HIGH FLUORIDE CONCENTRATION AND AMELIORATION EFFECT IN HAEMATOLOGICAL PARAMETER AFTER SUPPLEMENTARY DIET ALOE VERA IN ALBINO RAT (RATTUS NORVEGICUS)

ANIL CHOUHDARY, SHWETA PARIHAR & M.KASIM
Department of Zoology, J.N.V.University, jodhpur, 342001, Rajasthan.

ABSTRACT: Haematological parameters in Albino rats were altered after exposure to sodium fluoride. Static bioassay experiments were conducted to find out Lc50 with the administration of oral dose of 10.00ppm of Naf per kg of body weight to adult healthy albino rats. The experimental doses were set up for the three groups of animals each having 4 animals for 30, 60 & 90 days time interval. Blood samples were analyzed for haematological parameters after exposure of dose for particular duration. Alteration in different parameters viz. RBC count, WBC count, Haemoglobin, Acidphosphatase, Alkaline phosphatase, Protein, Cholesterol, glycogen, SGOT, SGPT, urea, creatinine were estimated in serum of treated animals and results were compared with control set of experiment. In present investigation, Aloe vera is significantly used to fluoride exposed rats to reduce the toxic risk of fluoride, exhibiting restoration.

Key words: Fluoride, Haematology, SGOT, SGPT, Creatinine

INTRODUCTION:

Environmental pollution has become a prominent and conspicuous global issue, some of components of environment have become the essential factor for living systems, especially for human beings. One of them is fluoride which has got a very significant role in human physiology as less than 0.6 ppm prevents dental carries while 1.0  ppm is the maximum permissible limit for fluoride and concentrations above this limit is responsible for its health hazardous effects.

High fluoride intake has proved a major health hazard (Romani & Goel 2001) WHO has prescribed the 0.6 mg/l of fluoride as an essential quantity while 1.0 to 1.5 mg/l is permissible limit and more than this is health hazardous for human beings (Susheela 2001). Recently some of the developing countries & people of developed nations have raised their concern against such limitations. The blood circulatory system plays an important role in the transportations of nutrients & other toxic substances in the body & with this the blood gets altered in its constituents. The present investigation deals with the effects of fluoride toxicity in adult male healthy Albino rat (Rattus norvegicus) after its long term exposure to sodium fluoride. Alterations in blood biochemistry due to Naf in case of test animal were compared with adult healthy albino rats. The RBC count was found decreased in all the three treated groups in comparison to control group after administering dose of 10ppm of sodium fluoride per kg of body weight for 30, 60 & 90 days. The decreased level was highly significant (P≥0.001) in all treated groups. Decreased trends of WBC count were observed, when compared with control after given 10ppm Naf administered for 30, 60 & 90 days. The decreased trend was highly significant (P≥0.001) in all treated groups. Haemoglobin contents were found to be decreased in all treated groups after the exposure to sodium fluoride in comparison to control group. Decreased in haemoglobin level was found non significant in group I (10ppm of Naf for 30 days); significant (P≥0.01) in group II (10 ppm of Naf for 60 days) while found highly significant (P≥0.001) in group III (10ppm of Naf for 90 days.)

In compare to control group, acid phosphatase level was found decreased in all treated groups after administering 10ppm of sodium fluoride per kg of body weight for 30, 60 & 90 days. It was non significant in group I (10 ppm of sodium fluoride per kg of body weight for 30 days.), significant (P≥0.001) in group II (10.00 ppm of Naf per kg of body weight for 60 days.) & highly significant in (P≥0.001) in group III (90 days).

Alkaline phosphatase level was found increased in all treated groups in comparison to control group. It was slightly significant (P≥0.05) in group I (10 ppm of Naf for 30 days.) while highly significant (P≥0.001) in group II & III (10 ppm of Naf per kg of body weight for 60 & 90 days.) Serum protein level after administrating 10ppm of Naf per kg of body weight for 30, 60 & 90 days to be decreased in all treated groups in comparison to control group. It was highly significant in group III (10ppm per kg of body weight for 90 days), significant in group II (60 days) & non significant in group I. The serum cholesterol level was also found to be decreased in all treated groups in comparison to control group. The decreased level was slightly significant (P≥0.05) in group I & II (30 & 60 days) while significant in group III (90 days). Serum glucose level was not significantly increased in group I (10ppm of Naf for 30 days) while it was significantly increased. (P≥0.01) in

MATERIALS AND METHODS:

Mature, healthy pathogen free Albino rats were obtained from Jaipur & acclimatized to laboratory conditions. They were maintained on normal diet i.e. wheat, gram & water ab libitum for a weak & then they were categorized into 3 groups. Each group having 4 animals.

First group was considered as control group in, which mice were given water without fluoride. In each group animals were exposed with the 10 ppm/kg body weight. The other groups were treated for 30, 60 & 90 days respectively, last group i.e.90 days treated rats were given Aloe vera to reduce toxicity of fluoride at the dose of 500 mg/kg of body weight. After the duration of exposure, rats were autopsied & blood of animal was extracted through cardiac puncture & collected for haematological estimation. Blood was collected in 2 test tubes for estimation of different parameters. One with EDTA anticoagulant estimations and another as clotted blood which is later centrifuged for separating serum for analysis of biochemical parameters.

Environmental pollution has become a prominent and conspicuous global issue, some of components of environment have become the essential factor for living systems, especially for human beings. One of them is fluoride which has got a very significant role in human physiology as less than 0.6 ppm prevents dental carries while 1.0  ppm is the maximum permissible limit for fluoride and concentrations above this limit is responsible for its health hazardous effects.

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In compare to control group, acid phosphatase level was found decreased in all treated groups after administering 10ppm of sodium fluoride per kg of body weight for 30, 60 & 90 days. It was non significant in group I (10 ppm of sodium fluoride per kg of body weight for 30 days.), significant (P≥0.001) in group II (10.00 ppm of Naf per kg of body weight for 60 days.) & highly significant in (P≥0.001) in group III (90 days).

Alkaline phosphatase level was found increased in all treated groups in comparison to control group. It was slightly significant (P≥0.05) in group I (10 ppm of Naf for 30 days.) while highly significant (P≥0.001) in group II & III (10 ppm of Naf per kg of body weight for 60 & 90 days.) Serum protein level after administrating 10ppm of Naf per kg of body weight for 30, 60 & 90 days to be decreased in all treated groups in comparison to control group. It was highly significant in group III (10ppm per kg of body weight for 90 days), significant in group II (60 days) & non significant in group I. The serum cholesterol level was also found to be decreased in all treated groups in comparison to control group. The decreased level was slightly significant (P≥0.05) in group I & II (30 & 60 days) while significant in group III (90 days). Serum glucose level was not significantly increased in group I (10ppm of Naf for 30 days) while it was significantly increased. (P≥0.01) in
group II & III (10ppm of Naf for 60 & 90 days). On administering 10ppm Naf per kg of body weight for 30, 60 & 90 days, the serum urea level was found increased in the entire treated group. It was significantly increased (P≥0.01) in all three treated groups.

Creatinine significantly increased after giving 10 ppm of sodium fluoride per kg of body weight in comparison to control rats. SGOT & SGPT level were also increased when treated with 10ppm of Naf per kg of body weight. It was also highly significant in all fluoride treated groups.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Groups</th>
<th>Dose</th>
<th>Duration</th>
<th>Autopsy Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Water without fluorid</td>
<td>15</td>
<td>Along with treated group</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>10ppm/kg/body weight</td>
<td>30</td>
<td>31 st</td>
</tr>
<tr>
<td>3</td>
<td>II</td>
<td>10ppm/kg/body weight</td>
<td>60</td>
<td>61 st</td>
</tr>
<tr>
<td>4</td>
<td>III</td>
<td>10ppm/kg/body weight</td>
<td>90</td>
<td>91 st</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSIONS:**

**Table 1:** Experimental Groups of Rattus norvegicus for the exposure with Sodium fluoride

**Table 2:** Experimental Groups of Rattus norvegicus after adopting food supplementation

**Table 3.1:** Showing the alteration in haematological parameters induced by fluoride concentration in Albino rat.

A  P ≥ 0.001 Highly significant
B  P ≥ 0.01 Significant
C  P ≥ 0.05 Slightly significant
D  Non significant

Fig 3.1: Alteration in haematological parameters induced by fluoride concentration in Albino rat.

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Table 3.1: Showing the alteration in haematological parameters induced by fluoride concentration in Albino rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration</th>
<th>Sodium Fluoride Treatment</th>
<th>Urea mg/dl</th>
<th>Creatinine mg/dl</th>
<th>SGOT U/L</th>
<th>SGPT U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Controlled</td>
<td>Water without sodium fluoride</td>
<td>23.41±0.21</td>
<td>0.51±0.08</td>
<td>28.2±1.40</td>
<td>17.6±1.33</td>
</tr>
<tr>
<td>II</td>
<td>30 days</td>
<td>10 ppm</td>
<td>A 28.56±0.38</td>
<td>B 0.66±0.03</td>
<td>A 30.1±1.7</td>
<td>A 20.1±1.20</td>
</tr>
<tr>
<td>III</td>
<td>60 days</td>
<td>10 ppm</td>
<td>A 35.50±0.47</td>
<td>B 0.97±0.09</td>
<td>A 33.1±1.35</td>
<td>A 23.2±1.19</td>
</tr>
<tr>
<td>IV</td>
<td>90 days</td>
<td>10 ppm</td>
<td>A 42.23±0.26</td>
<td>B 1.66±0.21</td>
<td>A 36.6±1.6</td>
<td>A 25.6±1.75</td>
</tr>
</tbody>
</table>

A  P ≥ 0.001 Highly significant  
B  P ≥ 0.01 Significant  
C  P ≥ 0.05 Slightly significant  
D  Non significant

Table 3.2: Showing the alteration in haematological parameters induced by fluoride concentration in Albino rat after amelioration of Aloe vera.

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration</th>
<th>RBC Million/m³</th>
<th>WBC Million/m³</th>
<th>Hb gm/l</th>
<th>AlpH IU/l</th>
<th>AcpH IU/l</th>
<th>Proteins mg/dl</th>
<th>Cholesterol mg/dl</th>
<th>Glucose mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>60 days</td>
<td>A 3.34 ± 0.05</td>
<td>A 3.26 ± 0.03</td>
<td>B 12.3 ± 0.06</td>
<td>A 2.81 ± 0.05</td>
<td>A 3.79 ± 0.07</td>
<td>B 6.60 ± 0.5</td>
<td>C 115.03 ± 2.21</td>
<td>B 112.50±0.03</td>
</tr>
<tr>
<td>II</td>
<td>90 days</td>
<td>A 3.45 ± 0.04</td>
<td>A 3.48 ± 0.05</td>
<td>B 12.9 ± 0.07</td>
<td>A 2.72 ± 0.04</td>
<td>B 3.81 ± 3.06</td>
<td>B 6.96±0.5</td>
<td>C 126.05±3.03</td>
<td>A 111.01±0.1</td>
</tr>
</tbody>
</table>

D  Non significant  
C  P ≥ 0.05 Slightly significant  
B  P ≥ 0.01 Significant  
A  P ≥ 0.001 Highly significant

Fig 3.2: Showing the alteration in haematological parameters induced by fluoride concentration in Albino rat after amelioration of Aloe vera.
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<table>
<thead>
<tr>
<th>Group</th>
<th>Duration</th>
<th>Urea mg/dl</th>
<th>Creatinine mg/dl</th>
<th>SGOT U/L</th>
<th>SGPT U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>60 days</td>
<td>A 40.92±0.21</td>
<td>B 1.23±3.2</td>
<td>A 23.6±3.1</td>
<td>A 21.4±2.4</td>
</tr>
<tr>
<td>II</td>
<td>90 days</td>
<td>A 37.40±0.20</td>
<td>B 1.01±3.0</td>
<td>A 21.0±1.9</td>
<td>A 15.8±1.65</td>
</tr>
</tbody>
</table>

D Non significant C P ≥ 0.05 Slightly significant
B P ≥ 0.01 Significant A P ≥ 0.001 Highly significant

Fig 3.2: Showing the alteration in haematological parameters induced by fluoride concentration in Albino rat after amelioration of Aloe vera.

Blood is important fluid connective tissues which transport the materials to different parts of body. The blood acts as a transport medium for fluoride. About 75% of the blood fluoride is present in the plasma; the rest is mainly in or on the red blood cells. (Carlson et. al., 1960 b, Hosking & Chamberlain 1977.) This part deals with estimation of different blood parameters after fluoride exposure viz, RBC count, total WBC count, Haemoglobin, Alkaline phosphatase, Acid phosphatase, SGOT, SGPT, Urea & Creatinine, Serum protein, cholesterol & Glucose. In present investigation the blood parameters in Albino rats were altered after exposure to sodium fluoride (10 ppm of Naf per kg of body weight) in comparison to control animals at an interval of 30, 60 & 90 days.

The RBC count, WBC count & Haemoglobin were decreased in all treated groups. Similar results were also reported by Mishra and Mohapatra 1987; Kristinson et. al, 1997; Jagdish et. al, 1998; Madal et. al, 1986, Shanthakumari & Subramaniam 2007; Banupriya et. al, 1997. Sushella et. al, 2001, Kahl et. al, 1973 and Pillai et. al , 1985 reported the inhibition of RBC on fluoride exposure. The decrease level of RBC and haemoglobin in fluoridated Channa punctatus has been reported by Saxena et. al, 2001. Fluoride damages erythrocytes and induces echinocyte formation (Jain & Susheela, 1986). These damaged erythrocytes are eliminated through the process of phagocytosis, this shown that fluoride decreases RBC & haemoglobin (Pillai & Mane 1985). Hylnyczak and Ubranksa (1987) reported a significant decrease in blood haemoglobin and haematocrit of Cat fish, Cows & rats exposed to fluoride.

A decrease in size of erythrocytes due to stressful conditions in Salmo grairdneri was also reported by Soivio et. al, 1974. The haematological parameters RBC, WBC count & Haemoglobin values were altered significantly after ingestions of fluoride Sharma et. al, 2006). Fluoride depletes the energy reserves & the ability of WBC to properly destroy foreign agents by the process of phagocytosis in turn reduced immunity leading to general weakness in animals, Gabbler and Leong 1979; Gabler et. al, 1985 & Kozylyuk., 1987). WBC count change by fluoride exposure to 100 ppm for four months rats by Eren et. al, 2005.

Plasma released some hydrolytic enzymes under stress condition results in decreasing of WBC count due to autolysis reported by Reddy et. al, 1993. Excessive intake of fluoride content reduced the level of haemoglobin concentration stated by Bano et. al, 1996 and Swaroop et. al, 1998. Reduced haemoglobin would be leaded to decrease in oxygen carrying capacity of blood reported by Chatterjee & Chand 1986, due to destruction of haemopoietic tissue, Patel & Dhande, 2000. Various enzyme activity in tissue & body fluid plays significant role in diagnosis of different disease reported by Malomo 2000.

Alkaline phosphatase (ALP) is the marker enzyme of fluoride toxicosis and bone pathology. An increase in serum alkaline phosphatase activity in animals treated with fluoride has been reported by Blood et. al, 1983. Similar observations were made by various authors, Farely et. al, 1983; Teotia & Teotia 1991. Alkaline Phosphatase acts as marker enzyme for plasma & endoplasmic reticulum (Wright & Plumner 1974). Shahjahan 2004 is often employed to asses integrity of plasma membrane (Akanji et. al, 1993). Singh & Swaroop, 1999 stated that the increasing level of this enzyme attributed to cell damage & disruption of cellular organization, physical & chemical changes in lysosomal membrane allowing release of hydrolytic enzymes & causing dissolution. Decreased level of alkaline phosphatase was reported by Portela et. al, 1974 in rat after prolonged intake.

The level of acid phosphatase is increased during fluoride intoxication which is also a early marker of tissue damage because of its specificity & catalytic activity by Marie
levels were increased after fluoride treatment in comparison to SGOT & SGPT substances and reabsorb the metal and non-metal ions. Associated with impairment of renal function(Kumar et. al, 2004; Maxwell & Bruinsma 2001; Shantakumari & Shivarajashankara et. al, 2003; Wang et. al, 2004; Shanthakumari et. al, 2004; Shan et. al, 2004; Karaoz et. al, 2004; Zhan et. al, 2006; Zhang et. al, 2006;) In blood serum, the protein contents were decreased in present investigation similar results were reported by Venkenteshwarlu et. al, 1994.

The average total serum protein level of the rats in treatment group decreased significantly compared to control reported by Qujeq et. al, 2002 & Krishnamachari, 1986; Chinoy & Memon 2001.Chinoy et. al, 1991, 1996, 1997; reported inhibition of protein synthesis due to fluoride. Decreased level of protein was also reported by Choudhary & Gaur 2001 in Cyprinus carpio. Fluoride is known to inhibit protein synthesis, mainly to impairment of peptide chain initiation, Hoeiz, Mc, Curty 1971 & by interfering with peptide chains on ribosomes Ravel et. al., 1966.

In present study after sodium fluoride treatment the serum glucose level was found to be increased as the action of fluoride impairs the carbohydrate metabolism and energy production by interacting with intracellular oxidative system. Same results were reported by Sondhi et. al, 1995; Bano et. al, 1996; Choudhary & Gaur 2001. A decreased level of cholesterol was found in blood serum after the exposure to sodium fluoride treated rats in comparison to control rats. Under stress conditions due to deposition of toxicants, fat is being utilized by the body which may lead to less cholesterol content in serum .Decreased level of lipid is due to the repression of the activity of a number of enzymes responsible for lipid transformation which ultimately causes disturbed lipid metabolism and the value of cholesterol drastically decreases reported by Chinoy et. al,1994.Similar trend of decreased cholesterol level was also reported by Venkenteshwarlu et. al, 1994 & Dronnuk 1999, Shivarajashankara et. al, 2003.

**Urea & Creatinine:** Urea & Creatinine were increased after exposure to sodium fluoride in present investigation. The same results were also reported by Singh et. al., 2002; Maiti & Das 2004; Maxwell & Bruinsma 2001; Shantakumari & Subramaniam 2007.

Fluoride intoxication induced elevation of Urea & Creatinine which are considered as significant markers for renal dysfunction. Changes in serum Urea, Creatinine are associated with impairment of renal function(Kumar et. al, 1988). The elevated serum levels of Urea & Creatinine indicate reduced ability of the kidney to eliminate the toxic metabolic substances and reabsorb the metal and non-metal ions.

**SGOT & SGPT:** In present investigation SGOT & SGPT levels were increased after fluoride treatment in comparison to control group. Michael et.al., 1996 also reported increasing trends of SGOT & SGPT in fluoride endemic population. The increased level of serum transaminases shows alteration in liver function. These levels increase several times if cellular damage occurs in the liver, so these enzymes are markers for assessing liver function. The same trends has also been supported by Kaur et.al., 1981; Flora et. al, 1991 & Chinoy et. al, 1993.

**Haematological investigation after amelioration of vitamin C & D (artificial source) and Aloevera (Natural) in fluoride treated rats:** The exogenous feeding of Vitamin C,D & Aloe vera after treatment for 60 and 90 days at the concentration of 500mg/kg of body weight, showed significant recovery in all altered parameters. The haematological parameters viz RBC count, WBC count, Haemoglobin, Acid phosphatase, serum protein, serum cholesterol were decreased after fluoride treatment but with withdrawal of fluoride and adopting food supplementation of vitamin C,D and Aloe vera, the above parameters tend to increase in significant manner as shown in table 3.2. While serum glucose Alkaline phosphatase, urea, Creatinine, SGOT & SGPT were decreased in a slightly significant manner after food supplementations as these parameters were increased after fluoride treatment.

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