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ORIGINAL ARTICLE

# Ameliorative or corrective effects of Fig "*Ficus carica*" extract on nickel-induced hepatotoxicity in Wistar rats

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## Abstract

Many heavy metals and metalloids (e.g., Pb, Cd, and Ni) can contaminate the environment and cause severe health problems. Through this study, investigated the possible corrective effects of *Ficus carica* extract (FCE) against nickel (Ni) induced stress response and damage on the liver of rats. Male Wistar rats were divided into four groups (8 rats per group) and co-treated with FCE (350 mg/kg) and exposed to Nickel chloride (10 mg/kg) for 4 weeks. The volatile compounds of FCE were characterized by solid phase micro-extraction (SPME) coupled with GC–MS, and the biochemical parameters of stress were determined. The SPME–GC/MS analysis of FCE indicated the presence of thirty (30) phyto-bioactive compounds including alcohols, aldehydes, organic acids, ketones, furans, terpenes, ester and others. The best capacity for scavenging DPPH free radicals and metal chelating were found with the $IC_{50}$ values of 0.49 and 2.91 mg/mL, respectively. Ni induced damage to various macromolecules. Malondialdehyde, protein carbonyls, alanine aminotransferase and gamma glutamyl transferarse levels were significantly increased in Ni exposed group compared to control group and co-treatment with FCE reduced the levels of these parameters. In conclusion, current findings showed that Ni-induced oxidative damage and the administration of FCE can improve correct and restore the alteration in the rat liver.

**Keywords** Nickel · *Ficus carica* · Oxidative stress · Liver · Wistar rats

## Introduction

Humans are exposed-daily to xenobiotics, drugs, and various toxic products [1]. Heavy metals mainly accumulate in the environment due to anthropogenic activities which may cause a number of disorders to both humans [2] and terrestrial biota [3]. Heavy metal pollution is becoming a potential harmful risk to human health. These xenobiotics induced the toxic effects and damage to the all organs and the liver is the most affected amongst them. Exposure to heavy metals causes lipid and protein oxidation, and oxidative DNA damage [2]. The metallic compounds of nickel (Ni) have many industrial applications because of their chemical and physical properties. Their concentrations have increased as a result of metal exploitation and industrial development [4].

Industrial waste is the main source of Ni contaminants in the environment. Various harmful effects of environmental Ni exposure and its compounds have been reported in humans [5], and considered as a type 1 carcinogen by the International Agency for Cancer Research [6]. Human exposure to nickel occurs primarily via inhalation and ingestion [7]. Drinking water and food are the two main sources of Ni exposure in humans and animals [8]. The environmental exposition of Ni induced the toxic effects in various organs [9, 10], such as immunotoxicity [11], and genotoxicity [12]. Ni intoxication results in loss of body weight and respiratory disorders, increased serum levels of kidney function biomarkers indicating the development of kidney failure [13]. Ni substitutes the metals (especially zinc) in the catalytic centers of enzymes, resulting alteration in the proteins function [14]. The liver is the primary organ managing

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homeostasis in the body, where metabolism and detoxification happen. It also plays a significant role in drug and heavy metals detoxification. Hepatotoxicity induced by Ni may be caused mainly by the oxidative stress reactions [15]. In vivo and in vitro studies revealed that Ni enhanced lipid peroxidationn, protein oxidation and DNA damage [9, 16].

In recent years, the genus *Ficus* has received more attention for their potential uses in the treatment and preventing diseases. These effects are linked to the antioxidant contents and various bioactive compounds. *Ficus carica* L. (Moraceae) is commonly known as figs. Leaves, fruits and latex of *F. carica* have been used as a source of food and health [17]. It's reported that figs contain high amounts of fiber and polyphenols [18]. Its content can play a significant role in protecting the liver tissue from injury and considerably increase plasma antioxidant capacity [18], and considered as an excellent source of nutrients [19], traditionally used for their therapeutic effects as a laxative and anti-inflammatory remedies [20]. Hepatoprotective, hypoglycemic, antifungal, antioxidant and antimutagenic activities have been reported [17, 21–24]. The current study was established to evaluate the potential ameliorative effect of Figs against oxidative stress induced by Nickel through biochemical assay and histopathology study.

## Materials and methods

### Reagents and chemicals

Nickel chloride ($NiCl_2$ 98% purity) was purchased from Sigma–Aldrich Chemical Co (St. Louis, MO, USA). Folin-Ciocalteu reagent, gallic acid, methanol, hydrochloric acid (37%), sulphuric acid ($H_2SO_4$), sodium carbonate ($Na_2CO_3$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), quercetin, polyvinyl polypyrolidone (PVPP), pyrogallol, and ferric chloride 6-hydrate were purchased from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH, 98%), 3-(2-Pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), iron(II) chloride ($FeCl_2$), ethylenediamine-tetra acetic acid (EDTA), bovine serum albumin (BSA), thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), and 2,4-dinitrophenylhydrazine (DNPH) were obtained from Sigma–Aldrich (Steinheim, Germany). All other chemicals used were analytical grade and were obtained from Merck, US.

### Plant material and extract preparation

Fig (*Ficus carica* L.) variety "*taamriout*" was collected from Ain Karma (Oran, Algeria). They were identified taxonomically and authenticated by the Herbarium of Botany Directorate in Ahmed Ben-Bella Oran 1 University (voucher specimen N° LB 0695). Preparation of fig fruit extract (FCE) was achieved by the method of Oliveira et al. [19]. Powder (50 g) was boiled for 15 min filtered and lyophilized.

### Determination of total phenol, flavonoids and tannins content

The Folin-Ciocalteu colorimetric method was used to assay the total phenolic content as described by Singleton and Rossi [25], and expressed as gallic acid equivalent per g dry extract. Using the aluminum chloride colorimetric assay according to Kim et al. [26], flavonoid contents were measured against the blank at 510 nm and expressed as mg quercetin equivalent $g^{-1}$ dry extract. The tannin content was determined according to the method described by Julkunen-Tiitto [27] and the results were expressed as mg catechin equivalent per g dry extract.

### Determination of volatile compounds: SPME extraction and GC–MS analysis

The volatile compounds of *Ficus carica* extract (FCE) were characterized by solid phase micro-extraction (SPME) coupled with GC–MS according to the method of Arthur and Pawliszyn [28] with slight modifications. The SPME fiber used was divinylbenzene (DVB), carboxen (CAR), polydimethylsiloxane (PDMS) (50/30 µm DVB/CAR/PDMS) (Supelco, Bellefonte, USA). The filter was preliminary conditioned at 250 °C for 1 h before each use. After extraction, SPME fiber was desorbed in the GC apparatus. Analyses of volatile compounds were performed on an Agilent Technologies 7890A GC9 System, and compounds were separated on a VF-WAXms column (Agilent Technologies, USA; 30 m × 0.250 mm I.D, × 0.25 µm film thickness). The identification of the compounds was performed on the basis of chromatographic retention indices (RI) and by comparison of the recorded spectra with a Pal 600 K® mass spectral database. A sample was analysed in triplicate and expressed in terms of relative peak area.

### In vitro activity

#### DPPH scavenging activity

*Ficus carica* extract (FCE) were tested for the scavenging effect on the DPPH radical according to Heimler et al. [29]. Briefly, 10 µL of the extract solutions was added to 1 mL of DPPH solution (4%), and incubated for 30 min. The absorbance was recorded at 515 nm. A control containing only DPPH solution is used as blank and the vitamin C as standard. The DPPH scavenging effect was calculated by the following formula:

$$\% \text{ scavenging effect} = \left[\left(A_{\text{blanc}} - A_{\text{sample}}\right)/A_{\text{blanc}}\right] \times 100.$$

#### Ferrous ion chelating capacity assay

The ferrous ion chelating of the extracts was evaluated by the method of Decker and Welch [30]. Briefly, 2 mL of extract (100 µg/mL) was added to 0.2 mL of 5 mM ferrozine and 0.1 mL of 2 mM $FeCl_2$ solution. The absorbance was recorded at 562 nm. The percentage of inhibition of ferrozine-$Fe^{2+}$ complex formation was calculated by the formula:

$$\% \text{ scavenging activity} = \left[\left(A_{\text{CT}} - A_{\text{FCE}}/A_{\text{S}}\right)/A_{\text{CT}}\right] \times 100.$$

Where, $A_{\text{CT}}$ was the absorbance of control reaction and $A_{\text{FCE}}$ was the absorbance of extract. $A_{\text{S}}$ was the Absorbance of standard (100 µg EDTA/mL).

#### Animals and experimental design

A total of 32 healthy male Wistar rats (*Rattus norvegicus*), weighing 165 ± 5 g, were maintained under following conditions (12 h light/dark cycle, 23 ± 1 °C, relative humidity 50 ± 15%), food and water ad libitum. All experiments were conducted in accordance with the ethical principles and institutional guidelines of the National Institutes of Health Guide for the care and use of laboratory animals.

Animals were randomly divided into 4 groups (8 rats per lot): Group "C": untreated control; Group "FC" treated with FCE (350 mg/kg BW) [21, 31] by gavage; Group "Ni": intoxicated group received dose of 10 mg Nickel chloride /kg body weight (BW) by intraperitonial injection (i.p), the exposure dose was selected based on the work of Das and Buchner [32]; Group "Ni + FC": Co-treatment with Ni and FCE for 4 weeks. At the end of the experiment, the rats were sacrificed and the liver was collected, rinsed and weighed. The samples and aliquots were stored at − 80 °C until analysis.

**Serum transaminases and ALP activities assay** Serum liver biomarkers: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were estimated using diagnostic kits (ChronoLab, Spain) according to the manufacturer's protocol.

#### Proteins measurement

Protein concentrations in homogenates were determined following the method of Bradford [33] with bovine serum albumin (BSA) as a standard.

### Antioxidant enzymes assay

#### Determination of catalase activity

Catalase activity was estimated by the method of Aebi [34]. The absorption was monitoring at 240 nm. Specific enzyme activity was expressed in nmoles of $H_2O_2$ consumed/min/mg of protein.

#### Determination of superoxide dismutase (SOD) activity

SOD activity was determined by the pyrogallol assay according to the method of Marklund and Marklund [35]. One unit of (Cu–Zn) SOD activity was defined as the amount of the enzyme required causing 50% inhibition of pyrogallol autoxidation at 25 °C.

#### Measurement of malondialdehyde (MDA)

The MDA level was estimated by the method of Yagi [36] and Ohkawa et al. [37], and expressed as nmol per mg of protein.

#### Measurement of protein carbonyls

The oxidative proteins damage was determined by the method of Levine et al. [38]. The protein carbonyl content was expressed as nmol per mg of protein.

#### Histopathological studies

Fresh tissue pieces of liver were instantly immersed and fixed in 10% formalin, dehydrated through graded alcohol, embedded in paraffin, sliced and stained with haematoxylin and eosin.

### Statistical analysis

The results obtained were analyzed by the one-way analysis of variance (ANOVA) followed by Tukey-multiple comparison test using SPSS program (version 23). A value of $p < 0.05$ was considered as statistically significant.

## Results

### Phenolic components and volatile compounds content

The results of total polyphenols and flavonoids in the FCE showed high amount estimated at 100.83 and 195.61 mg gallic acid equivalent per g dry extract, respectively. While the tannin dosage result showed a value of 0.75 mg of

catechin equivalent per gram of dry extract (mg EQC/g of dry extract). The SPME/GC–MS analysis of the FCE indicated the presence of 30 different compounds, including alcohols, aldehydes, organic acids, ketones, furans, terpenes, ester and others. The identified compounds are represented in the Table 1.

**Table 1** Volatile compounds identified in *Ficus carica* analyzed by SPME-GC/MS

| Compound name | RI[a] | RI[b] | Peak area |
|---|---|---|---|
| Alcohols | | | |
| 1. 2-cyclohexen 1 ol | 1713.6 | 1713 | 0.79 ± 0.08 |
| 2. Benzyl alcohol | 1866.2 | 1866 | 0.47 ± 0.13 |
| 3. 1-ethyldioxyindol | 1919.8 | – | 0.05 ± 0.01 |
| 4. 2-methoxy-4-vinyl-phenol | 2181.0 | 2181 | 0.27 ± 0.03 |
| Aldehydes | | | |
| 5. 2-butanal | 1028.8 | 1032 | 0.15 ± 0.06 |
| 6. Hexanal | 1071.5 | 1071 | 1.11 ± 0.38 |
| 7. 2-hexanal | 1211.0 | 1211 | 0.32 ± 0.13 |
| 8. Octanal | 1283.0 | 1283 | 0.25 ± 0.05 |
| 9. Benzene butyl | 1302.7 | 1304 | 52.35 ± 12.56 |
| 10. Nonanal | 1387.6 | 1387 | 0.59 ± 0.08 |
| 11. Octenal | 1420.4 | 1420 | 0.65 ± 0.29 |
| 12. Furfural | 1461.0 | 1461 | 1.41 ± 0.94 |
| 13. 2,4 Heptadienal | 1485.4 | 1485 | 0.42 ± 0.11 |
| 14. Benzaldehyde | 1509.4 | 1509 | 24.05 ± 0.39 |
| 15. 2 Furancarboxaldehyde | 1566.8 | 1566 | 0.11 ± 0.01 |
| 16. Furancarboxaldehyde | 2485.3 | 2485 | 1.76 ± 0.88 |
| 17. Vanilin | 2536.4 | 2538 | 0.31 ± 0.04 |
| Ketones | | | |
| 18. 3-octanone | 1248.2 | 1248 | 0.18 ± 0.04 |
| 19. 3,5 octadiéne -2-one | 1562.1 | 1562 | 0.05 ± 0.01 |
| 20. 2-cyclohexen 1-one | 1713.6 | 1713 | 0.24 ± 0.02 |
| Furans | | | |
| 21. 2(3H)-Furanone | 1611.6 | 1611 | 0.04 ± 0.02 |
| 22. 2-Furan methanol | 1660.4 | 1655 | 1.23 ± 0.75 |
| Acids | | | |
| 23. Acetic acid | 1450.4 | 1450 | 1.84 ± 0.73 |
| 24. Hexadecanoic acid | 2211.3 | 2210 | 0.05 ± 0,02 |
| 25. Myristic acid | 2699.2 | 2699.2 | 0.34 ± 0.17 |
| 26. Acid palmitic | 2898.0 | 2899 | 4.07 ± 0.05 |
| Terpenes | | | |
| 27. I-limonene | 1185.9 | 1183 | 0.55 ± 0.10 |
| 28. caryophyllène | 1573.6 | 1573 | 0.82 ± 0.08 |
| Other | | | |
| 29. Eugenol | 2151.4 | 2151 | 0.737 ± 0,009 |
| 30. 2,3 dihydroxy-6-methyl-4H-pyran-4 one | 2249 | 2240 | 3.04 ± 1.90 |
| Identified compound (%) | 98.35% | | |

RI[a]: Calculated retention index

RI[b]: Theoric retention index

## Antioxidant capacity of Ficus carica extracts (FCE)

The antioxidant capacities of the tested concentrations (0.1–0.8 mg/mL) of FCE were evaluated by the most commonly used antioxidant assays: DPPH and Ferrous iron chelating methods. In the DPPH method, the results indicated that the FCE showed the inhibition values (except standard) ranged from 40.61 to 49.26%, with the $IC_{50}$ values for scavenging activity of 495 µg/mL vs 142 µg/mL for ascorbic acid. The percentages of radical scavenging activities of the FCE at different concentrations within the range of 0.1–0.5 mg/mL are illustrated in Fig. 1. The chelating ability of ferrous ions of FCE was shown in Fig. 2. The absorbance of $Fe^{2+}$—ferrozine complex was observed to be linearly decreased with the concentration of the extract and EDTA (from 0.2 to 0.8 mg/mL). The results indicated that the extract has a good chelating activity compared to the positive control "EDTA". The percentages of the metal chelating assay at higher concentration

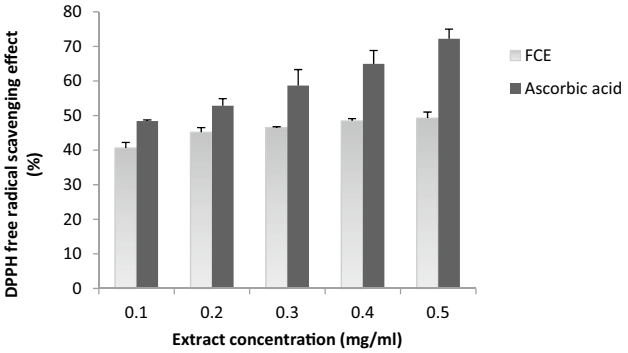



**Fig. 1** The scavenging effect on the DPPH radical of *Ficus carica* extracts at different concentrations compared with ascorbic acid. The values are given as mean ± SD ($n = 3$)

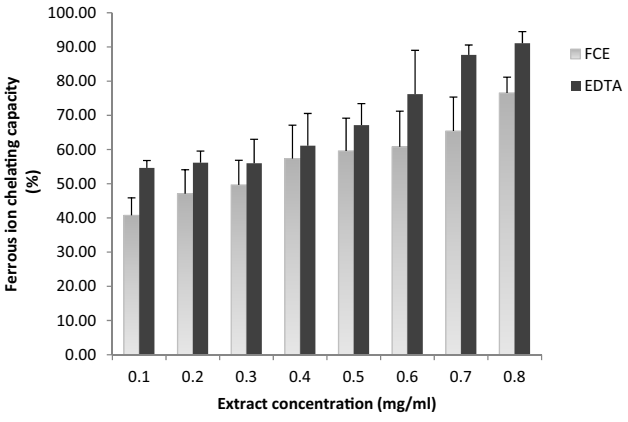



**Fig. 2** Iron chelating activities of *Ficus carica* extracts (FCE) at different concentrations compared with EDTA. The values are given as mean ± SD ($n = 3$)

**Table 2** Effects of *Ficus carica* extract (FCE) and Nickel chloride on the body weight, body weight gain, liver weight and liver body weight ratio (%) in control and experimental groups

| Parameters | Groups | | | |
|---|---|---|---|---|
| | Control | FC | Ni | Ni + FC |
| Initial body weight (g) | 164.57 ± 3.01 | 162.33 ± 2.65 | 166.06 ± 1.10 | 165.62 ± 1.79 |
| Final body weight (g) | 241.50 ± 6.5 | 280.33 ± 7.68** | 233.4 ± 6.94 | 291.66 ± 11.89### |
| body weight gain (g) | 82.50 ± 10.5 | 125.43 ± 8.62** | 67.00 ± 7.5 | 128.33 ± 7.96### |
| Liver weight (g) | 7.06 ± 0.18 | 7.27 ± 0.26 | 6.41 ± 0.15 | 7.36 ± 0.47 |
| Liver-body weight Ratio | 0.029 | 0.025 | 0.026 | 0.025 |

Each value is mean ± SD of eight observations in each group. Analysis of data was done by one-way ANOVA and post-hoc by Tukey test. Group "C": control, untreated group; Group "FC" treated with FCE (350 mg/kg BW) by gavage; Group "Ni": intoxicated lot with 10 mg Nickel chloride /kg body weight (BW) by intraperitonial injection (i.p); Group "Ni + FC": Co-treatment with Ni and FCE for 4 weeks

The * depicts comparison with group "C" (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), #depicts comparison with group "Ni" (#$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$)

**Table 3** Effects of *Ficus carica* aqueous extract (FCE) and Nickel chloride on the biochemical parameters in the control and experimental groups

| Parameters | Groups | | | |
|---|---|---|---|---|
| | Control | FC | Ni | Ni + FC |
| GGT (g/L) | 0.45 ± 0.23 | 0.91 ± 0.31 | 1.24 ± 0.12*** | 0.81 ± 0.23 |
| ALP (U/L) | 76.15 ± 7.25 | 80.50 ± 3.35 | 84.10 ± 2.90 | 75.11 ± 3.20 |
| AST (U/L) | 87.52 ± 1.95 | 79.53 ± 5.57 | 103.13 ± 5.56*** | 98.47 ± 3.00 |
| ALT (U/L) | 21.78 ± 1.13 | 23.06 ± 1.50 | 28.38 ± 0.62*** | 21.36 ± 0.86 ### |
| TP (g/L) | 52.36 ± 4.01 | 54.60 ± 1.18 | 57.10 ± 1.55 | 62.54 ± 0.93 |

Each value is mean ± SD of eight observations in each group. Analysis of data was done by one-way ANOVA and post-hoc by Tukey test. Group "C": control, untreated group; Group "FC" treated with FCE (350 mg/kg BW) by gavage; Group "Ni": intoxicated lot with 10 mg Nickel chloride /kg body weight (BW) by intraperitonial injection (i.p); Group "Ni + FC": Co-treatment with Ni and FCE for 4 weeks

*GGT* gamma glutamyl transferase, *ALP* alkaline phosphatase, *AST* aspartate transaminase, *ALT* alanine transaminase, *TP* total protein

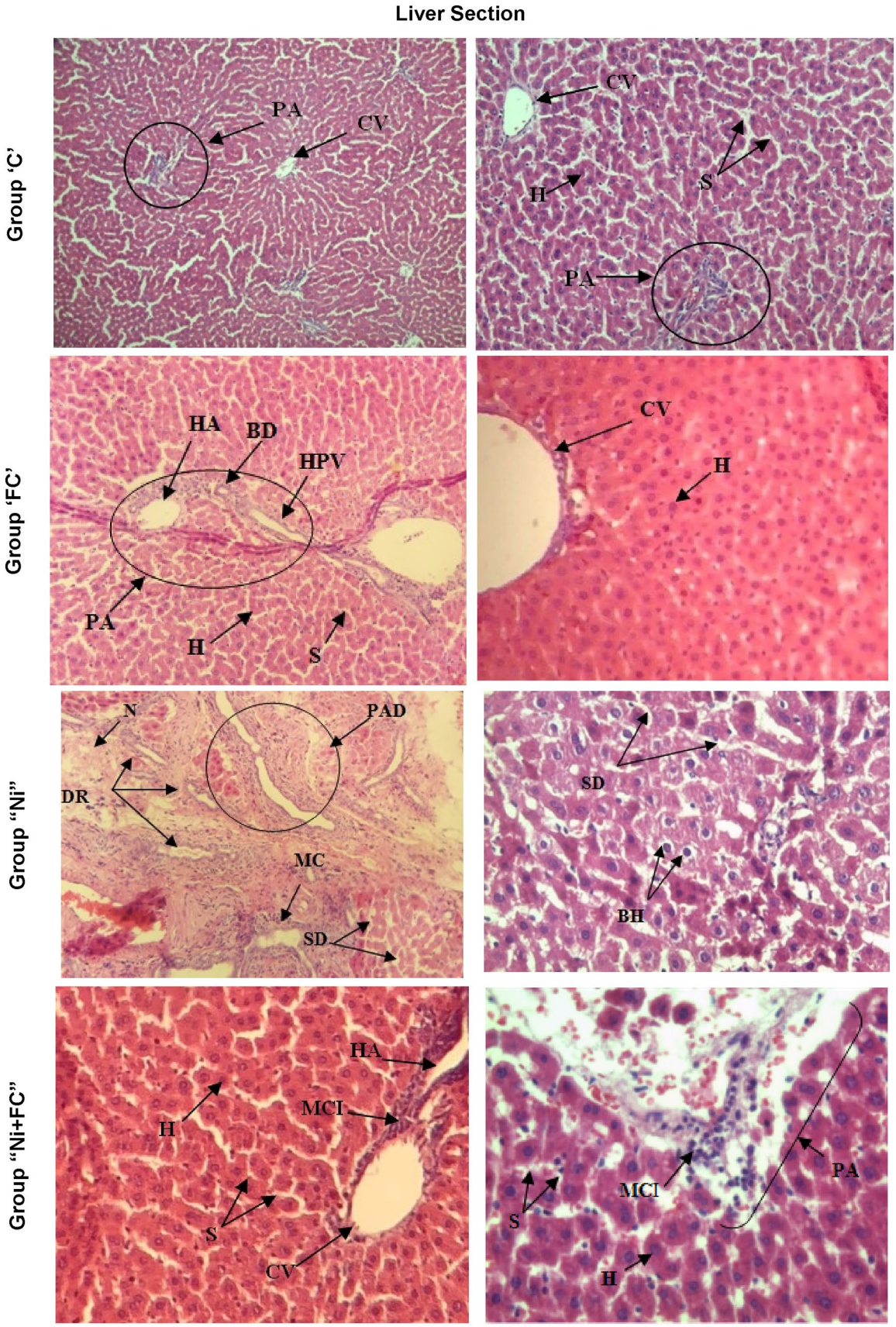
The * depicts comparison with group C (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; #depicts comparison with group Ni (#$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$)

**Table 4** Effects of *Ficus carica* aqueous extract (FCE) and nickel chloride on the antioxidant enzyme activities (catalase, CAT; superoxide dismutase, SOD), malondialdehyde (MDA) and carbonyls levels in control and experimental groups

| Parameters | Groups | | | |
|---|---|---|---|---|
| | C | FC | Ni | Ni + FC |
| CAT (mmol $H_2O_2$/mg of protein) | 366.47 ± 27.54 | 537.87 ± 16.13*** | 497.34 ± 35.79*** | 515.89 ± 31.75*** |
| SOD (U/mg of protein) | 862.12 ± 77.79 | 1255.61 ± 103.24*** | 735.11 ± 45.92 | 1362.03 ± 25.2### |
| MDA (nmol/mg of protein) | 1.41 ± 0.38 | 1.84 ± 0.09 | 2.43 ± 0.19*** | 1.62 ± 0.02### |
| Carbonyls (nmol/mg of protein) | 3.77 ± 0.51 | 2.25 ± 1.18 | 18.58 ± 7.22*** | 4.45 ± 0.96### |

Each value is mean ± SD of eight observations in each group. Analysis of data was done by one-way ANOVA and post-hoc by Tukey test. Group "C": control, untreated group; Group "FC" treated with FCE (350 mg/kg BW) by gavage; Group "Ni": intoxicated lot with 10 mg Nickel chloride /kg body weight (BW) by intraperitonial injection (i.p); Group "Ni + FC": Co-treatment with Ni and FCE for 4 weeks

The * depicts comparison with group C (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), #depicts comparison with group Ni (#$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$)


Liver Section
Group 'C'
PA
CV
CV
H
S
PA
Group 'FC'
HA
BD
HPV
PA
H
S
CV
H
Group "Ni"
N
PAD
DR
MC
SD
SD
BH
Group "Ni+FC"
HA
H
MCI
S
CV
PA
MCI
S
H

◂**Fig. 3** Effect of Nickel Chloride and *Ficus carica* aqueous extract (FCE) on the histological liver structure in control and experimental animals. *BD* bile duct; HPV, hepatic portal vein; *HA*, hepatic artery; *CV* central vein, *H* hepatocytes, *S* sinusoidal spaces, *PA* Portal artery, *MC* Mononuclear cells, *N* Necrosis, *DR* ductular reaction, *SD* sinusoidal dilatation, *PAD* portal area dilatation; *BH* Ballooned hepatocytes. Group "C": control, untreated group; Group "FC" treated with FCE (350 mg/kg BW) by gavage; Group "Ni": intoxicated lot with 10 mg Nickel chloride /kg body weight (BW) by intraperitonial injection (i.p); Group "Ni + FC": Co-treatment with Ni and FCE for 4 weeks

of testing FCE and EDTA were found to be 76.54% and 91.09% respectively.

### Effect of treatment on body weight and liver

The variations in the liver weight, body weight and liver body weight ratio of the rats subjected to different treatments are shown in Table 2. It observed that treatment with nickel chloride for one month induced a no significant variation in all this parameters compared to control group. But the FCE administrated rats showed progressive and significant ($p < 0.001$) increase in the body weight (+ 24%) and the body weight gain (+ 91%) in the Ni + FC group compared to the Ni group. The results showed also a significant ($p < 0.01$) increase of body weight gain (+ 52%) in FC group compared to untreated group. No significant difference in liver body weight ratio and liver weight was observed in rats of the different experimental groups.

### Effect of treatment on biochemical analysis

Table 3 showed the mean values of the biochemical indicators of liver function of the control and experimental groups. The levels of GGT and ALT were significantly ($p < 0.001$) increased in Ni group compared to control. A significant diminution ($p < 0.001$) (− 25%) induced by FC administration was only noted in ALT level in Ni + FC group compared to Ni group.

### Effect of treatment on the antioxidant enzyme activities

The activity of catalase was significantly ($p < 0.05$) increased by + 35% in the Ni group compared to the rats of the control group (Table 4). No significant variation in SOD activity was observed in this group. In contrast, administration of FCE was significantly ($p < 0.001$) increased SOD activity by + 85% in the Ni + FC group compared to the Ni group. A significant ($p < 0.01$) increase was also observed in the CAT and SOD activity in the FC group compared to the untreated group.

### Effect of treatment on the lipid peroxidation and carbonyls levels

Changes in MDA and carbonyls levels were illustrated in Table 4. The result showed a significant ($p < 0.001$) increase in MDA level in Ni group compared to control. There was also a 4.92-fold increase in carbonyl levels in this group compared to the control rat. The administration of FCE induce an improvement very significantly ($p < 0.001$) in MDA and carbonyls levels.

### Effect of treatment on histological changes

Microscopic examination of hepatic section (Fig. 3) revealed normal hepatic parenchyma architecture in the control and FC group (Table 5). However, in exposed group to Ni (Fig. 3, group "Ni") was observed dilatation in the portal area, deformed cellular organization, hypertrophic cells, and inflammation accompanied with the widening of intracellular sinusoids. The administration of FC extract ameliorates liver histology (Fig. 3, group "Ni + FC").

## Discussion

The fruits and vegetables, rich in antioxidants and different micronutrients, defend against varied types of xenobiotics induced hepatic injury and DNA damage [39]. Nickel is a potent toxicant with ability to disturb the cellular antioxidant defence system, induced damage to cell membranes [40]. In the present study we examined the possible protective effects of the FCE against Ni-induced hepatotoxicity in rats, and also we explored the antioxidant and the chelating activity of FCE in vitro using DPPH and ferrous ion-chelating assays.

The phytochemical composition reported by Debib et al. [41] indicates that figs were richer in polyphenols like gallic acid, chlorogenic acid and syringic acid. They also are abundant in flavonoids, specially -catechin, -epicatechin, and rutin [42], and anthocyanins [43]. It's known that polyphenols and flavonoids are able to reduce the hepatotoxicity induced by the xenobiotics [44]. Their positive effects can be linked to the inhibitory action on the free radical production [44]. The results of the phenolic components obtained are in agreement with those of Gilani et al. [45] who revealed that the aqueous extract of figs contains polyphenols, flavonoids, coumarins and terpenes. Similarly, the study of Veberic et al. [46] indicates that the figs harvested in the coastal zone to the north of the Mediterranean are very rich in flavonoid and phenolic acids. The flavonoids, a major constituent of our extract, have a wide spectrum of biological activities and chemical structure, including anti-free radical properties and contribute significantly to the taste, color, astringent flavors and aroma of figs [46]. Thirty (30) compounds have

**Table 5** Changes in the hepatocellular organization induced by *Ficus carica* extract (FCE) and Nickel chloride in control and experimental animals

| | Control and FC group | Ni group | Ni+FC group |
|---|---|---|---|
| Hepatocellular changes | Normal hepatic parenchyma architecture. The liver organ is composed of lobules which are roughly hexagonal in shape, with portal triads at the vertices and a clear central vein (CV) characterized by polygonal hepatocytes (H), which are separated by sinusoidal spaces (S) | Deformed cellular organization accompanied with distortion of the normal hepatic architecture, hypertrophic cells, mononuclear cell infiltration (MCI) extending into the hepatic parenchyma, appearance of a ductuliary reaction (DR) and a focal area of necrosis (N) widening of intercellular sinusoids (SD) with a granular aspect of the cytoplasm and congestion and dilatation in the portal area (PAD) | Showing marked decreased in portal mononuclear cell infiltration (MCI), which remains limited in the portal spaces; normal hepatocytes with mild portal inflammation. The liver organization appears normal with decreased widening of blood sinusoid (S), less lighting of the cytoplasm |

been identified by SPME GC–MS and distributed by distinct chemical classes (ketones, aldehydes, alcohols, furans acids, terpenes esters, and others). Our results are in accordance with those of Russo et al. [47] who identified 42 volatile compounds in white figs from southern Italy using the same technique compared to 55 compounds identified in Turkish figs; other studies have reported that figs contain between 46 and 59 volatile compounds [48]. Our data have shown that white figs of the "taamiriout" variety are distinguished by their abundance of aldehyde, among which are benzaldehyde (anti-carcinogenic and antimicrobial agent) and 2-methoxy-4-vinylphenol used as an antioxidant [49]. Vanillin is an aromatic aldehyde with antioxidant effect, followed by acid-type compounds, including palmitic acid and myristic acid. Our chromatogram also shows the presence of terpene compounds; limonene which is a powerful anti inflammatory agent with antioxidant and anticancer properties [50] and caryophyllene with analgesic and anti-inflammatory activity [51].

The FCE modulates the free radical DPPH compared with vitamin C. The content of FCE on the phenolic compounds may be related with their considerable activity of radical-scavenging. However, the structural variability of the phenolic compounds is extremely linked to the number and arrangements of hydroxyl groups [52]. Our results confirmed that FCE show the highest amount of antioxidant capacity. This ability is also seen in some other Ficus species like *Ficus bengalensis* and *Ficus racemosa* stem, bark, and fruit extracts. We have obtained that FCE has nearly the same chelating activity compared with EDTA. Viuda-Martosa et al. [53] reported that the Fig co-products obtained from peel showed higher ion-chelating activities at all concentrations and each cultivar studied. Concerning the in vivo analysis, our results don't show any significant variation in the liver weight, body weight gain and liver body weight ratio in animals exposed to nickel (Ni group) compared to control. Several studies indicate that nickel induces a decrease in the body weight gain and liver weight [54, 55], and combine this reduction to a lower food consumption [29], hormonal imbalance, and a decrease in protein synthesis [56]. The FCE administration induced a significant gain in body weight gain which may be due to an increased appetite and promotion of protein synthesis. No significant improvement is observed for liver weight and liver body weight ration after administration of FCE. These results showed also that Ni pretreatment increased the serum transaminases (ALT) and GGT. Sindhu et al. [57] reported similar results, and Winter et al. [58] observed that high levels of liver enzymes show hepatocellular injury. After FCE administration, no significant improvement was showed in the markers of liver function except for the activity of ALT, which recorded a

significant decrease in treated rats compared to pre-intoxicated and untreated rats.

A similar study proved that the administration of dried Fig supplements imparts protection against ethanol-induced oxidative injury [59]. We show also a significant increase in carbonyls and MDA levels in Ni- pre-intoxicated groups, which is the consequence of increased lipid peroxidation and proteins oxidation. Our results were in agreement with Pari and Amudha [60] who have shown that Ni-intoxication induces ROS formation leading to alterations in the redox status of several tissues in rats and mice. In the current study, a significant decrease in protein carbonyl and MDA level is shown after stopping metal intoxication and starting FCE administration. The antioxidant enzymes are the primary line of defence against oxidative stress that prevents bio-molecules from oxidative injury inside the cell. It's proved that Ni disturbs significant the antioxidant enzyme activities. The results confirmed that nickel treatment induced a significant increase in CAT activity contrary to the study of Hfaïedh et al. [61] which indicates that nickel induces the decrease in the activity of antioxidant enzymes due to the inhibition of free radicals [44]. Conversely, the treatment with FCE had a potent protective effect against liver damage caused by nickel. These results were already found by Saoudi and El Feki [62] which confirms that FCE increased the SOD activity after a hepatic damage. These results indicated that FCE decreases oxidative stress and the toxic effect of Nickel in liver tissue.

The liver histological structure of the Ni-intoxicated group showed significant typical morphological changes to those reported in the literature such as the dilatation, cytoplasmic vacuolization, inflammatory cell infiltration, sinusoidal dilatation, cell necrosis, and cellular hypertrophy. This is possibly due to the formation of highly reactive radicals because of oxidative threat induced by nickel. Rao et al. [56] reported that Ni-induced several changes in the histological structure characterized by dilated sinusoids, vacuolization and distorted nuclei. However, the liver of the rats treated with FCE had architecture nearly comparable to the control group, except some cell infiltration limited in central vein. FCE administration ameliorates the histological structure of the liver. Aghel et al. [63] showed that treatment with the FCE resulted in the less pronounced destruction of the liver architecture. Our study demonstrates that the administration of FCE could correct and accelerate the capacity of liver rat to regenerate after Ni-induced oxidative stress [59].

In conclusion, the present study showed that Nickel chloride induced histological and biochemical liver damage and altered antioxidant defense system and the administration of FCE accelerate the improvement of the parameters and liver regeneration. FCE increase the activities of antioxidant enzymes and decrease oxidative stress. These results suggested that FCE has a protective effect on hepatotoxicity induced by nickel. This protection is obviously due to proactive molecules and antioxidants in the Fig fruits tested in vitro and represent promising natural tools against Nickel toxicity.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethics approval** All animal experiments were conducted in accordance with the ethical principles and institutional guidelines of the National Institutes of Health Guide for the care and use of laboratory animals.

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