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Attenuation of sodium fluoride-induced hepatic injury by *Lactobacillus casei* through inhibition of NF-κβ signalling pathway with decreased expression of caspase-3 in rats

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Abstract

Lactic acid bacteria play an important role in the human and animal defense against liver damage. However, the potential mechanism of *Lactobacillus casei (L.casei)* on hepatic injury remains unclear. The objective of the present investigation was to assess the possible effect of *L.casei* against sodium fluoride (NaF) induced- hepatic injury in rats. Rats were allocated into three groups; first group received only distilled water thought the experiment. Second group received orally NaF (10mg/kg) for 4 weeks. Third group received NaF (10mg/kg; p.o) concurrently with *L.casei* (10^9CFU/kg.b.w.; p.o) for 4 weeks. Our study revealed that exposure to NaF increased the values of serum hepatic enzymes with increased levels of lactate dehydrgenase (LDH), nitric oxide (NO) and total bilirubin levels. In addition, NaF intoxication was associated with a reduction in the activities of glutathione (GSH) and an increment in malonaldheyde (MDA) in hepatic tissues. Moreover, a significant increment in the release of NF-κβ and caspase-3 with disturbed histopathological and ultrastructural architecture of liver cells of rats intoxicated with NaF. Conversely, *L.casei* supplementation (10^9CFU/kg.b.w.; p.o) could prevent NaF-induced hepatotoxicity, most likely as a result of possessing high antioxidant properties and anti-inflammatory activities. In conclusion, based on our findings, *L.casei* is effective treatment in hepatic injury and daily administration of *L.casei* is recommended to mitigate the deleterious effects of F-containing compounds.

Keywords: Hepatic injury, Sodium Fluoride, *Lactobacillus casei*, NF-κβ, Apoptosis.

1- Introduction

Fluoride anions (F) are one of the classes of xenobiotics that are environmentally distributed and widely used in industrial, agricultural and medical applications **[1, 2]**. Normally, F anions are considered the main constituent of bones, teeth, soft tissues and body fluids **[3]**. Water sources are chemically fluoridated in some areas, therefore, water is considered the most important source of F anions **[4]**. Sodium fluoride (NaF) is an in-organic chemical compound usually utilized in diverse applications such as wood preservations and insecticide formulations **[5]**. Therapeutically, F anions, especially in small doses, have a significant protective role in preventing osteoporosis and dental caries **[6]**.

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However, the excessive exposures induce pathological alterations mainly in the liver and consequently affecting its function leading to hepatic impairment **[7]**.

Reactive oxygen species (ROS) has been considered the main contributor in fluoride toxicity **[8]**. Where, NaF anions could triggers respiratory burst and potentiate the release of ROS that drastically interfere with the normal functions of the cells via altering the permeability and structure of cell membranes **[9]**. It was revealed by many investigators that an increase in the production of ROS has been observed in animals exposed to NaF toxicity **[10]**. Moreover, NaF affects the activity of cellular antioxidant enzymes leading to impaired mitochondrial functions, decreased antioxidant capacity and potentiate the oxidative damage to lipids, proteins and DNA of the cellular tissue **[11].**

Aside from oxidative stress imbalance, ROS produced by NaF toxicity are responsible for initiating the inflammatory cascade, that leads to release of numerous inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (il-6) due to activation of nuclear factor kappa beta (NF-κβ) **[12, 13]**. Finally, synergism between free radicals and cytokines occurs to enhance the synthesis of inflammatory mediators. However, NO has been shown to react with superoxide anion, resulting in the formation of peroxynitrite, an oxidative agent capable of causing tissue damage **[14]**. Oxidative stress could triggers apoptosis as a result of the noxious effects of ROS on inflamed tissue **[15]**. Therapeutic approaches that modulate the aforementioned signals may hold promise for treating a variety of pathological conditions.

Lactobacillus casei (L. casei) is recognized as a probiotic and is frequently used as a dietary supplement for their outstanding health benefits **[16]**. *L. casei* has sparked interest in research due to its potential immunoregulatory effect, which has been linked to increased resistance to diverse bacterial and viral infections. **[17, 18]**. Nevertheless, little literature discussing the roles of *L. casei* on hepatic toxicity. Consequently, the goal of the current study was to determine the role of *L. casei* against NaF induced- hepatic injury, via studying (i) the changes in serum hepatic indices, antioxidant status and inflammatory response in rats intoxicated with NaF. (ii) The mechanism of *L. casei* in modulating the deleterious effects of NaF in the liver. (iii) Studying the histopathological and ultrastructural findings in the liver of rats intoxicated with to NaF.

2. Materials and methods

2.1. Bacterial suspension *(L.casei)* and chemicals

L. casei DSM 20011 was prepared by growing the strain in a volumetric flask with 100 ml of milk before being incubated anaerobically for 48 hours at 37 °C. Then 1 ml of the bacterial suspension was added to 9 ml of De Man, Rogosa and Sharpe **(**MRS) broth then the mixture was incubated anaerobically at 37 °C/24hrs to produce 1 x 10^9 CFU per ml. For rats, 1 ml/day of *L. casei* DSM 20011 suspension was the recommended daily dose **[19]**. NaF was purchased from Sigma-Aldrich Co (USA). All kits were purchased from Biodiagnostic Co. (Egypt) and Sunlong Biotech Co. (China)

2.2. Animals

Eighteen adult male Wistar albino rats weighting 150 - 170 gm were housed in separated metal cages at the animal house, October 6 University, Giza, Egypt. Rats were housed in a well-ventilated room under ambient laboratory conditions (22±1°C temperature, 45-55% relative humidity) with a 12-hour light/12-hour dark cycle. Water and food were freely available. Under approval number (RECO6U/9-2022), our study was carried out in accordance with the ethical standards documented by the Research Ethics Committee, Faculty of Dentistry, October 6 University, Giza, Egypt.

2.3. Experimental design

Animals were divided into three groups; each contains six rats. Group 1: rats administered only normal drinking water. Group 2: rats intoxicated daily with doses of NaF (10 mg/kg, p.o.) for 4 weeks **[20]**. Group 3: rats received NaF (10 mg/kg, p.o.) concurrently with *L. casei* (10^9CFU/kg.b.w.; p.o) for 4 weeks **[18]**. 24 hrs after the last dose of NaF and *L. casei*, blood samples were collected from the retro-orbital venous plexus of all rats and placed in non-heparinized tubes. Serum was collected by centrifugation at 4000 rpm for 15 minutes and stored at -20 ^{0}C until analysis.

After sacrificing of rats by cervical dislocation, liver was excised immediately from each rat and reserved at - 80 ^{0}C for biochemical analysis. Another portion of tissues were kept in fixatives for histopathology, immune-histochemical and ultrastructural evaluation.

2.4. Evaluation of serum hepatic function values

Serum levels of AST, ALT and ALP were measured colorimetrically at 520 nm **[21] [22]**. LDH values were estimated at 340 nm **[23]**. Total bilirubin was quantified and measured at 535 nm **[24]**.

2.5. Determination of hepatic GSH and MDA levels

Levels of GSH in hepatic homogenate **[25]** were assessed via estimating the absorbance at 405 nm **[26]**. In addition, estimation of MDA values at 534 nm **[27]**.

2.6. Determination of hepatic NO levels

Through the Griess reaction, which involves reducing nitrate to nitrite, the concentration of nitric oxide (NO) in the liver homogenate was determined. This concentration was then determined spectrophotometrically at 540 nm **[28]**.

2.7. Determination of hepatic TNF-α and IL-6 levels

Hepatic results of TNF-α and IL-6 were assessed using the rat TNF-α ELISA kit (Sunlong Biotech Co., Catalog no. SL0722Ra, CHINA) and il-6 ELISA Kit (Sunlong Biotech Co., Catalog no. SL0411Ra, CHINA). The optical density (OD) for the concentration TNF-α and il-6 were measured spectrophotometrically at a wavelength of 450 nm **[29]**.

2.8. RT-PCR for NF-κB analysis

Total RNA was isolated from liver specimens, according to the manufacturer's recommendations for evaluation of of NF-κB. Results were expressed against, beta actin **[30]**. The sequence of the primer sequence was:

Forward 5_-GCG CAT CCA GAC CAA CAA TAA C-3_

Reverse 5_-GCC GAA GCT GCA TGG ACA CT- 3_

2.9. Histopathology picture and immunohistochemical analysis of caspase -3 in liver specimens

Liver samples were taken from each experimental group and fixed in 10% formalin for 48–72 h. The specimens then washed, dehydrated, and finally embedded in paraffin wax. Sections were taken using slide microtome (4-5μ thickness), deparaffinized, and stained with hematoxylin and eosin. **[31]**.

Immunohistochemical analysis was performed in liver specimens using caspase 3 antibody, where liver sections were stained with rabbit-anti-cleaved caspase-3 antibodies (Catalog no. 9661, Cell Signaling Technology, USA) **[32]**.

2.10. Electron microscopic examination of liver tissue

In each experimental group's liver samples were taken, and instantly fixed in 2.5 % glutaraldehydes for 4 hrs. After washing with phosphate buffer (pH 7.4), samples was post-fixed in 1% buffered osmium tetroxide. Tissues then dehydrated in a graduated series of alcohol, treated with propylene oxide and finally embedded in epoxy resin (Epon 812; Fluka Chemie, Switzerland). Polymerization were done in propylene oxide capsules at 60°C for 24 hrs. Ultra-sections were cut (60-70 nm),

stained with lead citrate and uranyl acetate. **[33]**.Sections were observed under transmission electron microscope (TEM) JEOL-JEM 2100 TEM operated at 80 KV. at Electron Microscopy Unit, Mansoura University (Egypt)

3. Statistical analysis

All measurable comparisons were done by using GraphPad Prism program v. 8.0 (GraphPad Software, Inc., CA, USA). One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test was the choice for our statistical analysis to document the significance (p value is ≤ 0.05). Results are expressed as mean ± SEM (six rats).

4. Results

4.1. Effect of *L. casei* on AST, ALT and ALP levels in rats intoxicated with NaF

Rats received orally NaF (10 mg/kg) for 4 weeks showed a marked elevation in serum hepatic indices (AST, ALT, and ALP) nearly 3 folds, 2.5 folds and 175% of the normal value, respectively. *L. casei* supplementation (10^9CFU/kg.b.w.; p.o) to intoxicated rats with NaF for 4 weeks could restore the aforementioned parameters to the normal values (figure.1.).

4.2. Effect of *L. casei* on LDH and total bilirubin levels in rats intoxicated with NaF

Hepatic injury induced by NaF (10 mg/kg) orally for 4 weeks showed a significant increment in LDH and total bilirubin levels nearly 151% and 4.5 folds of the normal value, respectively. *L. casei* supplementation (10^9CFU/kg.b.w.; p.o) to intoxicated rats with NaF for 4 weeks could normalize the levels of LDH and decrease the levels of total bilirubin about of 2 folds the normal values (figure.2.).

4.3. Effect of *L. casei* on GSH and MDA levels in rats intoxicated with NaF

Orally administered NaF (10 mg/kg) rats for 4 weeks resulted in an increase of hepatic MDA values that were nearly 4.5 times higher than normal and a drop in hepatic GSH activity that was almost 28% lower than normal. The hepatic values of MDA in intoxicated rats treated with *L. casei* (10^9CFU/kg.b.w.) orally for 14 days decreased to around 2 folds of the normal value, while the level of GSH activity increased to 72% of the normal values (figure.3.).

4.4. Effect of *L. casei* on NO levels in rats intoxicated with NaF

Figure.4. documented an increase in hepatic NO levels of about 144% of the normal value was seen after administration of NaF orally at a dose of (10 mg/kg) for four weeks. On the other hand, a 14-day oral *L. casei* (10^9CFU/kg.b.w.) treatment reduced hepatic NO levels to roughly 113% of normal levels.

4.5. Effect of *L. casei* on hepatic NF-κB levels in rats intoxicated with NaF

Figure.5. revealed that intoxicated rats received NaF (10 mg/kg) orally for four weeks revealed an increase in hepatic NF-κB levels reaching 5 folds of the normal value. On the other hand, oral treatment with *L. casei* (10^9CFU/kg.b.w.) for 14 days showed a reduction in hepatic values of hepatic NF-κB levels reaching about nearly 161% folds of the normal value.

4.6. Effect of *L. casei* on TNF-α levels and IL-6 levels in rats intoxicated with NaF

Figure.6. revealed that intoxicated rats given NaF (10 mg/kg) for four weeks showed an increase in hepatic results of TNF-α and IL-6 that were approximately 5 and 3 folds over normal, respectively. However, after 14 days of oral administration of *L. casei* (10^9 CFU/kg.b.w.), the values of TNF-α and IL-6 in the liver dropped to approximately 2 folds and 178% of normal levels, respectively.

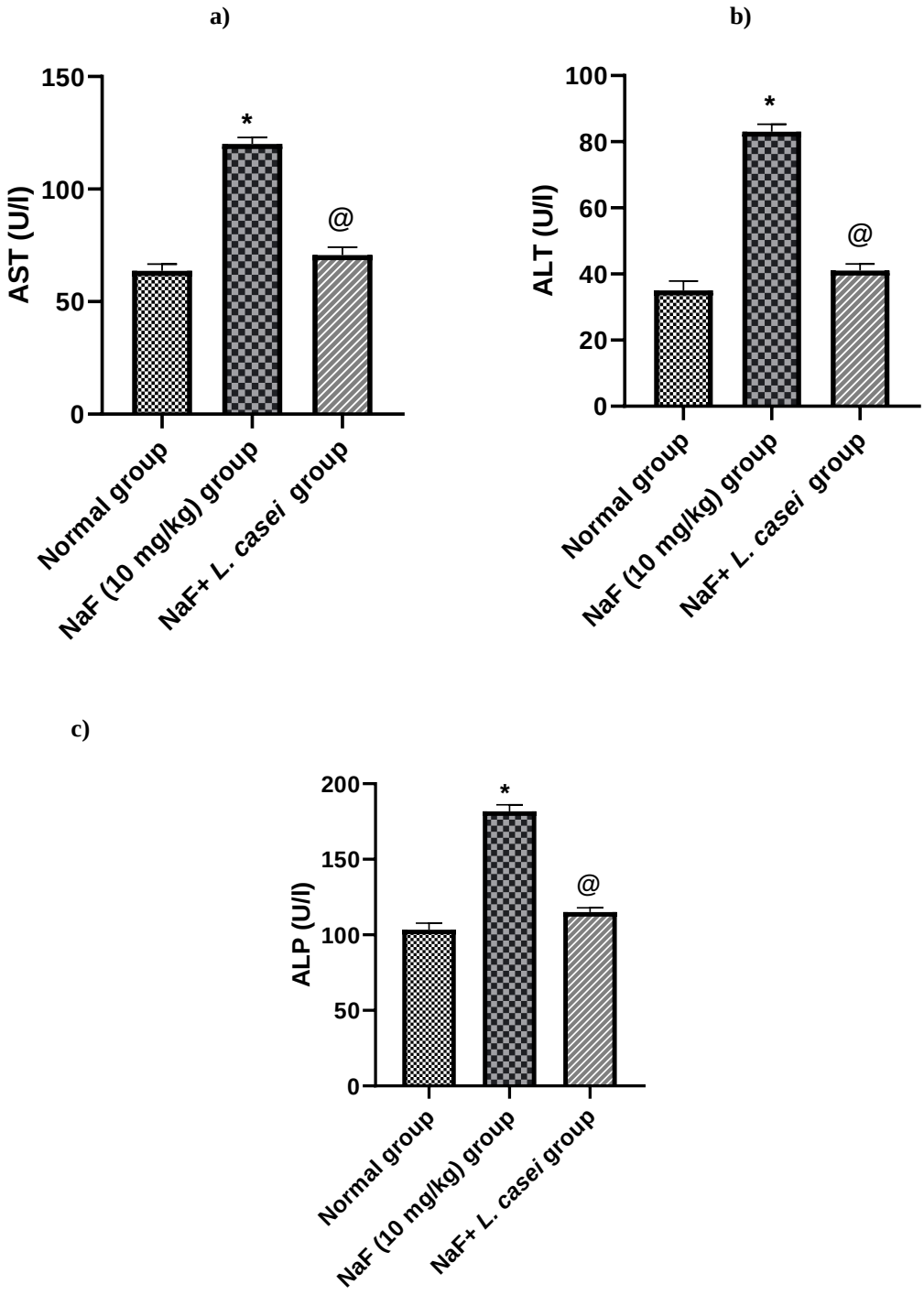


Figure.1. Effect of *L. casei* on AST, ALT and ALP levels in rats intoxicated with NAF

Hepatic injury was induced by daily administration of (NaF; 10mg/kg; p.o.) for 4 weeks. Oral treatment of NaF induced - hepatic injury with *L.casei* (10^9CFU/kg.b.w.; p.o) for 4 weeks. 24 hours after the last doses of NaF and *L.casei*, AST, ALT and ALP levels were estimated. Results are expressed as mean ±SEM (n=6). *Significant difference from normal control group $p < 0.05$. @ Significant difference from NaF intoxicated group.

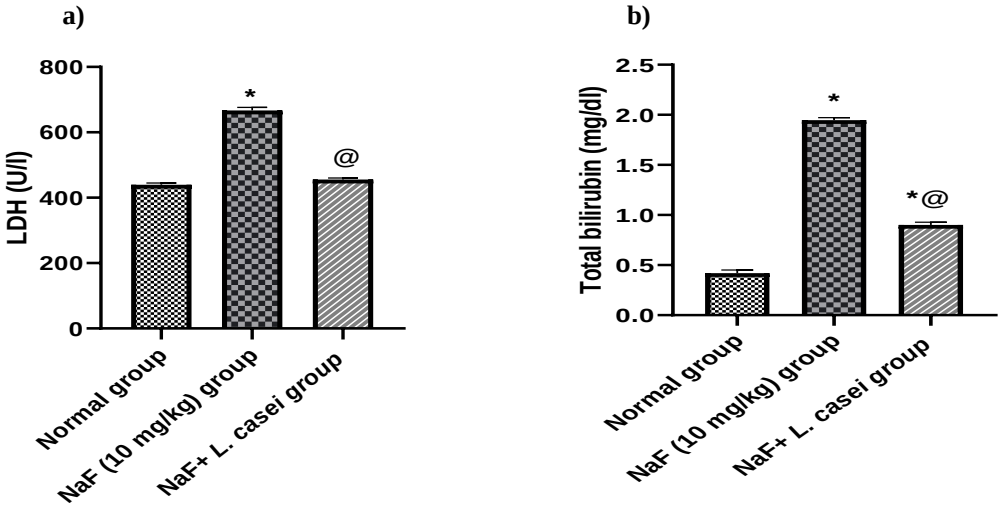


Figure.2. Effect of *L. casei* on LDH and total bilirubin levels in rats intoxicated with NAF

Hepatic injury was induced by daily administration of (NaF; 10mg/kg; p.o.) for 4 weeks. Oral treatment of NaF induced - hepatic injury with *L.casei* (10^9CFU/kg.b.w.; p.o) for 4 weeks. After 24 hours, the last doses of NaF and *L.casei*, LDH and total bilirubin levels were estimated. Results are expressed as mean ±SEM (n=6). *Significant difference from normal control group $p < 0.05$. @ Significant difference from NaF intoxicated group.

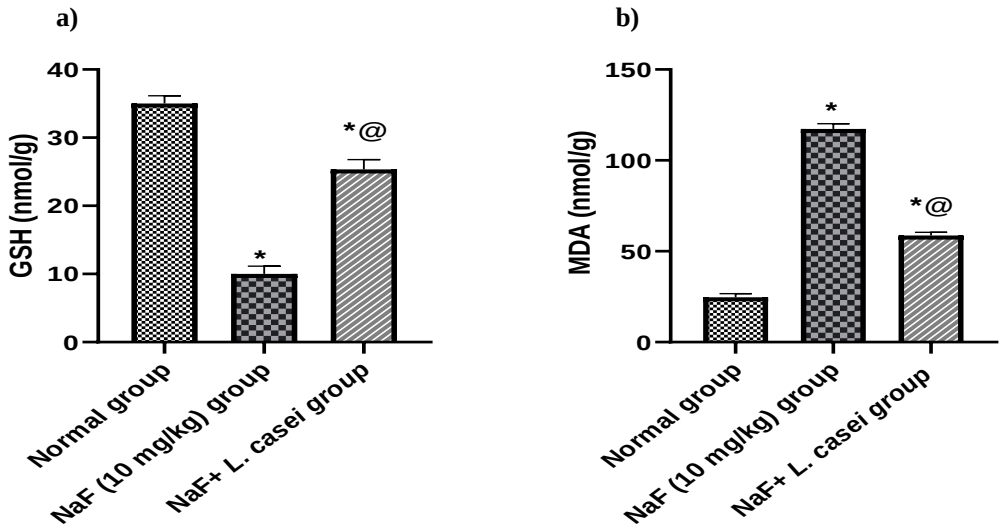


Figure.3. Effect of *L. casei* on hepatic GSH and MDA levels in rats intoxicated with NAF

Hepatic injury was induced by daily administration of (NaF; 10mg/kg; p.o.) for 4 weeks. Oral treatment of NaF induced - hepatic injury with *L.casei* (10^9CFU/kg.b.w.; p.o) for 4 weeks. 24 hours after the last doses of NaF and *L.casei*, hepatic levels of GSH and MDA were estimated. Results are expressed as mean ±SEM (n=6). *Significant difference from normal control group $p < 0.05$. @ Significant difference from NaF intoxicated group.

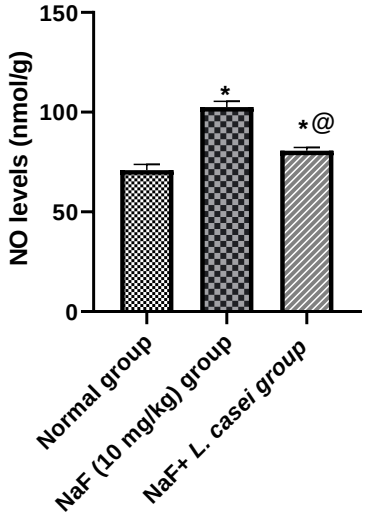


Figure.4. Effect of *L. casei* on hepatic NO levels in rats intoxicated with NAF

Hepatic injury was induced by daily administration of (NaF; 10mg/kg; p.o.) for 4 weeks. Oral treatment of NaF induced - hepatic injury with *L.casei* (10^9CFU/kg.b.w.; p.o) for 4 weeks. 24 hours after the last doses of NaF and *L.casei*, hepatic NO levels were estimated. Results are expressed as mean ±SEM (n=6). *Significant difference from normal control group $p < 0.05$. @ Significant difference from NaF intoxicated group**.**

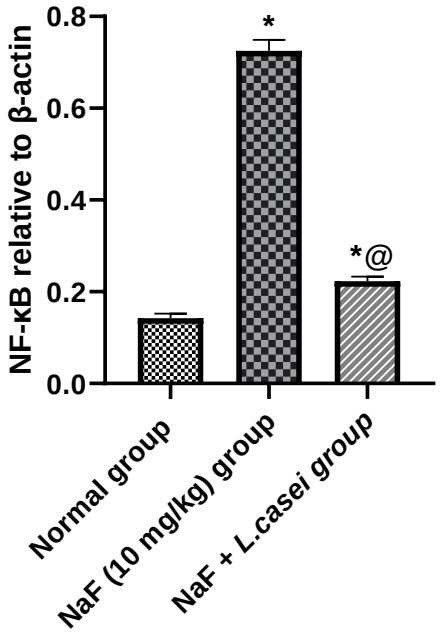


Figure.5. Effect of *L. casei* on hepatic NF-κB levels in rats intoxicated with NAF

Hepatic injury was induced by daily administration of (NaF; 10mg/kg; p.o.) for 4 weeks. Oral treatment of NaF intoxicated rats with *L.casei* (10^9CFU/kg.b.w.; p.o) for 4 weeks. 24 hours after the last doses of NaF and *L.casei*, hepatic NF-κB levels was estimated. Results are expressed as mean ±SEM (n=6). *Significant difference from normal control group $p < 0.05$. @ Significant difference from NaF intoxicated group**.**

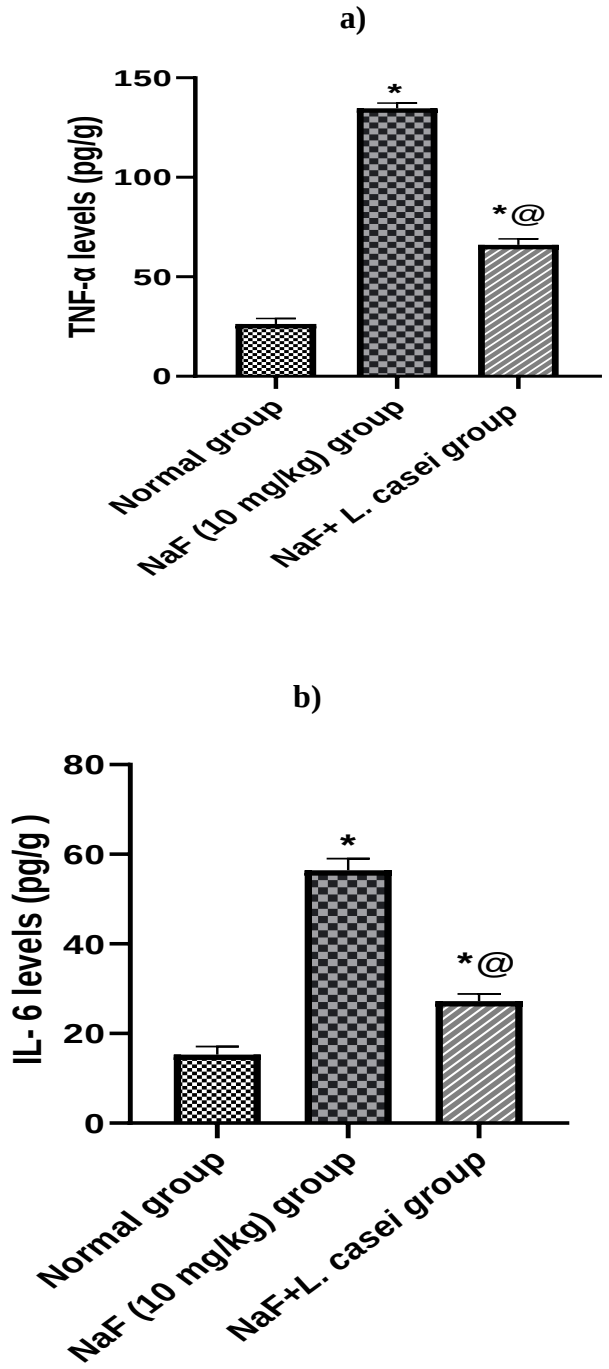


Figure.6. Effect of *L. casei* on hepatic TNF-α levels and IL-6 levels in rats intoxicated with NAF

Hepatic injury was induced by daily administration of (NaF; 10mg/kg; p.o.) for 4 weeks. Oral treatment of NaF intoxicated rats with *L.casei* (10^9CFU/kg.b.w.; p.o) for 4 weeks elevate the levels of TNF-α and IL-6 . 24 hours after the last doses of NaF and *L.casei*, Hepatic TNF-α levels and IL-6 levels were estimated. Results are expressed as mean ±SEM (n=6). *Significant difference from normal control group $p < 0.05$. @ Significant difference from NaF intoxicated group**.**

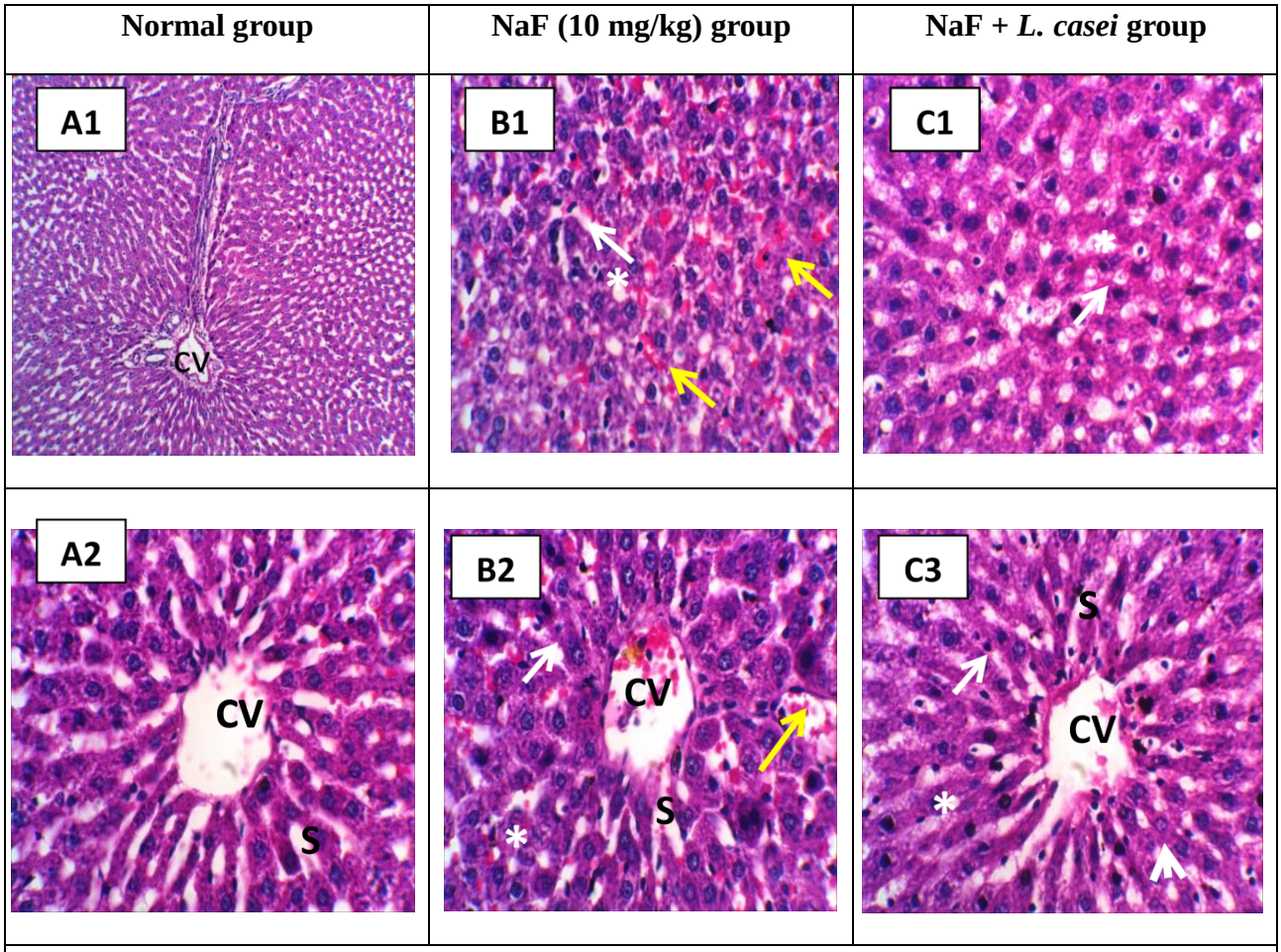


Figure.7. Photomicrograph of liver sections stained with H&E represents the different studied group; (A1, A2) showing normal organization of hepatic parenchyma with radially arranged hepatic lobules around central veins (CV), normal sinusoids (S) in control group, high magnification X: 400. (B1, B2) NaF group showing loss of normal hepatic cord architecture with narrowed or occluded sinusoids (S) with congested central veins (CV), intracellular vacuolization of hepatocytes (*) and marked hepatic necrosis and a pyknotic nuclei (white arrow) infiltrated with inflammatory cells (yellow arrow), High magnification X: 400. (C1, C2) NaF group + *L. casei* showing Improvement in morphology of histological structure of the hepatic lobules, naturally hepatocyte arrangements (head arrow) with approximately normal blood sinusoids (S). Mild to moderate microvacuolated hepatocytes (*) and plasma cells with few neutrophils individualized necrotic cells (white arrow) was also shown high magnification X: 400.

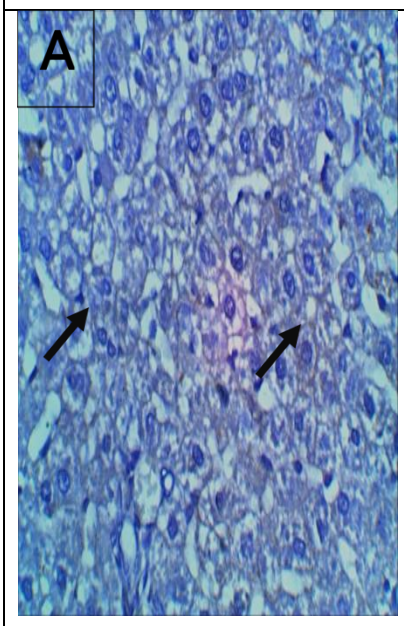
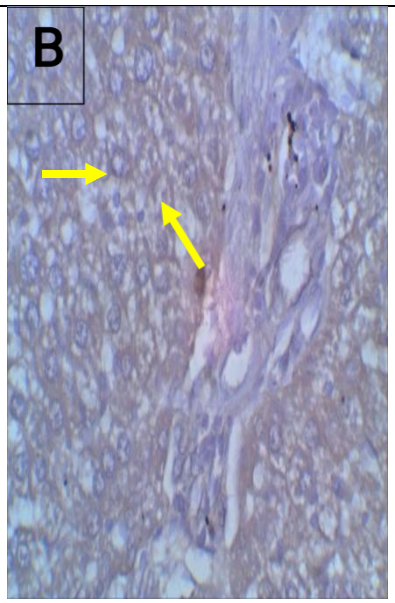
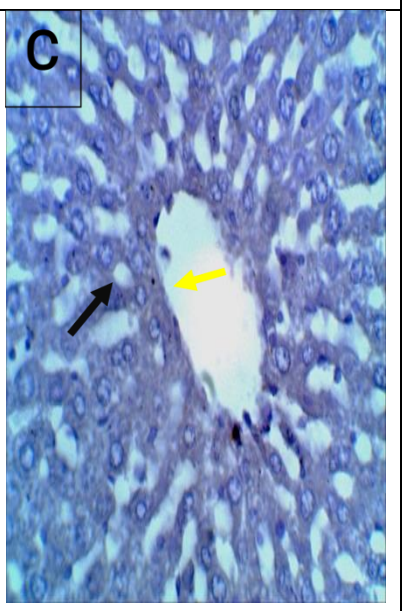
Normal group	NaF (10 mg/kg) group	NaF+ *L. casei* group
		

Figure.8. Photomicrograph of liver sections stained caspase-3 immunostaining represents the different studied group. (A): liver section from control rat showing the normal distribution of caspase-3 in the cytoplasm of hepatocytes (black arrow) (caspase-3 immunostaining, x400).

(B): liver section from NaF intoxicated group rat showing increase in caspase-3 distribution in the hepatic condensed chromatin of the nuclei which showing strong positive immunoreaction (yellow arrow) (caspase-3 immunostaining, x400). (C): liver section from NaF + *L. casei* group administered rat showing nearly normal distribution of caspase-3 in hepatic tissue (caspase-3 immunostaining, x400).

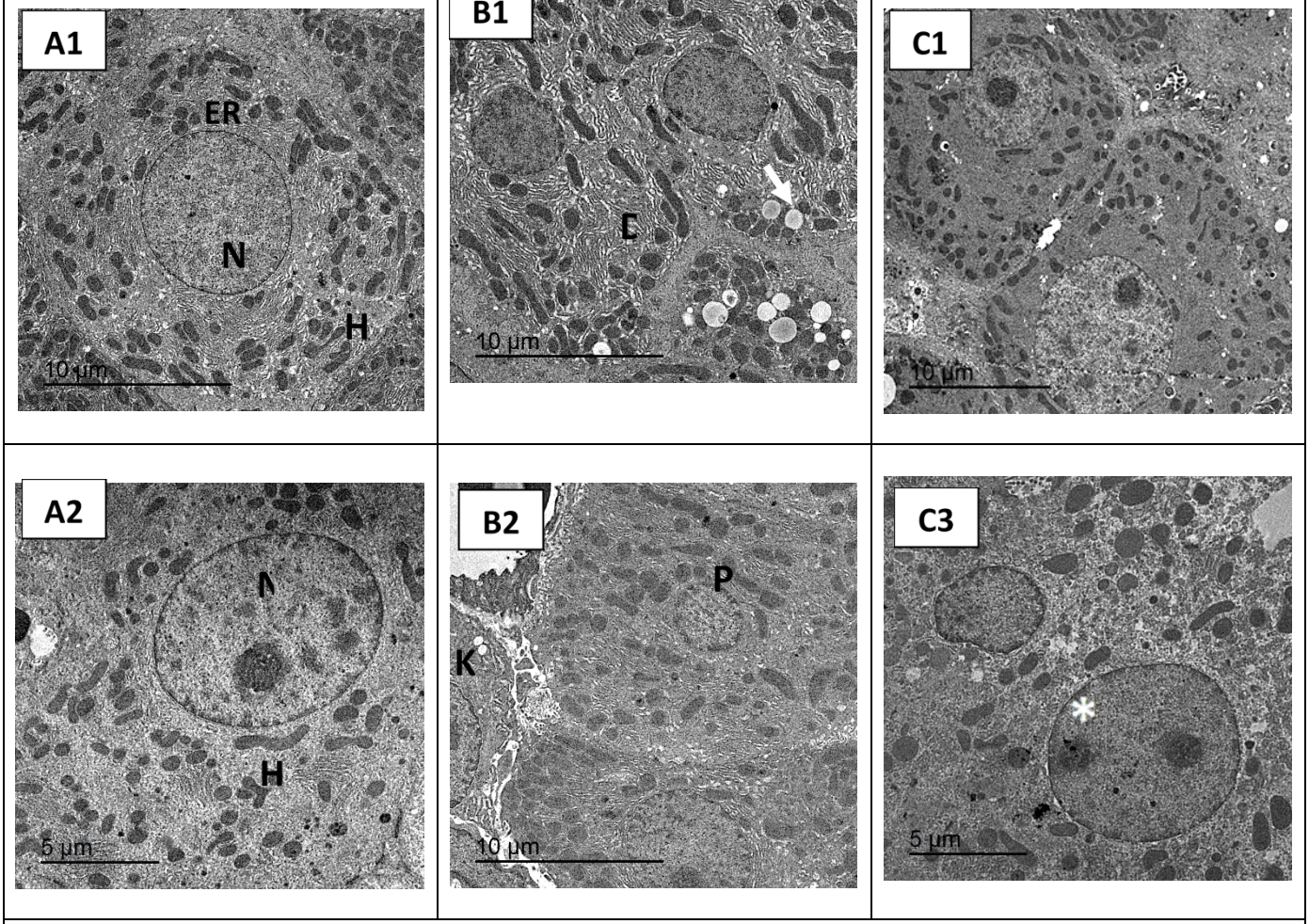


Figure.9. Electron micrograph of liver sections in different studied group; (A1, A2) showing typical hepatocyte with normal nucleus, nucleolus (nu) and nuclear membrane and organized cytoplasm containing healthy mitochondria and arrays of endoplasmic reticulum (ER). (B1, B2) NaF group showing irregular nuclear membrane, dense chromatin material and pyknotic nucleus (P). Active Kupffer cell (K) in sinusoidal space. The cytoplasm appears vacuolated (arrow), disorganized with dilated ER (D) (C1, C2) NaF + *L. casei* group showing mostly normal appearance of hepatocytes with minor changes. The actively dividing binucleated cell are shown (*) indicating the regeneration of hepatic tissue.

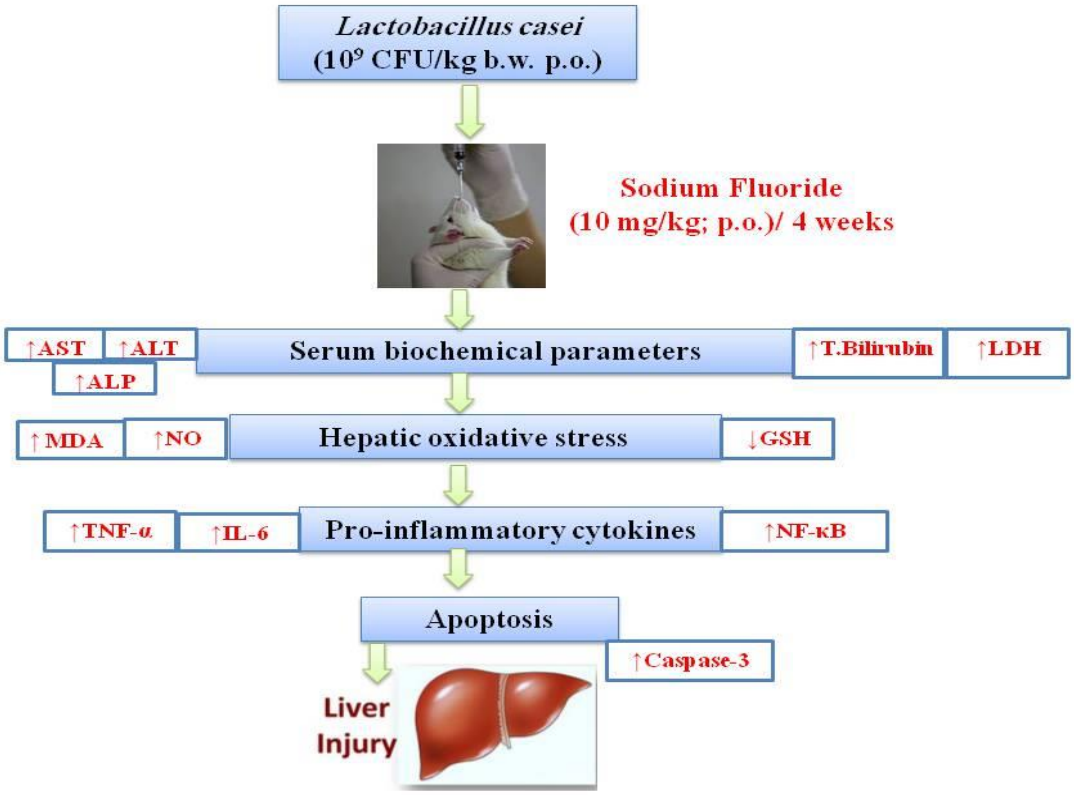


Figure.10. Diagram illustrating the effect of *lactobacillus casei* against sodium fluoride induced- hepatic injury in rats

4.7. Effect of *L. casei* on histopathological alterations in rats intoxicated with NaF

Figure.7. revealed the histopathological picture in different groups. Group 1 showed normal hepatic architecture with normal distribution of hepatic lobules around the central vein. NaF intoxicated group revealed the loss of normal hepatic organization with congested central vein and intracellular vacuolization of hepatocytes. Hepatic tissue was also infiltrated with inflammatory cells. In contrast, group two which treated with *L. casei* showed an improvement in the histological structure of the hepatic lobules with mild to moderate microvacuolated hepatocytes.

4.8. Effect of *L. casei* on caspase-3 in rats intoxicated with NaF

Figure.8. revealed the immunohistochemical analysis of caspase-3 in different groups. Normal group showed normal distribution of caspase-3 in the cytoplasm of hepatocytes. NaF group revealed huge distribution of caspase-3 distribution in the hepatic condensed chromatin of the nuclei which showing strong positive immunoreaction. On the other hand, *L. casei treated* group revealed a substantial decrease in caspase-3 distribution.

4.9. Effect of *L. casei* on the ultra- structural picture in rats intoxicated with NAF

Figure.9. revealed the ultra- structural picture of hepatic tissue in different groups. The hepatic parenchyma in the normal group had a normal hepatocyte while, NaF group showed many alterations represented by vacuolated cytoplasm, irregular nuclear membrane, and dense chromatin material with pyknotic nucleus. In contrast, *L. casei* group showed mostly normal appearance of hepatocytes.

5. Discussion

The liver serves as the primary organ for metabolizing and eliminating of xenobiotics that invades the organisms from the environment **[34, 35]**. The hepatotoxic action of xenobiotics is usually evidenced by cellular respiration dysfunction that interferes with the occurrence of oxidation and reduction reactions **[36]**. Fluoride is a known moderate pollutant that is non-biodegradable and causes major health issues when it is present in high concentrations **[37]**. Liver is the target organ of sodium fluoride (NaF) toxicity **[38]**. The aim of our study was to assess the therapeutic intervention of *L.casei* against the hepatic injury associated with NaF intoxication. Our findings reported that rats intoxicated orally with NaF (10 mg/kg) for four consecutive weeks showed a typical pattern of hepatotoxicity, as evidenced by marked elevation in the results of serum hepatic indices.

The increment in hepatic markers; AST, ALT, ALP, LDH, and total bilirubin could be attributed to the noxious effects of NaF leading to loss of functional integrity and leakage in hepatic cell membrane [39]. Our findings are matching with a study reported that rats received NaF possess an increment in the levels of serum hepatic indices **[40]**. Administration of *L.Casei* to rats intoxicated with NaF could restore the normal values of AST, ALT, ALP, LDH, and total bilirubin due to its role in maintaining the structural integrity of hepatic cell membrane and its was in line with **[41]**.

The mechanism of NaF toxicity is far from being fully understood, although it has been suggested that ROS is the main crucial factor in the pathogenesis of NaF-induced hepatic injury **[42]**. Our findings revealed that NaF could acts as an inducer of lipid peroxidation (MDA) with reduced the enzymatic levels of GSH, due to the ability of NaF to break hydrogen bonds in proteins, as well as increasing the mitochondrial generation of ROS resulting in oxidative stress, mitochondrial DNA damage and eventually cell death. The histopathological observations support the mentioned findings as the intoxicated group showed marked hepatic necrosis and a pyknotic nuclei in both light and TEM sections and it's in agreement with **[43]**. Administration of *L.casei* to intoxicated rats with NaF showed lower concentrations of MDA and potentiate the production of GSH. The ability of *L. casei* to inhibit the elevation in LPO may be the cause of its antioxidative effects. This ability stabilizes cellular membrane integrity and prevents or at least reduces hepatic enzyme leakage as revealed by biochemical analysis here and in previous literature **[44]**. This group also showed the same improvement in TEM sections as the hepatocyte appear almost normal.

Another goal of our research was to learn more about how *L.casei* affects the generation of NO, another significant oxidative stress mediator. Numerous investigations have discovered that NO has a contradictory function in tissues **[45]**. Our results showed intoxicated rats with NaF possess significant increase in NO that capable of reacting with superoxide to create peroxynitrite and causing tissue damage and it is in agreement with **[46]**. Treatment of NaF intoxicated rats with *L.casei* in our study results in a marked reduction in the generation of hepatic NO and it's in line with **[47]**.

In an attempt to elucidate the mechanism of *L.casei* in ameliorating the inflammatory cascade associated with NaF intoxication. Our study further examined the role of NF-κB signaling pathway in the liver of rats intoxicated with NaF. In the cytoplasm , NF-κB is usually conjugated to an inhibitory protein I-κB, where phosphorylation reaction occurs and leads to breakdown of I-κB that results in translocation of NF-κB to the nucleus

to activate the transcription of numerous genes of inflammation **[48]**. Our results confirmed that NaF intoxication resulted in increased the levels of NF-κB. Conversely, administration of *L.casei* could inhibit the phosphorylation of I-κB leading to decreased expression levels of NF-κB. Prior findings have revealed that treatment with lactobacilli could inhibit the signaling of NF-κB in lipoplysachharides model **[49]**. This study reports for the first time that *L.casei* supplementation reduces hepatic NF-κB activity in rats intoxicated with NaF. Intoxication activates multiple pathways, including up-regulation of NF-κB pathways and the production of pro-inflammatory cytokines TNF-α and IL-6, in addition to decreased antioxidant status and increased lipid peroxidation. TNF-α controls NF-κB signaling, resulting in the production of other cytokines responsible for involved in adhesion, cell proliferation, and inflammation **[50]**. Our findings reported a marked elevation in the hepatic contents of TNF-α and IL-6 in rats intoxicated with NaF. The results of the current investigation demonstrated that *L.casei* treatment considerably reduced the levels of TNF- and IL-6 in the liver of treated rats. Our findings are matching with a prior study where *L. plantarum* administration could decrease the levels of TNF-α in rats **[51]**. *L.casei* plays an important role in the downregulation of TNF-α and il-6 expression, as shown by the correlation between decreased NO levels and decreased TNF- and il-6 expression.

Previous studies have suggested that excessive F intake can induce generation of ROS and subsequently apoptosis **[52, 53]**. Caspases are present in cells as inactive zymogens and undergo a series of catalytic activation at the beginning of apoptosis **[53]**. Therefore, the activity of caspase-3 could be checked to detect apoptosis. Intoxicated rats with NaF showed an elevation in caspase-3 and this confirms the conclusion that fluoride induces apoptosis. Similar findings have been observed in Wistar rats intoxicated with NaF **[54]**. Fluoride-induced apoptosis may be caused by enhanced lipid peroxidation, oxidative stress, mitochondrial functional issues, downstream pathway activation, and imbalanced signals, according to the findings of previous study **[55]**. Administration of *L.casei* to intoxicated rats with NaF could reduce the elevated levels of caspase-3, due to its role in quenching the generation of ROS and restore the levels of GSH in the hepatic tissue of NaF intoxicated rats. Our findings are in line with prior study **[56]**.

The present findings were supported by histopathological examinations, which also offered crucial support for the biochemical study. When examined under a microscope, rats given NaF showed significant histological alterations, such as rupturing of the hepatic cords, vascular enlargement, and obstruction, Kupffer cell growth, and inflammatory cell infiltration. The hepatocytes additionally showed vacuolization, pyknosis, necrosis, and total lysis. Similar histological alteration have been reported by many investigators in liver of guinea pig intoxicated with NaF **[57] ,** rabbit **[58]** and rats **[59].** The treatement with *L.casei* could alleviate all these histopathological changes.

The ultrastructural changes of hepatic cells in the NaF-treated group confirmed the light microscopic findings of NaF toxicity, as shown in the current results. Furthermore, significant morphological features, particularly structural membrane damage of mitochondria, endoplasmic reticulum, nuclear, and plasma membranes, were identified. This study's data confirmed the findings of previous study **[60]**.

Finally, oral administration of *L. casei* (figure.10.) could ameliorate oxidative stress and inflammation caused by NaF induced- hepatic injury in rats. This was likely accomplished by lowering peroxidation levels, inflammatory markers and/or boosting the liver's enzymatic antioxidants' activity. Our research suggests administration of *L.casei* on a daily basis to

counteract the negative effects of fluoride-containing compounds.

6. Conflict of Interest

The authors declare no conflicts of interest exist.

7. Author contributions

Ahmed A. Sedik performed the experiments and was a major contributor in writing the manuscript. Ahmed A. Sedik analyzed and interpreted the raw data. Soha A. Hassan shared in histopathology and ultrastructural studies. All authors read and approved the final manuscript.

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