

Effects of formaldehyde intoxication on liver of Swiss albino mice

Afrin M¹, Amin T¹, Karim R¹ and Islam MR¹

¹Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

Abstract: Formaldehyde (FA) is a very reactive one-carbon compound, can react with lipids, proteins, and nucleic acids which are cellular components. FA induces cellular toxic effects in the liver by damaging hepatic parenchyma and impairment of functions. This study was carried out to evaluate the serum biochemical changes and cytotoxic effects on liver in Swiss albino mice caused by FA toxicity. For this purpose, mice were divided into three equal groups i.e. Control, oral and intraperitoneal. Oral and intraperitoneal groups were further divided into three subgroups which were subjected to exposure of FA for 30 and 10 consecutive days respectively. After exposure, blood and liver samples were collected and analyzed for biochemical and morphological studies. The serum biochemical parameters like Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were increased significantly ($P < 0.05$) in mice after FA exposure. The anatomical results revealed gross morphological changes i.e. congestion and petechial hemorrhages on the liver. Histologically, the liver showed scattered lymphocytic infiltration, dilatation of sinusoids, necrosis and degeneration of parenchymatous cells in orally exposed mice (10 mg/kg) and diffuse lymphocytic infiltration, necrosis were seen in intraperitoneally FA injected mice at the rate of 7 and 10 mg/kg body weight. All these findings revealed that, FA depending on specific dose leads to an irritant toxic effects on the liver of mice.

Keywords: Formaldehyde, Histomorphology, Liver, Mice, Serum biochemical test.

I. Introduction

Formaldehyde (FA) is colorless, flammable and highly reactive substances at normal temperature and pressure [1, 2]. Although it is readily broken down by sunlight in air but very stable in liquid over time [2, 3]. It is quickly diffuses in any tissues i.e. Liver when exposed by oral or intraperitoneal route [4] because it interacts with different cellular components [5]. While the main route of exposure is air [6], FA is also entering into body by food and drinking water. Using of formalin was started as fixative and embalming fluid, but nowadays it is using in all sphere of day to day life. The most alarming use of formalin (37% aqueous solution of FA) is as food preservative [7, 8] and that's why exposing to the risk of formalin intoxication is increasing. After ingestion, FA is readily absorbed from gastrointestinal tract [1]. Recent studies (FA exposure on animal) show hepatotoxicity and abnormal histopathological alteration in gastrointestinal tract [9]. FA is a mutagenic and carcinogenic even at low concentrations and produce toxicity in a variety of organisms [10]. There is also evidence of gastrointestinal cancer if FA taken in high concentration through drinking water [11].

In developing country like Bangladesh, indiscriminate use of FA in the form of formalin in different food items and in drinking water exposing a huge number of people to a great health hazard [12, 13]. Bangladesh is a country of tropical region where its weather is hot and humid. As a result, vegetables and perishable food items are tend to decay quickly. There is a report on wide range using of formalin in fruits, vegetables, perishable food items to keep them fresh [14]. The widespread use of 37% FA solution, in preservation of fish, fruit and other food items is posing a threat to public health. Although Bangladesh government issued a formalin control law in 2014 because of widespread use of FA to preserve food, it is using indiscriminately till today.

This study was conducted to investigate the serum biochemical, gross and histopathological changes in liver that can be caused by FA exposure and that reflect to create awareness by presenting the real health hazard.

II. Materials And Methods

2.1 Research animals and their management

The experimental Swiss albino mice (*Mus musculus*), weighing 30 ± 7 g and 100-120 days aged were collected from International Center for Diarrheal Disease Research (icddr), Mohakhali, Dhaka having apparently good health and devoid of any external deformities certified by the registered veterinarian from icddr. The mice were kept in cage made of galvanized iron sheet having 2 inches thick saw dust litter. Mice were reared under normal condition of temperature ($23-25^{\circ}\text{C}$) and humidity with a provision of feed and water *ad libitum*. All mice were handled according to the animal care in compliance with the Department of Anatomy and Histology under the Institutional Board Guidelines of Bangladesh Agricultural University on the care and use of laboratory animals.

2.2 Chemical preparation

FA solution was prepared from stock paraformaldehyde powder (Merck, Darmstadt, Germany) by thermal depolymerization according to the method described by Chang *et al* [15].

2.3 Experimental Design

For experimental purpose, the mice were randomly divided into three groups like; control (group A), oral and intraperitoneal groups. Each group had 5 mice. In oral and intraperitoneal groups, mice were exposed to toxic dose of 5.0 mg/kg (group B), 7.0 mg/kg (group C) and 10.0 mg/kg body weight (group D) FA solution once daily for 30 and 10 days respectively.

2.4 Serum biochemical assay

Mice were anaesthetized with ether during sacrifice. Thoracotomy was performed. Blood was directly collected from heart. Test tube containing blood with anticoagulant was placed in ice for 30 minutes. Sera were separated from unclotted blood by centrifuge at 3000 rpm for 20 minutes and again for 10 minutes. Then supernatant was collected in eppendorf tube by micro-pipette and stored in refrigerator at -20°C until use for biochemical test.

2.5 Histomorphological study

After completion of experimental period, liver was collected as soon as possible with the help of sharp scalpel and scissors without wreckage of the organ. Grossly observable abnormalities (shape, size, color, consistency) were taken into consideration and compared with the control by eye observation. Liver of each group were fixed in 10% FA for processing for light microscopy [16]. Hematoxylin and Eosin stain was done for preparing permanent slide. Necessary photographs were taken with Olympus BX 51 photographic light microscope and placed for better illustration of the result.

2.6 Statistical analysis

The data are presented as mean \pm SD. All the collected data from control and FA exposed mice were analyzed (student's *t*-test; one and two way ANOVA) to find the significant differences between values of various parameters. The differences will consider to statistically significant when the *P* values obtained will less than 0.05 or 0.01.

III. Results

3.1 Clinical signs

In the present study, no signs of irritation or intoxication, such as lacrimation, nasal secretion, or regurgitation was seen either during the exposure session or thereafter. Also no defensive or aggressive behavioral changes in the mice were evident. The clinical signs begun to appear after 1 week of experiment and included decreasing feed and water intake, dullness, staggering gait, sitting with closed eyes and decreased response on disturbance. These signs were more pronounced in morning soon after exposure to FA compared with rest of the day.

3.2 Serum biochemical assay

The present study was put in to assess hepatic function parameters (AST and ALT) using biochemical test and compared to control group. The mean concentrations of AST and ALT were significantly increased ($p < 0.05$) for 66.12 ± 0.43 and 21.43 ± 0.56 respectively in 10 mg/kg treated intraperitoneal group (Fig.1). There were also significant ($p < 0.05$) increased in AST in the oral group exposed to 10 mg/kg FA. The value was 59.43 ± 3 . (Fig.2)

3.3 Gross effects

The morphological appearance of the liver of control group A revealed normal brown color of the examined lobes. There is no change in size and consistency. The liver had smooth regular borders and normal shape of both the dorsal and ventral surfaces in all lobes. (Fig.3A and 4A)

The gross morphological changes in the liver of group D (10 mg/kg oral and intraperitoneal FA exposure) mice revealed dark color and decreased liver weight with irregular surfaces. Increased congestion in all lobes with petechial hemorrhages on the dorsal and ventral surfaces indicated prominent liver vasculature. (Fig.3D and 4D)

3.4 Histomorphological effects

Liver sections of hematoxylin and eosin stain of control group A revealed normal hepatic tissue architecture. There were no degeneration or necrotic changes observed in the liver of control group (Fig.5A and 6A).

Among the oral groups, only the liver of 10 mg/kg treated mice showed centrilobular necrosis and degeneration of parenchymatous cells. Dilated sinusoidal spaces were accompanied by vasculitis, this vascular reactivity characterized by scattered aggregation of lymphocytes (Fig. 5B).

Among the intraperitoneal groups no observable histopathological lesions were found in liver of 5 mg/kg treated group (Fig. 6B). There was a piecemeal necrosis in 7 mg/kg treated group by lymphocyte infiltration extends from portal areas and disrupts the limiting plate of hepatocytes undergoing necrosis (Fig. 6C). There was diffuse aggregation of lymphoid cells found in the liver parenchyma of both 7 mg/kg (Fig. 6C) and 10 mg/kg treated groups (Fig. 6D).

IV. Discussion

Liver has a central role in intermediary metabolism of carbohydrates, proteins, lipids, and amino acids in the body. In addition, liver renders a lot of processed metabolites for bioavailability in other tissues. Therefore, FA intoxication at different doses and routes affect that metabolic pathway of the liver. In the present study, revealed a relation between FA exposure and liver health by taking into account the biochemical, histological and the toxicological effects.

The study reported decreasing liver weight due to decreased body weight of mice after FA exposure which is consistent with that obtained by [17, 18] in rat. Mice fed or injected 10 mg/kg FA, showed more pronounced clinical signs like depression, staggering gait, decreased food and water intake. Similar clinical signs were reported by Babar *et al* [19] in broiler chicken while rat showed decreased responsiveness after FA exposure in drinking water [20].

Liver functions and tissue damage were determined by an increase in the activities of enzymes such as AST, ALT [21, 22] which are consistent with the findings of present study. The statistically significant differences in AST and ALT in comparison to control group showed impairment of liver functions.

Grossly in 10 mg/kg treated oral and intraperitoneally injected groups, presence of petechial hemorrhages and congestion in liver of mice are suggestive of an irritant effect of FA. Similar lesions in liver following oral administration of FA have been reported in rats [23, 17, 18].

FA causes cytotoxicity by presumably reacting directly with tissue constituents [24]. After intraperitoneal, oral, or inhaler administration, FA rapidly diffuses into many tissues including the liver, brain and testis [15]. In the present study, oral administration of FA causes lymphocytic aggregations, dilated sinusoidal spaces, centrilobular necrosis and degeneration of parenchymatous cells in liver. In FA exposed rat liver tissue, there were mild edema, mild degeneration in hepatocytes, and Kupffer cell hyperplasia evident. These findings obtained in the experiment of Uçmakli *et al* [25]. Indeed, in other studies of light and electron microscopy, FA exposed liver cells revealed: flat endoplasmic reticulum, hypertrophy and hyperchromatic nucleus [26], rough endoplasmic reticulum and mitochondrial damage [27] and impairment of membrane integrity [28]. It was also evident that FA caused damage to hepatocytes, intrahepatic and extrahepatic bile ducts [29]. This is due to its metabolic reactivity. We found that, in all animal species, FA is an essential metabolic intermediate in all cells and in the biosynthesis of purines, thymidine and certain amino acids [30]. Under physiological conditions, the level of endogenous FA is maintained at a low concentration being regulated by the expression and activity of both FA-generating and FA-degrading enzymes [31]. Free and reversibly bound FA is readily absorbed in the gastrointestinal tract when ingested and joins the pool of endogenous FA [32]. FA is rapidly oxidized in blood and liver to formic acid by the NAD-dependent FA dehydrogenase through a glutathione (GSH)-dependent process. In turn, formic acid partially enters the one-carbon pool of the body or is further oxidized to carbon dioxide and water in the liver and in the erythrocytes. In primates, this reaction occurs more slowly than in dogs or rats. The residual non-metabolized formic acid and other minor metabolites are excreted via urine, feces or expired air [33] and the relative amounts depending on the route of administration [34, 35, 36]. Owing to its chemical reactivity, FA is essentially present in reversibly and irreversibly bound forms, as free FA, representing 1 to 2% of total measurable amounts in tissues, and as FA irreversibly bound to proteins and nucleic acids, accounting for between 50% - 80% of endogenous FA [37]. General signs of toxicity occur if the exposure conditions (e.g. Concentrations in food and drinking water) lead to an extent of local lesions, which subsequently impair the general health of the exposed animals. This applies for the hepatotoxic effects after in vivo exposure [20].

Among the intraperitoneal groups necrosis and diffuse lymphatic aggregation in the liver parenchyma revealed in the result of present study being severe in mice of 10 mg/kg treated group. This is due to systemic toxicity and a local irritant effect of FA.

When using dose as a factor, histological changes found in groups D (10 mg/kg) were more intensive than in groups B (5 mg/kg) and C (7 mg/kg) in both oral and intraperitoneal groups. The results obtained in this study, pertaining to the relationship between histological changes and exposure dose, are in agreement with the study obtained by Babar *et al* [19] on broiler liver who determined the histological changes i.e. Hepatocytes have

foamy cytoplasm and scattered aggregation of lymphoid cells in the liver parenchyma, cytoplasm has multiple vacuole, sinusoidal spaces contain erythrocytes at highest exposure group of FA.

V. Conclusion

The present study revealed that FA exposure leads to irritant toxic effects on the liver of Swiss albino mice and the biochemical as well as histological changes had direct relationship with FA exposure concentrations.

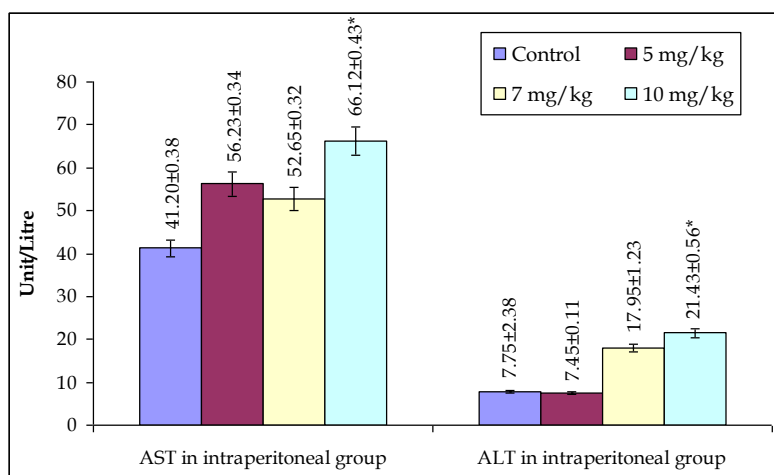
Acknowledgements

Authors are happy to express their gratitude to Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University and Ministry of Science and Technology of Bangladesh (Scholarship no. 66; June, 2014) for technical and financial support respectively.

References

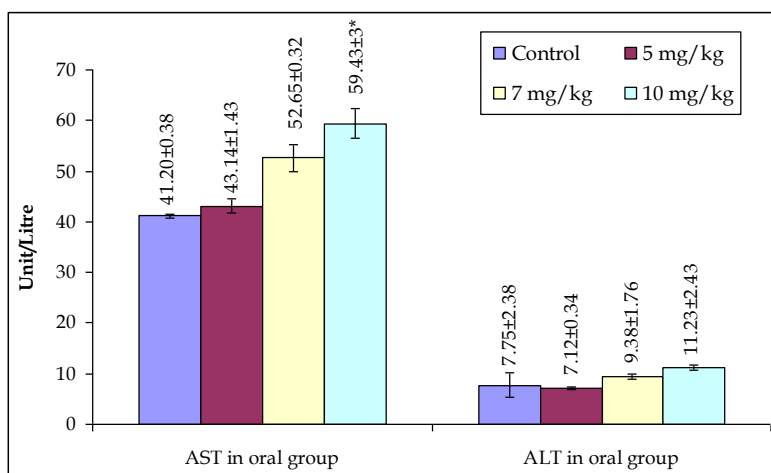
- [1]. ATSDR (Agency for Toxic Substances and Disease Registry), Toxicological Profile for Formaldehyde, US department of Health and Human Services. Atlanta, US, 1999.
- [2]. WHO (World Health Organization), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxy-2-propanol, *Volume 88*, 2006.
- [3]. WHO (World Health Organization), International Program on Chemical Safety, Environmental Health. Criteria 89: Formaldehyde, 1999.
- [4]. A.E. SMITH, Formaldehyde, *Occupational Medicine*, 42(2), 1992, 83–88.
- [5]. G. Cheng, Y. Shi, S.J. Sturla, J.R. Jallas, E.J. McIntee, P.W. Villalta, M. Wang, S.S. Hecht, Reactions of FA plus acetaldehyde with deoxyguanosine and DNA: formation of cyclic deoxyguanosine adducts and Formaldehyde cross-links, *Chemical Research in Toxicology*, 16(2), 2003, 145–152.
- [6]. US NRC, *Formaldehyde: an assessment of its health effects*, (Washington DC: US National Research Council, National Academy Press, 1980).
- [7]. P. Restani, A.R. Restelli, C.L. Galli, Formaldehyde and hexamethylenetetramine as food additives: chemical interactions and toxicology, *Food Additives and Contaminants*, 9(5), 1992, 597–605.
- [8]. B.A. Tomkins, J.M. McMahon, W.M. Caldwell, D.L. Wilson, Liquid chromatographic determination of total formaldehyde in drinking water, *Journal of AOAC International*, 72, 1989, 835–839.
- [9]. K.B. Rumchev, J.T. Spickett, M.K. Bulsara, M.R. Phillips, S.M. Stick, Domestic exposure to formaldehyde significantly increases the risk of asthma in young children, *European Respiratory Journal*, 20, 2002, 403–408.
- [10]. W.G. Nouh, A.G. Selim, Toxopathological studies on the effect of formalin and copper sulphate in tilapia as a commonly used disinfectant in aquaculture, *Journal of Applied Environmental and Biological Sciences*, 3(6), 2013, 7–20.
- [11]. M. Takahashi, R. Hasegawa, F. Furukawa, K. Toyoda, H. Sato, Y. Hayashi, Effects of ethanol, potassium metabisulfite, formaldehyde and hydrogen peroxide on gastric carcinogenesis in rats after initiation with N-methyl-N'-nitro-N-nitrosoguanidine, *Cancer Science*, 77, 1983, 118–124.
- [12]. G. Smedje, D. Norback, Incidence of asthma diagnosis and self-reported allergy in relation to the school environment a four year follow-up study in schoolchildren, *International Journal of Tuberculosis and Lung Disease*, 5, 2001, 1059–1066.
- [13]. P. Franklin, P. Dingle, S. Stick, Raised exhaled nitric oxide in healthy children is associated with domestic Formaldehyde levels, *American Journal of Respiratory and Critical Care Medicine*, 161, 2000, 1757–1759.
- [14]. The Daily Star, Fight against adulteration, Sunday, June 08, 2014.
- [15]. J.C. Chang, W.H. Steinhagen, C.S. Barrow, Effect of single or repeated formaldehyde exposure on minute volume of B6C3F1 mice and F-344 rats, *Toxicology and Applied Pharmacology*, 61, 1981, 451–59.
- [16]. J.D. Bancroft, A. Gamble, *Theory and practice of histological techniques* (5th ed), (Churchill Livingstone, Newyork, London, 2002) 165–180.
- [17]. H.P. Til, R.A. Woutersen, V.J. Feron, V.H. Hollanders, H.E. Falke, J.J. Clary, Two-year drinking-water study of formaldehyde in rats, *Food and Chemical Toxicology*, 27, 1989, 77–87.
- [18]. M. Tobe, K. Natio, Y. Kurokawa, Chronic toxicity study on formaldehyde administered orally to rats, *Toxicology*, 56, 1989, 79–86.
- [19]. A.M. Babar, M. Z. Khan, S. Ahmed, A. Khan, H. A. Bachaya, M. I. Anwar, Toxicopathological effects of formalin (37% formaldehyde) feeding in broiler chicks, *Pakistan Veterinary Journal*, 21(1), 2001, 13–16.
- [20]. H.V. Bhatt, G.M. Panchal, Behavioural change in rats due to chronic oral and systemic formaldehyde, *Indian Journal of Physiology and Pharmacology*, 36, 1992, 270–272.
- [21]. J.R. Beall, A.G. Ulsamer, Formaldehyde and hepatotoxicity: a review, *Journal of Toxicology and Environmental Health*, 14, 1984, 11–21.
- [22]. C. Euphoria, C.A.O. Akwiwu, J.O. Usoro, M. Akpotuzor, H. Etukudo, Hepatic Functions of Persons. Occupationally Exposed to Formaldehyde in Calabar, Nigeria, *Advances in Life Science and Technology*, 38, 2015, 64–69.
- [23]. H.P. Til, R.A. Woutersen, V.J. Feron, J.J. Clary, Evaluation of the oral toxicity of acetaldehyde and formaldehyde in a 4-week drinking-water study in rats, *Food and Chemical Toxicology*, 26, 1988, 447–452.
- [24]. J. Kimbell, J. Overton, R. Subramaniam, Dosimetry modeling of inhaled formaldehyde: Binning nasal flux predictions for quantitative risk assessment, *Toxicological Sciences*, 64, 2001, 111–121.
- [25]. E. Uçmakli, F. Armutcu, A. Öztürk, The effects of formaldehyde intoxication on the inducible nitric oxide synthase expression and nitric oxide level in the liver tissue of rats, *Turkish Journal of Medical Sciences*, 43, 2013, 52–56.
- [26]. S. Cizmaz, T. Kutoglu, M. Kanter, R. Mesut, Effect of formaldehyde inhalation on rat livers: a light and electron microscopic study, *Toxicology and Industrial Health*, 26, 2010, 113–9.
- [27]. O. Strubelt, M. Younes, R. Pentz, W. Kühnel, Mechanistic study on formaldehyde-induced hepatotoxicity, *Journal of Toxicology and Environmental Health*, 27, 1989, 351–66.
- [28]. R.H. Ku, R.E. Billings, Relationships between formaldehyde metabolism and toxicity and glutathione concentrations in isolated rat hepatocytes, *Chemico-Biological Interactions*, 51, 1984, 25–36.
- [29]. M. Dumont, C. D'Hont, H. Moreau, H. Mbape, G. Feldmann, S. Erlinger, Retrograde injections of formaldehyde into the biliary tree induce alterations of biliary epithelial function in rats, *Hepatology*, 24, 1996, 1217–23.

- [30]. A. Neuberger, The metabolism of glycine and serine, in A. Neuberger and LLM. Deenen (eds.), (Elsevier Amsterdam, Comprehensive Biochemistry, 19A, 1981) 257-303.
- [31]. S. Teng, K. Beard, J. Pourahmad, M. Moridani, M. Easson, R. Poon, P.J. O'Brien, The formaldehyde metabolic detoxification enzyme systems and molecular cytotoxic mechanism in isolated rat hepatocytes, *Chemico-Biological Interactions*, 2001, 130– 132, 285–296.
- [32]. WHO (World Health Organization), Formaldehyde in Drinking-water. WHO/SDE/WSH/04.08/48s, 1981.
- [33]. IARC (International Agency for Research on Cancer), Formaldehyde, 2-butoxyethanol and 1-tertbutoxypropan-2-ol, IARC Monographs on the Evaluation of Carcinogenic Risks to Human, Volume 88, 2006, 1–478.
- [34]. C.L. Galli, Toxicological evaluation in rats and mice of the ingestion of a cheese made from milk with added Formaldehyde, *Food and Chemical Toxicology*, 21, 1983, 313–317.
- [35]. R.K. Upreti, Toxicokinetics and molecular interaction of [14C]-formaldehyde in rats, *Archives of Environmental Contamination and Toxicology*, 16, 1987, 263–273.
- [36]. IPCS, *Formaldehyde*. Geneva, World Health Organization (Environmental Health Criteria 89), 1989.
- [37]. H.A. Heck, T.Y. Chin, M.C. Schmitz, Distribution of [14C] FA in rats after inhalation exposure, in JE. Gibson, (Ed), *Formaldehyde toxicity*. (Washington DC: Hemisphere Publishing Corp, 1983) 26-37.



Mean ± Standard deviation; * 5% level of significance (p < 0.05)

Fig. 1 Mean concentration of AST and ALT in the control & FA exposed intraperitoneal group mice.



Mean ± Standard deviation; * 5% level of significance (p < 0.05)

Fig. 2 Mean concentration of AST and ALT in the control & FA exposed oral group mice.

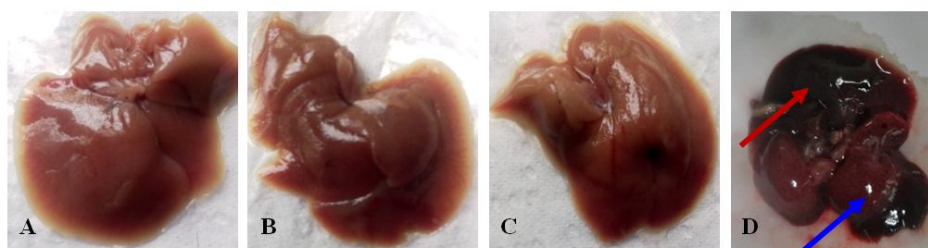


Fig. 3 Gross study of liver of control (A) and oral grouped (B-D) mice.

(A) Control (B) 5mg/kg and (C) 7mg/kg FA treated oral groups showing normal gross morphology of liver. No congestion and hemorrhage is found in those groups (D) Dark coloration of liver. Liver showing congestion (red arrow) and petechial hemorrhages (blue arrow) in 10mg/kg FA treated oral grouped mice.

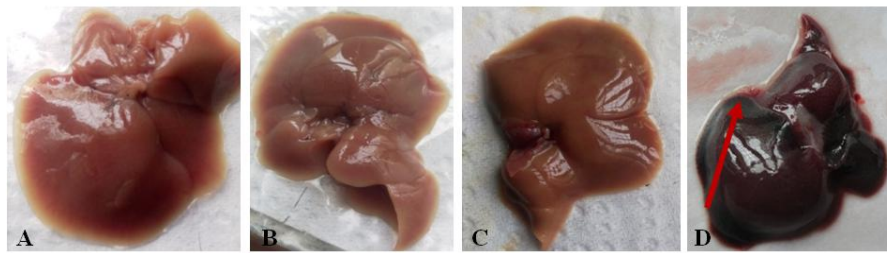


Fig. 4 Gross study of liver of control (A) and intraperitoneal grouped (B-D) mice.

(A) Control (B) 5mg/kg and (C) 7mg/kg intraperitoneally FA treated groups showing normal gross morphology of liver. No congestion and hemorrhage is found in those groups (D) Dark color liver showing congestion (red arrow) and petechial hemorrhages in 10mg/kg FA exposed intraperitoneal grouped mice.

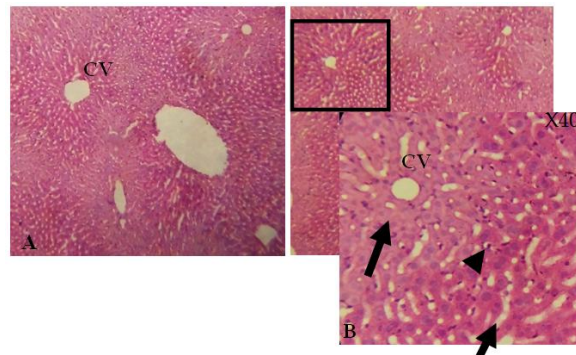


Fig. 5 (A-B) Histological features in liver of control and oral grouped mice in H and E stain (X10). CV = Central vein.

(A) No change observed in control mice liver. (B) The representative figure of 10 mg/kg FA treated oral group liver and showing centrilobular necrosis and degeneration of parenchymatous cells (long arrow), dilatation of sinusoids (short arrow) and scattered lymphatic aggregation (arrow head).

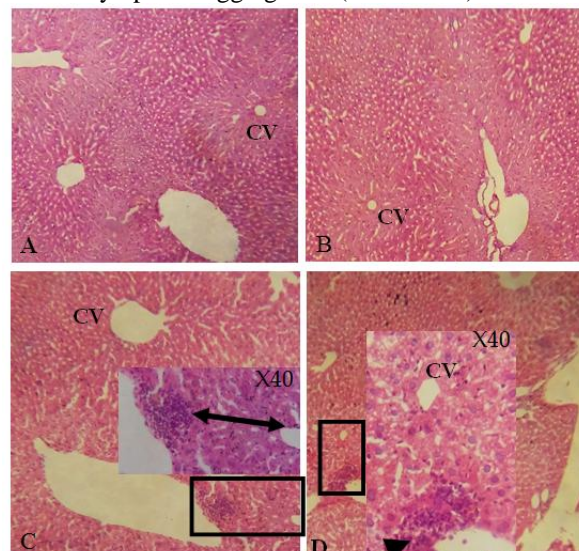


Fig. 6 (A-D) Histological features in liver of control and intraperitoneal grouped mice in H and E stain (10X). CV = Central vein.

(A) Normal architecture of liver in control group (B) Liver showing normal architecture in 5mg/kg FA exposed group (C) In 7mg/kg FA treated group, liver showing diffuselymphatic aggregation (arrow head) and piecemeal necrosis (two headed arrow) (D) Liver showing diffuselymphatic aggregation (arrow head) in 10mg/kg FA treated group.