

# AMELIORATION OF FLUORIDE INDUCED HEPATOTOXICITY IN ALBINO RAT BY PROBITIC BACTERIA *LACTOBACILLUS RHAMNOSUS*

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## Abstract

The aim of this experiment was to investigate the effect of sodium fluoride (NaF) on bilirubin, liver enzymes (SGPT and SGOT) and liver morphology and its amelioration by Probiotic bacteria *Lactobacillus rhamnosus*. Twelve weeks old, albino rats (*Rattus norvegicus*) with average weight of 120g were randomly distributed into three groups of four animals each. The first group was kept as control. The second group was given 5ml drinking water with 0.151mg NaF /kg- bw/day and the third group was fed the same dose of NaF and 5ml water with Probiotic bacteria *Lactobacillus rhamnosus*. After 60 days of experimental period Bilirubin, SGOT and SGPT were significantly ( $P<0.01$ ) increased in the NaF treated rat. Histological examination of the liver revealed focal necrosis, liver congestion and vacuolar degeneration. However, rats treated with *Lactobacillus rhamnosus* showed significant improvement in all these three parameters and in the liver morphology. From the study, it could be concluded that fluoridated water caused liver damage and these effects could be ameliorated by *Lactobacillus rhamnosus*.

Key words: Rat, NaF, Liver Toxicity, *Lactobacillus rhamnosus*, Amelioration.

## Introduction:

Natural and artificial fluoride sources including fluoridated foodstuffs, groundwater and toothpaste lead to an excessive fluoride exposure in daily life (Barbier et al., 2011). The long term high fluoride intake enhances oxidative stress, disturbs the antioxidant defense, suggesting increased oxidative stress as one of the mediating factors in the pathogenesis caused by fluoride (Trivedi et al., 2008). Besides skeletal and dental tissues, high fluoride permeability is known to allow fluoride ion penetrate cell membranes and accumulate in diverse soft tissues such as

stomach, small intestine, liver, kidney and brain pyruvic transaminase (Lech, 2011), threatening the health of human and animal. In rat liver, enzymes of the antioxidative system, such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH), were significantly inhibited after NaF exposure (Blaszczyk et al., 2011). Epidemiological investigations and animal experiments indicate that the histological structure and function of liver are altered in animals and humans with fluorosis (Chinoy and Menon, 2001).

Fluoride, depending on its dose, is likely to impair liver function and induce morphological changes in the liver (Anderson, 1985). Most frequently, the damage is expressed as parenchymal vacuolar degeneration, necrosis of hepatocytes or disorders in the activity of metabolic enzymes (DeValc et al., 1988). Liver contains considerable amounts of polyunsaturated fatty acids, which are prone to damage by free radicals (Xio-Ying et al., 2003).

Probiotics are “live” beneficial microbes that provide important health benefits in their hosts (Hardy, et al., 2013). Many scientific studies have reported that the regular intake of probiotics or their related products, especially *Lactobacillus* and *Bifidobacterium*, significantly improves human health through a range of effects, including the biosynthesis of vitamins (e.g. vitamin K) (Bentley and Meganathan, 2008), detoxification of xenobiotics (Maurice, et al, 2013), metabolic fermentation of indigestible dietary fiber (Nilsson, et al., 2008), competition with pathogenic microbes for binding sites on mucosal epithelial cells (Candela, et al.,2008), and the modulation, regulation, and improvement of the host’s immune response (Hardy, et al., 2013).

Liver is an important organ for metabolism and detoxification. SGPT and SGOT are markers of liver function. The present study was aimed to examine the ameliorative effect of *Lactobacillus rhamnosus* on sodium fluoride induced alteration on bilirubin, serum glutamate pyruvate transaminase (SGPT) and glutamate oxalate transaminase (SGOT) and liver morphology in Albino lab rat *Rattus norvegicus*.

**Materials of methods:****Bacterial strains**

Bacterial strain *Lactobacillus rhamnosus* was isolated from commercial probiotic formulation PreproKid in the Post graduate department of Microbiology, Kamaraj College, Thoothukudi and was grown aerobically in MRS broth at 22°C.

**Experimental animals**

Twelve weeks old, Albino lab rats (*Rattus norvegicus*) of 120±20gm weight were obtained from an inbred colony maintained in the animal house of Sankaralingam Bhuvaneshwari College of Pharmacy, Sivakasi. Rat were maintained according to the guidelines set by Institutional Animal Ethical committee (IAEC, India), under the controlled conditions of temperature (23±2°C), humidity (50±5%) and a 12-hours light- dark cycle. The present study was approved by the Institutional Animal Ethics Committee (Ref. No. SBCP/ 2012-2013/IAEC/CPCSEA/05) and conducted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi, India.

**Dosage of fluoride:**

The experimental rat was orally administered with 1/10<sup>th</sup> LD<sub>50</sub> dose (0.514mg/100g b.w) of fluoride days using a feeding tube attached to a hypodermic syringe. The dosage of fluoride was based on 96 hours LD<sub>50</sub> value of fluoride for albino rat and it was 51.45mg/Kg b.w (Vijaybaskara Rav, 1994). Oral administration was preferred in view of water being the main source of fluoride among the human population in endemic areas.

**Experimental design:**

Rat was divided into 3 groups of 10 animals each. One group served as control, Group I as antidote control and Group II as Fluoride and Probiotic treated experimental group. Control rat was given 5 ml distilled water. Group I rat received 5ml distilled water with 0.514mg NaF. Group II rat received 5ml distilled water with 0.514mg NaF for 60 days and were orally administered with *L. rhamnosus* culture containing 10<sup>7</sup>cells/ml (10ml/kg body weight) for further 30 days to study the amelioration effect of *L. rhamnosus* on fluoride toxicity. They were given standard diet and water *ad libitum*.

**Table.1 Experimental Design**

S.No	Sample	Treatment	No. of Animals	Duration
1	Control	Standard diet + Water <i>ad libitum</i>	10	60
2.	Antidote control	1/10 <sup>th</sup> LC 50 dose of F + Standard diet and water <i>ad libitum</i> .	10	60
3.	Fluoride & Probiotic treatment.	1/10 <sup>th</sup> LC 50 dose of F and 100mg/kg-bw/day Tamarind pulp, standard diet + water <i>ad libitum</i>	10	60+30

Control and Group I rats were sacrificed after 60 days of experimental period to collect blood and liver tissue. Blood samples were immediately brought to the laboratory for analysis. SGOT and SGPT were estimated by method recommended by German Society for Clinical Chemistry using Enzopak Diagnostic kit (Reckon Diagnostics, India). Jendrassik and Grof's (1938) method was adopted to estimate bilirubin and expressed as mg/dl blood. Liver tissues were removed aseptically from all the groups and cut into small pieces to fix in 10% buffered formalin. Five-micron thick sections were prepared and stained with hematoxylin. Photograph was taken to study the histopathology of the liver tissues. The same analysis was performed for Group II rats after 30 days of amelioration period.

### Statistical analysis

The statistical analysis was carried out by one way analysis of variance (ANOVA), followed by Duncan's multiple range test using sigma plot for windows, version 11.0; Build 11.2.0.11. Experimental data were expressed as mean  $\pm$  SD. Differences between groups were evaluated by one-way analysis of variance. Values of  $P < 0.05$  were considered statistically significant.

### RESULTS:

The altered values of Bilirubin, SGOT and SGPT of the experimental rats are indicated in table-1 and described as follows:

## Bilirubin

The bilirubin of control rat was  $0.11 \pm 0.04$  mg/dl. It was increased to  $0.85 \pm 0.14$  mg/dl in fluoride administered rat. The bilirubin was  $0.36 \pm 0.06$  mg/dl in fluoride and *L.rhamnosus* administered rats (Fig-1). Statistically significant difference was observed between group I and group II rat ( $P < 0.050$ ).

## SGOT (Serine Glutamic Oxaloacetic Transaminase)

The SGOT of control rat was  $52 \pm 7.0$  IU/l. It was increased to  $87.67 \pm 15.2$  IU/l in fluoride treated rat. The SGOT was  $76.66 \pm 13.65$  IU/l in fluoride and *L.rhamnosus* treated rats (Fig-2). Calculation of ANOVA showed that the values were statistically significant between the control and group II rat and also significant between group I and group II rat ( $P < 0.050$ ).

## SGPT (Serine Glutamic Pyruvate Transaminase)

The SGPT value was increased from the control value of  $76.67 \pm 11.67$  IU/l to  $104.34 \pm 11.67$  IU/l in the group I rat treated with fluoride. Group II rat treated with fluoride and *L.rhamnosus* showed the SGPT value of  $85.34 \pm 12.89$  IU/l (Fig-3). Statistically significant difference was observed between group I and group II rat ( $P < 0.050$ ).

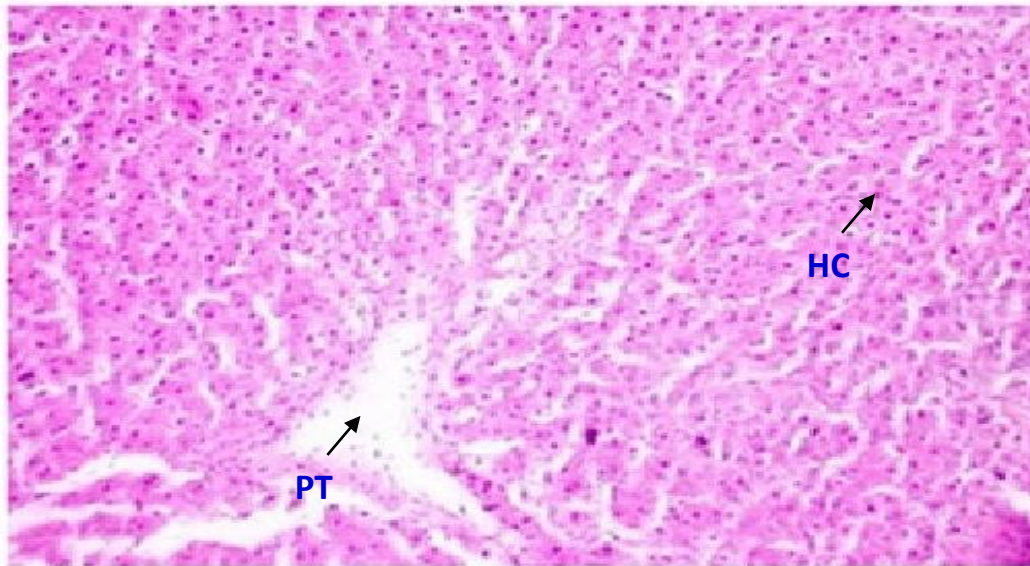
**Table-1 Serum biochemical factors of rat**

S.No	Sample	Bilirubin mg/dl	SGOT IU/l	SGPT IU/l
1	Control	$0.11 \pm 0.04$	$52 \pm 7.0$	$76.67 \pm 11.67$
2.	Group I	$0.85 \pm 0.14$	$87.67 \pm 15.2$	$104.34 \pm 11.67$
3.	Group II	$0.36 \pm 0.06$	$76.66 \pm 13.65$	$85.34 \pm 12.89$
4	F-Values	<b>46.32</b>	<b>6.52</b>	<b>4.11</b>

## Liver morphology of control rat

Control rat composed of polyhedral shaped hepatocytes, with defined cell lining. Nuclei were distinctly rounded with a distinctly marked nucleolus and peripheral chromatin. Hepatocytes were arranged in trabecules, running radiantly from the central vein and separated by sinusoids containing kupfer cells. (Plate-1).





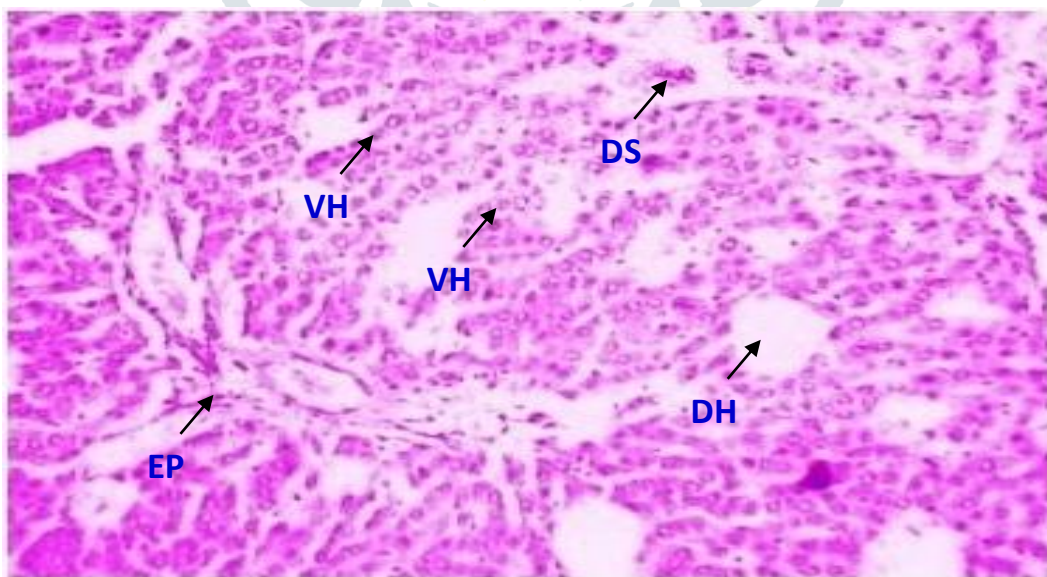
**PT: Portal Triad**

**HC: Hepatic Cell**

**Plate-1 Liver of control rat** shows normal hepatocytes with defined cell lining, nuclei, central veins and peripheral hepatic triads

### Liver morphology of group I rat

The liver tissue of the Group I rat indicated the presence of abnormal hepatocytes with a distorted shape and undefined cell lining. Dilated sinusoids and degeneration of hepatocytes was observed. Vacuolar degeneration-type changes occurred. Necrotic lesions were seen in hepatocytes (microfocal lesion (Plate-2)).



**DS: Dilated Sinusoid**

**VH: Vacuolated Hepatocyte**

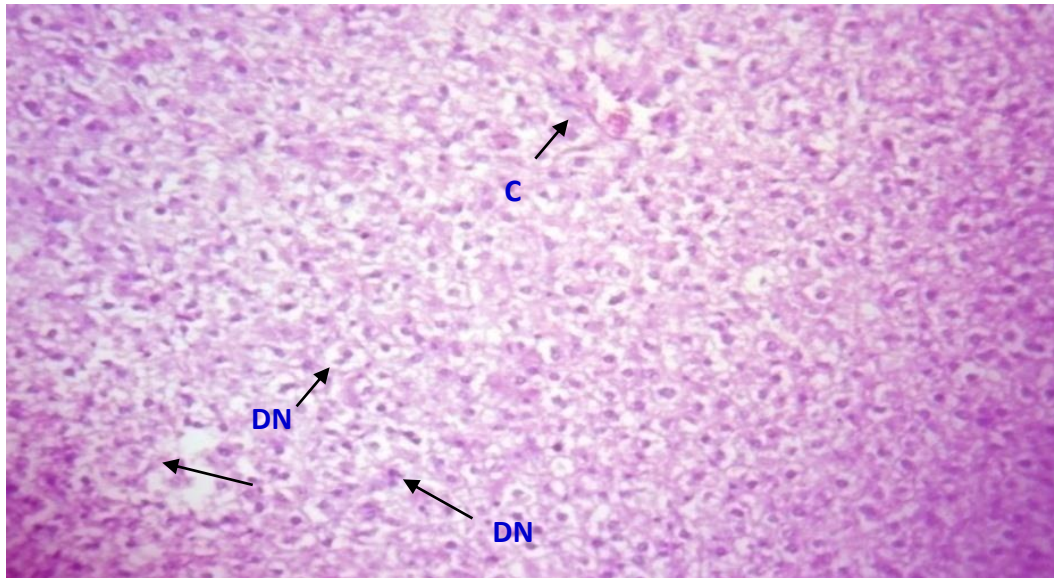
**DH: Degenerated Hepatocytes**

**EPT: Edematous Portal Triad**

**Plate-2 Liver of group I rat** shows abnormal hepatocytes with dilated sinusoids Vacuolar degeneration and necrotic lesions

## Liver morphology of group II rat

Normal hepatocellular arrangement was observed around the central vein and occasional binucleated hepatocytes indicate the regeneration of degenerated hepatocytes. Empty spaces were present at some places which had been occupied by hepatic cells. The central vein was normal without sinusoidal congestion.



**C: Congestion**

**N: Necrosis**

**DN: Double Nuclei**

**Plate-3 Liver of group II rat shows** normal hepatocytes, binucleated cells and normal sinusoids.

## DISCUSSION

The human microbiome exhibits many functions which are mostly beneficial to the organisms (Everts, 2010). O'Hara and Shanahan, (2006) reported that 'gut flora as a forgotten organ' to emphasize the essential role of intestinal microbes in eliciting the mucosal immune system.

The elevated level of bilirubin after fluoride treatment was recovered nearly to the control value in the probiotic treated group of rats. Bilirubin is a toxic and water insoluble end product of the catabolism of hemoglobin. In the intestine bilirubin conjugates are de-conjugated and transformed to a series of urobilinogens by de-conjugating enzymes called  $\beta$ -glucuronidase (Rod and Midtvedt, 1977). Some amount of deconjugating enzymes is derived from endogenous sources and large amount is produced by intestinal microbes (Gadelle et al., 1985). Studies on rat and man have shown that intake of ampicillin, bacitracin, clindamycin and erythromycin significantly suppress faecal excretion of urobilins (Steinbakk et al., 1992). Suppression of fecal

excretion of urobilins by fluoride is responsible for the elevated bilirubin in the present study and probiotic bacteria *L. rhamnosus* recover the ability of intestinal production of  $\beta$ -glucuronidase by colonizing in the gastro intestinal tract of the probiotic treated groups.

The recovery of SGOT and SGPT after probiotic treatment indicates amelioration of liver damage. Since the liver is implicated in almost all biological processes, its damage can have severe impacts on metabolism, the immune response and detoxification processes. In patients with liver failure (Hepatic encephalopathy), the blood concentrations of ammonia can reach toxic levels. Investigators have postulated that it may be possible to use probiotics to decrease intestinal urease activity and reduce the amount of ammonia. Probiotics can decrease oxidative stress and inflammation in hepatocytes which leads to increased function and capacity to clear and decrease uptake of toxins and ammonia (Martin et al., 2008).

*In vivo* studies of Kirpich et al., (2008), demonstrated that Combination of *Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3 for 5 days of treatment increased colonization of Bifidobacteria and Lactobacilli, thereby reduction in ALT, AST, LDH, and total bilirubin levels. In the same line study with dose of 15 g/kg/day ethanol consumption for two weeks normalized Alanine Aminotransferase; Aspartate Aminotransferase levels, liver function and endotoxin (Marotta et al., (2005). Thus the present study gives the clue that liver damage by fluoride could be efficiently restored by probiotic bacterium *L.rhamnosus*.

The liver histopathology of the fluoride treated rats indicated the presence of abnormal hepatocytes with centrilobular necrosis accompanied by dilated sinusoids and vacuolar degeneration. Ling-Fei He and Jian-Gang Chen, (2006) in their study confirmed that excess fluoride induces oxidative stress, DNA damage, apoptosis and cell cycle changes in rat oral mucosal cells and hepatocytes. The present study reports that probiotic bacterium *L. rhamnosus* repair the hepatic damages caused by fluoride. Imani Fooladi, et al., (2013) administered *Bifidobacterium animalis* NM2/ *Lactobacillus acidophilus* NMI/*Lactobacillus rhamnosus* /*Lactobacillus rhamnosus* DSM 6594/*Lactobacillus plantarum* DSM 9843 to rat with acute liver injury and observed prevention of alcohol induced dysbiosis. Probiotics also validated that it helps in decreasing ammonia production, which prevent hepatic encephalopathy



in patients with cirrhosis (Lunia, *et al.*, 2014). From this study it is evident that although cellular damage, necrosis and fibrosis occurred after fluoride administration, probiotics supplementation ameliorates these changes.

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