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Fluoride Induced Histo-pathological Alterations in Major Soft Organs of Wistar Albino Rat

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Abstract: **Liver and kidneys are the important organs for metabolism, detoxification, homeostasis and excretion in most of the higher animals, including man. Toxic substances cause histopathological changes in both of these major tissues. Because the kidney get easily exposed to higher concentrations of fluoride than any other soft tissues ,excess fluoride exposure contributes to kidney damage, thus damaged kidneys increase the retention of fluoride, causing in turn further damage to the kidney, bone, and other organs. In animals, kidney damage has been reported at levels as low as 1 ppm if the animals consume fluoridated water for long periods of time. In the present investigation status of various membrane bound enzymes and other biochemicals in liver and kidney were studied in both control and experimental rats, which were given NaF in drinking water for 180 days. Acid phosphatase, LDH, and SDH were depleted significantly in liver after 180 days of fluoride feeding through drinking water. Alkaline phosphatase (P< 0.01), G-6-PD (P < 0.01) and ATPase (P< 0.01) were elevated significantly. GOT and GPT in liver were also increased significantly except GPT which was significant (P< 0.1) only in male rats .Significant (P< 0.1) decrease in liver proteins and elevated glycogen content were noted in all the experimental rats after 180 days of exposure to fluoride in drinking water. In kidney of experimental rats, alkaline phosphatase, GOT, GPT and ATPase were significantly elevated after chronic treatment of fluoride through drinking water. However, the enzymes acid phosphatase, LDH and SDH were significantly (P< 0.01) depleted. Total protein was found to be depleted significantly (60.07%) in male rats and 55.72% in female rats.**

Keywords: **Sodium Fluoride, Biochemical, Histo-pathological alterations, Soft Tissues, Liver, Kidneys, Wistar Albino Rat.**

1. INTRODUCTION

Fluoride when absorbed is rapidly distributed by systemic circulation into the intracellular and extra- cellular water of tissues. More than 90% of the total body burden is retained in bones and teeth. The concentration of fluoride in soft tissues is reflected in blood. Fluoride is distributed from the plasma to all tissues and organs. The rates of delivery are generally determined by the blood flow to the tissues .Consequently, steady state fluoride concentrations are achieved more rapidly between plasma and well-perfused tissues, such as liver and kidney. The major route for the removal of fluoride from the body is by the kidney. Urinary fluoride is regarded as the best indicator of exposure to fluorine compounds, and usually it correlates well with the level of fluoride in drinking water (Czarnowski et al; 1996).

The health effects of ingested fluoride have been considered by a number of bodies, the most recent of which include the Royal Society of New Zealand and the Office of the Prime Minister's Chief Science Advisor (2014); European Food Safety Authority 2013; European Commission Scientific Committee on Health and Environmental Risks report (SCHER, 2011); National Academy of Sciences Report (2006). A monograph edited by Buzalaf (2011), also covered this topic. Approximately 90% of the fluoride ingested each day is absorbed from the alimentary tract, with higher proportions from liquids than from solids. The half-time for absorption is approximately 30 minutes, hence peak plasma concentrations usually occur within 30-60 minutes (Buzalaf and Whitford, 2011).

Fluoride which has been described as an essential nutrient is extremely useful for the normal development and growth of human beings (Dhar et al; 2009) As per WHO, 0.6 ppm fluoride ingestion is useful, when fluoride gets accumulated in hard tissues of the body it plays an important role in mineralization of bone and teeth (Adler et al;1970).

At high levels, fluoride causes dental and skeletal fluorosis. Dental fluorosis occurs due to exposure of more than 1-1.5 ppm, in developing enamel which is characterized mainly by mottling of the teeth (Dinman et al; 1984).The adverse effects of high fluoride intake are also observed in soft tissues which are known to affect collagen synthesis, inhibit enzymes such as those involved in the pentose pathway, antioxidant defense system and the myosin ATPase pathway (Sarkar et al; 2002). Fluoride exists as the fluoride ion or as hydrofluoric acid in the body fluids (Aoba et al;2002) Chronic poisoning from long term exposure is a serious health problem in many parts of the world where drinking water contains more than 1- 1.5 ppm of fluoride (Dinman et al;1984).Moderate amounts lead to dental defects, but long term ingestion of large amounts can lead to potentially severe skeletal problems (Ibrahim et al; 2011) .In human beings fluorosis is mainly caused by drinking fluorinated water, burning coal and drinking tea (Bhanu Prakash Reddy et al;2003) . Osseous and soft tissues are damaged due to fluoride intoxication (Strunecka et al; 2002 and Suwalsky et al; 2004). Effects of fluorosis can be skeletal or non skeletal (Shivarajashankara et al; 2003). Mucosal abnormalities are common with skeletal fluorosis .Higher concentrations of fluoride are known to affect collagen synthesis, inhibit enzymes involved in the pentose pathway, antioxidant defense system and the myosin ATPase pathway. Furthermore fluoride is also known to cross the cell membrane and enter soft tissues (Sarkar et al; 2002).

Liver is an important organ for metabolism and detoxification of foreign substances (Sodani, 2016). In the present study, liver histopathological changes varied with the concentration of fluoride in treated groups with respect to the control groups. Histopathological changes logical sectioning which indicated various degrees of hepatocellular necrosis and portal inflammation in the treated groups. Similar results were observed in the liver cells of animals exposed to 25 mg/kg F for 4 weeks (Thangapandiyan, 2014). Evidences of changes in liver may relate to that the liver has a central role as a detoxifying organ towards xenobiotics and chemicals (Sodani,2016) . The toxicants have been revealed by abnormal metabolic functions, reduced activity of detoxification reaction, and altered structure of sub cellular organelles (Parihar et al;2013) . These pathological alterations in the fluoride-treated liver tissues could be due to the accumulation of free radicals by fluoride ions (Thangapandiyan,2014) . Diagnostic evaluation of liver tissue is largely based on examination of sections stained with Hematoxylene and eosin . and it has been the most universal and traditional method for examination of formalin-fixed, paraffin-embedded tissue sections (Foureau et al;2014).The inflammatory cells could be lymphocytes, plasma cells, or macrophages, and they were stained as mononuclear inflammatory cells with Hematoxylene-eosin stain. Therefore, additional special stains such as immune-histochemical stains may be useful to highlight or identify features that are not easily seen on a Hematoxylene-eosin stain (Hall and Rojko; 1996).

The kidney helps to prevent the build-up of toxic fluoride levels in the body by excreting fluoride through urine. Excess fluoride exposure contributes to kidney damage, thus damaged kidneys increase the retention of fluoride, causing in turn further damage to the kidney, bone, and other organs. In animals, kidney damage has been reported at levels as low as 1 ppm if the animals consume fluoridated water for long periods of time. In humans, elevated rates of kidney damage are frequently encountered among populations with skeletal fluorosis. Individuals with advanced kidney disease are known to have a very high susceptibility to fluoride toxicity since their bones and other tissues accumulate fluoride at levels far higher than healthy individuals. This fluoride build-up places kidney patients at a quite enhanced risk of skeletal fluorosis. Fluoride intake can also contribute to and compound the complex bone and renal diseases (Whitford, 1997).

The present study was undertaken to investigate the effect of chronic fluoride ingestion for 180 days, and to find out the biochemically determined activities of acid phosphatase, LDH, SDH and GOT etc. both in liver and kidney (Table, 1)

2. MATERIALS AND METHODS

Experimental Animal Model:

In the present investigation the Wistar albino rats, *Rattus norvegicus* (male rats weighing about 90 ± 5 g and female rats weighing about 80 ± 5 g) were used as test animals.

Procurement of Rats:

The rats were procured from National Institute of Nutrition (NIN), Hyderabad, India. Animal experimentations were conducted according to INSA-Ethical guidelines for the use of animals for scientific research purpose, after getting permission from Animal Ethical Committee.

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Maintenance of Rats:

The animals were kept in vivariums throughout the period of experiment. They were regularly fed on standard pellet diet provided by National Institute of Nutrition, Hyderabad and water was given *ad-libitum*. The remaining food and waste matter was removed from the cages on the next day and proper care was taken to avoid any infection. Only healthy rats were used for the present experiments. Experimental animals were acclimatized for 8 days.

Experimental set-up:

After recording their initial body weights and temperatures, then were divided into two main groups: Group: I, as control and Group: II, as experimental. The duration of the experiment was 180 days i.e. chronic fluoride exposure. For control set-up five males with five females and for experimental setup ten males with ten females were selected. The animals were observed daily for any mortality and signs of intoxication upto 180 days i.e. 6 months (the period of experimentation). No mortality was recorded during the 180 days of experiment both in control and experimental animals.

Doses : The control group were given tap water *ad libitum* and the experimental groups were fed with 10mg F/kg/body weight/per day (i.e.22.22 mg NaF/ kg/body weight/per day) for 180 days.

Biochemical Methods Used:

Following standard methods were used during the present investigation of various aspects (Table No.1).

Table No.1: Methodologies used to study alterations various biochemical parameters of major soft organs of Wistar albino rats given NaF for 180 days

Sr.No.	Biochemical Parameters	Tissue	Methods
1	AChEase	Tissue	Biggs, CarreyandMorrison(1958)
2	Acid phosphatase	Tissue	King's(Varley 1980)
3	Alkaline phosphatase	Tissue	Kind and King's(1954)
4	GOT	Tissue	Reitman and Frankel(1957)
5	GPT	Tissue	Reitman and Frankel(1957)
6	Total Protein	Tissue	Biuret
7	Albumin	Tissue	Bromcresol green
8	ATPase	Tissue	Sickevitz and Potter (1953)
9	G-6-PD	Tissue	Qualitative(Elis *et al*. 1961)
10	SDH	Tissue	Marvin *et al*.,(1959)
11	Glycogen	Tissue	Montgomery(1957)
12	Na-K- ATPase	Tissue	Kaplay(1978)
13	Ca- ATPase	Tissue	Samaha and Yunis(1973)
14	Catalase	Tissue	Luke,H.(1974)
15	SOD	Tissue	Marklund and Marklund (1974)
16	Calcium	Bone	CPC
17	Inorganic phosphorous	Bone	Fiske and Subbarow , 1925
18	Fluoride	Bone	Spands (Marvin , 1979)

3. OBSERVATIONS AND RESULTS

Status of various membrane bound enzymes and other biochemicals in liver and kidney were studied in both control and experimental rats, which were given NaF in drinking water for 180 days. Acid phosphatase, LDH, and SDH were depleted significantly in liver after 180 days of fluoride feeding through drinking water. Alkaline phosphatase ($P< 0.01$), G-6-PD ($P < 0.01$) and ATPase ($P< 0.01$) were elevated significantly in both of these major organs. GOT and GPT in liver were also increased significantly except GPT which was significant ($P< 0.1$) only in male rats (Table 2 and 3).Significant ($P< 0.1$) decrease in liver proteins and elevated glycogen content were noted in all the experimental rats after 180 days of exposure to fluoride in drinking water (Table ,2and 3).In kidney of experimental rats alkaline phosphatase, GOT, GPT and ATPase were significantly elevated after chronic treatment of fluoride through drinking water. However, the enzymes acid phosphatase, LDH and SDH were significantly ($P< 0.01$) depleted (Table 3). Total protein was found to be depleted significantly (60.07%) in male rats and 55.72% in female rats (Table, 2and 3).

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In histological studies in this investigation (Hematoxylene-eosin staining), liver of experimental rats showed disarray of hepatic cords, intense vacuolization and hydropic degeneration (Fig.2 and 4),while kidneys of experimental male and female rats showed tubular degeneration, hypertrophied and lobulated glomerulus;vacuolization of renal tubular cells and thickened glomerular membrane(Fig.6 and 8).

Table 2: Alterations in liver biochemicals of the control and experimental male and female Wistar albino rats fed with sodium fluoride (NaF) for 180 days.

Sr. No	Liver biochemical parameters	Male			Female		
		Control	Experimental	Percent Variation	Control	Experimental	Percent Variation
1	Acid phosphatase (KA units)	45.76 ±2.66	28.15* ±2.08	-38.48	40.18 ±1.86	25.16* ±0.88	-37.38
2	Alkaline Phosphatase(KA units)	98.85 ±3.11	136.55** ±5.85	+38.13	90.33 ±2.55	145.18** ±3.89	+60.52
3	GOT (Units/ml)	52.20 ±2.16	62.88* ±5.28	+20.45	50.58 ±2.56	66.05* ±5.85	+30.58
4	GPT (Units/ml)	65.16 ±2.14	82.35* ±3.56	+26.38	69.00 ±3.69	75.59^{NS} ±6.58	+9.55
5	Total protein (gm/dl)	36.65 ±1.91	28.19* ±1.55	-23.08	35.88 ±2.11	29.65* ±1.56	-17.36
6	LDH (U/L)	256.15 ±12.18	206.65* ±8.15	-19.32	250.00 ±12.20	202.86* ±18.92	-18.85
7	Glycogen (mg/gm of tissue)	62.11 ±3.56	68.55* ±2.88	+10.36	60.55 ±8.15	70.16* ±3.86	+15.87
8	SDH (μ moles of Formazan mg protein /hr.)	26.92 ±1.66	12.85* ±1.24	-52.26	20.44 ±1.33	14.15* ±2.58	-30.77
9	G-6-PD (n moles of Pi libeated /mg protein /min.)	16.44 ±1.66	28.19** ±1.25	+71.47	17.86 ±1.06	30.58** ±2.86	+71.22
10	ATPase (n moles of Pi libeated /mg protein /min.)	106.33 ±3.15	148.9** ±2.89	+40.03	100.15 ±5.68	140.59** ±3.15	+40.37

All values are mean of six observations. ±SD.
* p < 0.01, **p < 0.1, NS-Not Significant.

Table 3: Alterations in kidney biochemicals of the control and experimental male and female Wistar albino rats fed with sodium fluoride (NaF) for 180 days.

Sr. No	Kidney biochemical parameters	Male			Female		
		Control	Experimental	Percent Variation	Control	Experimental	Percent Variation
1	Acid phosphatase (KA units)	23.11 ±1.99	16.88* ±1.08	-26.95	21.90 ±2.00	14.58* ±0.95	-33.42
2	Alkaline Phosphatase (KA units)	180.16 ±6.19	255.20* ±3.15	+41.65	175.75 ±10.00	189.62* ±2.18	+64.79
3	GOT (Units/ml)	75.20 ±2.62	105.28* ±4.15	+40.00	78.12 ±2.86	85.93* ±2.08	+9.99
4	GPT (Units/ml)	44.86 ±1.95	76.36** ±2.08	+70.21	40.44 ±1.56	82.28* ±1.32	+103.46
5	Total protein (gm/dl)	15.48 ±0.88	6.18** ±0.36	-60.07	18.86 ±0.14	8.35* ±0.28	-55.72
6	LDH (U/L)	615.38 ±11.16	472.30* ±12.15	-23.25	604.00 ±13.11	439..46* ±6.19	-27.24
7	SDH (μ moles of Formazan mg protein /hr.)	20.18 ±0.42	12.80* ±1.02	-42.29	20.15 ±1.26	14.82* ±1.08	-26.45
8	ATPase (μ moles of Formazan mg protein /hr.)	128.15 ±10.18	150.12* ±8.66	+17.14	120.00 ±9.25	145.29* ±8.63	+21.07

All values are mean of six observations. ±SD.
* p < 0.01, **p < 0.1, NS-Not Significant.

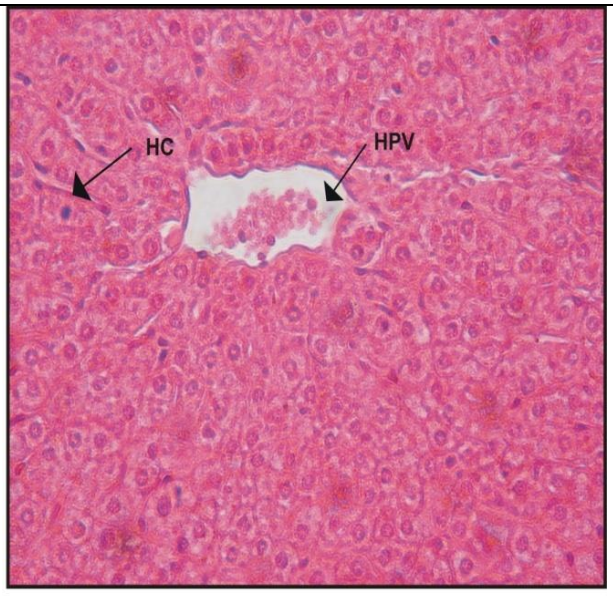

Fig.1:T.S. Liver of control albino rat (male), showing regular hepatic cords, Hematoxylene-eosin X400, HPV-Hepatic portal vein,HC-Hepatic cords

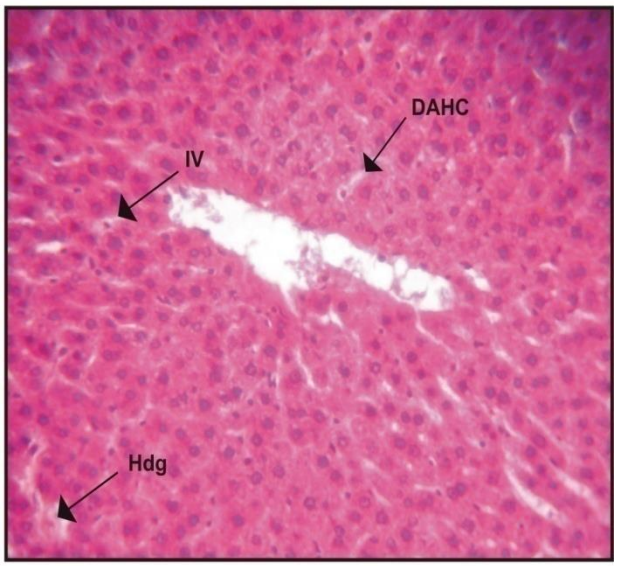

Fig.2:T.S. Liver of experimental albino rat (male),showing disarray of hepatic cords, Hematoxylene-eosin X400,DAHC- Disarray of hepatic cords, IV-Intense vacuolization,Hdg-Hydropic degeneration

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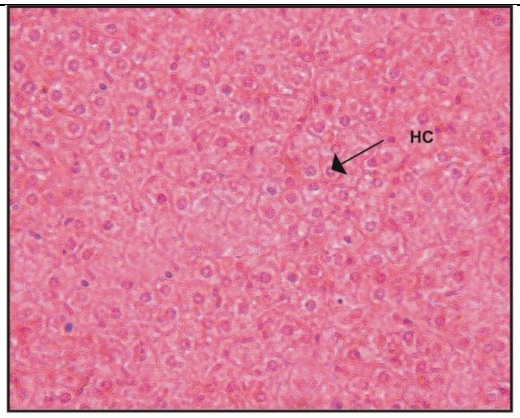

Fig. 3:T.S. Liver of control albino rat (female), Hematoxylene-eosin X400, HC-Hepatic cords

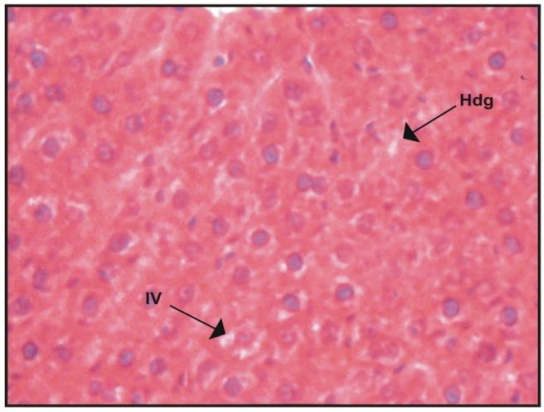

Fig.4:T.S. Liver of experimental albino rat (female),showing total disarray of hepatic cord, Hematoxylene-eosin X400, IV-Intense vacuolization,Hdg-Hydropic degeneration

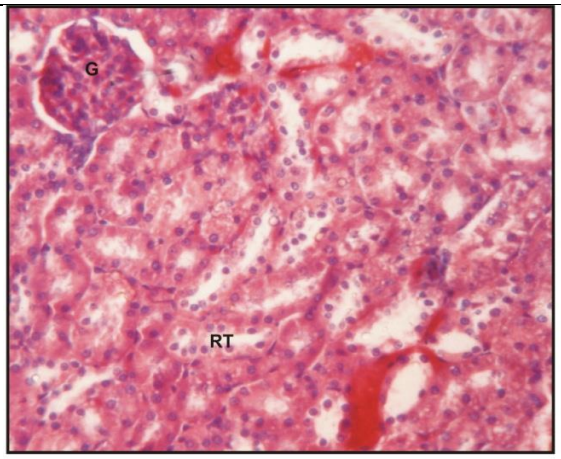

Fig.5: T.S. Kidney of control albino rat (male), Hematoxylene-eosin X400,G-glomerulus,RT-renal tubule.

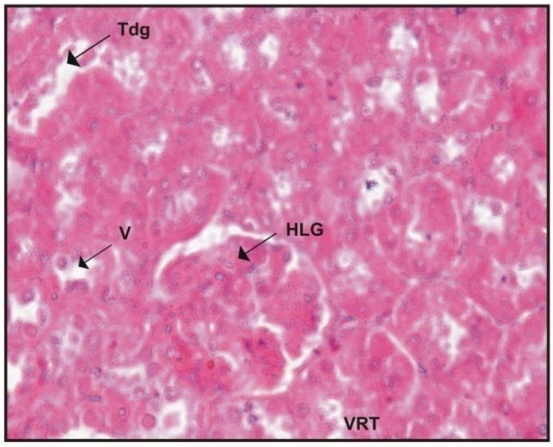

Fig.6: T.S. Kidney of experimental albino rat (male),Hematoxylene-eosin X400,Tdg Tubular degeneration ,HLG-Hypertrophied and lobulated glomerulus,VRT- Vacuolization in renal tubular cells.

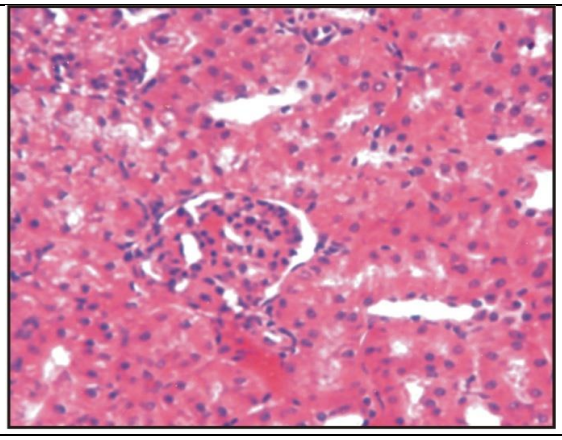

Fig.7: T.S. Kidney of control albino rat (female), Hematoxylene-eosin X400.

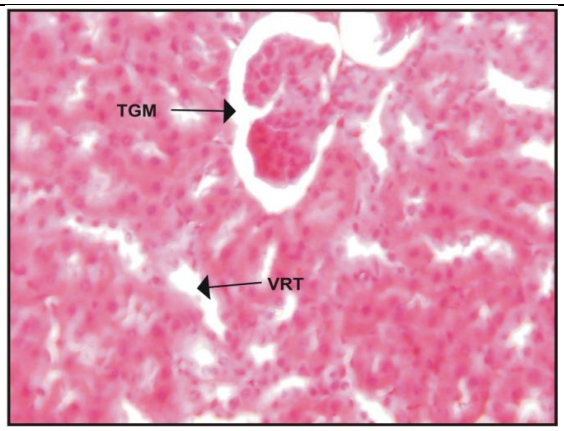

Fig.8: T.S. Kidney of experimental albino rat (female),Hematoxylene-eosin X400,TGM- Thickened glomerular membrane , VRT- Vacuolization in renal tubular cells.

4. DISCUSSION

In this study, the rats maintained on fluoridated water for 180 days showed an adverse effect on the level of different enzymes in the serum, liver and kidney. Ferguson, (1971); Riesktniece *et al.*, (1965), suggested changes in blood enzymes and proteins, due to ingestion of fluoride could result from a change in the membrane permeability and from the rate of loss of the enzymes from the different tissues. The present study reveals that following fluoride ingestion for 180 days, the biochemically determined activities of acid phosphatase, LDH, SDH and total protein content have decreased both in liver and kidney (Tables, 2 and 3). It is well known that fluoride inhibits many enzymes *in vitro* as well as *in vivo* (Wiseman, 1970; Chitra *et al*., 1983; Shahed *et al*., 1980). Furthermore, in fluoride toxicity, the fluoride content of liver and kidney gets appreciably elevated (Susheela and Singh, 1982; Shearer ad Ridlington, 1976). In fact, this elevated fluoride levels in liver might have inhibited enolase (glycolytic enzyme in glycolysis) as it is very sensitive to fluoride. These results are in accordance to the earlier reports of Stachowska *et al*., (2000). However, whether the reduced activities of these enzymes in the present study are due to direct inhibitory effect of fluoride remains to be investigated.

In the present study serum alkaline phosphatase level in fluoride fed rats is significantly increased. Higher activity of serum alkaline phosphatase reflects abnormal formation of bone due to stimulated osteoblastic activity as suggested by Blood and Radostits (1994). Decreased blood inorganic phosphate has also been observed in the present investigation, which indicates that it might have been retained in the cells of organs under investigation. The vacuolated hepatocytes, vacuolated cells of uriniferous tubules (Fig. 6 and 8) observed in the present investigation is nothing but the result of failure of active transport owing to diminished ATP, leading to sodium to accumulate in the interstitial spaces with the diffusion of potassium of the cell. In support to this decreased serum sodium and increased serum potassium is recorded in the present studies .

Many studies have shown that rats exposed to high fluoride concentrations in drinking water, the levels of renal and liver function enzymes in serum and caused severe histological changes of the liver and kidneys (Guo et al;2003 ;Zhan,2006) . In histological studies in present investigations , Hematoxylene-eosin staining; liver of experimental rats showed disarray of hepatic cords, intense vacuolization and hydropic degeneration (Fig.2 and 4),while kidneys of experimental male and female rats showed tubular degeneration, hypertrophied and lobulated glomerulus;vacuolization of renal tubular cells and thickened glomerular membrane(Fig.6 and 8).Our findings are in accordance with these findings. Sodium fluoride treated Wistar rats with 50, 100, and 150 mg NaF/L with their drinking water for 3 months have shown significant increase of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) activities and hepatic damages(Guo et al;2003). In another study, chicken were exposed to 10, 20, and 30 mg/g of NaF on weekly basis for 4 weeks have elevated the levels of the liver function indicators, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), (Anjum,2014). Mean time such studies have shown that fluoride has deleterious effects on kidneys exposed to high concentrations (50, 100, 125,150, 250 ppm, etc.) of fluoride (Tsunoda,2005 and Zhan,2006). Pigs exposed to fluoride concentrations of 100 and 250 mg/Kg showed significantly increased serum creatinine and urea levels and deleterious effect on kidney structure and function(Zhan,2006).

The present study also suggests a clear but differential inhibitory action of sodium fluoride on hepatic and renal enzyme system. Comparison between hepatic and renal response to fluoride toxicity clearly show that the kidney is a more sensitive target organ showing a significant decrease in LDH and significant increase in alkaline phosphatase and GPT (Table 2and 3) whereas the liver shows adaptive changes to some extent.

5. CONCLUSIONS

The prominent organs affected were liver and kidney. All most all the oxidative enzymes in liver were inhibited. Activity of alkaline phosphatase in liver and kidney was increased resulting into damage to liver and kidney architecture. Fluoride exposure impaired hepatocytes and hepatic function, which was strongly supported by the necrosis and portal inflammation histopathologically and increased serum AST, ALT, and ALP activities. Further, it has been demonstrated that there is a possibility of inducing renal damage by high fluoride levels for longer period of administration.

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