



AMELIORATING ROLE OF LYCOPENE AND TOMATO PASTE (WITH PEEL) ON GENERAL HEALTH AND LIVER HISTOPATHOLOGY OF FLUORIDE EXPOSED SWISS ALBINO MICE

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ABSTRACT

Present communication deals with ameliorating role of 2 diet supplements viz. lycopene and tomato paste with peel on general health and liver histopathology of Swiss albino mice exposed to sub-acute dose of fluoride (190 mg/kg body wt. prepared by dissolving sodium fluoride (Merck Ltd., Mumbai, India) in distilled water) for 7 days. Their recovery was also monitored after 48 days of fluoride withdrawal. The visible toxic effects of fluoride were observed on fur (less hairy & lustreless) and nails (black) of mice fed on standard diet were absent in the diet supplement groups. Fluoride exposure distorted hepatocytes and dilated sinusoids, portal and central veins in liver, particularly in the standard feed group. Fluoride withdrawal led to better recovery of general health and liver histology in the diet supplement groups.

INTRODUCTION

Fluorosis is a serious health problem in 23 districts (out of total 33 districts) of Rajasthan (Agarwal et al. 1997, Patra et al. 2000). Fluoride present in ionic form in the drinking water passes through intestinal mucosa passively. Because of higher electro negativity and small radius, it combines with Ca^{2+} to form calcium ionospheres that easily crosses cell membrane and enters into soft tissues such as muscle, kidney, liver, endocrine glands (thyroid, parathyroid and pituitary gland), testis and brain (Guan et al. 2000, Wang et al. 2000, Gupta et al. 2001, Ghosh et al. 2002, Sireli and Bulbul 2004, Yadav et al. 2016). This causes metabolic, functional and structural damage to soft tissue (Carlson et al. 1960, Jacyszyn and Marut 1986).

Liver is a vital organ of body which regulates flow of substances absorbed by digestive system into systematic circulatory system. It also regulates biosynthesis of plasma proteins {albumin, coagulation factors, α -1-antitrypsin, LDL (low density lipoprotein)} and gluconeogenesis and detoxifies injurious chemicals entering in body through pollution. Toxins bound with albumin enter into liver sinusoids and then to space of Disse where hepatocyte enzyme (cytochrome P-450) metabolize them. Although liver is blessed with regenerative ability but when damage exceeds a certain threshold, insufficient functions cause hepatic failure.

The excessive fluoride exposure damages redox balance of the cells in tissues, and increase toxic effects on visceral organs through ROS and lipid peroxidation (Rzeuski et al. 1998). A close association between chronic fluoride toxicity and increased oxidative stress has been reported in humans and in experimental animals (Shivarajashankara et al. 2001, Sharma et al. 2007). Several plant species rich in antioxidants such as flavonoids and phenolic compounds produce a definite physiological action in the human body (Edeoga et al. 2005). Antioxidants present in crude extract of tamarind fruit pulp (polyphenols and flavonoids) mobilize fluoride deposits of bone lost via urinary excretion (Khandare et al. 2002, 2004, Martinello et al. 2006). Extracts of *Tamarindus indica* fruit pulp and *M. oleifera* seeds mitigated toxic effect of fluoride in rabbits (Ranjan et al. 2009). Gupta et al. (2014) reported hepato and renoprotective role of *T. indica* fruit pulp in the experimental animal exposed to fluoride. Curcumin had protective role on lungs (Ibrahim et al. 2013) and liver (Harabi et al. 2014) while Spirulina + tamarind fruit pulp on hematology (Sharma et al. 2014) and kidney (Yadav et al. 2016) in fluoride exposed albino mice.

Lycopene is almost ten times more effective antioxidant than α -tocopherol in respect of singlet oxygen quenching ability (Rao et al. 2003). Tomato and its products (tomato juice, ketchup, tomato paste, tomato soup, pizza sauce and

spaghetti sauce) are the major source of dietary lycopene (80%) and total carotenoid (30%) intake in human diet (Rao and Agarwal 1999). The consumption of tomato paste with peels improves bioavailability of carotenoids compared to that without peels (Reboul et al. 2005). The processing of tomatoes also increased bioavailability of lycopene due to its release from tissue matrix (Rao et al. 1998). Considering easy availability of tomato fruits and their extensive use in preparation of culinary in the country, a comparative study was made to assess ameliorating role of lycopene and tomato paste on general health and histopathology of liver in fluoride exposed Swiss albino mice, including their recovery after fluoride with drawl.

MATERIALS AND METHODS

The pure inbred line of Swiss albino mice (*Mus musculus* L) maintained in a well-ventilated animal house of Zoology Department, University of Rajasthan, Jaipur (Temperature $24 \pm 3^\circ\text{C}$; humidity = 40 – 60 %; 12 h light: dark cycle) was the source of animals. All regulations of the Institutional Animal Ethical Committee of the University (1678/GO/a/12/CPCSEA) were strictly followed during experiments performed in the following three phases.

Phase -I

Following an acclimation period of one week, 20 healthy young male mice (age = 90-95 days, weight = $33 \pm 0.5\text{g}$) were allotted randomly to 4 groups of 5 mice each housed in a polypropylene cage [50 cm (length) x 25 cm (width) x 15 cm (height)].

Treatments

Mice of group I and II were administered orally through gavage (0.5 mL/mouse/day) sub-acute dose of fluoride {190 mg/kg body wt. prepared by dissolving sodium fluoride (Merck Ltd., Mumbai, India) in distilled water} for 7 days.

Sub-acute dose of fluoride was withdrawn from group II and these mice were reared in standard conditions for another 48 days for recovery. This group has been referred to as post- treated group hereafter in the text.

The respective controls of sub-acute (group III) and post-treated (group IV) studies received an equivalent amount of vehicle (distilled water) for the exposure period.

Mice had free access to potable water (pH = 7.1; ER = 0.55M Ω /cm; Total hardness = 198 mg/L; Chlorides = 30 mg/L; Fluoride = 0.9 mg/L; Aluminum = nil) and standard

feed (Ashirwad Ltd., Chandigarh, India) *ad libitum* and are referred to standard feed groups hereafter in the text.

Phase II and III

The general lay out of the experiment was similar to Phase -I. The only difference was that animals received diet supplement lycopene (@ 20 mg / kg body weight) in Phase-II and tomato paste with peel (@ 10%wt/wt) in Phase-III along with standard feed 45days prior to entry into experimental protocol.

Five lycored capsules (Jagsonpal Pharmaceuticals Ltd.) containing 10mg of lycopene (2mg lycopene/capsule) were dissolved in 5mL luke warm (40°) distilled water and 0.25mL/day/ mouse (@ 20mg/kg body weight) of this freshly prepared solution was administered through gavage. This group has been referred to as lycopene group hereafter in the text.

The deep red ripened tomato fruits (500g) were purchased from the local market. These were soaked for 15minutes in 250mL hot water (40°C) to soften fruits which were meshed and then sieved to remove seeds. The pulpy mass was finely crushed in the grinder adding 10g butter (Amul Ltd., Gujarat) to increase lycopene bio-availability (Stahl and Sies 1992). The crushed mass spread uniformly over a steel plate was dried in a hot air incubator at 45°C . Crusty layer was scalped and weighed amount of this was mixed (10% wt/wt) thoroughly with powered standard feed (Ashirwad Ltd., Chandigarh, India) adding water. Cakes made from mixture were dried in a hot air oven at 45°C and were fed to mice. This group has been referred to as tomato paste group hereafter in the text.

Animals were observed at least twice in a day for clinical signs and symptoms of toxicity. Their body and organ weights were recorded at the termination of experiment.

Autopsy

Mice were sacrificed by cervical dislocation at the termination of study i.e. on 8th and 49th day after sub-acute and post-treatment respectively. A midline abdominal incision was made to remove liver which was cleaned, blotted free of blood and weighed. Liver lobes cleaned in saline (0.90% NaCl) were quickly fixed in Bouins fluid, washed and dehydrated through graded alcohol series. These were embedded in paraffin and routine microtomy was carried out to obtain 6 μm thick sections which were stained by hematoxylin-eosin

(HE) and viewed under light microscope at multiple magnifications (100x, 400x, 1000x, Humason 1972).

Morphometric studies of liver included measurements of area of central veins, hepatocyte nuclei and Kupffer cell counting. All measurements were made using Digital Leica Microscope (Application Suite V4, Build: 877).

Data analysis

Results are expressed as mean \pm SEM. The differences between control and treated groups were determined applying one way analysis of variance (ANOVA) using a Systat 5.0 software program.

RESULTS AND DISCUSSION

The fur was more hairy and glowing white in diet supplement controls in comparison to standard feed control. Fluoride treated mice of standard feed group had lustreless poor hairy fur becoming pale white in the head region and their nails also turned black. Such abnormalities were not observed in F⁻ treated mice of diet supplement groups though fur was little inferior in comparison to respective controls. Yadav et al. (2016) made similar findings in fluoride treated mice of standard feed group, and absence of visible toxicity on fur quality and nails in mice fed with Spirulina supplement. Thus diet supplements had visible positive effects in both control and fluoride treated mice.

The body weights of F⁻ treated mice of standard feed group were higher than control mice but almost similar to controls in the diet supplement groups (Fig. 1). Other workers also reported little adverse effect of fluoride exposure on body weights (Tsunoda et al. 2005, Reddy and Pushpalatha 2007).

Body weights of post controls differed little with post treatments suggesting their recovery after F⁻ with drawl (Fig. 1).

Liver (7-10%) weights were higher (4-10%) than controls in the fluoride treatments (Fig. 2), but similar to post controls in the post-treated mice suggesting reversal of fluoride toxicity (Fig. 2). In contrast, other workers reported reduction in liver weights in fluoride treatments (Al-Hiyasat et al. 2000, Tsunoda et al. 2005, Chawla et al. 2008). During autopsy, internal organs of F⁻ treated mice were puffy and slimy, particularly in the standard group, possibly because of increase in water retention and this possibly increased their body and internal organ weights.

The liver in control mice had polygonal hepatocytes arranged in cords around central veins (Plate 1). Most of the hepatocytes had single large centrally placed rounded nucleus but binucleated hepatocytes were observed in about 17% hepatocytes of the standard feed group and in 12-13% hepatocytes of the diet supplement groups.

Hepatocytes with severe hydropic degeneration and fine vacuolization were observed in fluoride treatment of standard feed group (Chattopadhyay et al. 2010, Gupta et al. 2014, Atmaca et al. 2014, AL Harabi et al. 2014). Most of hepatocytes lost their polygonal shape becoming oval and also lost their characteristic cord like arrangement (Plate 1). Structural integrity and arrangement of hepatocytes in F⁻ treatments of diet supplement groups were however, almost similar to respective controls (Plate 1), as also reported in F⁻ treated mice receiving supplement tamarind fruit pulp extract (Gupta et al. 2014), resveratrol (Atmaca et al. 2014) and

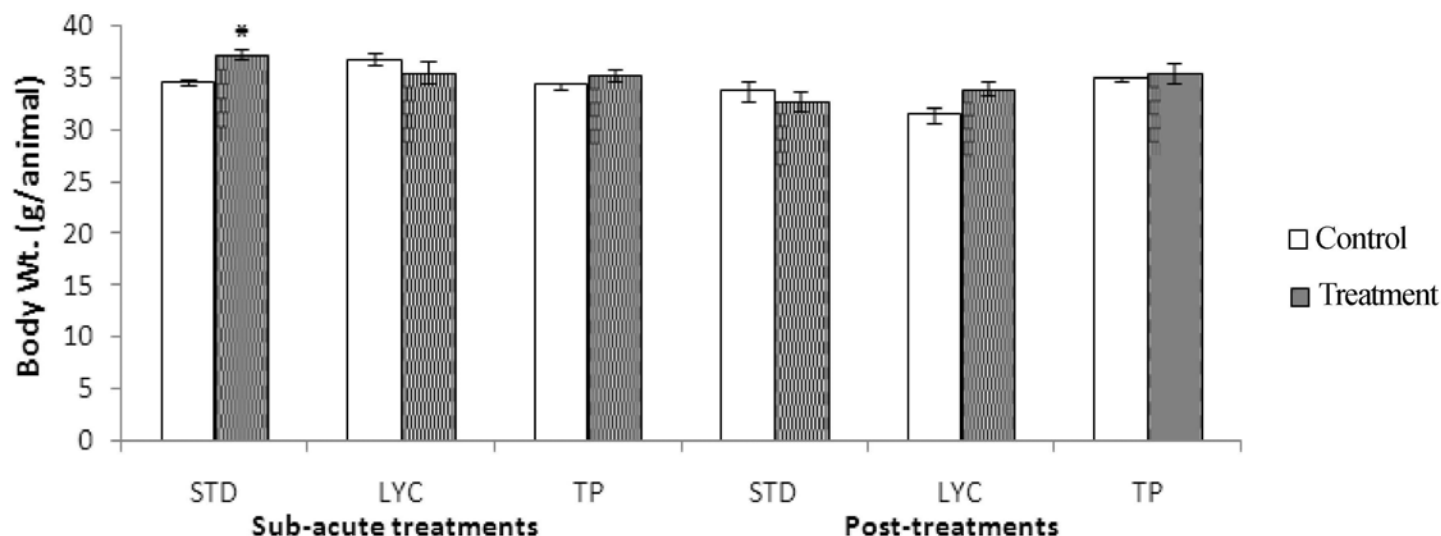


Fig.1. Body weights (Mean \pm SEM) of controls, fluoride treated and post-treated mice of different feeding groups (Std. = Standard feed, Lyc- Lycopene, TP- Tomato Paste), significant at * ($p < 0.05$).

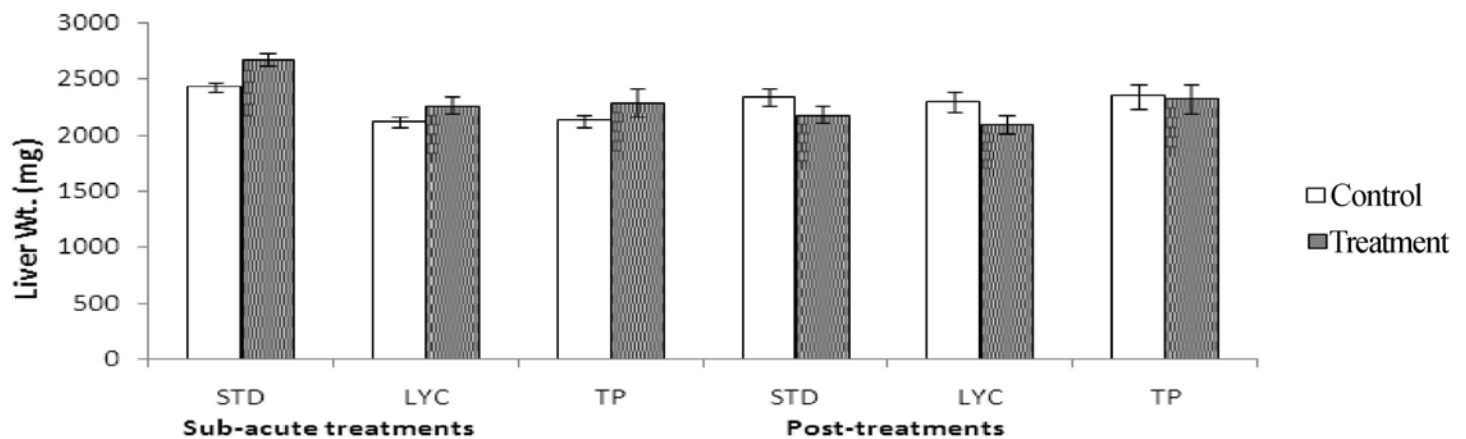


Fig.2. Liver weights (Mean \pm SEM) of controls, fluoride treated and post-treated mice of different feeding groups (Std. = Standard feed, Lyc- Lycopene, TP- Tomato Paste)

Se in combination with curcumin extract (AL Harrbi et al. 2014).

Fluoride exposure initiated necrosis of hepatocytes as evident by presence of smaller (15-23%) darkly stained nuclei (pyknotic nuclei) in about 38% of hepatocytes in standard feed group (Fig. 3, Atmaca et al. 2014, AL Harabi et al. 2014). The pyknotic nuclei found absent in tomato paste group were observed in about 10% of hepatocytes in the lycopene group (Plate 1).

The dilation of sinusoids was observed only in F-treatment of standard feed group but of central veins in all F-treatments (standard feed group = 43%, diet supplement groups = 35-40%, Plate 1, Fig. 4). Central vein dilation increased congestion in the liver that ruptured nearby hepatocytes releasing content within and around dilated veins, particularly

in the standard feed group (Plate 1). This has been reported in other studies also (Shashi and Thaper 2000, Chattopadhyay et al. 2010, Atmaca et al. 2014, AL Harabi et al. 2014). The erosion of epithelial lining found absent in the central vein of diet supplement groups was however, severe in the standard feed group (Plate 1). Chattopadhyay et al. (2010) also reported loss of integrity in the epithelial lining of central vein in F-treated mice.

The dilation of central veins and sinusoids in zone 3 (centrilobular) region of liver perhaps had role in flushing out of detoxified fluoride through intestine because of active involvement of hepatocytes of this region in glycolysis, lipogenesis and cytochrome P-450-based drug detoxification (Thoolen et al. 2010).

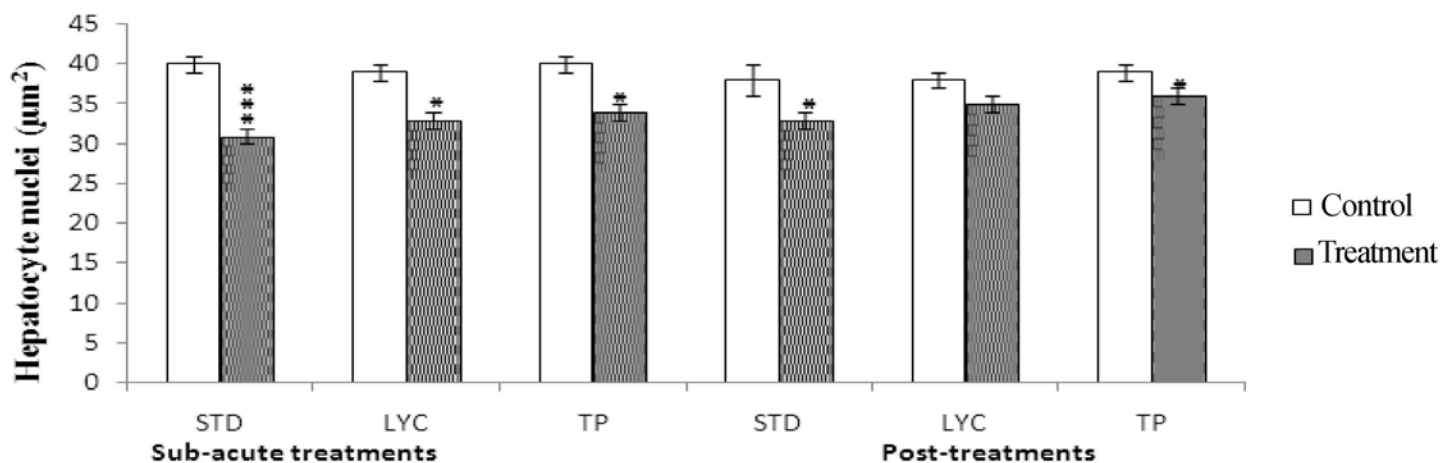


Fig.3. Area of hepatocyte nuclei (Mean \pm SEM) in the liver of controls, fluoride treated and post-treated mice of different feeding groups (Std. = Standard feed, Lyc- Lycopene, TP- Tomato Paste), significant at * ($p < 0.05$), *** ($p < 0.001$).

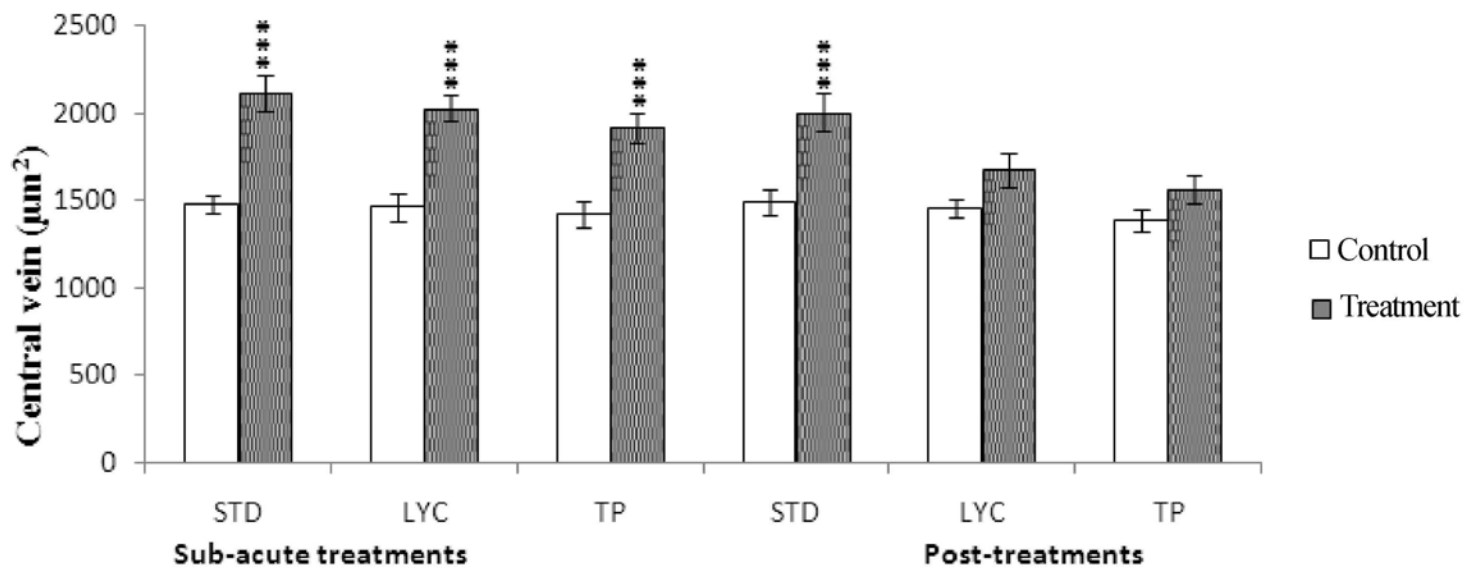


Fig. 4. Area of central vein (Mean \pm SEM) in the liver of controls, fluoride treated and post-treated mice of different feeding groups (Std. = Standard feed, Lyc- Lycopene, TP- Tomato Paste), significant at *** ($p < 0.001$).

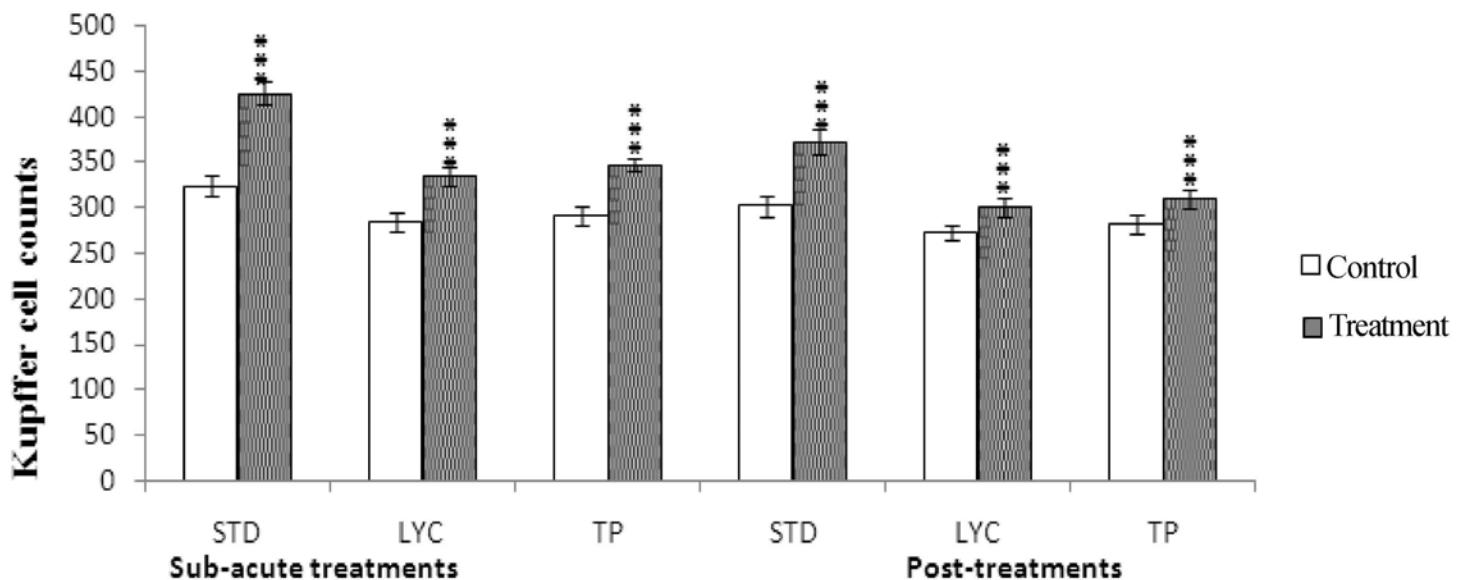


Fig.5. Kupffer cell counts (Mean \pm SEM/microscopic field at 400x) in the liver of controls, fluoride treated and post-treated mice of different feeding groups (Std. = Standard feed, Lyc- Lycopene, TP- Tomato Paste), significant at *** ($p < 0.001$).

Kupffer cells distributed uniformly in controls were concentrated around central and portal veins in F⁻ treatments of standard feed group and diet supplement groups (Plate 1). Their counts were higher in standard feed (31%) in comparison to diet supplement (18-19%) groups (Fig. 5). Khudiar et al. (2015) also reported increase in Kupffer cell counts in F⁻ treated rabbits receiving supplement grape seed oil. The increase in Kupffer cell counts indicates inflammatory

condition. Kupffer cells are tissutal macrophages with a crucial role in hepatotoxicity (Ding et al. 2003).

Post-treatments

Hepatocytes regained polygonal shape in the post treated mice of standard feed group (Plate 2). The recovery in size of nuclei and central veins were partial in the standard feed group but almost complete in the diet supplement groups

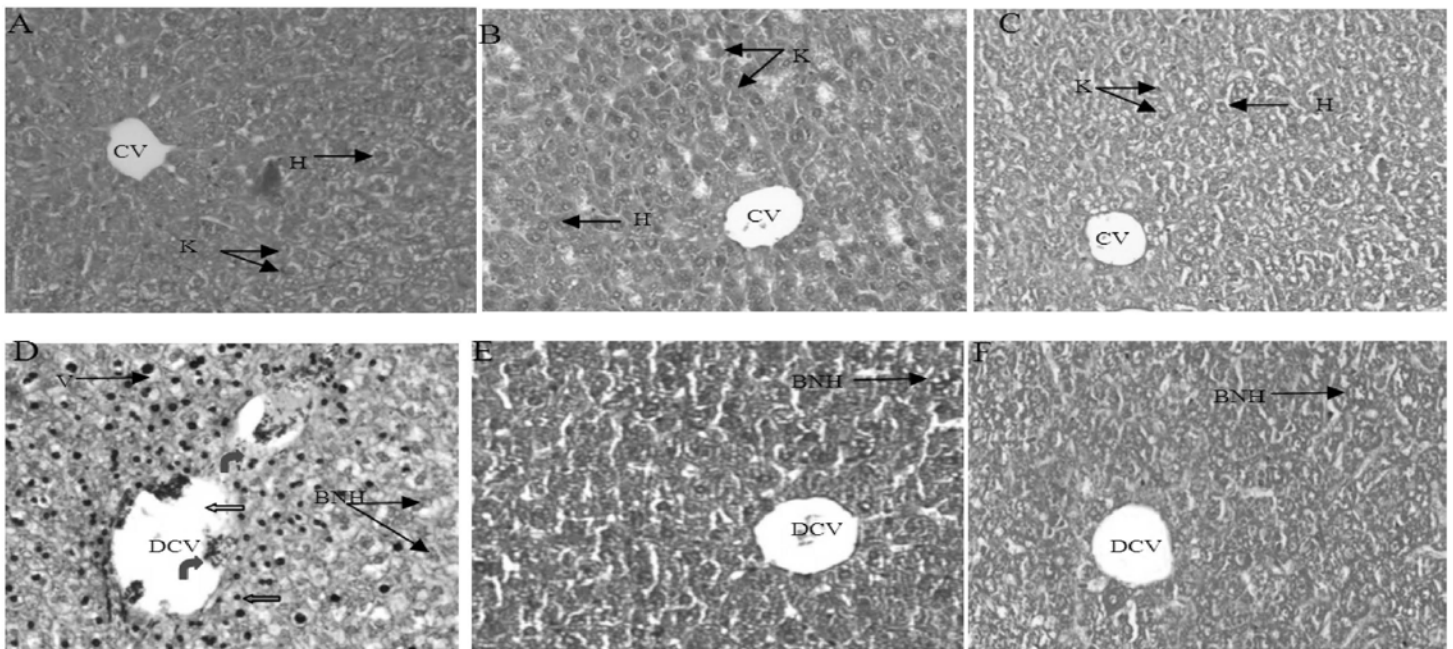


Plate. 1. T.S. of Liver of controls (A-C) and fluoride treated mice (D-F).

Controls (200X): Fig. A. Standard feed group, Fig. B. Lycopene group, Fig. C. Tomato paste group showing Hepatocyte (H), Central vein (CV) & Kupffer cells (K)

Fluoride treatments:

Standard feed group: Fig. D (200x) showing vacuolization (V), binucleated (BNH) hepatocytes, aggregation of kupffer cells at the site of cellular degeneration (➤), dilated central vein (DCV)

Lycopene group: Fig. E (200X) showing binucleated hepatocytes (BNH) and dilation in central vein (DCV)

Tomato paste group: Fig. F (200X) showing binucleated hepatocytes (BNH) and dilation in central vein (DCV)

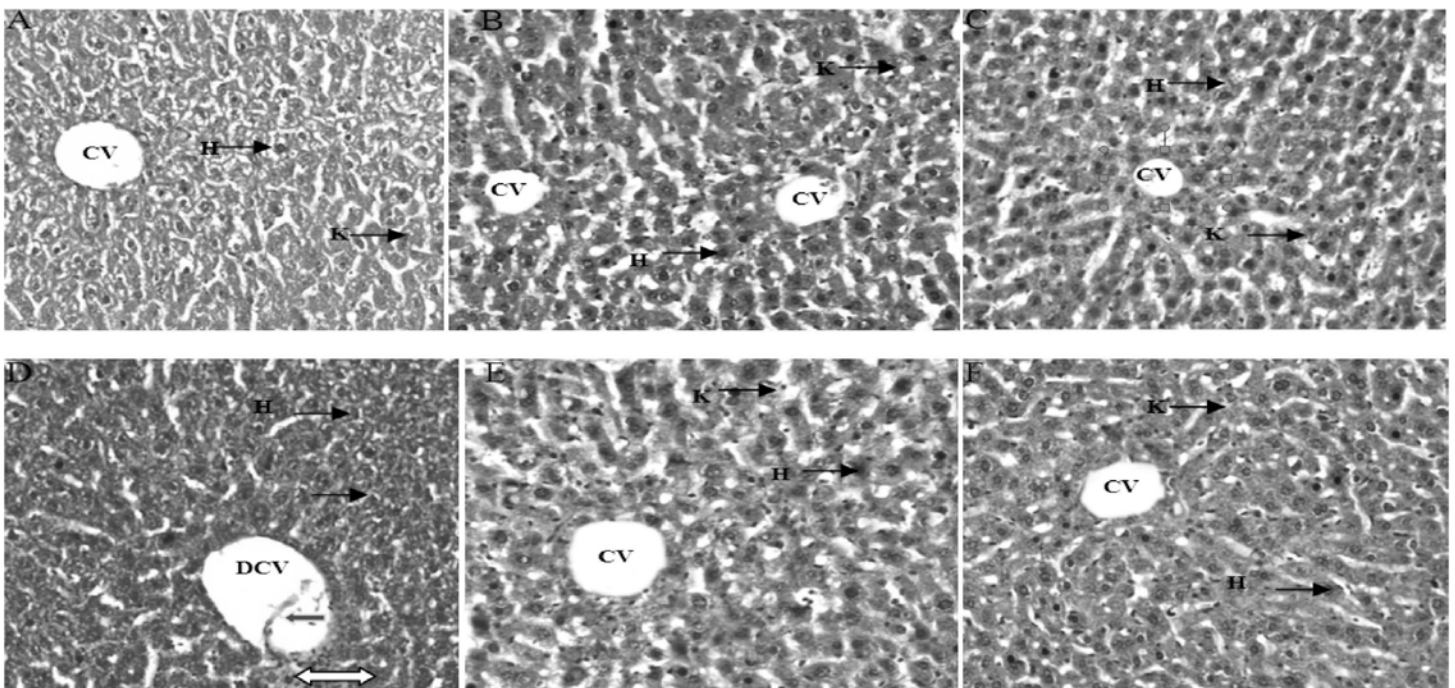


Plate. 2. T.S. of liver in post-controls (A-C) and post treated mice (D-F).

Post-controls (200X): Fig. A. Standard feed group, Fig. B. Lycopene group, Fig. C. Tomato paste group showing Central vein (CV), Hepatocyte (H) & Kupffer cells (K)

Post- treatments:

Standard feed group: Fig. D (200x) showing loss of cytoplasmic content of hepatocytes in intercellular spaces (⇔), and dilated central vein with ruptured cell wall (➤)

Lycopene group: Fig. E (200X) showing Central vein (CV), Hepatocyte (H) & Kupffer cells (K)

Tomato paste group: Fig. F (200X) showing Central vein (CV), Hepatocyte (H) & Kupffer cells (K)

(Fig. 3, 4). Kupffer cell counts were higher (10-24%) in the post-treatments particularly of standard feed group (Fig. 5) but decreased in comparison to F⁻ treatments suggesting partial recovery. It is evident that fluoride withdrawal led to recovery in histo-architecture of liver, particularly in the post-treatment of diet supplement groups.

Present study has thus revealed ameliorating role of lycopene and tomato paste on the general health and liver of fluoride treated mice. Further, their recovery was also better after fluoride withdrawal. Fluoride induced histopathological changes in the liver were minimum in tomato paste group. This may be related to better protection against ROS and peroxidation in comparison to lycopene since, tomato paste in addition to lycopene also contains vitamins A and C, folic acid, alpha-lipoic acid, choline, beta-carotene and lutein; all of which have antioxidants properties (Alshawi et al. 2010). We therefore recommend its increasing use in preparation of culinary in the fluoride affected areas in the country.

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