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QUANTITATIVE ESTIMATION OF HEAVY METALS IN RIVER WATER AND THEIR TOXICITY, HEMATOLOGY, GRAVIMETRIC, SERUM AND TISSUE BIOCHEMISTRY EFFECTS IN ALBINO RATS

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ABSTRACT

The aim of present study is to investigate on the concentration of heavy metals (Cu, Ni, Fe, Co, Mn, Cr and Zn) present in the water and their effects on Toxicity, Hematology, Gravimetric and Serum biochemistry of healthy albino rats. The results showed that 50% of toxicity and there was significant difference ($p<0.05$) in the Red Blood Cell (RBC) and White Blood Cell (WBC), It indicate the hemolytic anemia. This is due to injurious effect of the toxic heavy metals of water. Where in case of Gravimetric study the average weight reduced significantly ($p<0.05$) in test rats when compared with control, indicating that, the excess heavy metal water exposure to rats caused reduction in weight of liver, heart, kidney and adrenal. The serum biochemistry studies of protein, cholesterol, urea, and phospholipids were reduced in all the groups studied. Thus, it can be concluded that heavy metals of water as serious consequences on toxicity, hematology, Gravimetric, Tissue and serum biochemistry in rats and causes hemolytic anemia which is attributed to the toxic effect of heavy metals of the water in the experimental rats.

KEYWORDS: Heavy metals, Hematology, Toxicity and Gravimetric.

1. INTRODUCTION

Water is essential to the continued existence of all living organisms but is increasingly being threatened as human populations grow and demand for more water of high quality for domestic and economic activities. Water is vital to sustain life, and a satisfactory (adequate,

safe and accessible) supply must be readily available to all. Improving access to safe drinking-water can result in tangible benefits to health (Olatunji Matthew Kolawole,). The river systems may also be excessively contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents (Vander Oost et al., 2003).

The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). "Heavy metals" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3, or 5 times or more, greater than water (Huton and Symon, 1986; Battarbee et al., 1988; Nriagu and Pacyna 1988; Nriagu, 1989; Garbarino et al., 1995, Hawkes, 1997). However, being a heavy metal has little to do with density but concerns chemical properties. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) iron (Fe), and the platinum group elements.

The heavy metals such as lead, mercury, chromium, copper and cadmium together with other household chemicals and poisons can be concentrated in groundwater supplies beneath landfills (Wenger and Rhyner, 1984). These contaminants have been reported to possibly cause growth retardation, and hemoglobin abnormalities (Hogson, 2004). Furthermore, blood cell responses are important indicator of changes in the internal and /or external environment of animals (Adeyemi, 2007). Witeska (1998) and Kanu et al. (2006) reported that the major symptom associated with ingestion of high quantity of copper, lead, cadmium and chromium is destruction of blood cells which is followed by anemia. Metallic compounds on land and water pose potential health hazard not only to livestock and wild life but also to fishes, birds, mammals and even to human beings.

Heavy metallic compounds have emerged as a major class of industrial waste product and they can be produced synthetically in laboratories from their derivatives (Budavari, 1996). The most commonly used synthetic heavy metallic compounds are mercuric chloride, mercurous chloride and lead acetate of which mercuric chloride is most dangerous as it produces neurotoxicity, hepato-toxicity and nephrotoxicity in animals (Kavitha and Jagadeesan, 2006).

In the present study the effort has been made to know the Quantitative estimation, Toxicity, Hematology, Gravimetric, Tissue and Serum biochemistry of albino rats treated with heavy metals present in water sample.

2. MATERIALS AND METHODS

2.1. Collection of water sample

The water sample was collected at sampling points with 2000 mL plastic containers. The portion of the water sample for metal analysis were treated with 1ml of Hydrochloric acid (HCl) in 500ml sample to arrest microbial activities while those for non-metal analysis were freshly refrigerated in a cooler packed with, ice blocks to avoid microbial action affecting their concentration.

2.2. Quantitative estimation of Heavy metals

The heavy metal concentration were estimated using Atomic Absorption Spectrophotometric (AAS) method after acid digestion, sodium and potassium were determined with flame photometer using direct aspiration method (APHA,1998). The data generated were compared with World Health Organization (WHO, 2003) standards for drinking water.

2.3. Experimental animals

Experiment was performed on albino rats aged about seven weeks (100 g) obtained from Luqman Pharmacy College, Gulbarga. The animals were acclimatized for 1-2 weeks before being used for the experiment. Fed with Standard palliated diet (Amrut laboratory animal feed diet, Pune, Maharashtra, India) and water was given ad libitum. They were housed under standard condition of temperature (24 ^{o}C), humidity (65%) light and dark cycle (14:10 L), respectively. The initial body weight of each animal was recorded. All experimental procedures were carried out in strict accordance with the guidelines prescribed by the committee for the purpose of control and supervisor on experimentation on animals (CPCSEA Reg. No-34800/2001) and were approved by the institutional animal ethical committee.

2.4. Acute toxicity studies of Heavy metals

Toxicity studies were carried out in albino rats according to OECD guidelines. Water sample with heavy metals at different volume up to 100 mL b w was allowed to feed per day and animals were observed for behavioral changes, nervous, any toxicity and mortality up to 72 h. The blood sample was collected by retro-orbital method for hematology and serum

biochemical studies and vital organs like Heart, Liver, Kidney and Adrenal were collected for further detailed gravimetric and tissue biochemical studies [Turner, 1971].

2.5. Effect of Heavy metals on Hematology in albino rats

The blood sample was collected from retro-orbital venous plexus of the rats under light ether anesthesia using capillary tubes into Eppendorff tubes. The whole blood with anticoagulant was used for assay of the hematological parameters using the XF 9080 Animal Hematology Auto-analyzer (Hitachi, Germany). Parameters determined are packed cell volume (PCV), Red blood cells (RBC) count, White blood cells (WBC) count, mean corpuscular Hemoglobin concentration (MCHC), mean corpuscular volume (MCV) Lymphocytes, Monocytes and Neutrophils, using standard assay kits (Roche diagnostics Ltd, United Kingdom).

2.6. Gravimetric effect of Heavy metals in albino rats

The weight of the dissected organs was calculated for 100g- body weight of animal by using following formula.

$$\text{Weight of organ} = \frac{\text{Wet weight of organ}}{\text{Body weight}} \text{ X } 100\text{g}$$

= mg/100g body weight.

2.7. Serum Biochemistry

The blood was collected from retro-orbital venous plexus of the rats under light ether anesthesia using capillary tubes into Eppendorf tubes containing heparin. The plasma was separated by centrifugation (5 min, 5000 rpm) and was analyzed for serum cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, creatinine, urea, Hemoglobin and Glycosylated hemoglobin. The plasma profiles were measured by standard enzymatic methods with an Automatic analyzer (Phuong *et al.*, 2004 my thesis) and Glycosylated hemoglobin by colorimetric method.

2.8. Tissue Biochemistry

The biochemical analysis of heart, liver, kidney and adrenal of the treated rats were carried out to know the effect of heavy metals on the total protein, glycogen and cholesterol content of vital organs and estimated by the method as described previous (Lowry *et al.*, 1951, Good *et al.*, 1933].

2.9. Statistical analysis

Results are expressed as Mean ± SD. The statistical analysis was carried out using one-way ANOVA analysis. The *p*-value of 0.05 or less was considered significant for all experiments.

3. RESULTS

3.1. Quantitative estimation of various heavy metals

Quantitative estimation of various heavy metals was carried out from the water sample obtained from Bhīma River and the results are as shown in the Table 1. In the present study Arsenic was found to be 0.5 mg/L which is exiting the permissible limit prescribed by the World Health Organization (WHO) and BIS standards. According to a 1999 study by the National Academy of Sciences, Arsenic in drinking water causes Bladder, Lung, Skin, Kidney, & Liver cancers. The study further found that Arsenic effects on the central and peripheral nervous systems (CNS), Heart, Blood vessels, skin, birth and Reproduction. The concentration of Cadmium in the Bhīma river water sample was observed to be 0.04mg/L as it is more than the permissible range. The people who drink the water containing Cadmium excess level for many years could experience Kidney damage and disorder related to kidney.

TABLE 1: Quantitative estimation of various Heavy Metals present in water sample

Elements	Concentration of metal ion in water sample (in mg/L)	WHO (in mg/L)	BIS (in mg/L)	EU (in mg/L)
Arsenic (As)	0.5*	0.01	0.05	0.01
Cadmium (Cd)	0.04*	0.003	0.003	0.005 l
Copper (Cu)	2.5	2.0	0.05	2.0
Chromium (Cr)	0.5	0.05	0.05	0.03
Mercury (Hg)	0.1	0.001	0.00	0.001
Manganese (Mn)	0.3	0.5	0.1	0.05
Nickel (Ni)	0.01	0.02	0,02	0.02
Zinc (Zn)	4.0	3.0	5.0	0.00
Iron (Fe)	0.5	0.3	0.3	0.2
Selenium (Se)	0.00	0.01	0.01	0.01
Boron (B)	0.01	0.3	0.5	1.0
Molybdenum (Mo)	0.05	0.07	0.07	0.07
Lead (Pb)	0.5	0.01	0.01	0.01

3.2. Acute toxicity studies of Heavy metals

Over the study duration of 1-7 days, there was a toxicity effect and 50% mortality was recorded in the experimental group of animals while giving the dose ranging from 10 mL/kg to 100 mL/kg of b. w of Water sample having heavy metals. The animals also showed change in general behavior, skin effecting, defecation, loss of hairs or other physiological activities.

3.3. Effect of heavy metals on Hematology in albino rats

The hematological parameters WBCs, RBCs, hemoglobin content and hematocrit values were found to be altered when compared to normal range (Table 2). This indicates that the heavy metals of water sample have toxic effect on physiology of rat. Table 2 shows the levels of serum WBC, basophils, neutrophils, eosinophil's, lymphocyte and monocytes. The level of WBC was slightly increased as compared with the control group. The heavy metals also significantly increased the level of Lymphocyte, Eosinophil's, Monocytes and Platelet. The compound at tested dosage did not have any beneficiary effect on the level of neutrophils. How're the experimental animals produced more change on the packed cell volume, MCHC, MCV, and MCH count when compared to the control.

TABLE 2: Effect of heavy metals on Hematology in albino rats

Test	Control	1st Day	4th day	7th day	Normal Range
Hemoglobin	13.2 g/dl	11.7 g/dl	11.5	13.2	11.0-17.0
WBC Count	10,200/µL	5,700/µL	6,500/µL	5,600/µL	4,000-10,000
Neutrophils	20%	90%	92%	95%	27-55
Lymphocytes	79%	09%	07%	04%	36-51
Eosinophil's	01%	01%	01%	01%	00-05
Monocytes	00%	00%	00%	00%	00-04
Basophils	00%	00%	00%	00%	00-02
Platelet Count	5.50Lakhs/cmm	3.19 Lakhs/cmm	4.28 Lakhs/cmm	3.63 Lakhs/cmm	1,50,000-4,50,00
R.B.C. Count	7.62 10/µL	6.16 10/µL	6.45 10/µL	7.42 10/µL	3.2-5.5
P.C.V.	47.2 %	37.1%	40.9 %	49.2 %	37-47
M.C.V.	61.9 fl	60.2 fl	63.4 fl	66.3 fl	82-92
M.C.H.	17.3 Pg	19 Pg	17.8 Pg	17.8 Pg	27-37
M.C.H.C.	28%	31.5%	28.1 %	26.8 %	32-36
Absolute Eosinophil	180 Cells/µL	200 Cell/µL	150 Cells/µL	200 Cells/µL	40-440
Bleeding Time Min	4 min, 0 sec	4 min, 0 sec	3 min, 30 sec	3 min, 45 sec	2-5
Clotting Time Min	7 min, 15 sec	6 min, 30 sec	6 min, 15 sec	6 min, 30 sec	2-9

3.4. Gravimetric Studies

There is significant change was observed in the body weight of all both group animals when compared with control. Daily oral administration of heavy metal water sample at the dose level 10 -100 mL/kg of body weight was significantly ($p \leq 0.05$) decreased the body and vital organs (Heart, Liver, Kidney and Adrenal) weight when compared to control group. The results so obtained were presented as in the table 3.

TABLE 3: Gravimetric effect of heavy metals on body and various vital organs of rats

Treatments	Body weight (g)	Weight of Vital organs mg/100 g of b w			
		Liver	Kidney	Heart	Adrenal
Control WNHM	120	749.35±13.15	3141.35±138.75	367.02±36.65	23.02±1.90
WHM (1st day)	120	879.34±10.01	3655.07±0.04	4871.20±55.15	36.04±2.03
WHM (4thday)	115	780.15±15.0	3440.04±0.1	390.06±10.33	28.05±12.5
WHM (7thday)	110	751.02±11.45	3227.07±136.0	376.00±22.1	22.15±11.6

*WHM: Water with Heavy metal; WNHM: Water with No heavy metals

3.5. Serum Biochemistry

The biochemical study of blood was carried out to understand the manifestion and physiological status of animal body. If the heavy metals were toxic or interfere with any biochemical reactions, consequently there will be change in metabolic production. The treatment of heavy metal water at dose 10 mL/kg to 100 mL/kg body weight shown signification ($p \leq 0.05$) alteration in the blood sugar level, it reveals that heavy metals damaged the pancreas, which contains the sugar controlling cells (β-cells) in islests of Langerhans. There is significant ($p \leq 0.05$) alteration in serum cholesterol level when compared with control and which indicate that treatment of heavy metals affect the function of liver and kidneys.

TABLE 4: Serum biochemistry of rats treated with water sample congaing heavy metals

Biochemical Parameters	Normal values	1st day	4th day	7th day
Glucose(mg/dl)	90-140	57.00±	102.00±	68.00±
Cholesterol(mg/dl)	150-250	83.00±	82.00±	68.58±
Cretonne (mg/dl)	0.8-1.2	0.75±	87.00±	0.70±
Urea (mg/dl)	15-45	36.00±	51.00±	16.00±
Triglycerides(mg/dl)	124-156	75.00±	48.00±	31.85±
Total Bilirubin (mg/dl)	0.4-1.4	1.06±	0.89±	1.08±
Total protein(g/dl)	6.6-8.3	6.40±	06.19±	6.51±
Albumin(g/dl)	3.5-5.0	2.62±	3.04±	2.89±
Globulins(g/dl)		3.80±	3.6±	3.60±
SGPT(U/l)	12-67	96.00±	186. ±00	155.0±0
SGOT(U/l)	42-98	147.00±	138.00±	294.00±
Alkaline Phosphates (U/l)	30-110	119.00±	214.00±	173.00±

3.6. Tissue biochemistry

Protein content of the uterus was reduced 50 % significantly 131.66±2.88 with compound of both low and high doses respectively when compared to control group (239.33±0.57). Where in case of cholesterol content was reduced 30 % when compared to control group (301.15) as represented in Table 5. Cholesterol is the precursor for many of steroidal hormone due to this

un-utilization of cholesterol there will be less synthesis of steroidal hormones which leads hormonal imbalance in the rat body resulting to antifertility

TABLE 5: Biochemical changes in Ovary and Uterus of anti-ovulatory activity.

Group	Treatment mL/ kg of b w	Dosing Days	Organ type	Protein (mg/100g)	Cholesterol (mg/100g)	Glycogen (mg/100g)
I	Control WHM	1-7	Heart	190±1.52	60±2.06	60±2.05
			Liver	241±1.58	110±1.51	80±1.52
			Kidney	101±2.08	52±1.52	60±2.08
			Adrenal	80±1.52	36±1.61	28±1.54
II	1st day WHM	1-7	Heart	190±1.52	60±2.08	60±1.52
			Liver	241±1.52	110±2.11	80±1.08
			Kidney	101±2.08	52±2.30	60±1.52
			Adrenal	80±1.51	36±2.05	28±2.09
III	4th day WHM	1-7	Heart	154±1.03	65±2.00	58±2.11
			Liver	200±1.06	91±1.53	80±1.51
			Kidney	56±2.11	40±1.31	65±1.52
			Adrenal	48±1.23	31±1.52	32±1.50
IV	7th day WHM	1-7	Heart	82±1.20	31±2.08	58±2.06
			Liver	122±1.50	53±1.15	73±2.03
			Kidney	47±1.52	26±1.53	61±1.52
			Adrenal	32±2.06	19±1.09	32±2.06

4. DISCUSSION

Water pollution has become worldwide phenomenon. The underground water is polluted by many hazardous pollutants like colored dyes, nitrates, heavy metals, pesticides and fluoride. Heavy metals are one of the major concerns among these pollutants. The problem due to high concentration of heavy metals in ground water has now become one of the most important health related geo-environmental issues in India. The increase in the water pollution is a major and global problem. This is due to the use of toxic chemicals or xenobiotic substances or by certain synthetic compounds such as heavy metallic compounds (Foulkes, 1990; Jagadeesan and Pillai, 2007). Of these heavy metallic compounds few reveal potential effects. They reach the environment (water bodies like rever, pound, lake and well etc) after their liberation though industries (Migliore, 1999). Water parameters are one of the major factors responsible for individual variation in hematology. Since hematological parameters are necessary for clinical diagnosis of a disease and pathological conditions in human, these criteria should receive enough attention in assessing the health and has been accepted by many workers such as McCarthy et al. (1973) and Christensen et al. (1978). In the present investigation we observed a significant heavy metals associated decrease in R.B.C a W.B.C count and the levels of hemoglobin. Similar observation was also found previously in

rats [Leone, 1954], rabbits [Weber, 1969] and children [Balazova, 1969]. The heavy metals induced anemia observed in this study may result from inhibition of globulin synthesis, depression of erythropoiesis or a decrease in the level of blood folic acid [Kahl, 1973; Oser, 1965]. In the present study body weight and the weight of Kidney, Heart and Adrenal declined which may be due to a direct effect of heavy metals of water on vital organs. The ingestion of a high heavy metal content results in a decrease in food intake and reduction in growth rate of the rodents in mammals (Simon and Suttie 1968; Weber and Reid 1969). From our work it was observed that the values obtained for serum biochemical parameters like total protein, cholesterol, phospholipid showed significant reduction ($p \leq 0.001$) with higher heavy metals contents as compared to control. SGOT and SGPT are markers of liver function. The significant ($p<0.001$) decline in enzyme activity of SGOT and SGPT. Therefore, reduced activities of SGOT and SGPT may be due to heavy metals present in the treated water sample. The altered tissue biochemistry of Heart, liver, heart and adrenal may be due to toxic effects of heavy metals on vital organs, because ingestion of heavy metals causes decrease in the ionized calcium (Teotia and Teotia, 1972; Gupta, 1999; Srivastava et al., 1989). This hypocalcaemia lead to change in internal milieu of the body to maintain the calcium level and lead to secondary hyperparathyroidism. It was well known that ionic calcium was one of the important ions for the initiation and maintenance of the activity of the vital organs and muscular-skeletal system. Lowering of the ionized calcium was one of the important stimuli for the release of PTH (Schwartz et al., 1998). Increased parathyroid hormone causes increased activity of osteoclasts in bone by activating membrane bound 3’5' cyclic AMP (Tortora et al., 1990). Therefore, the present finding suggests that excess fluoride ingestion to rats caused adverse effects on soft tissues, hampering normal physiology of liver, heart, kidney and adrenal.

5. CONCLUSION

The present study concludes that, heavy metals present in water sample are toxic as they showed 50% of lethality in experimental animals and in hematology studies the significant ($p<0.05$) decrease in hemoglobin concentration, hematocrit value and red blood cell may be an indication of anemia while the increased platelet level shows ill-health thrombosis. The results of serum and tissue biochemistry also suggest that, heavy metals of water has effect on enzyme activity and also effects on protein, cholesterol and glycogen levels in the vital organs like heart, kidney, liver and adrenal. The present study strongly suggest that the water

having high content of heavy metals are not safe for drinking and domestic purpose as they cause adverse effect on health.

Conflict of Interests

The authors declare that there is no conflict of interests.

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