo (2007) collected drinking water samples from boreholes and pipes at 23 sampling sites, mostly villages within the district, for fluoride and other water physicochemical parameters during dry and rainy seasons of 2004 and 2005 respectively.

Alnos Easaand, (2010) collected groundwater samples at the pumping level. Harmful effects of waste water on the chemical compositions of groundwater were detected. In addition to that, toxicity and chemistry of heavy metals also increased in the groundwater. Ocheri and Ogwuche (2012) examined the concentration of lead, a toxic element in rural groundwater of Benue state.

Lead concentrations in the boreholes were noted to be higher in the wet season when compared to that of the dry season.

A study on drinking water in the Malaysian industrial area has been done by Razak, Praveena, Zaharin, and Hashim (2015) in order to observe the status of heavy metals in drinking water. A similar study was also conducted by Rahmanian et al. **(**2015) to evaluate the drinking water quality in the state of perak, Malaysia and to ensure the continuous supply of safe and clean drinking water for the protection of public health. Thus a number of parameters includes, pH, turbidity, conductivity, total suspended solids, total dissolved solids, and the determination of heavy metals such as Cu, Zn, Mg, Fe, Cd, Pb, Cr, As, Hg and Sn were analysed. The obtained values of all the parameters were found within the WHO standard limit.

A study for the analysis of microbial and chemical analysis of potable water in public water supply has been conducted by Ojo, Bakare, and Babatunde (2007) and observed the presence of coliform contamination which was far exceed than the WHO standards.

Napacho and Manyele (2010) have done a quality assessment of drinking water in Temeke District by the analysis of chemical parameters in drinking water collected from different sources. Various parameters that were studied include pH, chloride, nitrate, total hardness level and the study observed that the chemical parameters did not meet the allowable limits of WHO. A similar type of study was conducted in Abbottabad district of Pakistan for the qualitative and quantitative analysis of drinking water sample collected from different localities. In this study 15 drinking water samples were taken from the main source of water which was used by the maximum people for drinking water. The result observed certain sources of water borne disease which were also seen to be present within the localities.

A study was conducted on chemical effluent by Oloyede and Sunmonu (2008) on albino rats and the outcome of this study found to decrease the liver enzymes in the experimental rat due to the presence of soap, and detergent that has came from detergent industry. Another study was undertaken to assess the drinking water quality and determinants of household potable water consumption in Simada district of Ethiopia. Arunabh and Vasishta (2008) has done an experiment on different samples to examine the drinking water quality of various potable water sources of Anand district.

3. MATERIALS AND METHODS

3.1. Area of study:

The water sample was collected from Kathaldia village, which is located in sub-locality of Tongi, Gazipur district, Dhaka, Bangladesh. This village is in 5 km distance from the Turag River and 2 km distance from the Tongi khal. The Latitude and Longitude of Kathaldia is 23.9011 and 90.3878 respectively. The village has been chosen for this study due to the adjacent presence of several pharmaceutical industries that includes Beximco Pharmaceuticals (Approx 0.38 km away), Novartis Limited (Approx 0.10 km away), Eskayef Bangladesh Limited (Approx 0.10 km away), SK+F pharmaceuticals (0.32 km away), Drug International Ltd. (Approx 0.56 km away) and some textile industries. Therefore, Heavy metals such as Lead, Mercury, Cadmium, Nickel, Chromium, and other toxic organic chemicals or phenolic compounds discharged from Pharmaceutical industries are known to affect the surface and groundwater and thus may also affect the inhabitants by direct exposure to that water (Anyakora, Nwaeze, Awodele, Nwadike, Arbabi, and Coker, 2011). In this area, the dominant source of water used for drinking and household purposes is from tube wells and springs installed in the households of these villages away from other potential sources of contamination (fertilizers, animals, human sewage), and this water is also consumed by the inhabitants of that village without boiling. Therefore, the risk of getting exposure to the heavy metals discharged from adjacent pharmaceuticals and other industries has been considered as a major issue of concern in this study. Thus, groundwater collected from the tap which is consumed by the inhabitants was tested in order to focus on the impact of industrial contamination in the household or drinking water.

3.2. Collection of water:

Since the source of drinking and household water was same for the whole communities living in Kathaldia village of Tongi, therefore the sampling of drinking water was done randomly. Drinking water sample was taken from chlorine treated ground water source and it was collected in a double capped polyethylene bottles which has been prewashed with detergent, dilute HNO_3 and doubly de-ionized distilled water respectively. During the collection, the sampling bottles and caps were rinsed three times with the water to be sampled prior to sampling. The samples were obtained directly from the tap after allowing the water to run for at least 5 min in order to stabilize the variation in EC and temperature (Mebrahtu and Zerabruk, 2011). By following this way, 10 L of water samples has been collected for this study.

3.3. Study Animals:

The in life study was conducted in the Animal House of the Department of Pharmacy, Jahangirnagar University. All animal experiments were conducted in accordance with standard guidelines (CIOMS, 2012) on use of animals for experimental study. For this study, 18 healthy 8 weeks old male albino Sprague dawley rats weighed about 160 ± 25g were obtained from the animal house of Jahangirnagar University.

Prior to the experiment, rats were randomly divided into three groups of six rats each. Thus, six rats were taken for both control and experimental groups and they were acclimatized for a period of one week to adjust with the gradual change of the environment.

3.4. Distribution of rats and experimental design:

The rats were divided into Group A, B, and C of six rats in each. Group A served as Control of the study and Group B and C were served as the experimental groups. All the rats were marked for proper identification. The experimental study was conducted for 8 weeks (56 days).

The control groups A were given *ad libitum* access to mineral water in 100 ml bottle/day for 56 consecutive days.

Group B (Experimental group) were given *ad libitum* access to the sample tap water in 100 ml bottle/day for 56 consecutive days.

Group C (Experimental group) was given 5 times concentrated sample tap water by oral gavage (stomach tube; 2.9 inches length; 2.0 mm bulb) in order to see the effect in the long term exposure. Each animals of this group was gavaged with 2 ml of five times concentrated sample tap water for two times per day. That means, 2 ml was given by gavage in the morning and in the afternoon. So total 4 ml was administered to each rat along with *ad libitum* access to normal water and food for 56 consecutive days.

3.5. Preparation of 5 times concentrated sample water:

In order to prepare 5 times concentrated sample water for the oral gavage of group C, 100 ml of sample water that has been collected from the study area was taken in a beaker prewashed with the distilled water. Then it was heated by using a hot plate at 70 $^{\circ}$C. When the volume of 100 ml water decreased up to 20 ml, heating was stopped and the sample was kept at room temperature for cooling down.

3.6. Animal Care:

All of the rats were kept in plastic cages having dimensions of 30 x 20 x 13 cm and soft wood shavings were employed as bedding in the cages. Environmental condition was monitored strictly to maintain the temperature at 25°C and at 60% of relative humidity. Feeding of animals was done ad libitum, along with the experimented drinking water and maintained at natural day night cycle. The animals were housed in a well-ventilated hygienic experimental animal house. Thus, constant environmental parameters with adequate nutritional conditions were maintained. The rat ware fed with “mouse chow” (prepared according to the formula developed at BCSIR, Dhaka). All experiments on rats were carried out in absolute compliance with the ethical guidelines for care and use of laboratory animals. The experiment animals were marked carefully on the tail which helped to identify a particular animal. By using identification mark response were noted separately for a particular rat prior to and after the administration.

3.7. Body weight Measurement:

The body weight of each rat in the treatment and control group was measured in the beginning of the experiment and twice weekly during the exposure period by using an electronic analytical weight balance.

3.8. Blood collection and Organ weight measurement:

After 56 days of exposure, rats were fasted overnight. They were weighed before the collection of blood and then anesthetized with Ketamine (500mg/kg i.p). They underwent intracardiac aspiration puncture for euthanasia and sampling. Blood was collected from each of the rat through cardiac puncture by using 25G needle with 5ml syringe (Parasuraman, Raveendran, and Kesavan, 2010). After the collection, blood samples were immediately transferred to ethylene diamine tetra-acetic acid (EDTA) anticoagulant tubes (8.5%) was quickly returned by mixing with anticoagulant in the tube. All blood samples were labeled and immediately conveyed to the laboratory for analysis. The Heart, Kidney, lungs, liver, and spleen of each rat were surgically removed by using sharp blade and scissors, rinsed with physiological saline, blotted dried and then weight of individual organ was measured (Alimba, Bakare, and Aina, 2012).

3.9. Hematological analysis:

Hematological parameters that were analyzed includes, white blood cell count (WBC), red blood cells (RBC), hemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT) and the number of neutrophils, lymphocytes, Monocytes, Eosinophils, Basophils. All hematological parameters were analyzed in the medical laboratory of Medinova Hospital, Dhaka by using the automated method with the automatic analyzer “Hematology auto analyzer Sysmex XN 1000 System” (Hounkpatin et al. 2012)

Overview

The Sysmex XN 1000 System is a 28 parameter, automated hematology analyzer designed for *in vitro* diagnostic use in clinical laboratories. The System generates the following hematologic measurements on EDTA-anti coagulated whole blood:

The principles used by the Sysmex XN 1000 System to measure, count, and calculate the hematologic parameters are discussed generally in the first section of this chapter as part of an overview of the four measurement cycles. The parameters are then discussed individually in relation to the methodology used. At the end of the chapter is a discussion of operational messages and flags that pertain to the parameter measurements and data results.

Measurement Specifications

Four independent measurements are used in the Sysmex XN 1000 System to obtain the hematologic parameters.

- The WBC Optical Count (WOC) and the WBC Differential data are measured in the Optical Flow channel.
- The WBC Impedance Count (WIC) is measured in one Electrical Impedance channel.
- The RBC and PLT data are measured in a second Electrical Impedance channel.
- The HGB is measured in the Spectrophotometric channel.

During each instrument cycle, the sample is aspirated, diluted, and mixed, and the measurements for each parameter are performed.

Measurement Channels

- Laser Optics for WOC (WBC Optical Count) and WBC Differential
- Two impedance channels, one for WIC (WBC Impedance Count) and one for both RBC and PLT
- Hemoglobin Absorbance

WBC and Differential (Leonard and Ruben, 1986)

WIC:

Method	Electrical Impedance
Aperture Size	100 µm (diameter) x 77 µm (length)
Dilution	1:301 of blood in Diluent and WIC/HGB Lyse
Data Collection	256 channels, each channel = 0.5 fL

WOC:

Method	Laser light scatter
Light Source	Vertically polarized 5–10 mW helium-neon laser
Wavelength	632.8 nm
Dilution	1:51 of blood in Sheath Reagent
Data Collection	four angles measured: 0°, 10°, 90°, and 90° depolarized.

	Data collected in 256 channels for each angle of light scatter. (Leonard and Ruben, 1986)

RBCs and PLTs

Method	Electrical Impedance
Aperture Size	60 µm (diameter) x 72 µm (length)
Dilution	1:9,760 of blood in Diluent
Data Collection	256 channels for RBCs, each RBC channel = 1 fL
	256 channels for PLTs, each PLT channel = 0.137 fL

HGB

Method	Modified hemiglobincyanide or modified hemiglobinhydroxylamine
Light Source	Light Emitting Diode, wavelength: 555 nm
Filter	Interference Filter
	Center wavelength: 540 nm
	Bandwidth (at 1/2 peak): 22 nm
Dilution	1:301 of blood in Diluent and WIC/HGB Lyse or WIC/HGB Cyanide Free Lyse
Data Collection	Average of 5 absorbance readings for the detergent blank,
	Average of 5 absorbance readings for the sample dilution

WBC Analysis (Leonard and Ruben, 1986)

Two WBC values are provided by the Sysmex XN 1000 System:

- The WIC (WBC Impedance Count)
- The WOC (WBC Optical Count)

The WOC is the primary value reported as the WBC count. Whenever a clinically significant difference between WIC and WOC is present, the data is further evaluated to determine the most accurate value.

WBC Differential Analysis

The CELL-DYN 3700 System uses the scatter plots to differentiate the WBCs into five color-coded subpopulations:

Neutrophils (yellow)

Lymphocytes (blue)

Monocytes (purple)

Eosinophils (green)

Basophils (white)

RBC/PLT Analysis

Overview

An impedance channel is used for the determination of RBC and PLT data. A 1:9,760 dilution of the sample is made with the Diluent. The cells are counted and sized using the impedance method as they pass through the 60 x 70–µm aperture in the von Behrens RBC/PLT Transducer. Dynamic thresholding separates the PLTs from the RBCs. The 100-µL volume of sample that is analyzed is precisely regulated by the RBC/PLT metering assembly. Data is collected in 256 channels for both RBCs and PLTs.

Electrical Impedance Measurements

RBCs and PLTs are counted and sized by the Electrical Impedance method. This method is based on the measurement of changes in electrical current which are produced by a particle, suspended in a conductive liquid, as it passes through an aperture of known dimensions. An electrode is submerged in the liquid on either side of the aperture in order to create an electrical pathway through it.

As each particle passes through the aperture, a transitory change in the resistance between the electrodes is produced. This change produces a measurable electrical pulse. The number of pulses generated is indicative of the number of particles that traversed the aperture. The amplitude of each pulse is essentially proportional to the volume of the particle that produced it.

Each pulse is amplified and compared to internal reference voltage channels. These channels are delineated by calibrated size discriminators to accept only pulses of certain amplitude. Thus, the pulses are sorted into various size channels according to their amplitude.

RBC Parameters (Leonard and Ruben, 1986)

RBC Count

The red blood cell count (RBC count) is directly measured, gives the number of RBCs, and is expressed as follows:

$$RBC = \# \times 10^{6}/\mu L\ (10^{12}/L)$$

Counts below 1.0 x $10^{6}/\mu L$ ($10^{12}/L$) are displayed to three decimal places.

The RBC count is automatically corrected for the WBC count, and the corrected RBC count is displayed on the main **RUN** screen.

MCV

The mean corpuscular volume (MCV) is the average volume of the individual red blood cells. The MCV is derived from the RBC size distribution data and is expressed in femtoliters.

HCT

The hematocrit (HCT) is the ratio of red blood cells to plasma and is expressed as a percentage of the whole blood volume. The HCT is calculated from the RBC count and the MCV as follows:

$$HCT = (RBC \times MCV)/10$$

MCH

The mean corpuscular hemoglobin (MCH) is the average amount of hemoglobin contained in the red blood cell, expressed in picograms. The MCH is calculated from the RBC and the HGB as follows:

$$MCH = (HGB/RBC) \times 10$$

MCHC

The mean corpuscular hemoglobin concentration (MCHC) is the ratio of the weight of hemoglobin to the volume of the average red blood cell, expressed in percent. It is *calculated* from the HGB and the HCT as follows:

$$MCHC = (HGB/HCT) \times 100$$

PLT Parameters

Platelet count

The platelet count (PLT count) is derived from the PLT histogram after the PLT data have been analyzed by the platelet algorithm. The PLT count is expressed as follows:

$$PLT = \# \times 10^3/\mu L\ (10^9/L)$$

HGB Parameters

The Hemoglobin is directly measured and is expressed in grams of hemoglobin per deciliter of whole blood.

Erythrocyte Sedimentation Rate (ESR)

Westergren Method

When ant coagulated whole blood is allowed to stand in a narrow vertical tube for a period of time, the RBCs – under the influence of gravity - settle out from the plasma. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr).

The Westergren method requires collecting 2 ml of venous blood into a tube containing 0.5 ml of sodium citrate. It should be stored no longer than 2 hours at room temperature or 6 hours at 4 °C. The blood is drawn into a Westergren-Katz tube to the 200 mm mark. The tube is placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The distance of fall of erythrocytes, expressed as millimeters in 1 hour, is the ESR (Mohamed, 1969).

3.10. Serum Biochemistry:

Blood samples was taken in a lithium coated serum separator tubes and centrifuged at 4000 rpm for 10 minutes to separate the serum (supernatant) and the serum obtained (± 2 ml/rat) was stored in closed test tubes inside a freezer at (-20^{o}C) prior to bio-chemical analysis within 48 hours. The stored serum samples were used to determine the following biochemical parameters: creatinine, urea, and total Bilirubin were measured as functional marker for nephro-toxicity; and aspartate aminotransferase (AST), alanine aminotransferase (ALT) as marker for hepatotoxicity. This was done by using automated Biochemistry analyzer “Dimension RxL Max integrated Chemistry system”.

3.11. Histopathological study:

Histopathology is an important medical tool for the microscopic study of diseased tissue. It is performed by examining a thin tissue section under light microscopes. It consists of a number of procedures that allow visualization of tissue and cell microscopic features and recognize specific microscopic structural changes of disease (Slaoui and Fiette, 2011).

After obtaining the fresh organs weight of Heart, Kidney, lungs, liver, spleen, and brain of each rat, the organs was preserved in 10% formaldehyde (pH 7.2 to 7.4) and sections of tissues were cut for histological procedures.

Preparation of Tissue for Histopathological Analysis:

- For the histological study, a slice of tissue (2-3 mm) from each of the organ (Such as slices of the right lobe of the liver and the left kidney, Cardiac Muscle fibers for the histopathological approach of Liver, Kidney and Heart) that has been collected from exposed and control Rats were fixed in 10% neutral buffered formalin (10% formaldehyde in Phosphate buffered saline).
- After 48 hours of fixation, the tissues was placed in 70% isopropyl alcohol for 3 hours and then in each ascending strength (80%, 90%, 100% isopropyl alcohol) for 2 hours each. The amount of alcohol used was 15 times of the size of the tissue.
- Then the tissues were dipped in acetone for a period of 1 – 2 h with periodical shaking.

- Then acetone was removed and xylene was added to check for the appearance of milkyness. If milkyness appears then repeat the dehydration procedure.
- The dehydrated tissue was then soaked in paraffin wax (M.P = 56°C) for a period of 1h at 58 – 60 °C.
- The molten paraffin was then poured into L-block along with the tissues and allowed to become hard.
- Tissue sections of 3–5µm thick were cut by using a microtome and then mounted on the slides with Mayer's albumin solution (a mixture of equal parts of egg white and glycerin, beaten and filtered with the addition of 1% sodium salicylate) and keep in warm oven for 2 h at 60°C.
- The slides containing paraffin sections were placed on a slide holder.
- The tissue was deparaffinize with Xylene for 20 – 30 minutes
- The tissue was then rehydrated successively with 100%, 90%, and 80% isopropyl alcohol for 2 – 3 min. each and put it into water for 3 min.
- The excess water was blotted and the tissue was put into Hematoxylin stain for 1 – 2 min.
- It was removed from Hematoxylin stain and then again put it into tap water for 1 – 2 min.
- the slides containing tissue sections was dipped into 1N HCl followed by Scott's water (Sodium Bicarbonate 3.5 g, Magnesium sulphate 20 g, distilled water 1 liter) for 1 min each.
- The tissue was immersed in Eosin stain for 30 sec.
- The tissue was dehydrated successively with 80%, 90%, 100% isopropyl alcohol and finally with Xylene for 20 – 30 min.
- The cover slip was placed on the slides using one drop of DPX, taking care to leave no bubbles and dry overnight to make the permanent slide and then assessed in light microscope (Pattanayak and Majumder, 2009).

3.12. Statistical Study:

Statistical analysis was performed on a PC using SPSS, V.22. Results are expressed as Mean ± SE of n experiments (where n represents the number of animals used). The differences between the treated and control rats were evaluated using the Students t-test p (T > t) = 0.05. The differences were statistically significant if the value of p was less than 0.05 and not significant if the value of p was greater than 0.05.

3.13. Determination of Heavy metals in the sample water:

From various literatures, it has been found that in Tongi, Gazipur areas river water (Turag River, Tongi Khal) and sediments the concentration of some heavy metals such as Pb, Cr, Cd, Zn, Mn, Cu found more than the permissive limit when compared with the National and International Organizations such as WHO, USEPA, EPA, EUC (Mokaddes, Nahar, and Baten, 2013; Zakir et al. 2006; Bakali et al. 2014). These metals again found to have some physiological effect when present in more than the desired limit, especially in kidney and liver (Sabath and Robles-osorio, 2012).

Since the level of toxic and heavy metals in Tongi areas drinking water has not been investigated yet, therefore the concentration of heavy metals in the water resources of Kathaldia village of Tongi, Gazipur district was measured which will be a great concern for proper assessment of drinking water quality and possible hazards to public health.

The concentration of Heavy metals that were determined in this study includes Cd, Pb, Cr, Zn, As, Mn, Cu, Se, Cr, and Ni. The concentrations of resulting heavy metals were then compared with the national and International organizations. (WHO-2008, USEPA, EPA, EUC). The effects of heavy metals found more/less than the maximum admissible limits were noted.

3.14. Preparation of Sample:

The sample treatment prior to the analysis of heavy metals in the sampled water was done according to the approved guidelines of WHO, 2008 for Drinking water Quality analysis and United States environmental protection Agency, 1983 for chemical analysis in water and wastes. 100 ml of sample water was taken in beaker (prewashed with distilled water). Then 3 ml of concentrated HNO_3 (extra-pure) was added into the samples and mixed. The beaker was then covered and heated in a hot plate until the volume has been reduced to 20-30 ml. After this treatment, the volume was adjusted to 100 ml by adding deionized water. The sample was then stored at 4°C for a short period of time to minimize the physicochemical alteration. Then the sample was delivered for analysis (WHO, 2008; Pirsaheb, Khosravi, Sharafi, Babajani, and Rezaei, 2013).

3.15. Reagents and Chemicals:

Deionized double distilled water was used throughout the testing procedures. The standard solutions contained 1 g/L of all elements were prepared by proper dilution of 1000 parts per million (ppm) of stock standard with de-ionized double distilled water. For the analysis of all other elements no modified medium was used. All solutions were prepared with de-ionized water and the daily standards were prepared. The stock solution of selected heavy metals, containing 1000 ppm and nitric acid was used without additional purification (Pirsaheb et al. 2013).

3.16. Analytical Method:

The determination of the concentration of selected Heavy metals was done in the Wazed Miah Science Research Center of Jahangirnagar University by using an atomic absorption spectrophotometer (AAS) (Shimadzo, AA7000, Japan) because it is widely accepted as the standard technique for metals determination since they offer satisfactory sensitivity and fairly low acquisition cost (Es'haghi et al., 2011). Mono element hollow cathode lamp was employed for the determination of each heavy metal of interest. The analysis for the majority of the trace metals like chromium (Cr), cadmium (Cd), nickel (Ni), zinc (Zn), copper (Cu), lead (Pb), manganese (Mn), Selenium (Se) was done by Shimadzo, AA7000 Flame Atomic Absorption Spectrophotometer. Only the analysis of Arsenic (As) was done by Shimadzo, AA7000 Graphite Furnace Atomic Absorption Spectrophotometer. It was used because through that even very minute amount of metal can be determined.

The calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. A reagent blank sample was analyzed and subtracted from the samples to correct for reagent impurities and other sources of errors from the environment. Average values of three replicates were taken for each determination. A standard curve was prepared by plotting the absorbance reading on Y-axis versus the concentration of each standard solution of metal on X-axis. Then, the concentration of metal was calculated in the sediment samples of interest by plotting the AAS reading on the standard curve (Lokhande et al. 2011; Mokaddes et al. 2013; Islam, 2014).

4.RESULTS

4.1. Body and Organ weight:

Figure 4.1(a) represent the growth curve of rats exposed to sample water over a period of 56 days. All the Animals found to be increased in body weight after 56 days relative to their initial weight at the beginning of the experiment. But statistically no significant differences in body weight of the animals in each group were observed (p >0.05) relative to one another and relative to the control. This observation compared favorably with the observation of Oloyede *et al.* (2003) which revealed that polluted water supports growth in rats.

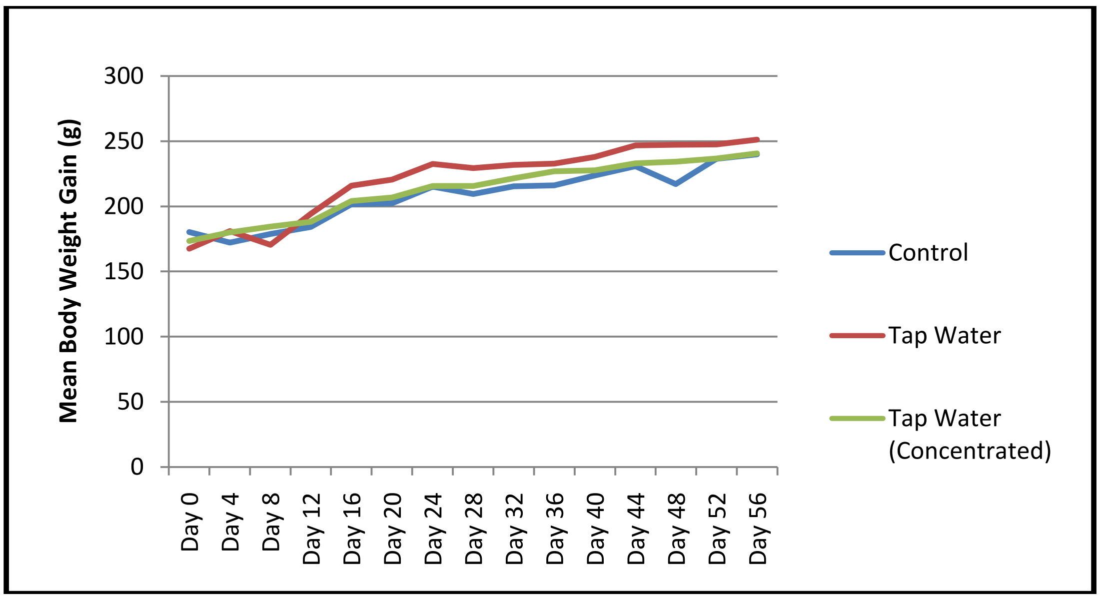

Figure 4.1(a): Growth response curve of male rat orally exposed to sample water. Results were expressed as mean body weight of rats after 56 days of experiment and changes are statistically non-significant when compared with control rats (P> 0.05). All the values are Mean ± SE; n= 6.

There was small decrease in the weight of liver in the treated group exposed with Concentrated Tap water which was statistically significant (p< 0.05) in comparison to control group. A slight decrease in the weight of liver in treated group exposed with normal Tap water has also been found but is not statistically significant (p>0.05) in comparison to control group. Thus, it may be considered as variation close to or within the normal range. Weights of other organs are not changed significantly and are present in Figure 4.1 (b) and Data is presented in Table 4.1.

Table 4.1 Individual Organ weight of Rat (n=6) after 8 weeks of experiment.

Organ	Group 1 (Control)	Group 2 Tap water (Normal)	Group 3 Tap water (Concentrated)
Heart	0.63 ± 0.03	0.60 ± 0.03	0.58 ± 0.02
Kidney	0.71 ± 0.04	0.66 ± 0.03	0.60 ± 0.04
Lungs	0.97 ± 0.05	1.04 ± 0.07	0.97 ± 0.04
Liver	6.45 ± 0.29	6.01 ± 0.18	5.73 ± 0.27 *
Spleen	0.49 ± 0.01	0.56 ± 0.02	0.48 ± 0.05

All values are expressed in Mean ± SE; * Value (P= 0.005) is significantly different from the control group (P<0.05)

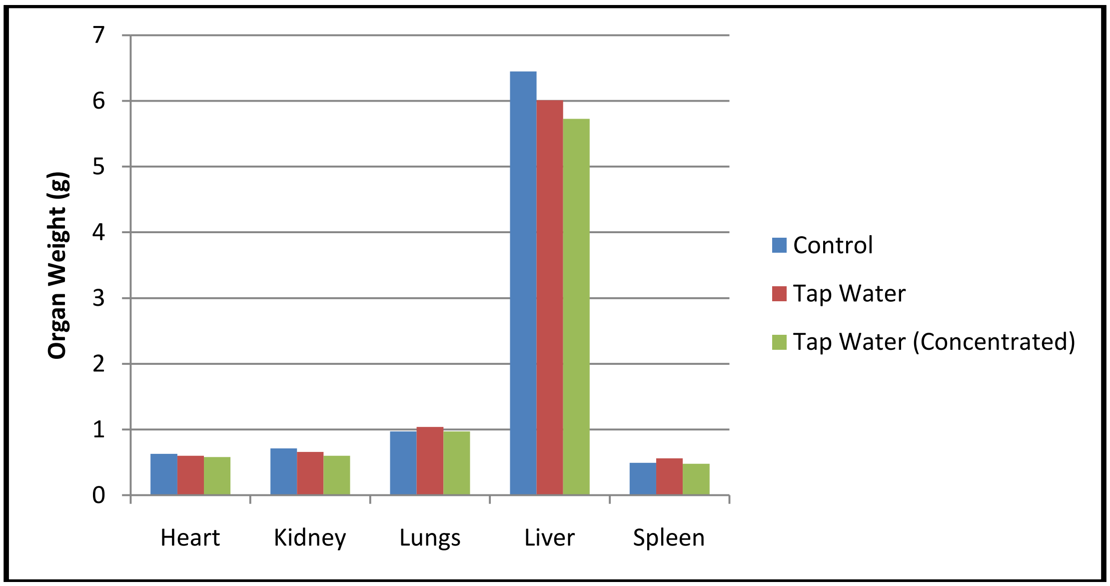

Figure 4.1(b): Individual Organ weight of Rats (n=6) after 8 weeks of experiment. Results were expressed as mean SEM (n = 6) of rats after 56 days of experiment. Tap water (Normal) exposed group showed non-significant changes as compared with control group (p>0.05). Means of 6 rats only.

4.2. Hematological parameters:

Hematological results of this study are as presented in Table 4.2. It was observed that hematological parameters, Hemoglobin, HCT, MCV, MCHC, ESR, WBC, and RBC in both control and experimental group showed no significant difference (p>0.05) after the period of

56 days experiment. The value of platelets were found to decrease significantly ($p<0.05$) in the experimental group exposed with normal tap water as compared with the control.

In the differential leukocyte count, neutrophils showed to decrease in the experimental group exposed with Concentrated Tap water but statistically no significant difference was obtained as compared with control group. The lymphocyte, Monocytes and Eosinophils counts showed no significant difference ($p>0.05$) in the experimental group as compared to control (Table 4.2).

Table 4.2 Hematological Values (Mean ± SE) of Sprague dawley rats (n=6) after 8 weeks of experiment.

Parameters	**Group 1 (Control)**	**Group 2 Tap water (Normal)**	**Group 3 Tap water (Concentrated)**
Hemoglobin	13.75 ± 0.30	13.75 ± 0.29	13.92 ± 0.15
HCT/PCV	10.03 ± 9.57	0.46 ± 0.01	0.46 ± 0.007
MCV	58.22 ± 0.53	50.75 ± 6.77	48.23 ± 9.57
MCH	17.18 ± 0.16	16.95 ± 0.13	17.35 ± 0.40
MCHC	29.5 ± 0.16	29.58 ± 0.20	30.10 ± 0.31
ESR	6.17 ± 0.48	6.83 ± 1.07	6.50 ± 0.62
WBC	4616.66 ± 913.39	4433.33 ± 202.76	5050 ± 469.57
RBC	7966.66 ± 223.11	8033.33 ± 170.62	8000 ± 228.03
Platelet	838700 ± 28406.50	418350 ± 151163.89*	795716.66 ± 50197.61
Differential Count			
Neutrophils	29.83 ± 9.19	28 ± 2.43	21.83 ± 2.21
Lymphocytes	74.17 ± 1.90	68 ± 2.77	74.66 ± 2.33
Monocytes	2.33 ± 0.21	2.167 ± 0.30	2.167 ± 0.30
Eosinophils	2 ± 0.25	1.83 ± 0.30	1.33 ± 0.21
Basophils	0.00	0.00	0.00

WBC, White blood cells ($X10^3/\mu L$); MCV, mean corpuscular volume (fL); RBC, red blood cells ($X10^6/\mu L$);MCH, mean corpuscular hemoglobin (pg); HGB. hemoglobin (g/dL); PLT, platelets ($X10^3/\mu L$); HCT, hematocrit (%); LYM: lymphocytes (%); MCHC, mean corpuscular hemoglobin concentration (g/dL). *Value (P = 0.035) is significantly different from the control group ($P<0.05$)

4.3. Biochemical indicators of hepatic and renal functions:

Estimation of activities of various enzymes in tissues and body fluids is a critical and well known aid in disease investigation and identification (Malomo, 2000). Such estimation will give an understanding to the site of cellular damage as an after effect of strike by medication or other chemicals. The estimation of enzyme activity is especially imperative since it sums up the catalytic impact of different variables such as inhibitors and activators during such pathological conditions. The specific activity of aspartate amino transferase (AST) has found to decrease in the experimental group but showed no significant difference (P>0.05) in comparison with the control group. The activity of alanine amino transferase (ALT) showed significant decrease (P<0.05) in the experimental group in comparison with the control group. The level of Urea (mg/dl), creatinine (mg/dl) and total Bilirubin (mg/dl) showed no significant changes in the activity in comparison with the control group. All values are given in Table 4.3 and changes are presented in Figure 4.3 (A- E).

Table 4.3 Biochemical values (Mean ± SE) of Sprague dawley Rats (n=6) after 8 weeks of experiment.

Parameters	**Group 1 (Control)**	**Group 2 Tap water (Normal)**	**Group 3 Tap water (Concentrated)**
S.Urea (mg/dl)	36.35 ± 2.32	36.54 ± 3.57	34.01 ± 3.03
S.Creatinine (mg/dl)	0.84 ± 0.02	1.03 ± 0.09	0.84 ± 0.08
S.ALT (U/L)	47.17 ± 3.75	34.33 ± 1.69 *	38.67 ± 3.59
S.AST (U/L)	186 ± 8.78	183 ± 3.53	169.66 ± 12.11
S.Total Bilirubin (mg/dl)	0.29 ± 0.03	0.292 ± 0.02	0.28± 0.02

* value (P=0.045) is significantly different from the control group (p <0.05);(n=6)

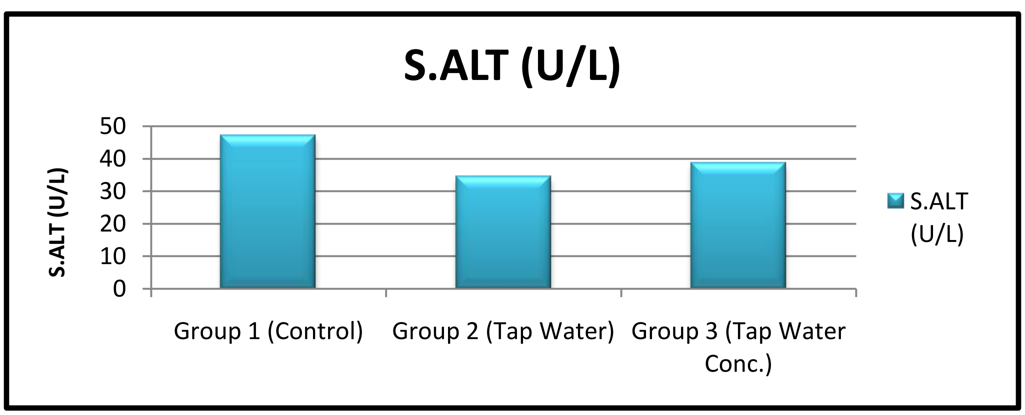

Figure 4.3 (A): The levels of alanine amino transferase (ALT) in rat blood serum. Significant changes obtained as compared to the control (p<0.05).

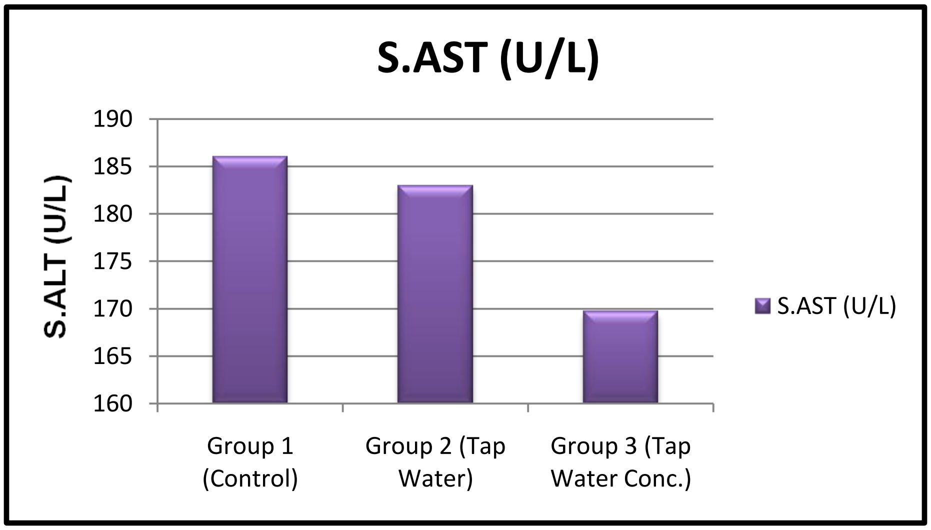

Figure 4.3 (B): The levels of aspartate amino transferase (AST) in rat blood serum. No significant changes as compared to the control (p>0.05).

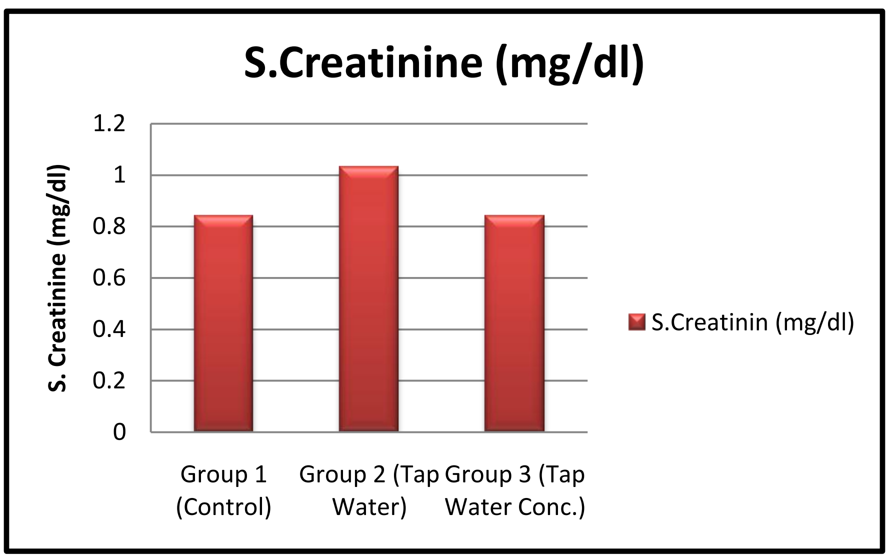

Figure 4.3 (C): The levels of Creatinine in rat blood serum. No significant changes as compared to the control (p>0.05)

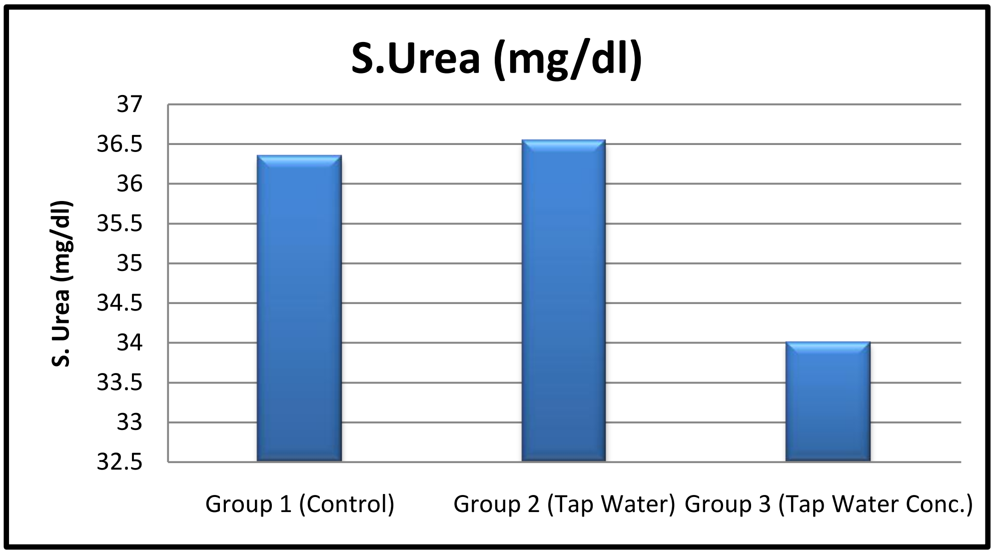

Figure 4.3 (D): The levels of Urea in rat blood serum. No significant changes as compared to the control (p>0.05).

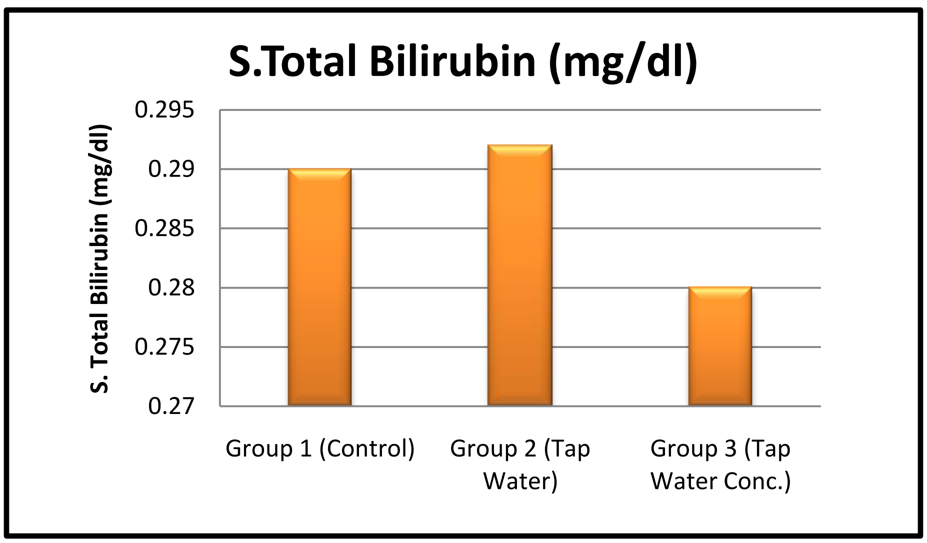

Figure 4.3(E): The levels of Total Bilirubin in rat blood serum. No significant changes as compared to the control (p>0.05).

4.4. Histopathological Findings:

The result of the histopathological responses of the Brain, Heart, Kidney, Lungs, liver and spleen after the period of 8 weeks study are presented in plates 4.4(1-6). By using light microscopy (x100 magnifications), the histopathological changes especially prominent lesions and observed recoveries were photographed.

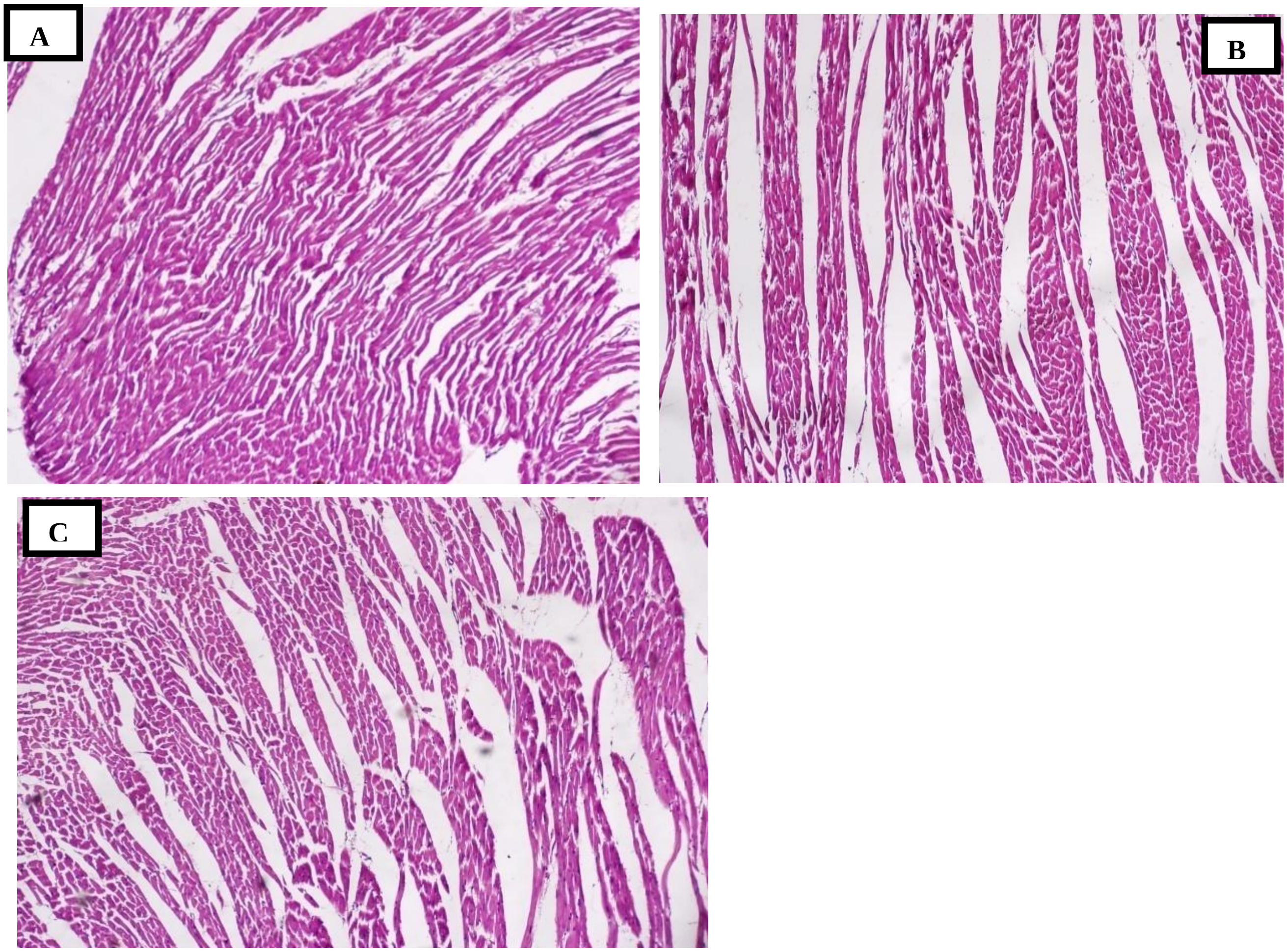

Plate 4.4.1:

A: Histological section of Heart of the control rat (H&E x100): Section showing well developed and normal distribution of cardiac muscle fibers; normal appearance of cardiac tissue.

B: Histological section of Heart of the rat treated with Normal Tap water (H&E x100): showing somewhat normal appearance of collagen fibers in the cardiac muscle fibers. Showing irregular distribution of nuclei of myocytes of cardiac tissue.

C: Section of Heart of rat treated with concentrated Tap water (H&E x100): Showing somewhat normal appearance of the cardiac tissue and somewhat normal appearance of collagen fibers of cardiac muscle fibers.

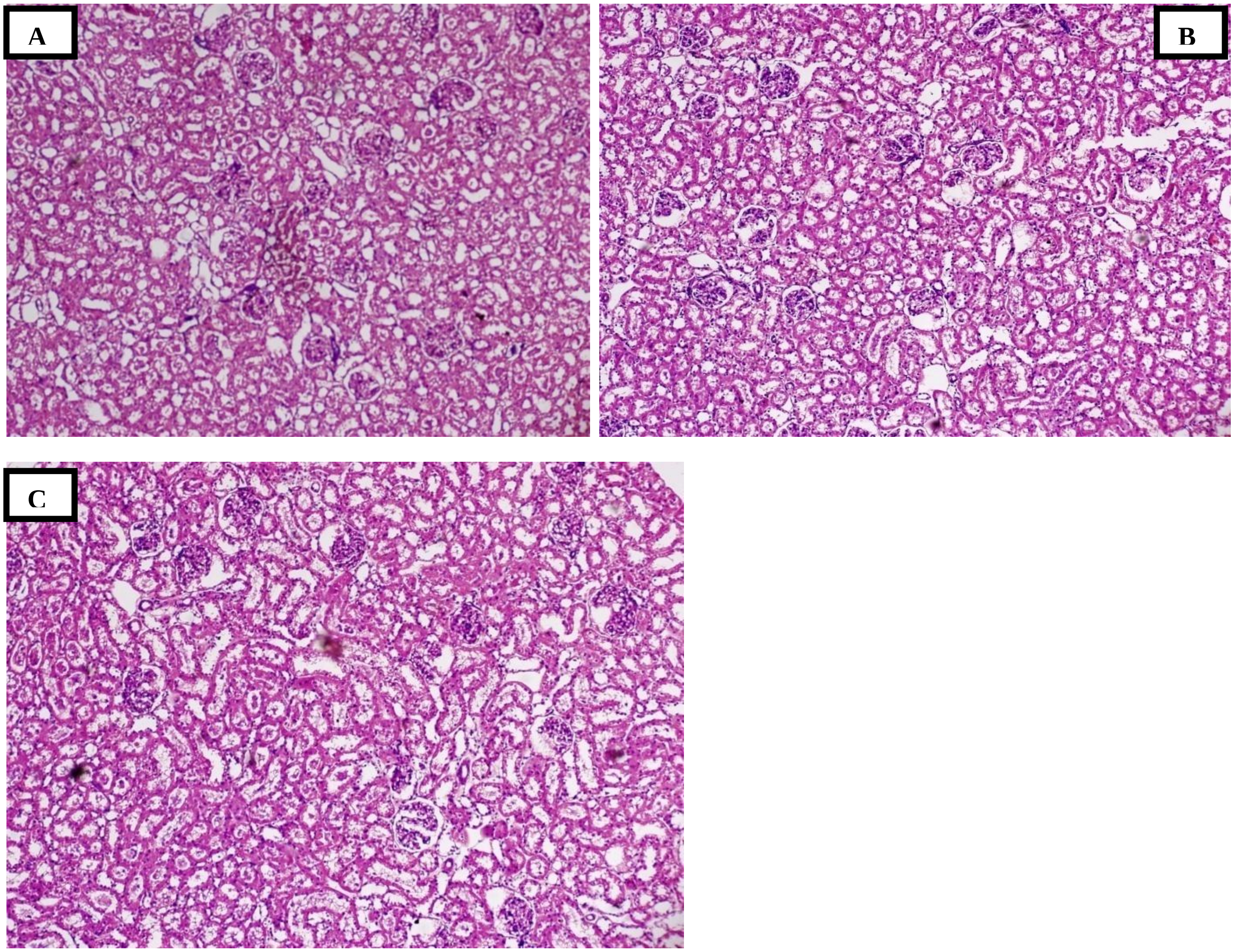

Plate 4.4.2:

A: Histological section of kidney of control rat (H&E x100): Section showing normal histological features including glomerulus and tubules. Glomeruli are normal and tightly filing the Bowman's corpuscle. Renal tubules are lined with typical thick cubic epithelium.

B: Histological section of Kidney of the rat treated with normal Tap water (H&E x100): section showing mild interstitial hemorrhage and tubular necrosis at the renal cortex.

C: Histological section of Kidney of the rat treated with concentrated Tap water (H&E x100): Section showing an intact glomerulus and tubules. Mild tubular necrosis and severe congestion at the renal cortex.

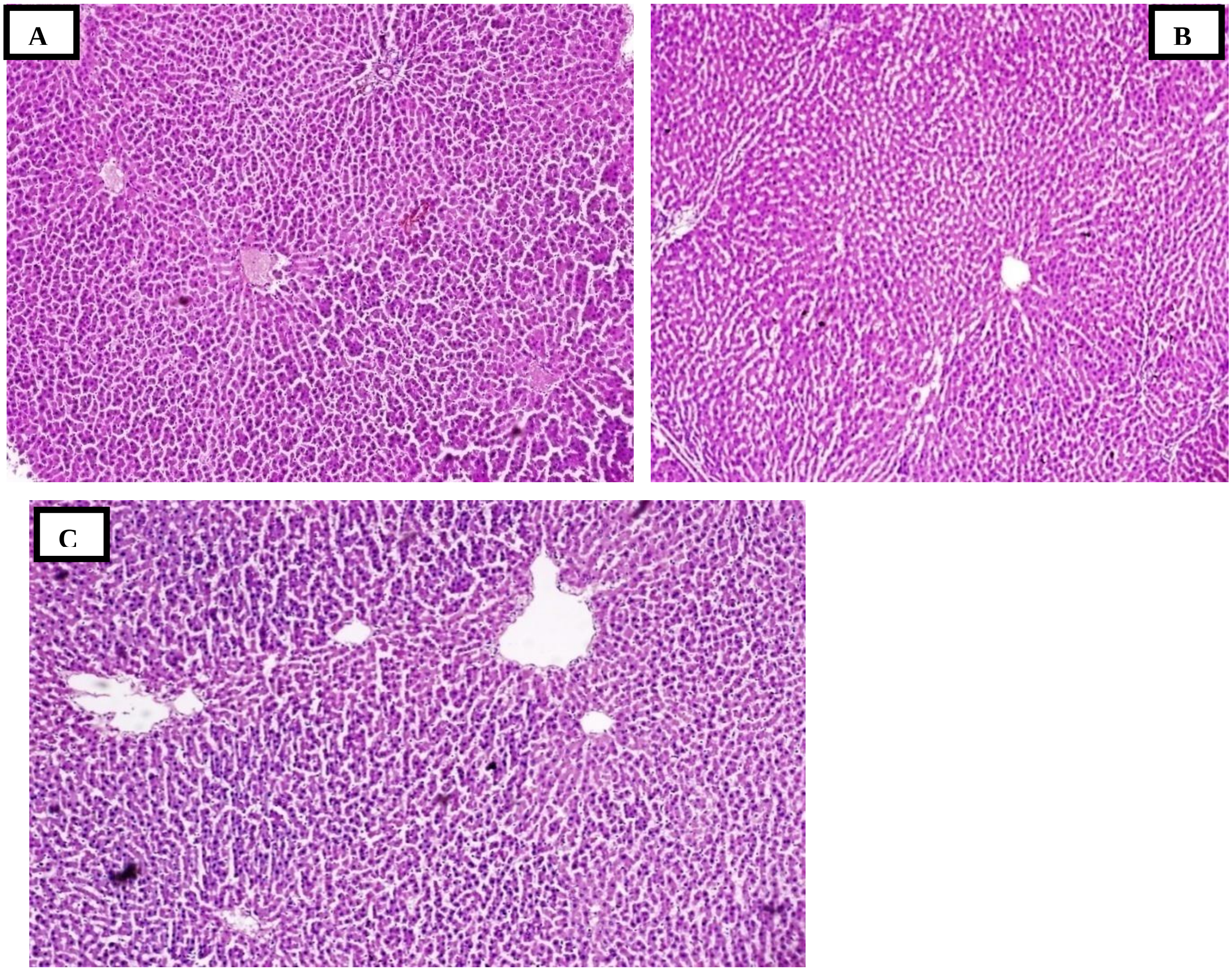

Plate 4.4.3:

A: Histological section of Liver of control rat (H&E x100): Section showing normal hepatocytes and red cell stasis within the central vein and sinusoids.

B: Histological section of Liver of the rat treated with normal Tap water (H&E x100): section showing mild disturbance of the sinusoidal structure and sinusoidal narrowing as compared to the control.

C: Histological section of Liver of the rat treated with concentrated Tap water (H&E x100): Section showing mild narrowing of sinusoids along with cytoplasmic vacuolation. It also shows the presence of hepatocytes binucleation and irregular shaped stained nuclei.

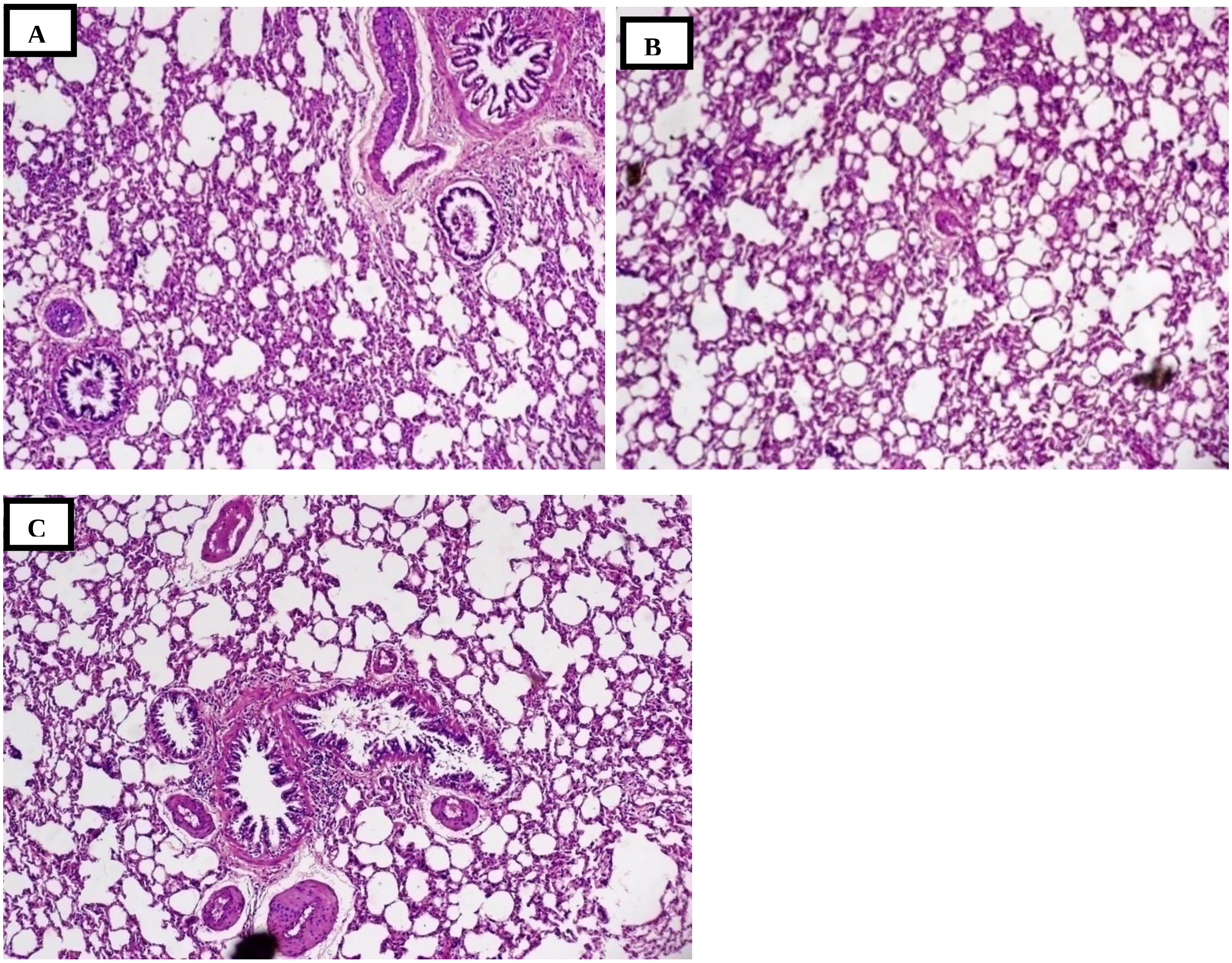

Plate 4.4.4:

A: Histological section of Spleen of control rat (H&E x100): Section showing normal histology of spleen, Region of the periarterial lymphoid sheath.

B: Histological section of Spleen of the rat treated with normal Tap water (H&E x100): section showing the presence of mild white pulp hyperplasia.

C:Histological section of spleen of the rat treated with concentrated Tap water (H&E x100): section showing the presence of white pulp hyperplasia as compared with the control.

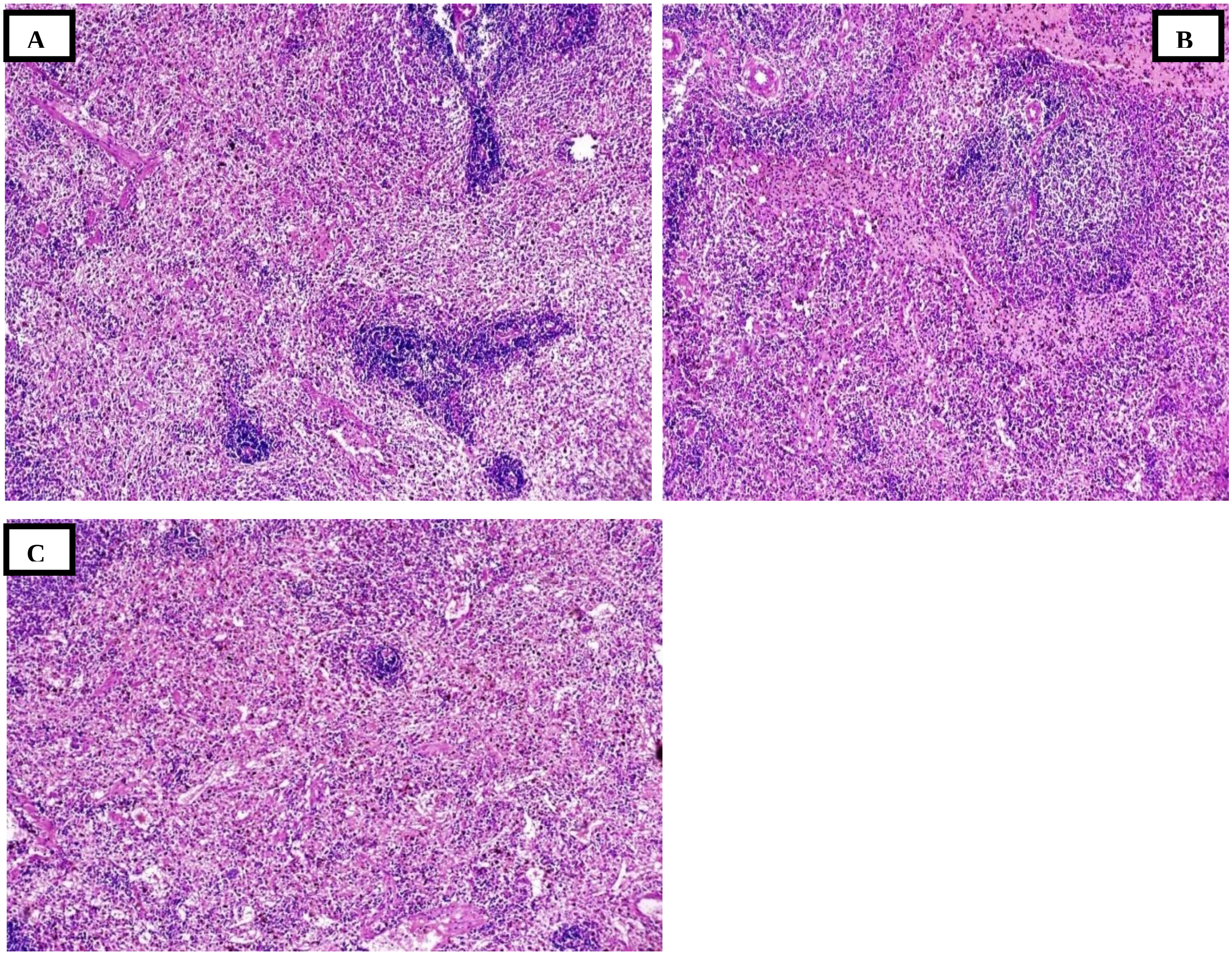

Plate 4.4.5:

A: Histological section of lungs of control rat; **B:** Histological section of lungs of the rat treated with normal Tap water, **C:** Histological section of lungs of the rat treated with concentrated Tap water (H&E x100). In section B and C, alveolar fibrosis is observable in contrast to the well-stained branching alveolar in the group A and no significant change is found.

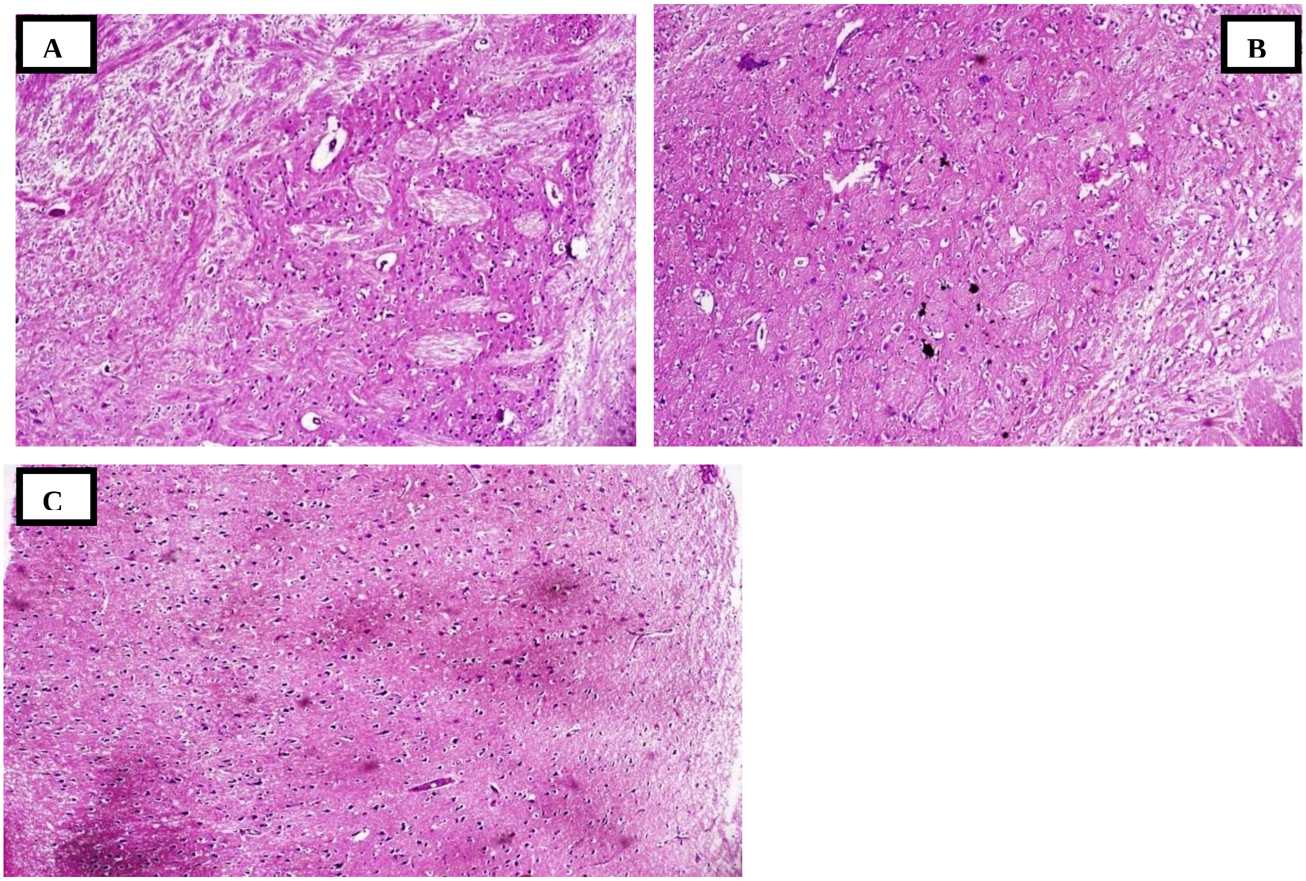

Plate 4.4.6:

Histological section of brain: The cortical layers, neuronal cells and blood vessels are well delineated in the control group (A). No ovbious changes were noted in the treated groups (B & C) as compared to the control group.

4.5. Concentration of Heavy Metals:

The concentration of Heavy metals found in the normal sample tap water with the help of Atomic Absorption Spectroscopy are given in Table 4. The obtained result shows the concentration of Manganase (Mn), Arsenic (As), and Cadmium (Cd) high than the maximum recommended limits of WHO (2011) guideline of drinking water quality (Table 4.5; Figure 4.5).

Table 4.5 Concentration of Heavy Metals found in the sample Tap water of Kathaldia Village and maximum admissible limit set by WHO(2011).

Metals	Concentration Found by AAS (ppm)	WHO (2011) Maximum admissible limits (mg/l)
Cr	0.0074	0.05
Mn	0.1249	0.05
Ni	0.0211	0.07
Cu	0.05	1.0
Zn	0.33	5.0
As	0.89	0.05
Se	0.544	0.01
Pb	0.037	0.05
Cd	0.013	0.005

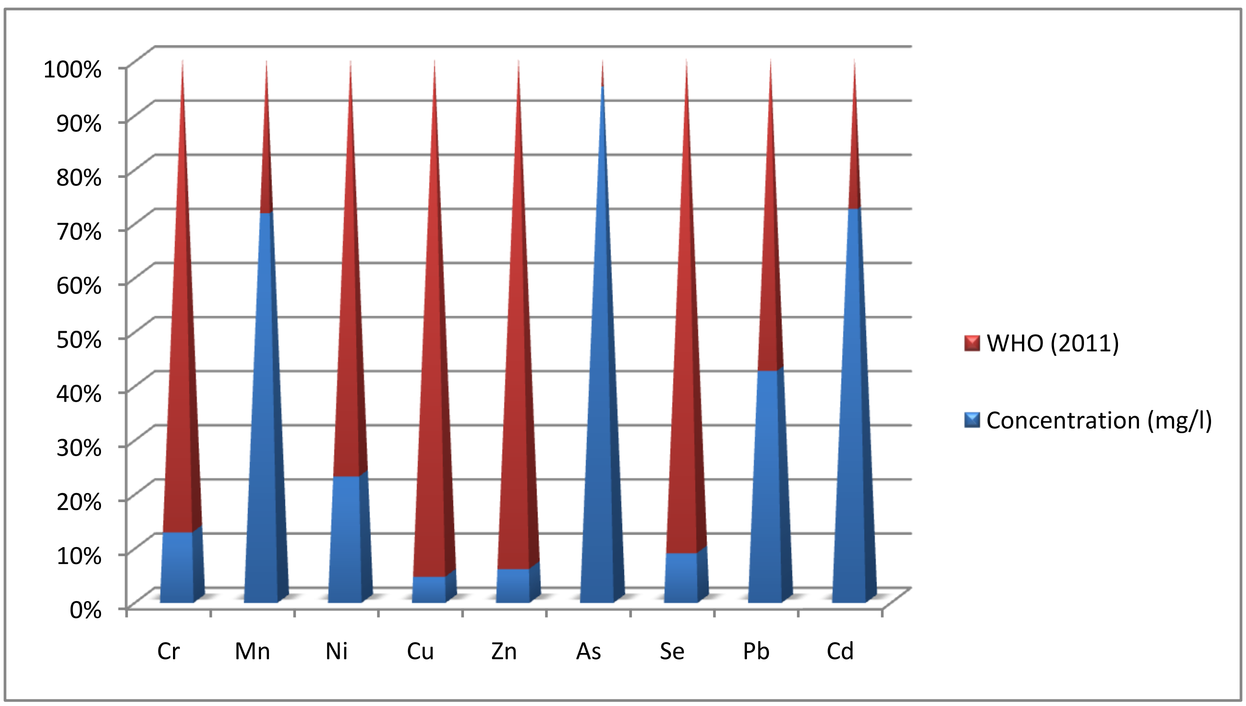

Figure 4.5: The concentration of Heavy metals in the sample water and comparison with the WHO (2011) standard limit.

5.DISCUSSION

After the completion of 56 days period of study on industrial areas drinking water and observing the effect of the study on white albino Sprague Dawley by several statistical analysis the possible implications that can be drawn to justify all of the parameters of the result has been discussed in this section.

5.1.Effect on Body weight and Organ weight:

During the study period, normal growth and increase of body weights of rat have been observed relative to their initial weight at the beginning of the experiment. Also, statistically, no significant difference has been observed as compared to the control. These findings favor the observation of Oloyede, Sunmonu, Adeyemi, and Bakare, (2003) that polluted water supports the normal growth of the rat (Figure 4.1.b).

The statistical analysis of the individual organ weight of rat after the completion of 8 weeks of study includes Heart, Kidney, Liver, Spleen, and lungs. The result showed a small decrease in the weight of liver in the experimental group treated with normal Tap water. But in the experimental group that has been treated with Concentrated Tap water showed a significant decrease in liver weight as compared to their control (Table 4.1). This observation can be correlate with the study of Zahid and Abidi, (2003) which demonstrate that the decrease in liver weight may occur due to the presence of free radicals which interfere with the development and growth of hepatocytes and causes the destruction of already formed parenchymal tissue. Free radicals are cytotoxic and act as a mediator of tissue injury by causing direct damage to the tissue or by initiating additional immunological reactions that result in damage. Thus, in this study in order to have a conspicuous idea the histological observation of liver tissue needs to be considered which will be discussed in later part. However, the weight of other organs does not show any significant changes as compared to their control (Table 4.1; Figure 4.1.b).

5.2.Effect on Hematological Parameters:

The hematological examinations could be utilized to assess the physiological status of an animal and an evaluation of hematological parameters can be utilized to decide the degree of pernicious impact of foreign compounds on the blood.

In the statistical analysis of hematological parameters, no significant changes were observed in the case of Hemoglobin, HCT, MCV, MCHC, ESR, WBC and RBC in the experimental group as compared to their control. But the value of platelets was found to decrease significantly in the experimental group exposed to normal tap water as compared to the control (Table 4.2). Platelets are considered as the smallest formed element of blood which plays a vital role in maintaining the hemostasis. To be more specific, they form a plug at the sites of endothelial cell injury. Moreover, it also plays a role as mediators of inflammation and helps in coagulation (Travlos, 2007). In many studies, it has been observed that the decrease of platelets may occur due to some reasons such as entrapment of platelets in an enlarged spleen, endothelial injury of hepatocytes, several autoimmune diseases (which causes Immune thrombocytopenia), presence of bacteria in blood, viral infections of hepatocytes, and in some types of anemia (Gauer and Braun, 2012; George, 2000; Moffat, 2013). Since in this study, no significant changes in red blood cell (RBC), and in mean corpuscular volume (MCV) as compared to control was observed, thus presence of anemia cannot be considered as a reason for the decrease of platelets (Moffat, 2013).

Again in this study, the value of platelets was found to increase in the experimental group exposed to concentrated tap water in comparison with the group treated with normal tap water even though statistically no significant changes was observed when compared with the control (Table 4.2). But the observation indicates that the process of boiling/ heating used in making concentrated tap water somehow helped in the removal of pathogenic bacteria and viruses (Fawell and Nieuwenhuijsen, 2003). In the differential leukocyte count, neutrophils showed to decrease in the group treated with concentrated tap water even though statistically no significant difference was obtained as compared with their control. But the decrease of neutrophils somehow indicates that presence of Endotoxin (French et al. 2013) and this finding can be correlate with the study of Vuaden, Furstenaua, Savio, and Sarkisa (2009) that presence of Endotoxins in blood decrease platelets but these findings are not enough to reach into a conspicuous conclusion for which further observations is highly recommended. The lymphocytes, Monocytes, and Eosinophils count showed no significant changes as compared with the control (Table 4.2).

5.3. Effect on Serum Enzymes:

Serum alanine aminotransferase (ALT) and aspartate aminotransferase are considered as well known biomarkers to predict the liver condition. ALT is usually considered as a more sensitive indicator of hepatocellular injury than AST. In the statistical analysis of the biochemical enzymes, the level of ALT was found to decrease significantly in the group treated with normal tap water as compared with the control. The value of ALT also showed a normal decrease in the experimental group exposed to concentrated tap water but statistically no significant differences was observed as compared to the control (Table 4.3). The observation can be correlated with the study of Abdelhalim and Moussa, (2013) which demonstrate that the decrease of ALT and AST might indicate liver dysfunction, liver injury or presence of any viral infection of hepatocytes. It also proposed that the significant decrease of ALT and AST might indicate the exposure to free radicals by inducing oxidative stress which is again linked to organ damage. The level of creatinine and urea which is considered as an indicator of kidney function has been observed to decrease in the group treated with concentrated tap water even though statistically, no significant changes as compared to the control was observed. Thus, it again somehow corroborates the previous findings that the decrease of biochemical enzymes might occur due to the induced of oxidative stress that is linked with the oxidative damage of the organ by the exposure of radiation or free radicals (Abdelhalim and Moussa, 2013). Therefore, it can be predicted that in the experimental water there might have some chemicals or compounds present which is somehow directly or indirectly involved in inducing oxidative stress.

5.4. Observation of Heavy metals:

The investigation of the presence of heavy metals in drinking water and the associated hazards on biological system is considered as an important parameter in the study of drinking water quality. In this study, the observation of the heavy metals showed a high concentration of Manganese (Mn), Arsenic (As) and Cadmium (Cd) than the maximum permissible limit need to be present in drinking water according to the WHO guidelines for drinking water (WHO 2011).

The concentration of manganese in the sample tap water was found 0.1249 mg/l which is very high than the maximum permissible limit (0.05 mg/l) of WHO (2011). Manganese is an essential trace element which is found to produce some toxic effect upon the long-term exposure. It has been reported that chronic exposure to manganese causes serious liver function and kidney damage (Indravathi, K, and Devi.C, 2014). Arsenic and cadmium are the metals which are found to be toxic at a very trace level. The concentration of Arsenic found in the sample water was 0.89 ppm which is much higher than the (0.05 mg/l) WHO permissible limit. On the other hand, the concentration of cadmium also found in a higher concentration (0.013 mg/l) whereas the maximum permissible limit of Cadmium is 0.005 mg/l (WHO 2011). The cadmium has always been considered as one of the most toxic metal since it produces a toxic effect even at a very low concentration and can easily bio-accumulate in the body. Therefore, it has always been considered as one of the major pollutants in the water by several researchers (Mohod and Dhote, 2013).

In several studies, it has been observed that the presence of Arsenic, cadmium and lead greatly damage the immune responses of the body which in turn effect the hematology of the body by decreasing the platelets. Moreover, the presence of arsenic alone in a higher amount has been found to alter the biochemical enzymes and usually reduces the level of AST (Mahaffey and Fowler, 1977; Islam et al. 2011). These findings somehow corroborate the previous observation to justify the reason for the decreased platelet and AST value in the experimental rat of this study.

Again in several studies, it has been observed that cadmium in a trace amount instigates oxidative stress in several organs especially in the liver cells. Because cadmium has a high affinity for sulfhydryl (-SH) groups, disabling numerous enzymatic reactions, amino acids, sulfur-containing antioxidants that include N-acetylcysteine, glutathione, and alpha-lipoic acid which in turn follows up with a subsequent decrease in antioxidants and thus increases oxidative stress. In the case of long term of exposure cadmium increases the production of reactive oxygen species (ROS) such as superoxide radical, peroxide, hydroxyl radical, thus inhibits the serum superoxide dismutase (SOD) enzyme which acts against free radicals.

Thus, it ends up with causing platelet aggregation, oxidative damage of the liver, kidney and testis (Fahim et al. 2012). Moreover, in some studies it was demonstrated that oxidative stress decrease the liver weight along with the decrease of serum ALT and AST and also interferes with the body's normal immune response (Latinwo, Badisa, Ikediobi, Odewumi, Lambert, and Badisa 2006; Kheradmand, Alirezaei, and Dezfoulian, 2013; M, Abdelmouleh, Ellouze, Jamoussi, and El Feki 2009). These findings to some extent validate the previous observation of this study.

5.5. Histopathological observation:

Through the microscopic histological observation of the tissue the presence of any lesions, inflammation, swelling, damage or any alteration in the tissue due to the presence of some disease or exposure of the organ to any pernicious compound can easily be determined. Even though the 8 weeks of study is too early to create any conspicuous alteration in the histology of the organs but in some sections few alteration of the tissue has been observed as compared with the control. The histopathological observation of Heart showed normal distribution of cardiac muscle along with the normal appearance of cardiac tissue. Even though in the experimental group irregular distribution of cardiac tissue has been observed but no distinct alteration has been observed as compared to the control to justify the reason.
The histopathological observation of kidney in the experimental group manifested severe congestion at the renal cortex, intact glomerulus, and tubules as compared to the histopathology of the control group. Therefore, it might predict that due to the short term of study distinct alteration of the kidney tissue does not occur but this mild alteration might validate the presence of kidney dysfunction at an initial stage (Awe and Banjoko, 2013).

In the histopathological section of the liver of the experimental groups few alterations has been noticed as compared to the control, which might indicate the presence of mild liver dysfunction to corroborate the previous observation of biochemical enzymes. In the experimental group mild disturbance of the sinusoidal structure along with sinusoidal narrowing and the presence of irregular shaped stained nuclei has been noticed as compared to the control group, which indicates the presence of reperfusion injury in the liver tissue as a result of oxidative damage through the induction of oxidative stress and adhesion of platelets in the kupffer cells (Ogawa et al. 2013).

The histopathological observation of spleen showed the presence of mild white pulp hyperplasia as compared with the control which again somehow validate the decrease of platelets due to the enlarged spleen but to have a distinct result long-term study is needed (Elmore, 2006). The histopathological study of Brain and lungs showed no obvious changes as compared to the control group.

5.6 Overall Observation:

Therefore, from the overall discussion it can be predicted that the presence of some toxic heavy metals in the sample water in a high amount might have created some negative effect on the experimental Sprague-Dawley rat. Somehow those effect is linked with the oxidative damage of some of the organs to some extent due to the instigate of oxidative stress by some toxic metals. Even though eight weeks of study is a very short period to develop distinct damage in the organs and to know the effect of some heavy metals in the drinking water, but the outcomes of this study can be used as an important tool for assessing the possible health consequences in the long term of study.

6. CONCLUSION

To conclude the whole study, it can be added that this study to some extent has validated the findings of several researchers who have enlighten the fact that industrialization is one of the major reason behind the deterioration of water sources. Since Tongi is considered as an industrial hub, thus the impact of those industries has been seen to affect the standard of water. However, due to the presence of very few work on drinking water of industrial areas especially in Bangladesh, a preliminary study was undertaken to evaluate the health consequences of drinking water consumed by the dwellers living in the tongi industrial area. Even though a large number of sample Due to the presence of some limitations, one of the rural areas of Tongi was focused and the outcomes of this study observed the presence of Cadmium, Arsenic and Manganese in a very high amount in their drinking water which has also found to initiate some negative alteration in the experimental animal. This observation somehow indicates that the community members living in the study area are being affected by this water since their drinking water contains some heavy metals more than the permissible limit of WHO. However, for the detailed analysis of water quality and to reach into a conspicuous conclusion this study demands for a longer period of study to confirm the study reliability. Therefore, the observations of this study can be used to implement the long-term study by broadening the sampling areas.

Since this study predicted the presence of free radicals due to the induced oxidative stress of Cadmium, therefore, further studies can be done to have transparent outcomes of the effect of using this water and the extent of damage in the long run due to the presence of high amount of Cadmium. Moreover due to the time and economic constraints the chemical and microbial parameters of this sample water could not be done, which also can be taken accounted for the purpose of future work. Moreover, the investigation of radiological materials for a longer period of time, including human body fluids can also be done.

At the very end, it can be said that the outcomes of this study indicates that the water that is used for the drinking and household purposes in the rural areas of tongi is not in a good condition and thus, the concern of various national and international organizations who works for the supply of safe drinking water need to be increased along with the authority of DoE to control the pollution of water in the industrial areas. Even though in this study, some of the heavy metals found within the permissible limit but they are in the red line and can be raised as the days are passed by along with the increased manner of industrialization.

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