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Hepato-destructive effect of chemically disordered drinking water

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ABSTRACT

The present research work deals with the identification of liver damages in human beings due to the consumption of chemically disordered water. The Chang Liver cell lines were used for in-vitro assay using the MTT assay method. The chemically damaged water were collected from the nearby well of a chemical industry and the disorders were confirmed using the methods as per the environmental protection agency protocols of USA. The study reveals the role of chemicals in the liver damage in human body.

Key words: Drinking water, Chemical Contamination, Liver Damage, MTT Assay, Liver Cell Lines.

INTRODUCTION

Water is essential for life, and maintaining hydration is important for physical and mental performance. The human body is largely made of water. Body water content declines with age, from about 75% in babies to 60% in adults. Although we can live for up to 50 days without food, without water we will survive only a few days, even in a cool climate [1]. Testing of wastewaters for genotoxicity may become a routine requirement for some industrial wastewater discharge permits, not unlike the more common requirement for routine aquatic toxicity tests [2]. Drinking water quality has a heavy impact on human health. A recent assessment of major health risk factors attributed over two million deaths to unsafe water, which represents the leading environmental risk factor on a global scale [3]. Recent statements on water requirements have been based on retrospective recall of water intake from food and beverages among healthy non-institutionalized individuals. We provide examples of water intake assessment in populations to clarify the need for experimental studies. Beyond these circumstances of dehydration, we do not truly understand how hydration affects health and well-being, even the impact of water intakes on chronic diseases [4]. Pollution of water bodies is one of the areas of major concern to environmentalists. Water quality is an index of health and well being of a society. Industrialisation, urbanisation and modern agriculture Practices have direct impact on water resources. These factors influence the water resources quantitatively and qualitatively[5].

MATERIALS ANAD METHODS

Collection of sample

The chemically disordered samples were collected from the near by well of a chemical factory in Malappuram district of Kerala. The disorder was confirmed using the following methods.

ESTIMATION OF CHLORIDES IN WATER

Materials

- Burette
- Conical flask
- Potassium chromate
- Silver nitrate
- Sodium Chloride

Reagents

a) 5% Potassium Chromate: Dissolve 50g of potassium chromate in little amount of distilled water, add silver nitrate solution until definite red precipitate is formed, stand for 12 hours, filter and dilute to 1L with distilled water

b) 0.0141N silver nitrate: Dissolve 2,395g of AgNO3 (MW – 169.87) in 1000ml of chloride free distilled water (1ml=500µg of chloride)

c) Standard NaCl Solution (0.0141N): Dissolve 824mg of NaCl dried at 140°C in distilled water and make up the volume to 1000ml (1ml=500µg of chloride)

Standardization of 0.0141N silver nitrate: Standardize against standard NaCl solution using 1-2ml of 5% potassium chromate as indicator. The end point is appearance of pinkish yellow color. Use the value as reagent blank (Vb).

Procedure

- Take 100ml of water in conical flask (Adjust the PH between 7-10 with sulphuric acid or sodium hydroxide)
- Add 1 ml of 5% potassium chromate
- Titrate with 0.0141N silver nitrate (Va)
- End point is appearance of pinkish yellow color
- Establish reagent blank value by titration (Vb)

Calculation:

Chloride (as Cl) in ppm = $\frac{(Va-Vb)x\ N\ x\ 35,450}{Vol.of\ sample}$

Specification: Should be less than 50 ppm.

DETERMINATION OF IRON IN WATER[7]

Preparation of standards

Dissolve 0.7022 grams of ferrous ammonium sulfate, hexahydrate in distilled water. Dilute to 1.00 L. This solution is 100 mg/L Fe^{+2} (same as 100 ppm).Prepare standard solutions of 0.0, 2.0, 4.0, 6.0, and 8.0 ppm by respectively diluting 0.0, 2.0, 4.0, 6.0, and 8.0 mL of the 100 ppm stock solution into five separate 100.0 mL volumetric flasks. To each flask add 5 mL of a 0.25% ortho-phenanthroline solution. Dilute with deionized water to 100.0 mL. Clean and dry a set of cuvette. Label the cuvettes 0ppm (or blank), 2.0ppm, 4.0ppm, 6.0ppm, 8.0ppm. Fill each cuvette with the appropriate solution.

Preparation of the water Sample

Obtain an unknown sample from the instructor or prepare your own unknown using a collected water sample. If using a collected water sample, add 5 mL of the 0.25% ortho-phenanthroline solution to a 100.00 mL volumetric flask. Dilute to mark with collected water sample.

Forming the standard curve[8]

Turn on the spectrophotometer. Press the **A/T/C** button on the Spec 20 Genesys to select absorbance. Adjust the wavelength to **510** nm by pressing the **nm** arrow up or down. Insert the blank (0ppm) into the cell holder and close the door. Position the cell so that the light passes through clear walls. *****Remember to wipe off the cuvette with a Kimwipe before inserting it into the instrument. Press **0 ABS/100% T** to set the blank to 0 absorbance. Record the absorbance of the 0ppm solution. Obtain absorbance readings for each of the other standard solutions. If using a collected water sample as an unknown, use collected water without the 0.25% ortho- phenanthroline solution as the blank and re-zero the absorbance. If not, proceed to the next step. Obtain an absorbance reading for the unknown

sample. Make a graph of Concentration (x-axis) vs. absorbance (y-axis).From the standard curve, determine the concentration of iron in this unknown sample.

MTT Assay (Liver-Cell Lines)[9]

For the Liver cells, remove the medium and replace it with 100 µL of fresh culture medium. For non-adherent cells, centrifuge the microplate, pellet the cells, carefully remove as much medium as possible and replace it with 100 µL of fresh medium. Add 10 µL of the 12 mM MTT stock solution (prepared in step 1.1) to each well. Include a negative control of 10 µL of the MTT stock solution added to 100 µL of medium alone. Incubate at 37°C for 4 hours. At high cell densities (>100,000 cells per well) the incubation time can be shortened to 2 hours. Add 100 µL of the SDS-HCl solution (prepared in step 1.2) to each well and mix thoroughly using the pipette. Incubate the microplate at 37°C for 4– hours in a humidified chamber. Longer incubations will decrease the sensitivity of the assay. Mix each sample again using a pipette and read absorbance at 570 nm.

RESULTS

CHLORIDE CONTENT

SAMPLE	CONTENT FOUND	DESIRABLE AMOUNT
WATER	1254 mg/L	250 mg/L

IRON CONTENT

SAMPLE	CONTENT FOUND	DESIRABLE AMOUNT
WATER	1.50 mg/L	0.30 mg/L

MTT ASSAY

Water Sample Volume in ml	MTT assay (Chang Liver) % death
Control	0.00%
0.50	12.21%
1.00	54.50%
1.50	65.20%
2.00	98.45%
2.50	98.95%

CONCLUSION

From the above investigation we found that the chemically disordered water is a major reason for the liver damage in human beings. The water which we collected from a source was very bad in terms of chlorides and iron. The content was around 10 times the desirable limits. This water samples was used for the MTT assay for the identification of the hepato-destructive activity. The results were significant as in the 2.5 ml of the water samples are capable of killing the liver cells around 98%. Thus we conclude that the quality of the water should be maintained for the better health.

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