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# The protective effect of naringenin against pyrazinamide-induced hepatotoxicity in male Wistar rats

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## Abstract

**Background:** Pyrazinamide (PZA) is efficient antituberculosis drug. However, PZA-induced hepatotoxicity mediated by oxidative damage is documented. Naringenin (NGN) is a common flavanone with antioxidative properties. Thus, the present work aimed to elucidate the protective role of NGN against PZA-induced toxicity in rats. Eighty adult male Wister rats were randomly divided into four groups: control, PZA, NGN and NGN+PZA. Rats were orally administered 155 mgPZA/kg or 50 mgNGN/kg or NGN 1 h before PZA daily. After 1, 2, 3 and 4 weeks, blood and liver were collected for hematological, biochemical, and histopathological investigations.

**Results:** Administering PZA alone caused remarkable declines in the white and red blood cell counts, hemoglobin content, packed cell volume, and serum levels of albumin, albumin/globulin ratio, high-density lipoprotein cholesterol, and hepatic activities of superoxide dismutase, and glutathione reductase and glutathione level. Serum levels of total cholesterol, low-density lipoprotein cholesterols, triglycerides, globulin, glucose, total and indirect bilirubin, malondialdehyde, and aminotransferases activities were markedly elevated. Additionally, the liver of PZA group exhibited considerable histopathological alterations. Inversely, in the NGN+PZA group, all the aforesaid disturbances in the studied parameters were ameliorated.

**Conclusions:** The current study revealed that NGN can be successfully utilized during treatment with PZA to prevent its side actions.

**Keywords:** Histopathology, Hepatotoxicity, Naringenin, Pyrazinamide, Wistar rats

## Background

Tuberculosis (TB) is an epidemic contagious disease that is caused by Mycobacterium tuberculosis (M. tuberculosis). By 2018, the estimated number of TB cases was about 10 million people worldwide (MacNeil et al., 2020). TB is considered as one of the most common leading causes of death. Globally, an estimated 1.5 million TB deaths were recorded on 2018 (MacNeil et al., 2020). The most preferred TB treatment regimen consists of an intensive phase for 2 months of isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) followed by a continuation phase for 4 months of INH and RIF (Dartois & Rubin, 2022), to avoid the occurance of drug-resistant mutants.

PZA is a first-line anti-TB drug that can inhibit M. tuberculosis in an acidic medium (Peterson et al., 2015). Unfurtunately, asymptomatic alterations in the hepatic functions after TB treatment with PZA were documented (Hussain et al., 2021). With improper detection of these alterations, PZA can induce severe hepatotoxicity (Zhao et al., 2017). It is noteworthy that PZA-induced hepatotoxicity is more frequent in females than males (Zhao et al., 2017). It should be emphasized that PZA-induced hepatotoxicity is mainly assoiated with its metabolites such as pyrazinoic acid (Zhao et al., 2017). Thus, there is

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a real need to protect the liver from the PZA-associated toxicity. Natural products are mostly good choices.

Naringenin (NGN) is a promising natural flavonoid that is abundant in the citrus fruits (Abdel-Ghaffar et al., 2018a). NGN protected the liver of rats against the isonizide-induced toxicity (Abdel-Ghaffar et al., 2018a). Generally, NGN exerted beneficial impacts against various liver diseases caused by drug intake, alcohol, viruses, or malnutrition (Hernández-Aquino & Muriel, 2018). The hepatoprotective action of NGN may be linked to its anti-inflammtory, antioxidative, and anti-apoptotic properties (Stabrauskiene et al., 2022). Recently, Sahu et al. (2018) and (2019) studied the effect of NGN on the toxicity induced by a combination of anit-TB drugs. Nevertheless, it was not clear which drug was responsible for the resultant toxicity. Accordingly, the present study aimed to evaluate the potential protective role of NGN against PZA-induced hepatotoxicity in male Wistar rats.

## Methods

All the present experimental procedures were held in the laboratory of Zoology Department, Faculty of Science, Cairo University, Giza, Egypt.

### Chemicals

Pyrazinamide (PZA; CAS no. 98-96-4, purity ≥ 99%) and naringenin (NGN; CAS no. 67604-48-2, purity ≥ 95%) were obtained from Merck KGaA, Darmstadt, Germany. Kits for assessment of all the studied biochemical parameters, oxidative stress biomarker, and endogenous antioxidants were purchased from Bio-diagnostics company, Dokki, Giza, Egypt.

### Experimental model

Eighty adult healthy male Wistar rats, Rattus norvegicus, were used as experimental animals. The rats were purchased from the animal house of the National Research Center (NRC). At the onset of experiments, rats of 4–5 weeks of age were weighting from 130 to 150 g. During acclimatization period, rats were housed in clean polyacrylic cages, with five individuals per cage, and were supplied with normal rodent chew and clean water ad libitum. Acclimatization period continued for two weeks in the animal house. The rats were kept in conventional conditions of 12-h/12-h light–dark cycle and at room temperature (22–25 °C).

### Study design

The experimetal rats were randomly divided into equal four main groups (with 20 rats per main group). The main groups were treated as clarified in Fig. 1.

The applied dose of PZA (155 mg/kg) was calculated based on the human effective dose (25 mg/kg) recommended by Alsultan et al. (2017). The calculation of PZA

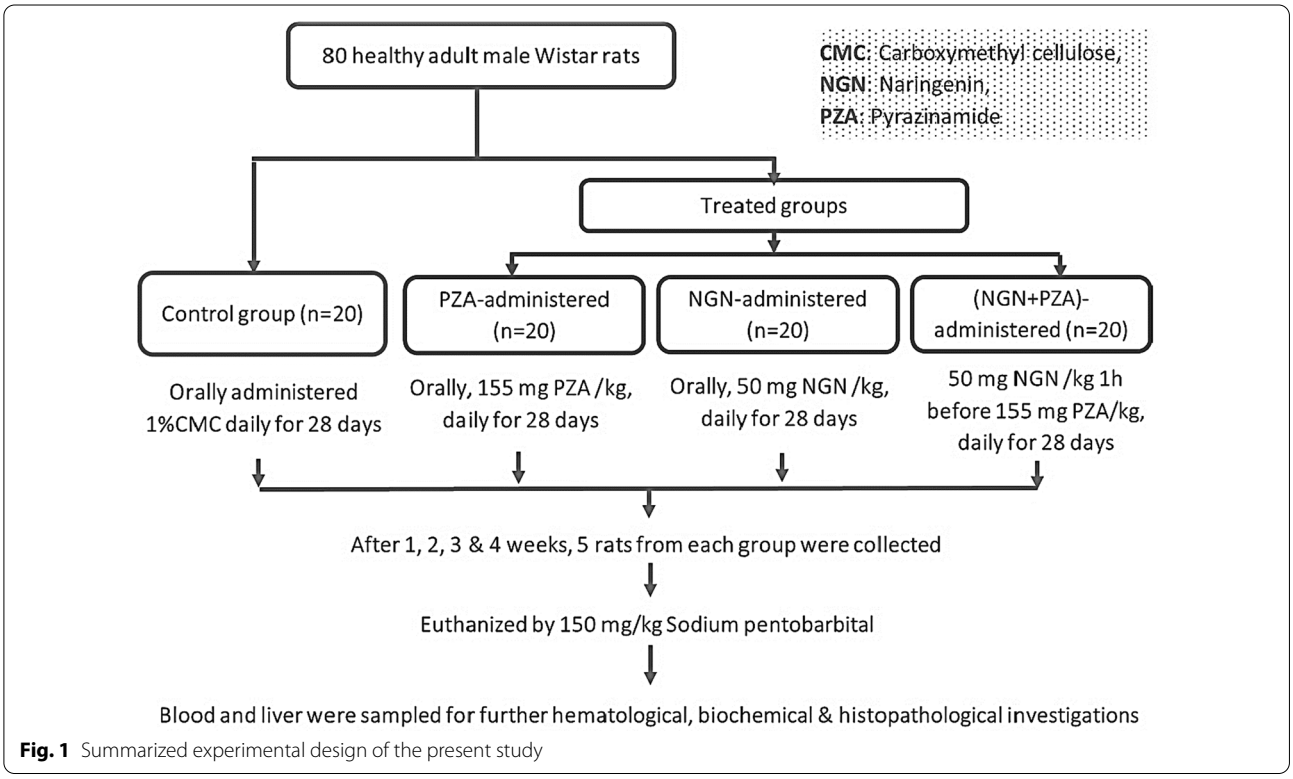


**Fig. 1** Summarized experimental design of the present study

dose was done according to the following equation as provided by Nair and Jacob (2016).

$$\text{Animal dose } (\text{mg/kg}) = \text{Human equivalent dose}(\text{mg/kg}) \times K_m \text{ ratio}$$

where $K_m$ ratio represents the ratio of human constant (KH) divided by animal constant (KA) and equals 6.2 for rats.

The applied dose of NGN was 50 mg/kg according to Abdel-Ghaffar et al. (2018a). The rats of NGN+PZA group were orally administered NGN 1 h prior to the administration of PZA throughout the experiment.

### Sampling

After 1, 2, 3 and 4 weeks, five rats from each group were collected. Rats were euthenized via intraperitoneal injection of an overdose of 150 mg $kg^{-1}$ of sodium pentobarbital and then were killed. Blood was collected from the jugular vein into two types of test tubes. The first type of tubes was dry to separate serum after centrifugation at 1200×*g* for 5 min at room temperature. Serum was then stored at − 20 °C for further biochemical analysis. The other tubes were coated by ethylenediaminetetraacetic acid (EDTA) for regular hematological analyses. A small piece of the extract liver was homogenized in 50 mM cold potassium phosphate buffer of pH 7.5 and then was centrifuged at 1467×*g* (Sigma 3-30Ks centrifuge, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at temp 4 °C, for 15 min for deterimnation of malonedialdehyde (MDA) levels (Sayed et al., 2018). Another part of liver was homogenized in 50 mM cold potassium phosphate buffer of pH 7.5 containing 1 mM EDTA and then centrifugated at 7426×*g* (Sigma 3-30Ks centrifuge, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) for 15 min at temp 4 °C, for the estimation of level of glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione reductase (GR) (Sayed et al., 2018).

### Hematological assay

Hematological variables including red and white blood cells (RBC & WBC) count, Hb content, and PCV were estimated via Blood analyzer of Medonic M-Series (Clinical Diagnostics solutions Inc, Florida, USA).

### Serum biochemical assay

The serum levels of total cholesterol (TC), low- and high-density lipoprotein cholesterol (LDL-C & HDL-C), triglycerides (TG), total proteins (TP), albumin, glubulin, albumin/globulin ratio, total and indirect bilirubin (TBil & IBil), and glucose as well as the serum activities of alanine and aspartate aminotransferases (ALAT & ASAT) were colorimetrically measured using Biodiagnostics kits (Dokki, Giza, Egypt).

### Lipid peroxidation assay

The hepatic levels of MDA were measured according to the method described by Ohkawa et al. (1979). This technique was based on the reaction of MDA, a by-product from cell membrane lipid peroxidation, with thiobarbituric acid in acidic medium.

### Endogenous antioxidant assay

GSH content and SOD and GR activities were analyzed in homogenate of the liver. The GSH content measurment was based on the reaction of 5,5′ dithiobis-2-nitrobenzoic acid with GSH (Tipple & Rogers, 2012). The SOD activity assessment was based on the ability of SOD to inhibit the reduction reaction of nitroblue tetrazolium dye mediated by phenazine methosulphate (Nishikimi et al., 1972). The GR activity estimation was based on its ability to catalyze the reduction of glutathione (GSSG) as described by Faizal et al. (2017).

### Histopathological investigation

Hepatic tissue was fixed in 10% formalin for one day. Liver was then dehydrated by ethanol. Xylene was then used for clearing the fixed liver. Thereafter, the hepatic tissue was mounted in molten paraplast and was cut using microtome into sections of 4–5 µm. Finally, the resultant sections were stained using hematoxylin and eosin (H&E). Investigation of slides was blindly done the pathologist using a Nikon light microscope (Eclipse E200-LED, Tokyo, Japan).

### Statistical analysis

Statistical analysis was executed using Statistical Package of the Social Sciences; SPSS version 22 (copyrighted by IBM SPSS software, USA). Kolmogorov–Smirnov test was applied to ensure the normal distribution of the data. One-way analysis of variance (ANOVA) was applied to study the effects of time (7, 14, 21 and 28 days) and type of treatment (control, PZA alone, PZA + NGN and NGN alone) on the studied parameters. Post hoc Duncan's test was utilized to examine statistcial differecnes among the studied groups. Least significant difference (LSD) test was utilized to study the statistical differences as compared to different time intervals. Pearson's correlation coefficient was used to examine the correlation of time with the studied varaibles. Data were expressed as a mean ± standard error of mean (SEM). $P < 0.05$ is considered significant effect.

## Results

### Effect on hematological parameters

The results of all the studied hematological parameters of the experimental groups are displayed (Table 1). By the 14th, 21st and 28th days, WBC count was significantly affected by treatment type. WBC count in PZA was significantly lower the control group, at most experimental periods. However, in PZA + NGN group, WBC count was significantly higher than in PZA group and insignificantly different from the controls. WBC count was significantly ($P = 0.007$) affected by the experimental time and showed negative correlation ($r = -0.67$) with the time intervals. In comparison with the 7th day, WBC count in PZA group showed significant decline at the 28th day.

RBC count, Hb content, and PCV were significantly affected by the type of treatment on the 21st and 28th days. By the 21st and 28th days, RBC count, Hb content, and PCV in PZA group were significantly lower than the control and PZA + NGN groups.

### Effect on serum biochemical parameters

The levels of TC, LDL-C, HLD-C, and TG in the serum of the experimental rats are presented (Table 2). At the 21st and 28th days, TC, LDL-C, HDL-C, and TG levels were significantly ($P = 0.000$) affected by treatment type. On the 21st and 28th days, the PZA group showed significant elevations in the TC, LDL-C, and TG levels, whereas a marked reduction in HDL-C level, as compared to the control and PZA + NGN groups. The experimental time showed a significant ($P = 0.000$) effect on the TC, LDL-C, HDL-C, and TG levels in the PZA group. In the PZA group, experimental time showed strong positive correlations with TC ($r = +94$), LDL-C ($r = +90$), and TG ($r = +86$) levels, which showed gradual elevations from the 21st day till the end

**Table 1** The white and red blood cells (WBC & RBC) counts, hemoglobin (Hb) content and packed cell volume (PCV) of the control rats and those orally administrated PZA alone, NGN 1 h prior to PZA, and NGN alone, every day for 7, 14, 21 & 28 days. Data are displayed as mean (of 5 rats) ± standard error of mean

| Parameter | Time (days) | Experimental group | | | | Treatment effect |
|---|---|---|---|---|---|---|
| | | Control | PZA | PZA + NGN | NGN | |
| WBC ($X10^9L^{-1}$) | 7 | $9.23 \pm 0.53^a$ | $8.31 \pm 0.19^a$ | $8.63 \pm 0.36^a$ | $8.95 \pm 0.42^a$ | $F_{3,16} = 1.01, P = 0.414$ |
| | 14 | $9.33 \pm 0.38^b$ | $8.20 \pm 0.30^a$ | $9.32 \pm 0.35^b$ | $9.62 \pm 0.27^b$ | $F_{3,16} = 3.675, P = 0.035$ |
| | 21 | $9.18 \pm 0.28^b$ | $7.88 \pm 0.25^a$ | $9.51 \pm 0.15^b$ | $9.55 \pm 0.12^b$ | $F_{3,16} = 13.79, P = 0.000$ |
| | 28 | $9.21 \pm 0.49^b$ | $7.09 \pm 0.15^{a*}$ | $9.07 \pm 0.34^b$ | $9.03 \pm 0.25^b$ | $F_{3,16} = 9.167, P = 0.001$ |
| | *r* | – | −0.67 | +0.24 | +0.03 | |
| | Time effect | $F_{3,16} = 0.023, P = 0.995$ | $F_{3,16} = 5.757, P = 0.007$ | $F_{3,16} = 1.446, P = 0.267$ | $F_{3,16} = 1.462, P = 0.262$ | |
| RBC ($X10^{12}L^{-1}$) | 7 | $7.42 \pm 0.26^a$ | $7.06 \pm 0.07^a$ | $7.04 \pm 0.05^a$ | $7.37 \pm 0.33^a$ | $F_{3,16} = 0.837, P = 0.493$ |
| | 14 | $7.42 \pm 0.14^a$ | $7.05 \pm 0.16^a$ | $7.48 \pm 0.17^a$ | $7.42 \pm 0.10^a$ | $F_{3,16} = 2.12, P = 0.138$ |
| | 21 | $7.46 \pm 0.20^b$ | $6.57 \pm 0.13^a$ | $7.41 \pm 0.21^b$ | $7.62 \pm 0.15^b$ | $F_{3,16} = 7.407, P = 0.002$ |
| | 28 | $7.67 \pm 0.25^b$ | $6.56 \pm 0.24^a$ | $7.49 \pm 0.14^b$ | $7.62 \pm 0.20^b$ | $F_{3,16} = 8.434, P = 0.001$ |
| | *r* | – | −0.54 | +0.40 | +0.20 | |
| | Time effect | $F_{3,16} = 0.308, P = 0.819$ | $F_{3,16} = 2.968, P = 0.063$ | $F_{3,16} = 1.98, P = 0.158$ | $F_{3,16} = 2.89, P = 0.068$ | |
| Hb content (g $dL^{-1}$) | 7 | $13.42 \pm 0.36^a$ | $13.38 \pm 0.20^a$ | $13.82 \pm 0.24^a$ | $13.57 \pm 0.20^a$ | $F_{3,16} = 0.597, P = 0.626$ |
| | 14 | $13.62 \pm 0.15^a$ | $13.34 \pm 0.20^a$ | $13.53 \pm 0.19^a$ | $13.83 \pm 0.13^a$ | $F_{3,16} = 1.395, P = 0.280$ |
| | 21 | $13.72 \pm 0.27^b$ | $12.86 \pm 0.13^a$ | $13.72 \pm 0.16^b$ | $13.76 \pm 0.14^b$ | $F_{3,16} = 5.484, P = 0.009$ |
| | 28 | $13.64 \pm 0.32^b$ | $12.80 \pm 0.18^a$ | $13.84 \pm 0.36^b$ | $13.91 \pm 0.13^b$ | $F_{3,16} = 3.697, P = 0.034$ |
| | *r* | – | −0.55 | +0.05 | +0.33 | |
| | Time effect | $F_{3,16} = 0.199, P = 0.895$ | $F_{3,16} = 2.889, P = 0.068$ | $F_{3,16} = 0.318, P = 0.812$ | $F_{3,16} = 0.905, P = 0.46$ | |
| PCV (%) | 7 | $40.24 \pm 1.03^a$ | $40.32 \pm 0.74^a$ | $40.44 \pm 0.87^a$ | $40.80 \pm 0.45^a$ | $F_{3,16} = 0.095, P = 0.962$ |
| | 14 | $41.10 \pm 0.84^a$ | $40.60 \pm 0.42^a$ | $41.18 \pm 0.47^a$ | $41.30 \pm 0.58^a$ | $F_{3,16} = 0.263, P = 0.851$ |
| | 21 | $41.68 \pm 0.59^b$ | $38.44 \pm 0.91^a$ | $41.67 \pm 0.21^b$ | $41.28 \pm 0.39^b$ | $F_{3,16} = 7.149, P = 0.003$ |
| | 28 | $41.64 \pm 0.51^b$ | $38.46 \pm 0.54^a$ | $41.58 \pm 0.31^b$ | $41.44 \pm 0.41^b$ | $F_{3,16} = 11.73, P = 0.000$ |
| | *r* | – | −0.51 | +0.38 | +0.22 | |
| | Time effect | $F_{3,16} = 0.754, P = 0.536$ | $F_{3,16} = 2.954, P = 0.064$ | $F_{3,16} = 1.123, P = 0.369$ | $F_{3,16} = 0.367, P = 0.778$ | |

$P < 0.05$, $P < 0.01$, $P < 0.000$: represents significant effects. *: represent significant ($P < 0.05$) differences as compared to 7th day

*r*: Pearson's correlation coefficient of the studied parameters with the experimental time

In the same row, according to Duncan's test, means marked with the same *small* superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

**Table 2** The levels of total cholesterol (TC), low- and high-density lipoprotein cholesterols (LDL-C & HDL-C), and triglycerides in serum of the control rats and those orally administrated PZA alone, NGN 1 h prior to PZA, and NGN alone, every day for 7, 14, 21 & 28 days. Data are displayed as mean (of 5 rats) ± standard error of mean

| Parameter | Time (days) | Experimental group | | | | Treatment effect |
|---|---|---|---|---|---|---|
| | | Control | PZA | PZA + NGN | NGN | |
| TC (mg $dL^{-1}$) | 7 | $120.60 \pm 0.76^{a}$ | $120.68 \pm 0.43^{a}$ | $120.51 \pm 1.13^{a}$ | $120.61 \pm 0.78^{a}$ | $F_{3,16} = 0.008, P = 0.999$ |
| | 14 | $120.55 \pm 0.42^{a}$ | $122.68 \pm 0.81^{a}$ | $120.72 \pm 0.94^{a}$ | $120.69 \pm 0.97^{a}$ | $F_{3,16} = 1.554, P = 0.239$ |
| | 21 | $120.71 \pm 0.78^{a}$ | $135.81 \pm 1.78^{b*}$ | $120.60 \pm 0.62^{a}$ | $120.79 \pm 0.68^{a}$ | $F_{3,16} = 49.12, P = 0.000$ |
| | 28 | $120.70 \pm 1.01^{a}$ | $149.03 \pm 1.56^{b*}$ | $120.79 \pm 0.35^{a}$ | $120.66 \pm 0.83^{a}$ | $F_{3,16} = 187.9, P = 0.000$ |
| | *r* | – | +0.94 | +0.05 | +0.02 | |
| | Time effect | $F_{3,16} = 0.011, P = 0.998$ | $F_{3,16} = 107.08, P = 0.000$ | $F_{3,16} = 0.023, P = 0.995$ | $F_{3,16} = 0.008, P = 0.999$ | |
| LDL-C (mg $dL^{-1}$) | 7 | $60.66 \pm 0.52^{a}$ | $62.30 \pm 2.29^{a}$ | $60.83 \pm 2.57^{a}$ | $60.90 \pm 1.06^{a}$ | $F_{3,16} = 0.175, P = 0.912$ |
| | 14 | $60.84 \pm 0.66^{a}$ | $64.89 \pm 1.95^{b}$ | $60.54 \pm 0.75^{a}$ | $60.80 \pm 0.81^{a}$ | $F_{3,16} = 3.206, P = 0.051$ |
| | 21 | $60.72 \pm 0.97^{a}$ | $79.03 \pm 3.26^{b*}$ | $60.79 \pm 0.86^{a}$ | $60.57 \pm 1.10^{a}$ | $F_{3,16} = 24.93, P = 0.000$ |
| | 28 | $60.70 \pm 0.80^{a}$ | $98.11 \pm 2.61^{b*}$ | $60.91 \pm 0.56^{a}$ | $60.97 \pm 0.33^{a}$ | $F_{3,16} = 175.9, P = 0.000$ |
| | *r* | – | +0.90 | +0.02 | −0.001 | |
| | Time effect | $F_{3,16} = 0.011, P = 0.998$ | $F_{3,16} = 40.81, P = 0.000$ | $F_{3,16} = 0.012, P = 0.998$ | $F_{3,16} = 0.039, P = 0.989$ | |
| HDL-C (mg $dL^{-1}$) | 7 | $39.04 \pm 0.35^{a}$ | $37.41 \pm 2.17^{a}$ | $38.98 \pm 1.61^{a}$ | $38.73 \pm 0.53^{a}$ | $F_{3,16} = 0.305, P = 0.821$ |
| | 14 | $38.82 \pm 0.22^{b}$ | $36.34 \pm 1.62^{a}$ | $39.32 \pm 0.29^{b}$ | $38.85 \pm 0.17^{b}$ | $F_{3,16} = 2.609, P = 0.087$ |
| | 21 | $39.08 \pm 0.65^{b}$ | $33.99 \pm 1.85^{a}$ | $38.74 \pm 0.59^{b}$ | $39.45 \pm 0.76^{b}$ | $F_{3,16} = 5.509, P = 0.009$ |
| | 28 | $39.04 \pm 1.66^{b}$ | $22.29 \pm 1.92^{a*}$ | $38.91 \pm 0.59^{b}$ | $38.72 \pm 0.46^{b}$ | $F_{3,16} = 39.36, P = 0.000$ |
| | *r* | – | −0.75 | −0.05 | +0.06 | |
| | Time effect | $F_{3,16} = 0.016, P = 0.997$ | $F_{3,16} = 13.45, P = 0.000$ | $F_{3,16} = 0.071, P = 0.975$ | $F_{3,16} = 0.432, P = 0.733$ | |
| TG (mg $dL^{-1}$) | 7 | $104.55 \pm 0.98^{a}$ | $104.89 \pm 1.48^{a}$ | $103.48 \pm 0.64^{a}$ | $104.91 \pm 1.09^{a}$ | $F_{3,16} = 0.379, P = 0.77$ |
| | 14 | $104.44 \pm 0.80^{a}$ | $107.21 \pm 2.10^{a}$ | $104.29 \pm 1.43^{a}$ | $105.20 \pm 1.50^{a}$ | $F_{3,16} = 0.772, P = 0.527$ |
| | 21 | $104.57 \pm 1.02^{a}$ | $113.93 \pm 1.63^{b*}$ | $105.38 \pm 1.39^{a}$ | $103.85 \pm 1.27^{a}$ | $F_{3,16} = 12.25, P = 0.000$ |
| | 28 | $104.86 \pm 1.07^{a}$ | $143.15 \pm 2.45^{b*}$ | $104.87 \pm 1.68^{a}$ | $104.87 \pm 1.00^{a}$ | $F_{3,16} = 133.24, P = 0.000$ |
| | *r* | – | +0.86 | +0.21 | −0.07 | |
| | Time effect | $F_{3,16} = 0.034, P = 0.991$ | $F_{3,16} = 81.71, P = 0.000$ | $F_{3,16} = 0.365, P = 0.779$ | $F_{3,16} = 0.231, P = 0.874$ | |

$P < 0.05$, $P < 0.01$, $P < 0.000$: represents significant effects. *: represent significant ($P < 0.05$) differences as compared to 7th day

*r*: Pearson's correlation coefficient of the studied parameters with the experimental time

In the same row, according to Duncan's test, means marked with the same *small* superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

of the experiment. On the 28th day, the HDL-C level in the PZA group was negatively correlated with time intervals ($r = -0.75$) and showed a significant decline, as compared to the rest of the experimental intervals.

The serum protein profile of all experimental rats is recorded (Table 3). On the 21st and 28th days, treatment type significantly affected albumin ($P = 0.005$, $P = 0.000$), globulin ($P = 0.002$, $P = 0.000$), and albumin/globulin ratio ($P = 0.000$, $P = 0.000$), respectively. By the 21st and 28th days, PZA groups showed marked declines in albumin level and albumin/globulin ratio, whereas a remarkable elevation in globulin level, as compared to the controls and PZA + NGN groups. In the PZA group, the time intervals remarkably affected albumin ($P = 0.001$), globulin ($P = 0.000$) levels, and albumin/globulin ratio ($P = 0.000$). On the 21st and 28th days, the PZA group exhibited marked reductions in albumin level and albumin/globulin ratio whereas remarkable elevation in globulin level, as compared to the 7th and 14th days.

The glucose, bilirubin levels, and aminotransferase activities in serum of all the studied groups are reported (Table 4). Treatment type remarkably affected glucose, bilirubin levels and aminotransferase activities, by the 21st and 28th days. On the 21st and 28th days, the PZA group showed marked elevations in the blood glucose, TBIL, IBIL, as well as ASAT and ALAT activities, as compared to the rest of the groups. In the PZA group, on the 21st and 28th days, meaningful elevations in the blood glucose, TBIL, IBIL, as well as ASAT and ALAT activities was recorded, as compared to the rest of time intervals. Strong direct correlations were observed between the time intervals and blood glucose ($r = +0.77$), TBIL ($r = +0.75$), and IBIL ($r = +0.70$) levels, as well as ASAT ($r = +0.92$) and ALAT ($r = +0.90$) activities in the PZA group.

**Table 3** The levels of total protein (TP), albumin, globulin and albumin/globulin ratio in serum of the control rats and those orally administrated PZA alone, NGN 1 h prior to PZA, and NGN alone, every day for 7, 14, 21 & 28 days. Data are displayed as mean (of 5 rats) ± standard error of mean

| Parameter | Time (days) | Experimental group | | | | Treatment effect |
|---|---|---|---|---|---|---|
| | | Control | PZA | PZA + NGN | NGN | |
| TP ($g\ dL^{-1}$) | 7 | $6.58 \pm 0.21^{a}$ | $6.83 \pm 0.17^{a}$ | $6.74 \pm 0.13^{a}$ | $6.57 \pm 0.19^{a}$ | $F_{3,16} = 0.509, P = 0.681$ |
| | 14 | $6.53 \pm 0.21^{a}$ | $6.72 \pm 0.12^{a}$ | $6.88 \pm 0.18^{a}$ | $6.86 \pm 0.20^{a}$ | $F_{3,16} = 0.792, P = 0.516$ |
| | 21 | $6.61 \pm 0.16^{a}$ | $6.95 \pm 0.05^{a}$ | $6.88 \pm 0.14^{a}$ | $6.86 \pm 0.17^{a}$ | $F_{3,16} = 1.126, P = 0.368$ |
| | 28 | $6.80 \pm 0.09^{a}$ | $6.67 \pm 0.10^{a}$ | $6.69 \pm 0.08^{a}$ | $6.86 \pm 0.08^{a}$ | $F_{3,16} = 0.997, P = 0.419$ |
| | *r* | – | −0.11 | −0.06 | +0.27 | |
| | Time effect | $F_{3,16} = 0.447, P = 0.723$ | $F_{3,16} = 1.056, P = 0.395$ | $F_{3,16} = 0.49, P = 0.694$ | $F_{3,16} = 0.75, P = 0.538$ | |
| Albumin ($g\ dL^{-1}$) | 7 | $3.72 \pm 0.17^{a}$ | $3.69 \pm 0.04^{a}$ | $3.56 \pm 0.08^{a}$ | $3.58 \pm 0.07^{a}$ | $F_{3,16} = 0.595, P = 0.627$ |
| | 14 | $3.77 \pm 0.14^{a}$ | $3.67 \pm 0.11^{a}$ | $3.68 \pm 0.10^{a}$ | $3.63 \pm 0.14^{a}$ | $F_{3,16} = 0.246, P = 0.863$ |
| | 21 | $3.72 \pm 0.15^{b}$ | $2.99 \pm 0.23^{a*}$ | $3.67 \pm 0.07^{b}$ | $3.74 \pm 0.06^{b}$ | $F_{3,16} = 6.326, P = 0.005$ |
| | 28 | $3.70 \pm 0.15^{b}$ | $2.89 \pm 0.16^{a*}$ | $3.80 \pm 0.05^{b}$ | $3.84 \pm 0.09^{b}$ | $F_{3,16} = 14.51, P = 0.000$ |
| | *r* | – | −0.72 | +0.46 | +0.46 | |
| | Time effect | $F_{3,16} = 0.044, P = 0.987$ | $F_{3,16} = 8.33, P = 0.001$ | $F_{3,16} = 1.704, P = 0.206$ | $F_{3,16} = 1.469, P = 0.26$ | |
| Globulin ($g\ dL^{-1}$) | 7 | $2.87 \pm 0.09^{a}$ | $3.14 \pm 0.13^{a}$ | $3.18 \pm 0.06^{a}$ | $2.99 \pm 0.12^{a}$ | $F_{3,16} = 1.838, P = 0.181$ |
| | 14 | $2.96 \pm 014^{a}$ | $3.05 \pm 0.04^{a}$ | $3.20 \pm 0.09^{a}$ | $3.23 \pm 0.09^{a}$ | $F_{3,16} = 1.749, P = 0.197$ |
| | 21 | $3.09 \pm 0.09^{a}$ | $3.76 \pm 0.15^{b*}$ | $3.21 \pm 0.07^{a}$ | $3.12 \pm 0.12^{a}$ | $F_{3,16} = 7.94, P = 0.002$ |
| | 28 | $3.10 \pm 0.07^{a}$ | $3.79 \pm 0.07^{b*}$ | $3.00 \pm 0.10^{a}$ | $3.02 \pm 0.01^{a}$ | $F_{3,16} = 27.54, P = 0.000$ |
| | *r* | – | +0.74 | −0.31 | −0.01 | |
| | Time effect | $F_{3,16} = 1.199, P = 0.342$ | $F_{3,16} = 13.09, P = 0.000$ | $F_{3,16} = 1.303, P = 0.308$ | $F_{3,16} = 1.348, P = 0.294$ | |
| Albumin / globulin (%) | 7 | $1.21 \pm 0.04^{a}$ | $1.19 \pm 0.04^{a}$ | $1.12 \pm 0.02^{a}$ | $1.20 \pm 0.03^{a}$ | $F_{3,16} = 1.69, P = 0.209$ |
| | 14 | $1.22 \pm 0.03^{a}$ | $1.20 \pm 0.04^{a}$ | $1.15 \pm 0.02^{a}$ | $1.16 \pm 0.03^{a}$ | $F_{3,16} = 1.01, P = 0.415$ |
| | 21 | $1.16 \pm 0.01^{b}$ | $0.86 \pm 0.08^{a*}$ | $1.15 \pm 0.01^{b}$ | $1.20 \pm 0.03^{b}$ | $F_{3,16} = 12.96, P = 0.000$ |
| | 28 | $1.20 \pm 0.05^{b}$ | $0.77 \pm 0.05^{a*}$ | $1.18 \pm 0.05^{b}$ | $1.20 \pm 0.04^{b}$ | $F_{3,16} = 19.27, P = 0.000$ |
| | *r* | – | −0.80 | +0.36 | +0.03 | |
| | Time effect | $F_{3,16} = 0.411, P = 0.748$ | $F_{3,16} = 15.91, P = 0.000$ | $F_{3,16} = 0.952, P = 0.439$ | $F_{3,16} = 0.351, P = 0.789$ | |

$P < 0.05$, $P < 0.01$, $P < 0.000$: represents significant effects. *: represent significant ($P < 0.05$) differences as compared to 7th day

*r*: Pearson's correlation coefficient of the studied parameters with the experimental time

In the same row, according to Duncan's test, means marked with the same *small* superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

### Effect on oxidative stress biomarker and endogenous antioxidants

Table 5 shows the MDA and GSH levels as well as SOD and GR activities in the liver. Treatment type considerably affected MDA and GSH levels at most time intervals. By the 14th day till the end, the PZA group showed significant elevation in MDA level, whereas a marked decline in GSH content, as compared to the control and PZA+NGN groups. The SOD and GR activities were significantly ($P = 0.000$) affected by treatment type on the 21st and 28th days. On the 21st and 28th days, SOD and GR activities in the PZA group were remarkably lower than the control and PZA + NGN groups. In the PZA group, time intervals had significant ($P = 0.000$) effects on all the studied oxidative stress parameters. In rats of PZA group, the experimental time was correlated positively with MDA level ($r = +0.94$), whereas inversely with GSH level ($r = -0.92$) and SOD ($r = -0.92$) and GR ($r = -0.93$) activities in PZA group.

### Effect on liver histology

In control, NGN, and PZA+NGN groups, histopathological investigation of the hepatic tissue revealed the regular histological structure of liver parenchyma, showing normal hepatocytes distributed around the portal area (Figs. 2, 3 and 5). On the other hand, the PZA group showed some histopathological alterations such as excessive vacuolation of the hepatic parenchyma, expanded periportal edema, and thickening of the bile duct walls (Fig. 4).

## Discussion

The present work aimed to study the protective role of NGN against PZA-induced toxicity using hematological, biochemical, and histopathological investigations. In the

**Table 4** The levels of glucose, total and indirect bilirubin (TBIL & IBIL) as well as the activities of aspartate and alanine aminotransferases (ASAT & ALAT) in serum of the control rats and those orally administrated PZA alone, NGN 1 h prior to PZA, and NGN alone, every day for 7, 14, 21 & 28 days. Data are displayed as mean (of 5 rats) ± standard error of mean

| Parameter | Time (days) | Experimental group | | | | Treatment effect |
|---|---|---|---|---|---|---|
| | | Control | PZA | PZA + NGN | NGN | |
| Glucose (mg $dL^{-1}$) | 7 | $120.20 \pm 1.54^{a}$ | $118.70 \pm 2.64^{a}$ | $119.54 \pm 0.42^{a}$ | $118.40 \pm 2.67^{a}$ | $F_{3,16} = 0.161, P = 0.921$ |
| | 14 | $120.24 \pm 1.13^{a}$ | $120.70 \pm 1.44^{a}$ | $120.28 \pm 1.00^{a}$ | $120.02 \pm 0.92^{a}$ | $F_{3,16} = 0.062, P = 0.979$ |
| | 21 | $121.58 \pm 0.91^{a}$ | $127.30 \pm 1.62^{b*}$ | $121.98 \pm 0.91^{a}$ | $121.42 \pm 1.02^{a}$ | $F_{3,16} = 6.021, P = 0.006$ |
| | 28 | $123.04 \pm 1.21^{a}$ | $132.26 \pm 2.45^{b*}$ | $121.60 \pm 1.01^{a}$ | $122.18 \pm 1.02^{a}$ | $F_{3,16} = 10.59, P = 0.000$ |
| | *r* | – | +0.77 | +0.44 | +0.41 | |
| | Time effect | $F_{3,16} = 1.217, P = 0.336$ | $F_{3,16} = 8.756, P = 0.001$ | $F_{3,16} = 1.714, P = 0.204$ | $F_{3,16} = 1.099, P = 0.378$ | |
| TBIL (mg $dL^{-1}$) | 7 | $0.67 \pm 0.01^{a}$ | $0.68 \pm 0.02^{a}$ | $0.66 \pm 0.01^{a}$ | $0.69 \pm 0.03^{a}$ | $F_{3,16} = 0.554, P = 0.653$ |
| | 14 | $0.68 \pm 0.01^{a}$ | $0.74 \pm 0.04^{a}$ | $0.69 \pm 0.04^{a}$ | $0.67 \pm 0.03^{a}$ | $F_{3,16} = 0.787, P = 0.519$ |
| | 21 | $0.68 \pm 0.01^{a}$ | $0.80 \pm 0.02^{b*}$ | $0.70 \pm 0.02^{a}$ | $0.68 \pm 0.02^{a}$ | $F_{3,16} = 13.14, P = 0.000$ |
| | 28 | $0.69 \pm 0.01^{a}$ | $0.83 \pm 0.01^{b*}$ | $0.69 \pm 0.02^{a}$ | $0.68 \pm 0.03^{a}$ | $F_{3,16} = 16.32, P = 0.000$ |
| | *r* | – | +0.75 | +0.27 | −0.06 | |
| | Time effect | $F_{3,16} = 0.764, P = 0.531$ | $F_{3,16} = 7.267, P = 0.003$ | $F_{3,16} = 0.681, P = 0.577$ | $F_{3,16} = 0.061, P = 0.98$ | |
| IBIL (mg $dL^{-1}$) | 7 | $0.57 \pm 0.01^{a}$ | $0.58 \pm 0.01^{a}$ | $0.55 \pm 0.01^{a}$ | $0.58 \pm 0.03^{a}$ | $F_{3,16} = 0.397, P = 0.757$ |
| | 14 | $0.58 \pm 0.01^{a}$ | $0.63 \pm 0.04^{a}$ | $0.59 \pm 0.03^{a}$ | $0.57 \pm 0.04^{a}$ | $F_{3,16} = 0.598, P = 0.626$ |
| | 21 | $0.58 \pm 0.02^{a}$ | $0.69 \pm 0.01^{b*}$ | $0.59 \pm 0.01^{a}$ | $0.58 \pm 0.01^{a}$ | $F_{3,16} = 12.37, P = 0.000$ |
| | 28 | $0.59 \pm 0.01^{a}$ | $0.71 \pm 0.02^{b*}$ | $0.59 \pm 0.02^{a}$ | $0.58 \pm 0.02^{a}$ | $F_{3,16} = 12.98, P = 0.000$ |
| | *r* | – | +0.70 | +0.25 | −0.02 | |
| | Time effect | $F_{3,16} = 0.518, P = 0.676$ | $F_{3,16} = 5.381, P = 0.009$ | $F_{3,16} = 0.694, P = 0.569$ | $F_{3,16} = 0.023, P = 0.995$ | |
| ASAT (U/mL) | 7 | $34.50 \pm 1.15^{a}$ | $35.60 \pm 1.23^{a}$ | $34.10 \pm 0.79^{a}$ | $33.68 \pm 0.82^{a}$ | $F_{3,16} = 0.657, P = 0.59$ |
| | 14 | $36.10 \pm 0.94^{a}$ | $37.72 \pm 1.37^{a}$ | $36.58 \pm 1.01^{a}$ | $35.30 \pm 1.28^{a}$ | $F_{3,16} = 0.76, P = 0.533$ |
| | 21 | $32.60 \pm 1.08^{a}$ | $46.32 \pm 1.52^{b*}$ | $35.60 \pm 1.30^{a}$ | $33.10 \pm 1.12^{a}$ | $F_{3,16} = 25.55, P = 0.000$ |
| | 28 | $34.92 \pm 1.17^{a}$ | $55.26 \pm 1.29^{b*}$ | $33.52 \pm 1.29^{a}$ | $34.00 \pm 1.76^{a}$ | $F_{3,16} = 57.56, P = 0.000$ |
| | *r* | – | +0.92 | −0.12 | −0.05 | |
| | Time effect | $F_{3,16} = 1.797, P = 0.188$ | $F_{3,16} = 43.78, P = 0.000$ | $F_{3,16} = 1.558, P = 0.238$ | $F_{3,16} = 0.521, P = 0.674$ | |
| ALAT (U/mL) | 7 | $25.80 \pm 0.39^{a}$ | $25.38 \pm 1.18^{a}$ | $25.16 \pm 1.09^{a}$ | $25.18 \pm 1.41^{a}$ | $F_{3,16} = 0.075, P = 0.973$ |
| | 14 | $24.16 \pm 0.69^{a}$ | $29.00 \pm 2.12^{a}$ | $25.42 \pm 1.38^{a}$ | $22.68 \pm 1.16^{a}$ | $F_{3,16} = 1.768, P = 0.194$ |
| | 21 | $24.76 \pm 0.65^{a}$ | $38.28 \pm 1.28^{b*}$ | $24.64 \pm 1.42^{a}$ | $23.80 \pm 1.37^{a}$ | $F_{3,16} = 32.49, P = 0.000$ |
| | 28 | $25.86 \pm 1.32^{a}$ | $41.22 \pm 0.72^{b*}$ | $26.82 \pm 1.29^{a}$ | $23.94 \pm 1.15^{a}$ | $F_{3,16} = 47.89, P = 0.000$ |
| | *r* | – | +0.90 | +0.17 | −0.11 | |
| | Time effect | $F_{3,16} = 0.984, P = 0.425$ | $F_{3,16} = 46.03, P = 0.000$ | $F_{3,16} = 0.512, P = 0.68$ | $F_{3,16} = 0.643, P = 0.598$ | |

$P < 0.05$, $P < 0.01$, $P < 0.000$: represents significant effects. *: represent significant ($P < 0.05$) differences as compared to 7th day

*r*: Pearson's correlation coefficient of the studied parameters with the experimental time

In the same row, according to Duncan's test, means marked with the same *small* superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

PZA group, the reported disturbances in most studied parameters evidenced PZA-associated toxicity. On the contrary, administering NGN before PZA attenuated all the PZA-induced alterations.

In the PZA group, the WBC and RBC counts were significantly lower than in the controls, indicating PZA-induced leucopenia and anemia. Similar findings following PZA administration were reported (Aderemi & Oluwatosin, 2015). The WBC infiltration to the inflamed tissues contributes to the reduced WBC count (Abdel-Ghaffar et al., 2017), as evidenced by histopathological investigation of the liver. PZA-associated sideroblastic anemia may be due to insufficient erythropoiesis (Colucci et al., 2012). In addition, PZA can induce excessive production of the reactive oxygen species (ROS) (Zhao et al., 2017), leading to disruptions in RBC membranes and the Hb molecules (Abdel-Ghaffar et al., 2018b). Alternatively, in PZA+NGN group, all the studied hematological parameters were not significantly different from the controls. NGN can effectively stabilize Hb within RBCs (Maity et al., 2017). NGN may

**Table 5** The levels of malondialdehyde (MDA), glutathione (GSH), as well as the activities of superoxide dismutase (SOD) and glutathione reductase (GR) in the liver of the control rats and those orally administrated PZA alone, NGN 1 h prior to PZA, and NGN alone, every day for 7, 14, 21 & 28 days. Data are displayed as mean (of 5 rats) ± standard error of mean

| Parameter | Time (days) | Experimental group | | | | Treatment effect |
|---|---|---|---|---|---|---|
| | | Control | PZA | PZA + NGN | NGN | |
| MDA (mg $g^{-1}$ liver) | 7 | $5.30 \pm 0.11^{a}$ | $5.28 \pm 0.15^{a}$ | $5.35 \pm 0.12^{a}$ | $5.35 \pm 0.13^{a}$ | $F_{3, 16} = 0.078$, $P = 0.971$ |
| | 14 | $5.13 \pm 0.08^{a}$ | $5.64 \pm 0.16^{b}$ | $5.15 \pm 0.08^{a}$ | $5.26 \pm 0.10^{a}$ | $F_{3, 16} = 4.55$, $P = 0.017$ |
| | 21 | $5.16 \pm 0.08^{a}$ | $7.70 \pm 0.14^{b*}$ | $5.18 \pm 0.07^{a}$ | $5.14 \pm 0.10^{a}$ | $F_{3, 16} = 157.4$, $P = 0.000$ |
| | 28 | $5.30 \pm 0.10^{a}$ | $8.28 \pm 0.12^{b*}$ | $5.14 \pm 0.07^{a}$ | $5.15 \pm 0.08^{a}$ | $F_{3, 16} = 273.9$, $P = 0.000$ |
| | *r* | – | +0.94 | −0.35 | −0.36 | |
| | Time effect | $F_{3, 16} = 0.907$, P = 0.459 | $F_{3, 16} = 109.53$, $P = 0.000$ | $F_{3, 16} = 1.295$, $P = 0.31$ | $F_{3, 16} = 0.916$, $P = 0.455$ | |
| GSH (mg $g^{-1}$ protein) | 7 | $44.00 \pm 1.53^{a}$ | $45.24 \pm 1.30^{a}$ | $44.72 \pm 1.38^{a}$ | $44.44 \pm 1.14^{a}$ | $F_{3, 16} = 0.149$, $P = 0.929$ |
| | 14 | $45.38 \pm 1.09^{b}$ | $41.10 \pm 0.98^{a*}$ | $46.04 \pm 1.02^{b}$ | $45.96 \pm 1.16^{b}$ | $F_{3, 16} = 4.93$, $P = 0.013$ |
| | 21 | $45.06 \pm 1.19^{b}$ | $34.92 \pm 1.43^{a*}$ | $44.54 \pm 1.38^{b}$ | $44.38 \pm 1.06^{b}$ | $F_{3, 16} = 14.71$, $P = 0.000$ |
| | 28 | $46.04 \pm 1.31^{b}$ | $30.90 \pm 0.73^{a*}$ | $45.84 \pm 1.11^{b}$ | $45.32 \pm 1.20^{b}$ | $F_{3, 16} = 44.60$, $P = 0.000$ |
| | *r* | – | −0.92 | +0.081 | +0.05 | |
| | Time effect | $F_{3, 16} = 0.433$, $P = 0.732$ | $F_{3, 16} = 31.14$, $P = 0.000$ | $F_{3, 16} = 0.384$, $P = 0.766$ | $F_{3, 16} = 0.44$, $P = 0.727$ | |
| SOD (U $g^{-1}$ protein) | 7 | $9.41 \pm 0.13^{a}$ | $9.31 \pm 0.12^{a}$ | $9.30 \pm 0.12^{a}$ | $9.34 \pm 0.12^{a}$ | $F_{3, 16} = 0.145$, $P = 0.932$ |
| | 14 | $9.41 \pm 0.08^{b}$ | $9.04 \pm 0.05^{a}$ | $9.28 \pm 0.11^{b}$ | $9.25 \pm 0.12^{b}$ | $F_{3, 16} = 2.709$, $P = 0.08$ |
| | 21 | $9.39 \pm 0.15^{b}$ | $7.33 \pm 0.11^{a*}$ | $9.32 \pm 0.12^{b}$ | $9.30 \pm 0.14^{b}$ | $F_{3, 16} = 59.87$, $P = 0.000$ |
| | 28 | $9.48 \pm 0.11^{b}$ | $7.19 \pm 0.11^{a*}$ | $9.33 \pm 0.14^{b}$ | $9.20 \pm 0.10^{b}$ | $F_{3, 16} = 88.33$, $P = 0.000$ |
| | *r* | – | −0.92 | +0.056 | −0.17 | |
| | Time effect | $F_{3, 16} = 0.101$, $P = 0.958$ | $F_{3, 16} = 122.7$, $P = 0.000$ | $F_{3, 16} = 0.031$, $P = 0.992$ | $F_{3, 16} = 0.266$, $P = 0.849$ | |
| GR (U $g^{-1}$ protein) | 7 | $82.46 \pm 1.03^{a}$ | $84.02 \pm 1.38^{a}$ | $83.52 \pm 1.27^{a}$ | $83.08 \pm 1.04^{a}$ | $F_{3, 16} = 0.31$, $P = 0.818$ |
| | 14 | $82.64 \pm 0.79^{a}$ | $81.94 \pm 1.23^{a}$ | $83.54 \pm 1.10^{a}$ | $83.52 \pm 0.84^{a}$ | $F_{3, 16} = 0.587$, $P = 0.632$ |
| | 21 | $83.86 \pm 1.13^{b}$ | $70.34 \pm 0.90^{a*}$ | $83.70 \pm 0.97^{b}$ | $83.66 \pm 1.42^{b}$ | $F_{3, 16} = 35.74$, $P = 0.000$ |
| | 28 | $84.02 \pm 0.72^{b}$ | $55.94 \pm 1.60^{a*}$ | $83.22 \pm 0.81^{b}$ | $83.12 \pm 1.23^{b}$ | $F_{3, 16} = 144.8$, $P = 0.000$ |
| | *r* | – | −0.93 | −0.04 | +0.01 | |
| | Time effect | $F_{3, 16} = 0.753$, $P = 0.536$ | $F_{3, 16} = 98.41$, $P = 0.000$ | $F_{3, 16} = 0.036$, P = 0.99 | $F_{3, 16} = 0.063$, P = 0.979 | |

$P < 0.05$, $P < 0.01$, $P < 0.000$: represents significant effects. *: represent significant ($P < 0.05$) differences as compared to 7th day

*r*: Pearson's correlation coefficient of the studied parameters with the experimental time

In the same row, according to Duncan's test, means marked with the same *small* superscript letters are insignificantly different ($P > 0.05$), whereas those marked with different ones are significantly different ($P < 0.05$)

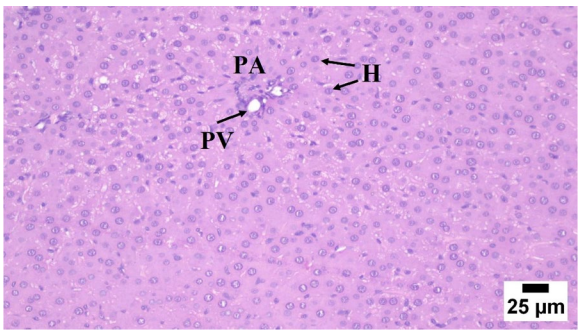


**Fig. 2** Photomicrograph of hepatic sections of control rats showing histologically normal hepatocytes (H) surrounding portal area (PA) (Hematoxylin, H &Eosin, E). PV: portal vein

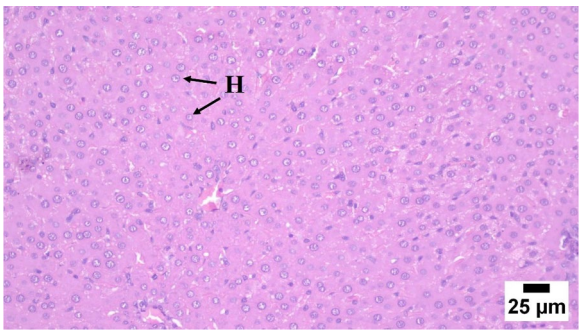


**Fig. 3** Photomicrograph of hepatic sections of rats administered naringenin (NGN, 50 mg/kg BW) daily for 4 weeks showing histologically normal hepatic parenchyma (Hematoxylin, H &Eosin, E). H: hepatocyte

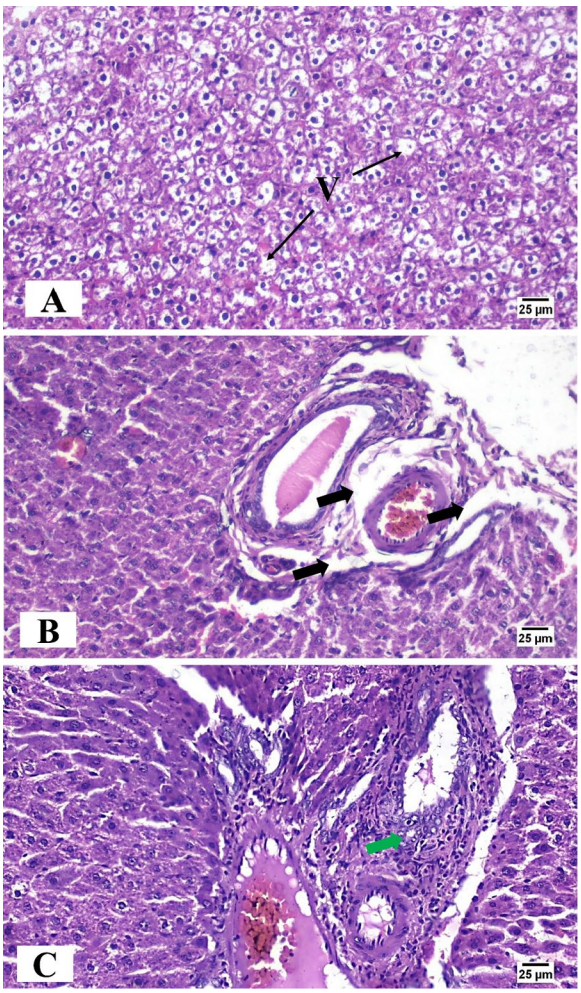


**Fig. 4** Photomicrograph of hepatic sections of rats administered pyrazinamide (PZA, 155 mg/kg BW) daily for 4 weeks showing **A** excessive vacuolated (v) hepatic parenchyma, and excessive periportal fibroplasia, **B** expansion of the portal area with perivascular edema (black arrows), and **C** thickening of the bile duct wall (green arrow) (Hematoxylin, H &Eosin, E)

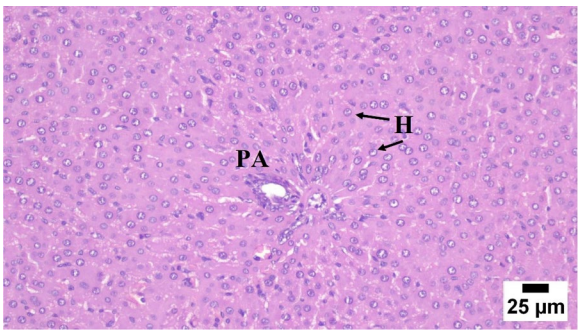


**Fig. 5** Photomicrograph of hepatic sections of rats administered naringenin (NGN, 50 mg/kg BW) 1 h prior to pyrazinamide (PZA, 155 mg/kg BW) daily for 4 weeks showing (a) apparently normal hepatocytes (H) around portal area (PA) (Hematoxylin, H &Eosin, E)

also increase membrane fluidity of blood cells, preventing their rupture (Ajdžanovic et al., 2015).

The observed elevation in the glucose levels in the PZA group suggests stressful conditions. Tasduq et al. (2007) also reported a remarkable increase in glucose level following treatment with PZA. These findings may be due to ROS-induced insulin resistance (Di Meo et al., 2017). Also, elevated glucose levels might be considered transient hyperglycemia. In contrast, the glucose level in the PZA+NGN group returned to normal levels. NGN can increase insulin sensitivity (Li et al., 2019), leading to enhanced glucose uptake by the liver and muscles (Hartogh & Tsiani, 2019).

In the serum of the PZA group, the reported elevations in ASAT and ALAT activities imply PZA-induced hepatotoxicity. Previous studies also reported similar findings in the activities of transaminases after treatment with anti-TB drugs (Abdel-Ghaffar et al., 2017; Basheer et al., 2017). These findings may be due to the altered permeability of hepatocytes due to PZA, its metabolites, or ROS (Zhao et al., 2017). On the other hand, in the PZA+NGN group, ASAT and ALAT activities were significantly lower than in the PZA group and insignificantly different from the controls, indicating that NGN protected hepatocytes against PZA-induced toxicity. The NGN protective action may be through stabilizing their membranes or scavenging ROS (Stabrauskiene et al., 2022).

The reported elevation in TBIL level was associated with a marked elevation in the IBIL level. Similarly, Abdel-Ghaffar et al. (2018a) reported significant elevations in TBIL levels after administering isoniazid. The excessive hemolysis of RBCs and the breakdown of Hb molecules contribute markedly to the increased bilirubin level in the blood (Jayachandra & Devi, 2012). In addition, the recorded depletion of albumin can delay the transportation of bilirubin to the hepatocytes (Abdel-Ghaffar et al., 2018a). On the contrary, the bilirubin levels in the PZA+NGN group were insignificantly higher than in the controls. These results can be due to that NGN stabilizes erythrocytic membranes and their Hb content.

The reported marked decline in the albumin level, whereas an elevation in the globulin level in the PZA-treated group resulted in a significant reduction in the albumin/globulin ratio, indicating PZA-induced alteration in the hepatic function. Previous studies reported the same results following treatment with various anti-TB drugs (Abdel-Ghaffar et al., 2017, 2018b). The reported hypoalbuminemia may reflect exhaustion of albumin in ROS scavenging (Ali, 2019). However, the increased globulin level may reflect an immune response against PZA, which acts as a hapten (Lin et al., 2015). The reported normal protein profile in the PZA+NGN group indicates regular liver functions. It is noteworthy that NGN

can maintain protein biosynthesis by ensuring the energy required by mitochondrial enzymes (Abdel-Ghaffar et al., 2018a).

The reported increases in the TG, TC, and LDL-C levels in the PZA group indicate the risk of vascular diseases. Pal et al. (2008) also detected remarkable elevations in TC levels in serum of rats after administering anti-TB drugs. The enhanced production in the cholesterol and TG levels may reflect the ability of PZA as other anti-TB drugs to alter the expression of genes involved in their synthesis (Abdel-Ghaffar et al., 2018a). In the PZA+NGN group, TG, TC, LDL-C, and HDL-C levels exhibited similarity with the controls. These findings imply the hypolipidemic and hypocholesterolemic action of NGN. NGN induces lipid β-oxidation and inactivates fatty acid and cholesterol synthesis (Goldwasser et al., 2010). Additionally, NGN can inhibit the very low lipoprotein (VLDL) that is the prime transporter of TG from the liver (Soppert et al., 2020).

In the liver of the PZA group, the reported disturbances in MDA and GSH levels and SOD and GR activities indicate PZA-induced oxidative damage. Likewise, Zhao et al. (2017) detected PZA-associated lipid peroxidation in the liver of male and female rats. They linked that to the ability of PZA to induce overproduction of ROS. Excessively produced ROS can damage proteins and alter the expression of genes responsible for antioxidants biosynthesis (Morsy et al., 2016), leading to a subsequent depletion in albumin and GSH levels as well as SOD and GR activities. The MDA and GSH levels and SOD and GR activities in the PZA+NGN group were insignificantly different from the controls, indicating NGN-induced protection against oxidative stress conditions. NGN can substantially reduce the ROS level by scavenging them (Stabrauskiene et al., 2022) or reducing their production by inactivating xanthine oxidase (Alam et al., 2014). Additionally, NGN may enhance the expression of the glutamate-cysteine gene to maintain GSH production (Ramprasath et al., 2014). NGN can also upregulate nuclear factor erythroid-related factor 2 (Nrf2) involved in the SOD and GR gene transcription (Abdel-Ghaffar et al., 2018a).

In the PZA group, remarkable structural alterations were observed in the hepatic tissue. In the same line, Shih et al. (2013) reported PZA-related histopathological modifications in the liver. They correlated these disturbances to the active metabolite of PZA, which is hydroxy pyrazinoic acid. Inflammatory cell infiltration can be due to the ROS ability to initiate immune response via increasing levels of proinflammatory cytokines (Choudhury & MacNee, 2017). The observed vacuolation represents a critical sign of oxidative damage that stimulates necrosis (Abdel-Khalek et al., 2018). Edema is an excessive water filtration due to impaired permeability of the blood capillaries, which may increase the water content of the tissue to reduce the concentration of xenobiotics. The regular appearance of hepatic tissue in the PZA+NGN group supports all the abovementioned ameliorative actions of NGN against PZA-induced hepatotoxicity. NGN can downregulate the cytochrome P450 expression, leading to an inhibited drug biotransformation (Alam et al., 2014). Moreover, NGN can protect the hepatic tissue from necrosis by reducing the activity of caspases (Sahinoğullari et al., 2021). Likewise, NGN has vasodilator activity (Alam et al., 2014).

## Conclusions

The present work revealed that administering PZA alone adversely influenced all the studied hematological parameters, indicating incidence of leucopenia and anemia. Besides, marked alterations in the serum activities of transaminases, lipid variables, protein profile, glucose, and bilirubin levels were recorded. Furthermore, the liver of the PZA group showed substantial modifications as ensured by the reported enhanced lipid peroxidation accompanied with disturbances in the endogenous antioxidant system. Histologically, meaningful disturbances were observed in the hepatic tissue of the PZA group. On contrary, the prior administering with NGN efficiently ameliorated all the abovementioned PZA-induced disturbances. These findings can pave the way for the use of NGN as a protective agent during treatment with PZA.

#### Abbreviations

ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; GR: Glutathione reductase; GSH: Glutathione; Hb: Hemoglobin; HDL-c: High-density lipoprotein cholesterol; IBil: Indirect bilirubin; LDL-c: Low-density lipoprotein cholesterol; MDA: Malondialdehyde; NGN: Naringenin; PCV: Packed cell volume; PZA: Pyrazinamide; RBC: Red blood cell; SOD: Superoxide dismutase; TB: Tuberculosis; TBil: Total bilirubin; TC: Total cholesterol; TP: Total proteins; WBC: White blood cell.

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#### Author contributions

AAA contributed to sharing in the experimental designing, experimental procedures, statistical analysis of data, writing and editing of the manuscript. OAG contributed to suggesting the point, putting the experimental design, supervising the work, and sharing in preparation of paper for publication. DAMA contributed to data collection and carrying out the practical part of the work. All authors have read and approved the manuscript.

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It is not applicable.

#### Availability of data and materials

Data obtained by the present study are included in this manuscript. Data are available upon request.

## Declarations

### Ethics approval and consent to participate

All the used experimental procedures on the rats were allowed by the committee of Institutional Animal Care and Use Committee (CU-IACUC), Cairo University, Egypt. The protocol number was CU/I/F/14/18, at March 2018. Animal handling was according to the standard international guidelines for experimental animal care and use. Consent to participate is not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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