

Toxicity of fluoride in liver of Albino rat and Mitigation after adopting artificial (Vitamin C and D) and natural (Aloe vera) food supplementations.

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ABSTRACT:-Fluoride is a widespread non biodegradable and relatively persistent pollutant, which at low levels of contamination causes serious health problems difficult to cure. Fluoride pollution in India is mainly due to natural sources reported so far. Its concentration in water is much higher than the recommended values by different authorities. More than half a million people are affected by the disease called fluorosis which is due to chronic fluoride intoxication. Fluoride toxicity is characterized by a variety of signs and symptoms. Poisoning most commonly occurs following ingestion either accidental or intentional of fluoride containing products. In present study effects of oral administration of sodium fluoride(Naf) at a dose of 10mg/l of Naf per kg of body weight for 30, 60 & 90 days respectively were investigated in liver of mice. Naf treatment resulted in liver few focal areas, necrosis of hepatic cells were observed along with proliferation of hepatic cords. These changes were appeared after exposing rats to 10mg/l of sodium fluoride per kg of body weight for 30 days. While after 60 days of treatment, the normal lobular pattern of hepatic cords start distorted showing degenerative changes with necrosis and hepatic cells in certain areas were found to be vacuolated. After 90 days treatment enlarged & dilated hepatic vein were observed along with degeneration of hepatocyte with and excessive hepatic cell necrosis. These changes were recovered too much extent after withdrawal of Naf and mitigated by Artificial source (vitamin C & D) and Natural source (Aloe vera) food supplementation.

KEY WORDS: - Fluoride, contamination, necrosis, hepatic cells, Naf, degenerative changes, mitigation, food supplementation.

INTRODUCTION: - Sodium fluoride is used in fluoridating municipal water supplies, resulting in chronic exposure of millions of people worldwide. The wide spread distribution of fluoride in nature is a direct source of adverse health effects in human populations. The main source of fluoride is tap water. Other sources were food and drugs. Fluorosis is an endemic public health problem in nearly 22 nations around the world. The World Health Organization (WHO) guideline is that 1.5 ppm of fluoride is the desirable upper limit in drinking water. Accidental exposures to high level of toxic substances are known to cause liver damage. Liver plays a vital role in mammalian metabolism. The primary function of liver is secretion of bile & it also detoxifies most of toxic substances. The vertebrate's liver is made up of polygonal cells, the hepatocytes, which are arranged in branched columns called hepatic cords. These cords are separated from each other by small blood spaces or sinusoids. The sinusoids are formed from branches of the hepatic portal veins & empty into central veins. The hepatocyte is also known as liver cells. Each liver cell has granular cytoplasm & a prominent nucleus. In present investigation an attempt has been made to study the ill effects of fluoride on liver of albino rat and compared with controlled rats and also study amelioration effect after providing artificial and natural food supplementation.

MATERIALS AND METHODS: - Healthy Albino rats (*Rattus norvegicus*) weighing between 130 to 150g were obtained for experiments. Animals were housed in polypropylene cages measuring 12"x10"x8" with sawdust to soak the excretory fluids and ejected debris, cages provided ample spaces for movement under controlled environmental conditions with provisions of 12:12 hrs darkness & light and temperature between $22 \pm 2c$. The rats were maintained on standard diet & given soaked maize, wheat and water ad libitum. Static bioassay tests were conducted to find out the value of Lc 50. The present study reveals the lethal concentration for the 50% of the populations for 96 hours was 10mg/l so it was undertaken for study. This value of Lc 50 was determined by conducting the static bioassay experiment, APHA (1985). Fluoride is slow toxic element and causes health hazardous effects after long term exposures to living organisms. As time passes by its continuous exposures, it affects slowly. So an attempt was made to find out the effect of a sub-lethal concentration of fluoride in liver. Animals were exposed to a conc. of 10mg/l of fluoride for different time interval. All the treatments were given orally sodium fluoride were administered to the group 1st 2nd and 3rd. After exposure of dose for particular duration, animals were dissected out under light anesthesia. Liver organs were dissected out immediately and kept in fixative. Neutral formaline was used as fixative. Tissues were processed for paraffin sections. 3-5 μ thick of different tissues were cut with the help of microtome and stained in Harris haematoxylene and eosine.

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

S.No.	Groups	Dose	Duration	Autopsy Day
1.	Control	Water without fluoride	15	Along with treated group
2.	I	10mg/l/kg/body weight	30	31 st
3.	II	10mg/l/kg/body weight	60	61 st

4.	III	10mg/l/kg/body weight	90	91 st
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Table 2: Experimental groups of Rattus norvegicus after adopting food supplementation.

S.No.	Experimental Protocol	Dose	Duration	Autopsy Day
01	Control	Water without fluoride	60	61 st
02	Doses of Vitamin C & D	500 mg	60	61 st
03	Doses of Aloe vera juice	500 mg	90	91 st

Plate I

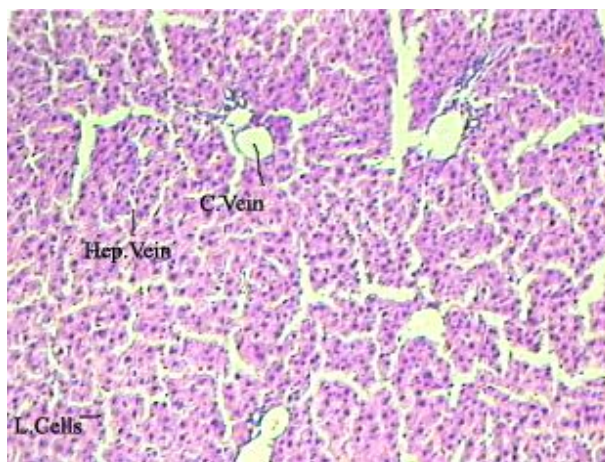


Fig. 1 Microphotograph of Liver of controlled animals

Plate II



Fig. 2 Microphotograph of Liver of fluoride treated animals

for 30 days 10x

Plate III

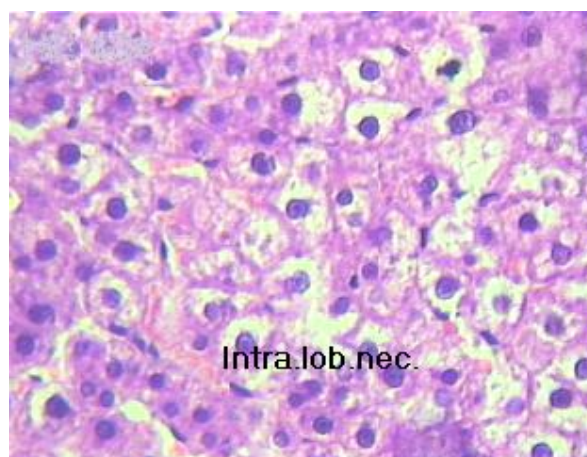


Fig .3 Microphotograph of Liver of fluoride treated animals
for 60 days 40x.

Plate IV

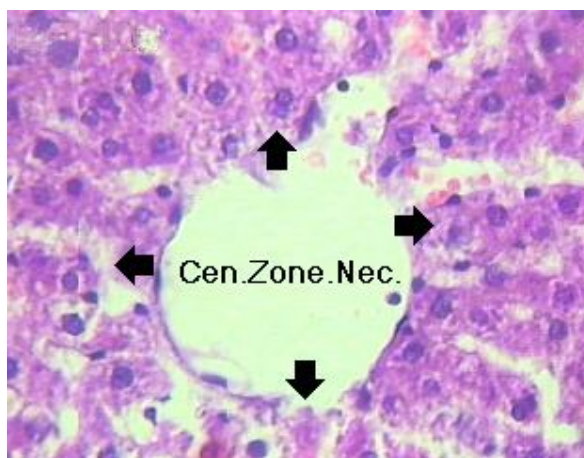


Fig. 4 Microphotograph of Liver of fluoride treated animals
for 90 days 100x.

Plate V

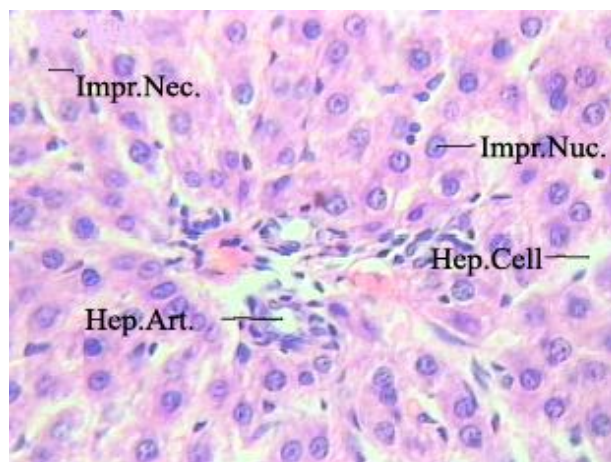


Fig. 5 Microphotograph of Liver of Albino Rat after amelioration of
Vit. C & D (60 days) 40x

Plate VI



Fig. 6 Microphotograph of Liver of Albino Rat after amelioration of
Aloe vera (90 days) 40x

RESULT

In present study, liver showed marked histopathological changes in all sodium fluoride treated groups in comparison to control groups of Albino rats. In few focal areas, necrosis of hepatic cells was observed along with proliferation of hepatic cords and dilation in central vein after treated rats with 10mg/l of Naf per kg of body weight for 30 days. The spaces normally occupied by hepatic cells in certain areas were found to be vacuolated in 60 days treated rats. Degeneration of hepatocyte with nucleus & excessive hepatic cell necrosis could be observed in 90 days Naf treated rats along with extensive vacuolization of cytoplasm. Hepatic vein appeared to be enlarged & dilated exhibiting distortion with centrilobular necrosis at places.

The exogenous feeding of Vitamin C, D and Aloe vera to fluoride treated rats can play prophylactic role to maintain normal physiology. After giving the food supplements the structural abnormalities could not be observed peculiarly as compared to the treated groups. The artificial food supplementation of vitamin C & D were given at the concentration of 500 mg/kg of body weight for 60 days while natural food supplementation of Aloe vera were given for 90 days treated rats with the same concentration of 500 mg /kg of body weight.

The hepatic cells of liver were initialized to recover after the administration of vitamin C & D for 60 days. The structural abnormalities could not be observed peculiarly as compared to the treated groups. While natural food supplementation of Aloe vera were given to 90 days treated rats showing hepatic cells of liver begin to recover the hepatocyte revealed structure similar to normal pattern.

DISCUSSION

Fluoride is most abundantly present in the environment & in the earth crust & an important environmental toxicant originating from natural & industrial sources (Whitford 1983). The consumption of food stuffs & drinking water is the principal route of exposure to fluoride. When a large proportion of fluoride were ingested & inhaled by humans or laboratory animals than it is rapidly absorbed through the gastrointestinal tract. Absorbed fluoride is carried by the blood causes metabolic disturbances in the body and is excreted via the renal system (Carlson et. al, 1960, NAS 1971 and Sahay 1986).

Liver is associated with metabolism and elimination of toxicant from the body and its histologic & biochemical parameters are considered as key points to elucidate toxicity of the chemicals. Evidences of changes in liver due to toxicants has been revealed by abnormal metabolic functions, reduced activity of detoxication reaction & altered structure of sub cellular organelles (Wang et. al, 2000). In present study structural alteration, vacuolization, dilation & degeneration of hepatic cell nucleus, necrosis in central vein in liver was appeared after fluoride treatment. Necrosis of hepatic cells was also observed by Hirano & Ando 1997 in mudskipper *Boleophthalmus dussumierei*. In present investigation histoarchitecture of liver showed radially arranged hepatic cords around the central vein.

Fluoride can produce deformation in the liver architecture including degenerative & inflammatory changes. Similar results were also reported by Chinoy et. al, 1993. Hepatic cells necrosis of experimental animals was observed in present study. Similar results were also reported by Philips et. al, 1934, Kour et. al, 1981, Muehlberger et. al, 1930 reported degenerative changes in liver of animals after administrating excessive amount of fluoride.

Shashi & Thapar 2001 observed increasing degree of hepatocellular necrosis, degenerative changes, hepatic hyperplasia, extensive vacuolization in hepatocytes & centrilobular necrosis in liver of the fluoride exposed animals. The dilation and congestion of sinusoids, ballooning of hepatocytes with pycnotic nuclei & focal necrosis was observed by Dikshit et. al, 1980.

Amelioration by Vitamin C & D and Aloe vera in fluoride treated rats

The toxic effects of fluoride could be reversed or mitigated by feeding rats with appropriate amount of artificial source i.e. Vitamin C & D for 60 days and by natural Aloe vera after 90 days of sodium fluoride treated rats. The feeding of Vitamin C & D and Aloe vera along with fluoride water for 60 & 90 days showed recovery in all altered parameters. Vitamins are natural substances found in plants & animals and essential nutrients for human beings. Vitamin C is very essential as it protects our bones, teeth & gums. Vitamin C significantly reduced the severity & incidence of fluoride induced toxicity in

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rats (Verma & Sherlin 2001). Vitamin C is also known as ascorbic acid having antibacterial, anti viral & anti fungal properties (Frie et. al., 1989). Vitamin D required for teeth & bone formation. It improves absorption & utilization of phosphorous & calcium in blood and it also helpful in maintaining stable nervous system. Food rich in protein, vitamins, essential amino acids & minerals exhibited protection from fluoride induced oxidative stress to various organs in rats (Blaszczyk et.al, 2008). Vitamin D also increase the calcium absorption & maintaining normal blood levels of calcium & phosphorus, toxicity of fluoride can be ameliorated by Vitamin E & D (Chinoy and Aarti 1998).

The hepatic cells of liver were initialized to recover after the administration of vitamin C & D. After giving the food supplements the structural abnormalities could not be observed peculiarly as compared to the treated groups. The artificial food supplementation of vitamin C & D were given at the concentration of 500 mg/kg of body weight for 60 days while natural food supplementation of Aloe vera were given to 90 days treated rats of the same concentration of 500 mg /kg of body weight. The hepatic cells of liver begin to recover the hepatocyte revealed the structure similar to normal pattern. Medicinal plants with long history human are ultimately yield novel drug prototypes. Systematic & intensive search for plants for new drugs to treat fluorosis seems to be of great utility by Madhusudhan et. al, 2010. Aloe is widely used to treat several diseases as it is known to exhibit antioxidant, antibacterial, anti-inflammatory, antistress, antipyretic, antimicrobial, antihypertensive, analgesic properties observed by Raja Sekaran et. al, 2004, Sreejayan & Rao 1994 & Yagi et. al., 2003.

In present study Aloe vera is used against fluoride toxicity for 90 days treated rats. The plant Aloe vera is selected in present investigation because of the following qualities:-This plant has great ability to reach deep inside the tissue, it detoxifies & normalize body's metabolism, it effectively solve problem of body's nervous system & it helpful in regeneration of tissue.

In present study after the administration of Aloe vera supplementation, the organs which were damaged due to toxicity of fluoride were showed more recovery. Similar results have also been reported by Madhusudhan et. al., 2010. Histopathological observation showed that Aloe vera preserves the organ architecture, changes induced by various toxic chemicals treating the patients suffering from hepato cellular damage.

CONCLUSION: - Intake of high fluoride in diet increases the toxic manifestations of fluorosis, whereas intake of diet rich in vitamin C & D & Aloe vera helps in overcoming the toxicity of fluorosis, because the diet can have two fold actions: a. Reduce the intake of fluoride by avoiding the intake of fluoride rich food. As it is always not possible to avoid the intake of fluoride from food, the simultaneous ingestion of adequate Protein, Vitamin C (ascorbic acid) and Calcium diet plays a vital role in prevention of fluorosis.

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