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Contaminated fish meal diet induces hematological alterations in albino rat, *Rattus norvegicus* (Album)

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ABSTRACT

Fish inhabiting polluted waters accumulate various metals in tissues. The transfer of these into human beings through the food chain has adverse effects. The present study was undertaken to elicit the effect of consumption of contaminated fish meal on the hematological parameters of albino rats, *Rattus norvegicus* (Album). Thirty rats, weighing 45-50gms and randomly divided into 5 groups (n=6), were used for this study. Group I served as control and were given normal diet, group V were given only contaminated fish meal diet while group II, III and IV were given a mixture of normal and contaminated fish meal diet in the ratio of 1:1, 1:2 and 1:3, respectively. All experiments were carried out for 90 days. Results of blood parameters revealed significant reduction ($P< 0.05$) in Total erythrocyte count (TEC), Hemoglobin (Hb) and packed cell volume (PCV) in group IV and V when compared to control. Leukocytes count showed a significant decrease ($P<0.05$) in group IV and V. These effects may be due to the presence of heavy metals in the fish meal given to experimental rats. It is therefore concluded that consumption of fish from polluted waters is always associated with health risks.

KEY WORDS: Fishmeal, Haematology, *Rattus norvegicus*, *Tilapia mossambicus*, toxicity

INTRODUCTION

Rapid population growth and urbanization in developing countries has led to production of large quantities of wastes which are discharged into water bodies thereby polluting them (Gungum *et al.*, 1994; Unlu *et al.*, 1996; Nagendran *et al.*, 2006). Many lakes on the outskirts in the vicinity of Bangalore are contaminated with heavy metals released by industries (Varalakshmi and Ganeshmurthy, 2010).One such water body present in the peri urban region of Bangalore that has been polluted is the Nagavara lake which covers an approximate area of 43-86 sq ft, located down stream side of Hebbal lake on outer ring road. Industrial wastes, atmospheric deposition from crowded cities and other domestic wastes are among the major sources of heavy metals in the urban sewage (Sorme and Lagervist, 2002). Increase in use of heavy metals in the past few decades has resulted in an increased influx of metals into water bodies causing serious damage to aquatic life (Yang and Rose, 2003; Karabasi *et al.*, 2001). Contamination of water with heavy metals may have devastating effects on the ecological balance of the aquatic environment and the diversity of aquatic organisms becomes limited with the extent of contamination (Suziki *et al.*, 1988). Among animal species that inhabit the aquatic system and which cannot escape the detrimental effect of pollutants are the fishes (Olaifa *et al.*, 2004; Witeska *et al.*, 2006; Clarkson, 1998; Dickman and Leung, 1998).

Studies have shown that fish are able to accumulate and retain heavy metals from their environment and it depends upon the exposure to concentration and duration (Pagenkopf, 1983; Cusimano *et al.,* 1986; Heath, 1987; Canli and Furness, 1993; Allen, 1995). The metal contamination in aquatic eco system is considered to be unsafe not only to fishes but also for human beings because, they consume fishes which are the best sources of proteins. In mammals most of the heavy metals burdened from the aquatic eco system by way of their food especially where, fish are present so there exists the potential for considerable biomagnifications (Mance, 1987; Langston, 1990; Cumbie, 1975, Farkas *et al.,* 2002). Hence, the entry of such potentially harmful substances into the food chain destined for human consumption is receiving great importance (Lacher and Goldstein, 1997). In any living tissue toxic influences exert their effect first at the molecular and then at the biochemical level (Robbins and Angell, 1976). Toxicants in the environment on entering the body of the vertebrates induce changes in structure and functions of blood (Juneja and Mahajan, 1983; Ranzani-Paiva *et al.,* 1997, Javed and Usmani, 2012). Alterations in blood factors serve as the earliest indicators of toxic effect on tissues (Paprika and Sharma, 2003). Hematological parameters have thus been used as indicators of metal toxicity as they provide information on the physiological response of animals due to the close association of the circulatory system with the external environment (Wepener *et al.,* 1992). Hence, the present study was undertaken to elicit the effect of consumption of contaminated fish meal on the hematological parameters of albino rats.

MATERIALS AND METHODS

Albino rats, *Rattus norvegicus* procured from Raghavendra enterprises, Bangalore during 2010 – 2011 and maintained in the animal house in the Department of Zoology, Bangalore University, Karnataka, India, were used for the studies. Weanling rats thirty in number and weighing 45 – 50 g was divided into 5 groups of 6 each (n=6). Group I were given rat feed procured from Amruth mice and feed, India (crude protein 20%, crude fiber 10%, energy 2700 Kcal/Kg, common salt 2%, calcium 2%, potassium 1%), served as control diet. The remaining four Groups served as test groups. Group II, III and IV were fed control diet and contaminated fish meal diet in different ratios (1:1, 1:2 and1:3).Group V was given only contaminated fish meal diet *ad libitum*. Rats were housed in plastic cages for 90 days and maintained at room temperature of 24°C$\pm$2° C and photoperiod of 12L/12 D.

Preparation of fish meal:

Tilapia mossambicus (Peters) was collected from the polluted Nagavara lake (13° 04' N, 77° 60' E, elevation 921 masl) Bangalore, brought to laboratory, sundried, powdered, made into pellets and used as experimental feed, water samples were also collected for analysis of heavy metals.

Collection of blood samples:

Blood samples were collected at the end of 90 days from both control and test groups from abdominal aorta in heparinised capillary tubes. Prior to blood collection rats were fasted for 12hrs. RBC content was carried out in Neubauer hematocytometer after dilution of blood 1:200 with Hayem's solution. Packed cell volume (PCV) was determined by spinning blood samples contained in heparized capillary tubes in hematocrit centrifuge. Hemoglobin (Hb) was determined by cyano-methanoglobin method. Mean corpuscular volume (MCV), Mean erythrocyte concentration (MCH) were analyzed using the method described by (Hesser, 1960) and Mean corpuscular

haemoglobin content (MCHC) was calculated as the product of hematocrit and haemoglobin concentration. White blood cell count, platelets and differential white blood count were made according to method described by (Stoskopf, 1993). WBC sub populations were quantified and expressed in absolute numbers and as percentage (%) of the white blood cell count.

Statistical analysis:

Data were expressed as mean ±standard deviation (SD) and subjected to one way Anova, followed by Tukey's HSD using SPSS Version 17. Values at P<0.05 were considered significant.

RESULTS AND DISCUSSION

Mean heavy metal concentration (mg/l) in the water body of Nagavara lake showed 0.42 mg/l Lead, 0.03 mg/l Chromium, 0.026mg/l Cadmium and 0.028mg/l Arsenic. It was found that mean contents of all metals exceeded the recommended levels, in comparison with the standard guide lines (Pescod, 1992).The higher levels of Cr, Cd and As in Nagavara lake can be attributed to rapid industrialization, urbanization, electroplating industries, number of small scale industrial units release waste waters into the lake through water drains. Similar results were also reported by Varalakshmi and Ganeshmurthy (2010).

Haematological changes in control and experimental groups are presented in table I and II. In the present study significant differences (P<0.05) among groups for all the hematological parameters were observed. There was significant reduction in Hemoglobin, RBC and PCV in the experimental groups compared to control and more significant reduction was in group IV and V (F=328.22; df=4,25; P<0.05, F=570.55; df=4,25; P<0.05 and F=114.33; df=4,25; P<0.05). The relatively lower hemoglobin, RBC and PCV concentration in the blood of experimental rats suggests metal pollutants in the fish meal might be destroying RBC directly or indirectly and there by lowering haemoglobin concentration. Heavy metals alter properties of hemoglobin by decreasing their affinity towards oxygen binding capacity rendering RBC more fragile and permeable (Witeska and Kosciuk, 2003; Karuppasamy, 2000). Significant difference in MCV and MCH content was noticed among different groups and more significant reduction was found in group IV and V (F=476.5; df=4, 25; P<0.05 and F=203.1; df=4, 25; P<0.05). This result is an indicative of hypochromic microcytic anemia due to hypoxia or shrunk RBC. Similar hemalogical abnormalities have also been studied in fish exposed to mercuric chloride (Shakoori *et al.,* 1991). Reduction in MCHC was observed in experimental groups indicate decrease in hemoglobin synthesis there by lowering oxygen carrying capacity by the animal (F=58.47; df=4, 25; P<0.05). Progressive decrease in MCHC has also been reported in bifenthrin treated rabbits (Shakoori *et al.,* 1990). Differences in Platelet count were significant among the groups (F=101.2; df=4, 25; P<0.05) except Group III and IV (P>0.05) (table I). Reduction in platelet count suggests the presence of hyper coaguable substances in the fish meal diet and increasing risk of thrombosis. Similar findings have been obtained in rats subjected to chronic Cadmium toxicity (Mehtap Koçak and Ethem Akçıl, 2006). There were significant differences in percentage (%) of neutrophils (F= 109.34; df= 4, 25 P<0.05), eosinophils (F= 158.78; df= 4, 25; P<0.05) basophils (F= 468.91; df= 4, 25; P<0.05) among the treated groups. There were significant differences in percentage (%) of monophils (F=407.58; df= 4, 25; P<0.05). However, there was no significant difference between

Group I and II, Group II and III respectively (P>0.05) (table II). Reduction in differential white blood cell counts caused leucopenia and lympopenia in treated rats, which leads to lowering of resistance to infectious agents and markedly elevated the animal's defense mechanism. The reduction of WBC caused leucocytosis which is directly proportional to severity of stress condition. Similar results were also noticed in sewage fed fish (Hardikar and Gokhale, 2002.)

CONCLUSION

The present study showed that fishes inhabiting polluted waters may contain heavy metals which when consumed by rats will induce hematological disturbance like erythrocytic destruction, leucocyctisis making animal vulnerable to diseases which in turn affects the immune status of the animal.

Table 1: Effect of fishmeal on haematological parameters in albino rat, *R. norvegicus*

Parameters	GROUP I Control	GROUP II 1:1	GROUP III 1:2	GROUP IV 1:3	GROUP V Fishmeal
HB (g/dl)	17.4±0.37 a	15.8±0.17 b	14.2±0.54 c	13.8±0.12 d	13.6±0.21 d
RBC(x10^{12}/L)	9.38±0.14 a	8.61±0.24 b	6.80±0.23 c	5.85±0.10 d	4.47±0.25 e
PCV (%)	50.1±0.60 a	46.7±0.58 b	45.8±0.31 b	42.6±1.09 c	42.3±0.82 c
MCV(Fl)	87.8±0.98 a	85.4±1.40 b	80.1±0.41 c	70.2±1.08 d	65.4±1.26 e
MCH(pg)	32.9±1.61 a	31.4±0.67 b	28.8±0.40 c	26.0±0.45 d	20.2±0.56 e
MCHC(g/dL)	35.6±1.24 a	32.6±0.85 b	32.0±0.54 bc	30.8±0.52 c	28.9±0.52 d
Plateletcount(l/cu)	5.50±0.44 a	4.78±0.55 b	3.18±0.08 c	2.76±0.28 c	2.09±0.11 d

Mean numbers in rows followed by the different small letters indicate significant differences among the different groups within the same parameters (ANOVA-Tukey HSD test, P<0.05)

Table 2: Effect of fishmeal on differential white blood cell count in *R. norvegicus*

Parameters	GROUP I Control	GROUP II 1:1	GROUP III 1:2	GROUP IV 1:3	GROUP V Fishmeal
Neutrophils(%)	57.1±1.17 a	53.5±1.51 b	50.1±1.47 c	46.0±1.78 d	42.0±0.89 e
Lymphocytes(%)	44.3±1.63 a	43.1±0.81 ab	41.6±1.16 b	39.0±0.89 c	37.1±0.75 c
Eosinophils(%)	6.64±0.45 a	5.44±0.22 b	4.61±0.26 c	3.66±0.08 d	2.92±0.19 e
Monophils(%)	1.07±0.00 a	1.01±0.02 ab	0.96±0.05 b	0.60±0.05 c	0.34±0.03 d
Basophils (%)	0.55±0.01 a	0.45±0.01 b	0.36±0.00 c	0.36±0.00 c	0.26±0.01 d

Mean numbers in rows followed by the different small letters indicate significant differences among the different groups within the same parameters (ANOVA-Tukey HSD test, P<0.05)

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