Biochemistry Letters, 15(5) 2019, pages 64- 74

Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Ameliorative effect of Nringenin against Zinc oxide nanoparticles hepatotoxicity in rats

Eassam A.[1], Haytham A. Ali [2, 3], Keshta A.[1]

(1) Biochemistry Division, Chemistry Department, Faculty of Science, Zagazig university, Egypt
(2) Biochemistry Department, Faculty of Veterinary Medicine, Zagazig university, Egypt
(3) Biochemistry Department, faculty of Science, University of Jeddah. Saudi Arabia.

ARTICLE INFO

Keywords:

Nano-Zinc oxide

Antioxidant

Toxicity

Naringenin

ABSTRACT

Background: The speedy growth of the nanotechnology industry has led to the wide-scale production and application of engineered nanoparticles(NPs). **Aim:** The aim of the study is to evaluate the toxicity of oral exposure to zinc oxide nano-particles (ZnO-NPs) on liver tissue of albino rats, and hepatoprotective effect of Naringenin (N) against such ZnO-NPs induced toxicity. **Materials &Methods:** ZnO-NPs were administered orally in two doses (125 mg or 300mg/Kg body weight/ day for 21 consecutive days) to rats. Co-administration of Naringenin (20mg/Kg body weight daily for three weeks to rats. In order to detect the protective effects of the studied antioxidants against n-ZnO induce hepatotoxicity, different biochemical parameters were investigated. Moreover, histopathological examination of liver tissue was performed. **Results**: Nano zinc oxide-induced hepatotoxicity was confirmed by the elevation in the levels of serum Aspartate transaminase (AST), Alanine transaminase (ALT), and alkaline phosphatase (ALP) were considered as biomarkers to indicate hepatotoxicity. Additionally, a significant increase in oxidative stress through the increase in lipid peroxidation marker Malondialdehyde (MDA), a significant decrease in Glutathion peroxidase (GPx) activity and a significant decrease in non-enzymatic antioxidant reduced glutathione (GSH) in liver tissue. Moreover, elevation in inflammatory gene expression of cytokines (Tumor Necrosis Factor alpha (TNFα), Interleukin-2 (IL-2) and Interleukin-6 (IL-6). These biochemical findings were supported by a histopathological examination of liver tissue. **Conclusion*:*** The data suggested that Naringenin protects the liver from the hepatotoxicity caused by ZnO-NPs.

Biochemistry letters, 14(5) 2019, pages 64-74

Introduction:

Metal nanoparticles (NPs) and their oxides have a considerable number of present and upcoming uses in the medical and industrial fields. The smaller size and unique properties of the NPs has greatly improved NPs are considered to be more highly absorbed into the respiratory, skin, and gastrointestinal systems than micron-sized particles because of their unique physicochemical properties, such as surface modifications and their size [(4)]. Previous studies discovered that administration of NPs to rodents resulted in their accumulation in the a number of tissues including liver, brain and spleen [(5, 6)]. One of the most common industrial applications of NPs is sun cream [(7)]. The major component of sunscreens is zinc oxide nanoparticles, which can effectively absorb ultraviolet light [(8)]. A number of of the NPs can be swallowed into and reach the gastrointestinal tract when they are expelled from the mucociliary system of the lungs after inhalation [(9)]. ZnO-NPs are being used in the food manufacturing as additives and in packaging due to their antimicrobial properties. They are also being explored for their potential used as anticancer drugs and as fungicides in agriculture and imaging in biomedical applications [(10)]. With the frequent use of ZNO NPs, exposure to these nanoparticles has increased steadily, resulting in increased attention to their potential toxicity, including cytotoxic, genotoxic, and inflammatory effects [(11, 12)].

It has been reported that ZnO-NPs administered orally dissolve in the stomach, with Zn ions then absorbed to enter into systemic circulation [(13)]. Some researchers ZnO-NPs consider as a material of low toxicity, because zinc is an essential trace element in the human body and are usually present in foods or added as a nutritional Supplement, so zinc interests little attention during assessment of toxicity of nanoparticles [(14)]. On the other hand, it is well-known that a high concentration of zinc is responsible for toxic effects [(15)]. Flavonoids belong to a category of polyphenol compounds that are produced exclusively in plants. These compounds are capable of leading to several biological and pharmacological activities in animal cells. It is generally believed that most mammals need to consume polyphenols from plants due to their antioxidant properties in order to stay healthy [(16)]. Naringenin is a predominant flavonone abundant in fruits such as grapes, tangelo, blood orange, lemons, pummelo and tangerines [(17)]. Naringenin is also the main metabolite of Naringin which is the important flavonoid in *Exocarpium citri grandis*. Naringenin is used as a traditional medicine in China [(18)]. It has been reported to have several biological effects such as anticancer [(19)], anti-mutagenic [(20)], anti-inflammatory [(21)]. This study aimed to investigate the effect of Naringenin against hepatotoxicity induced by ZnO-NPs in rats.

Materials and Methods

Chemicals

The 27-nm ZnO-NP powders were purchased from faculty of science of Beni-Suef University in EGYPT. Naringenin (N) was obtained from Sigma-Aldrich Co. (USA).

Animals

Sixty male albino rats weighting at the beginning of the experiment (150±20g.) were randomly divided into six groups (10 rats in each). Animals were housed in groups of ten in cages at 25±0.5°C, under a 12:12 light/dark cycle, with free access to standard diets and water *ad libtums.* Animals from all groups were kept under similar environmental conditions of temperature, illumination, acoustic noise, and ventilation, and received the determined

diet during the course of the experiment in animal house of Faculty of Veterinary medicine, Zagazig University, Egypt.

Experimental Design

Swiss Albino rats were divided into 6 groups (n=10).

G1: Normal healthy animals.**G2:** Animals orally administered N (20 mg/kg/day) [22] for three weeks. **G3, G4:** Serve as toxic groups, animals orally administered ZnO-NPs (125 mg/kg/day) [23] & (300mg/kg/day) [24] for three weeks.**G5:** Serve as preventive groups, animals orally administered (N 20 mg/kg/day + ZnO-NPs 125 mg/kg/day) for three weeks. **G6:** Serve as preventive groups, animals orally administered (N 20 mg/kg/day + ZnO-NPs 300 mg/kg/day) for three weeks. All groups were kept on the same condition for twenty one days under different treatments then one day after last treatment all rats are sacrificed and blood samples collected in a clean dry capped tubes.

Blood Collection and Tissue Samples

The blood samples were about 5 ml collected without anticoagulant, left to clot at room temperature then centrifuged at 4000 rpm for 5 min. according to **Joslin** [24] to separate serum for biochemical analysis of ALT, AST and ALP.

Liver from all rats were collected and divided into 3 parts as following:

First part were taken only from 3 rats per group as possible as before rats death, weighted (30 mg) and washed in normal saline and immediately kept in liquid nitrogen until be used for determination of gene expression of immunologic pro-inflammatory bio-markers Tumor Necrosis Factor alpha (TNFα) , Interleukin-2 (IL-2) and Interleukin-6 (IL-6). **Second part** were taken from all rats in all groups weighted (1 g.), washed and kept on -20 till homogenized in distilled water using electrical homogenizer, centrifuged at 3000 r.p.m. for 15 minutes, the resulting supernatant were collected and used for determination of lipids peroxidation (Malondialdehyde MDA), antioxidant levels (reduced Glutathione GSH) and antioxidant enzyme activity (Glutathione peroxidase (GPx). **Third part** was collected from rats and preserved in 10% neutral buffered formalin, processed and stained with haematoxylin and eosin (H&E) dyes for histopathological studies using a light microscope according to **Bancroft & Gamble** [25].

Biochemical Analysis

Serum samples collected from different groups were analyzed for ALT, AST and ALP using kits supplied by **SPINREACT Kit (Ctra. Santa1 Coloma,SPAIN)**. Liver tissue homogenates were used for estimation of the content of GSH according the method of **Beutler et al.** [26], GPx activity were determined according to **Paglla and Valentine** [27].The extent of lipid peroxidation was assayed by the measurement of MDA according to **Satoh** [28].

Molecular determinations

Determination of TNFα, IL-2 and IL-6 gene expression. Using a semi- quantitative RT-PCR according to **Meadus** [29]. The gene expression of TNFα, IL-2 and IL-6 genes were determined using RT-PCR technique. Total RNA was extracted from separated tissues using RNeasy Mini Kit **(Qiagen, Cat. No.74104)**. First strand cDNA was synthesized using Revert Aid TM H Minus **(Fermentas, life science, Pittsburgh, PA, USA)**. The PCR reaction was started by using **SYBR® Green PCR Master Mix Catalog Number 2501130** (Master Mix) supplied by applied bio systems in a rotor gene apparatus **(Biometra-Germany)**. The housekeeping gene β-actin was used as a constitutive control for normalization. Primers were provided by Sigma Aldrich **(Sigma-Aldrich Chemie GmbH, Steinheim, Germany)** and were listed in

table (1). The quantitative fold's changes in mRNA expression were determined relative to the housekeeping controls (ß-actin mRNA) levels in each corresponding group and calculated using the 2-DD CT method. (2-ΔΔCT the relative Quantification level of target genes calculation) [(30)].

Statistical Analysis

The obtained data were analyzed and graphically represented using the statistical package for social science (**SPSS** Inc. Released 2007. SPSS for windows, Version 16.0. Chicago, SPSS Inc.) [(31)] for obtaining means and standard error. The data were analyzed using one way ANOVA to determine the statistical significance of differences among groups.

Results

Result were presented as means ± SE of ten rats in each group. Values of $p < 0.05$ were regarded as statistically significant The statistical significance of differential findings between the experimental groups and control were determined and that are represented by symbols (a,b,c,d,e,f,g).

Data presented in **table (2)** showed the effect of Naringenin (N) on ALT, AST, ALP, MDA, GSH, and GPX in the studied groups.

There was a significant increase in the serum ALT, AST, ALP activities and increase in the hepatic MDA levels in the groups G3, G4 administered ZnO-NPs at a dose of (125 and 300 mg/Kg b.wt.) compared to control group. This increase was dose dependent. While G5, and G6 (preventive groups Naringenin in a dose of (20 mg/kg b.wt.) recorded a reduction in ALT, AST, and ALP activities and decrease in MDA levels compared to G3, and G4 **table (2)**.

The hepatic reduced GSH levels and the hepatic GPx activities were decreased from10.60 ± 1.13, and 25.28 ± 1.12 in G1 to 4.13 ± 0.40, and 16.77 ± 0.61 in G3 and to 2.39 ± 0.21, and 12.96 ± 2.33 in G4; respectively. The reduction in GSH, and GPX was dose dependent (ZnO-NPs). Meanwhile, GSH levels and GPX activity in G5, and G6 (treatment with Naringenin at dose of 20 mg/kg b.wt.) were elevated to 5.99±0.32, and 20.77±1.73 in G5 and to 8.59±1.19, and 22.69 ± 2.33 respectively, **table (2)**

Molecular results

The relative gene expression of TNF-α, IL-2, IL-6 in the studied groups were illustrated in Fig. (1). The mean transcriptional level of mRNA of TNF-α, IL-2, IL-6 genes in negative groups were 1.00±0.04, 1.00±0.06, 1.00±0.02 Pg/ml; respectively. The transcriptional levels of these genes were increased in G3, and G4 to [2.63± 0.12 & 3.95± 0.13], [3.03±0.09 & 4.23±0.09], and [2.10±0.11 and 3.79±0.09]; respectively. While, Naringenin treatment (G5, and G6) recorded a decrease in transcription of TNF-α [1.47± 0.12 & 1.90± 0.14], of IL-2 [1.54±0.09 & 2.43±0.12], and of IL-6 [1.32±0.08 & 1.86±0.10]; respectively.Fig. (1)

Histological Investigation

In the histopathological study, the liver sections of normal group (G1) and rats treated with Naringenin (G2) revealed preserved hepatic lobular architecture and normal hepatocytes with rounded vesicular nuclei **Fig.2 (A, B)**. In G3, and G4, there were a dilated congested central vein surrounded by hepatocytes with fatty change **Fig.2 (C, D)**. While, in G5, and G6, the liver sections revealed that there were a mild fatty change in liver cell and no cell necrosis **Fig.2 (E, F)**.

Discussion

ZnO-NPs were used in a variety of different applications including cosmetics, paints, as drug carrier and filling in medical materials [(32)], including sunscreens and environmental remediation, direct and indirect release of these NPs into aquatic environments via bathing, sewage effluent and other engineering application, said that feeding of ZnO-NPs suspension through digestive tract at a dose 0. 6mg daily which leads to damage of some

primary organs (heart, lung, liver & kidney) of mice [33, 34]. Because of their small size, nanoparticles can diffuse throughout the body when consumed, and can cross the small intestine and further distribute into the blood, brain, lung, heart, kidney, spleen, liver, intestine and stomach [35]. When cells are exposed to any insult (chemical/physical), it results in the production of reactive oxygen species (ROS) [36]. ZnO-NPs may promote the formation of pro-oxidants which, in turn, destabilizes the delicate balance between the biological system's ability to produce and detoxify the ROS. ROS include free radicals such as the superoxide anion ($O2^-$), hydroxyl radicals (OH) and the non-radical hydrogen peroxide (H_2O_2), which are constantly generated in cells under normal conditions as a consequence of aerobic metabolism.

In the present study, the decreased GPx and GSH activity and elevated MDA levels in liver indicated that the presence of oxidative stress and lipid peroxidation response were generated by ZnO-NPs administration. It has been reported that oxidative stress mediated DNA damage and cytotoxicity induced by ZnO-NPs [37]. In contrast, a marked increase in the antioxidant enzyme activities was seen when rats were treated with Naringenin after ZnO-NPs administration.

Furthermore, oxidative stress activates specific signaling pathways including mutagen activated protein kinase (MAPK) and Nuclear factor-κB (NF-kB), which together with the depletion of antioxidant defenses that leads to release of pro-inflammatory cytokines. The overall result of this signaling cascade is the triggering of inflammation, a defensive reaction that leads to further ROS release from inflammatory cells (e.g. neutrophils) [37]. So, this explained elevation in level of pro-inflammatory cytokines TNFα, IL-2 and IL-6. in ZnO-NPs orally administrated group when compared to the control group. Nringenin attenuated inflammation, necrosis and also reduced oxidative stress, as it possesses hydroxyl groups at the 4^{th}, 5^{th} and 6^{th} positions. This substitution appears to aid the flavonone to inhibit the production of TNF-α [38] and other cytokines and decreased the oxidative stress.

Due to damaged liver cells, such enzymes ALT, AST, and ALP in hepatocytes are released into the blood. Therefore, a high amount of these enzymes indicates the destruction of liver cells. As a result of the imbalance among antioxidants/ oxidants ratio in the cells, the levels of hepatic enzymes (ALT, AST and ALP) elevate in serum due to tissue necrosis or membrane damage and subsequent leakage of enzymes into the serum [40]. Serum aminotransferases (ALT & AST) are cytosolic enzymes of hepatocytes; an increase in their activities reflecting an increase in the plasma membrane permeability of hepatocyte which in turn associated with cell death [41]. The reversing of hepatotoxic effect induced by ZnO-NPs, herewith observed after treatment with Naringenin which evaluated by significant decreasing in liver enzyme ALT, AST and ALP comparing with Zinc oxide NPs groups. The present results revealed that treatment with Naringenin after ZnO-NPs administration was able to normalize the activities of liver enzymes. These demonstrated by significant decrease in ALT, AST and ALP as compared with ZnO-NPs treated group, as Naringenin ameliorates the hepatic functions. This was confirmed by histopathological study of liver tissue where the hepatotoxic alterations were improved by Naringenin administration due to its antioxidant and anti-inflammatory characters of Naringenin.

CONCLUSION

ZnO-NPs 125 mg/kg and 300 mg/kg showed hepatotoxicity, and the toxic effect was dose dependent. Naringenin as a bioflavonoid antioxidant and anti-inflammatory properties reduced the nanoparticle toxicities .

References:

1- Adams LK, Lyon DY, Alvarez PJJ. Comparative eco-toxicity of nanoscale TiO_2, SiO, and ZnO water suspension. Water Res. 2006; 40 (19): 3527-3532.

2- Mody VV, Siwale R, Singh A. and Mody HR. Introduction to metallic nanoparticles. J Pharm Bioallied Sci. 2010; 2(4): 282-289.

3- Warheit DB. Nanoparticles: Health impacts? Materials Today.2004; 7(2) 32-35.

4- Fubini B, Ghiazza M. and Fenoglio I. Physico-chemical features of engineered nanoparticles relevant to their toxicity.Nanotoxicol. 2010; 4: 347-363.

5- Borm PJ. and Kreyling W. Toxicological hazards of inhaled nanoparticles potential implications for drug delivery. J Nanosci Nanotechnol. 2004; 4(5): 521-531.

6- Chen Y, Xue Z, Zheng D, Xia K ZhaoY. and Liu T. Sodium chloride modified silica nanoparticles as a non-viral vector with a high efficiency of DNA transfer into cells.Curr Gen Ther. 2003; 3(3): 273-279.

7- Burnett ME. and Wang SQ. Current sunscreen controversies: a critical review. Photodermatol Photoimmunol Photomed. 2011; 27(2): 58-67.

8- Nohynek GJ, Lademann J, Ribaud C. and Roberts MS. Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety.Crit Rev Toxicol. 2007; 37(3): 251-277.

9- Oberdorster G, Oberdorster E. and Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005; 113(7): 823-39.

10- Rasmussen JW, Martinez E, Louka P.and Wingett DG. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opin Drug Deliv 2010; 7: 1063-1077.

11- Hackenberg S, Zimmermann FZ, Scherzed A, Friehs G, Froelich K. and Ginzkey C, Koehler C, Burghartz M, Hagen R and Kleinsasser N. Repetitive exposure to zinc oxide nanoparticles induces dna damage in human nasal mucosa mini organ cultures. Environ Mol Mutagen. 2011; 52: 582-589.

12- Teow Y, Asharani PV, Hande MP. and Valiyaveettil S. Health impact and safety of engineered nanomaterials. Chem Commun (Camb). 2011; 47: 7025-7038.

13- Cho WS, Kang BC, Lee JK, Jeong J, Che JH. and Seok SH. Comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. Part Fiber Toxicology. 2013; 10: 9

14- Wang B, Feng W. and Wang M. Acute toxicological impact of nano- and Submicro-scaled zinc oxide powder on healthy adult mice. J Nanopart Res. 2008; 10: 263-276.

15- Plum LM, Rink L. and Haase H. The essential toxin: impact of zinc on humanhealth. Int J Environ Res Public Health. 2010; 7: 1342-1365.

16- Nadia Salem Alrawaiq. A review of flavonoid quercetin: Metabolism, Bioactivity and antioxidant properties. PharmTech Res.2014; 6(3): 933-941.

17- Holden JM, Bhagwat SA. and Patterson KY. Development of a multinutrient data quality evaluation system. *J. Food. Comp. Anal. 2002;* 15:339–348.

18- Fang T, Wang Y, Ma Y, Su W, Bai Y, Zhao P. 2006: A rapid LC/MS/MS quantitation assay for naringin and its

two metabolites in rats plasma. *J Pharm Biomed Anal* 40:454–459.

19- **Takahashi T, Kobori M, Shinmoto H, Tsushida T.** Structure-activity relationships of flavonoids and the induction of granulocytic- or monocytic-differentiation in HL60 human myeloid leukemia cells. *Biosci Biotechnol Biochem. 1998;* 62:2199–2204.

20- **Choi JS, Park KY, Moon SH, Rhee SH, Young HS.** Antimutagenic effect of plant flavonoids in the Salmonella assay system. *Arch Pharm Res.1994;* 17:71–75.

21- **Raso GM, Meli R, Di Carlo G, Pacilio M. and Di Carlo R.** Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A.1. *Life Sci.2001;* 68:921–931.

22- **Karnakar Redy Y, Saritha Ch, Saridhar Y and Shankaraiah P.** Naringenin prevents the Zinc Oxide Nanoparticles Induced Toxicity in Swiss Albino Mice. J Pharmacol Clin Toxicol. 2014; 2(1):1021.

23- **Chung HE, Yu J, Baek M, Lee JA , Kim MS, Kim SH, Maeng EH, Lee JK, Jeong J. and Choi SJ.** Toxicokinetics of zinc oxide nanoparticles in rats. Nanosafe 2012: International conferences on safe production and use of Nanomaterials, Journal of Physics: Conference Series 429 (2013) 012037

24- **Joslin J.** Blood Collection Techniques in Exotic Small Mammals. Journal of Exotic Pet Medicine 2009; 18:117 – 139.

25- **Bancroft, JD, Gamble M.** Theory and Practice of Histological 551 Techniques. 6th ed Philadelphia: Churchill Livingstone/Elsevier. eds. 2008.

26- **Beutler, E., Duron, O., and Kelly B.M.** Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 1963; 61: 882-888.

27- **Pagalla, D.E. and Valentine, W.N.** Studies on qualitative and quantitative characterization of erythrocytes glutathione peroxidase. J.lab. clin. Med. 1967; 70,158.

28- **Satoh K.**, Serum Lipid Peroxide in Cerebrovascular Disorders Determined by a New Colorimetric Method. Clinica Chimica Acta., **1978**, 90, 37-43. .

29- **Meadus W.J.** A semi- Quantitative RT-PCR method to measure the in vivo effect of dietary conjugated linoleic acid on protein muscle PPAR gene expression. Biol. Proced. On line. 2003; 5 (1): 20-28.

30- **Livak K, Schmittgen KD.** Analysis of relative gene expression data using real time quantitative PCR and the 2 (-Delta Delta C (T)) method. Methods. 2001;25(4):402–408

31- **Levesque R.**, SPSS. 2007; Programming and Data Management: A Guide for SPSS and SAS Users, Fourth Edition, SPSS Inc., Chicago Ill.

32- **Jiang J., Jiang Pi, and Cai J.,** The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications. Bioinorganic Chemistry and Applications. 2018. Article ID 1062562, 1:18.

33- **Venkatachari P, Hopke PK, Brune W H, Ren X, Lesher, R, Mao J. and Mitchell M.** Characterization of wintertime reactive oxygen species concentrations in flushing, New York. Aerosol Science and Technology, 2007; 41: 97–111.

34- **Jiang J, Oberdörster G, Elder A, Gelein R, Mercer P. and Biswas P.** Does Nanoparticle Activity Depend

upon Size and Crystal Phase? Nanotoxicology. 2008; 2: 33-42.

35- **Sharma V, Singh P, Pandey AK. and Dhawan A.** Induction of oxidative stress, DNA damage and apoptosis in mouse liver after sub-acute oral exposure to zinc oxide nanoparticles. Mutat Res. 2012; 745: 84-91.

36- **Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, Yeh JI. and Nel AE.** Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano. 2008; 2: 2121-2134.

37- **Sharma V, Anderson D. and Dhawan A.** Zinc oxide nanoparticles induce oxidative stress and genotoxicity in human liver cells(Hep G2). J Biomed Nanotechnol.2011; 7(1): 98-99.

38- **Wang B, Feng WY, Wang M, Wang TC, Gu YQ, Zhu MT, Ouyang H, Shi JW, Zhan F, Zhao YL, Chai ZF, Wang HF and Wang J.** Acute toxicological impact of nanoand submicro-scaled zinc oxide powder on healthy adult mice. J. Nanopart. Res. 2008; 10 : 263–276.

39- **Jayachitra J., Victor Antony Santiago Jesudoss, Venugopal P. Menon, and Nalini Namasivayam.** Anti-inflammatory role of Nringenin in rats with ethanol induced liver injury. Informa Healthcare USA,Inc. 2012; 22(7):568-76.

40- **Casalino E, Calzaretti G, Sblano C. and Landriscina C.** Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium, Toxicology, 179, 2002, 37-50.

41- **Rosen, H.R. and Keeffe, E.B.** Laboratory evaluation of the patients with signs and symptoms of liver disease. In: Brandt, L. J., (Ed.) Clinical practice of gastroenterology. 2000; 812-820.

Table (1): Primers used in determination of the gene expression of the selected genes:

Gene	Primers	Target(bp)	Mimic(bp)
β-Actin	**Sense 5`**-CCTGCGTCTGGACCTGGCTG**3`**	477	256
	Antisense 5-CTCAGGAGGAGCAATGATCT-**3`**		
TNF-α	**Sense 5`**-ACGCTCTTCTGTCTACTG-**3`**	592	292
	Antisense 5`**-GGATGAACACGCCAGTCG-**3`		
IL-2	**Sense 5`**-AACAGCGCACCCACTTCAA-**3`**	400	292
	Antisense 5`**-TTGAGATGATGCTTTGACA-**3`		
IL-6	**Sense 5`**-GAAATGAGAAAAGAGTTGTGC-**3`**	321	256
	Antisense 5`**-GGAAGTTGGGGTAGGAAGGAC-**3`		

Table (2): Effect of Naringenin on ALT, AST, ALP, GSH, MDA, and GPX

Group	ALT (U/l)	AST (U/l)	ALP (U/l)	GSH (mg/g.t)	MDA (nmol/g.t)	GPX (U/g.t)
Group 1: (Control)	26.33 ± 0.88^{g}	52.33 ± 1.45^{e}	137.83 ± 1.48^{f}	10.60 ± 1.13^{c}	34.03 ± 0.33^{d}	$25.28 \pm 1.12^{b,c}$
Group 2: (Naringenin)	$30.33 \pm 0.88^{f,g}$	56.43 ± 0.99^{e}	143.10 ± 1.02^{f}	14.55 ± 1.97^{b}	$45.95 \pm 3.66^{c,d}$	32.22 ± 2.39^{a}
Group 3: (ZnO 125)	$44.33 \pm 2.33^{b,c}$	112.23 ± 1.13^{b}	274.33 ± 3.48^{c}	$4.13 \pm 0.40^{f,g}$	91.51 ± 1.54^{b}	$16.77 \pm 0.61^{e,f}$
Group 4: (ZnO 300)	60.66 ± 1.76^{a}	129.83 ± 1.01^{a}	316.00 ± 4.93^{a}	2.39 ± 0.21^{g}	161.21 ± 22.33^{a}	12.96 ± 2.33^{f}
Group 5: (N + ZnO 125)	37.66 ± 1.45^{d}	97.70 ± 1.37^{c}	245.83 ± 2.20^{d}	$5.99 \pm 0.32^{d,e,f}$	65.80 ± 3.99^{c}	$20.77 \pm 1.73^{c,d,e}$
Group 6: (N + ZnO 300)	48.00 ± 1.52^{b}	117.03 ± 1.06^{b}	295.33 ± 2.90^{b}	$8.59 \pm 1.19^{c,d,e}$	104.29 ± 1.18^{b}	$22.69 \pm 2.33^{c,d}$

Values are means ± SE of ten rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Duncan′s multiple range test).

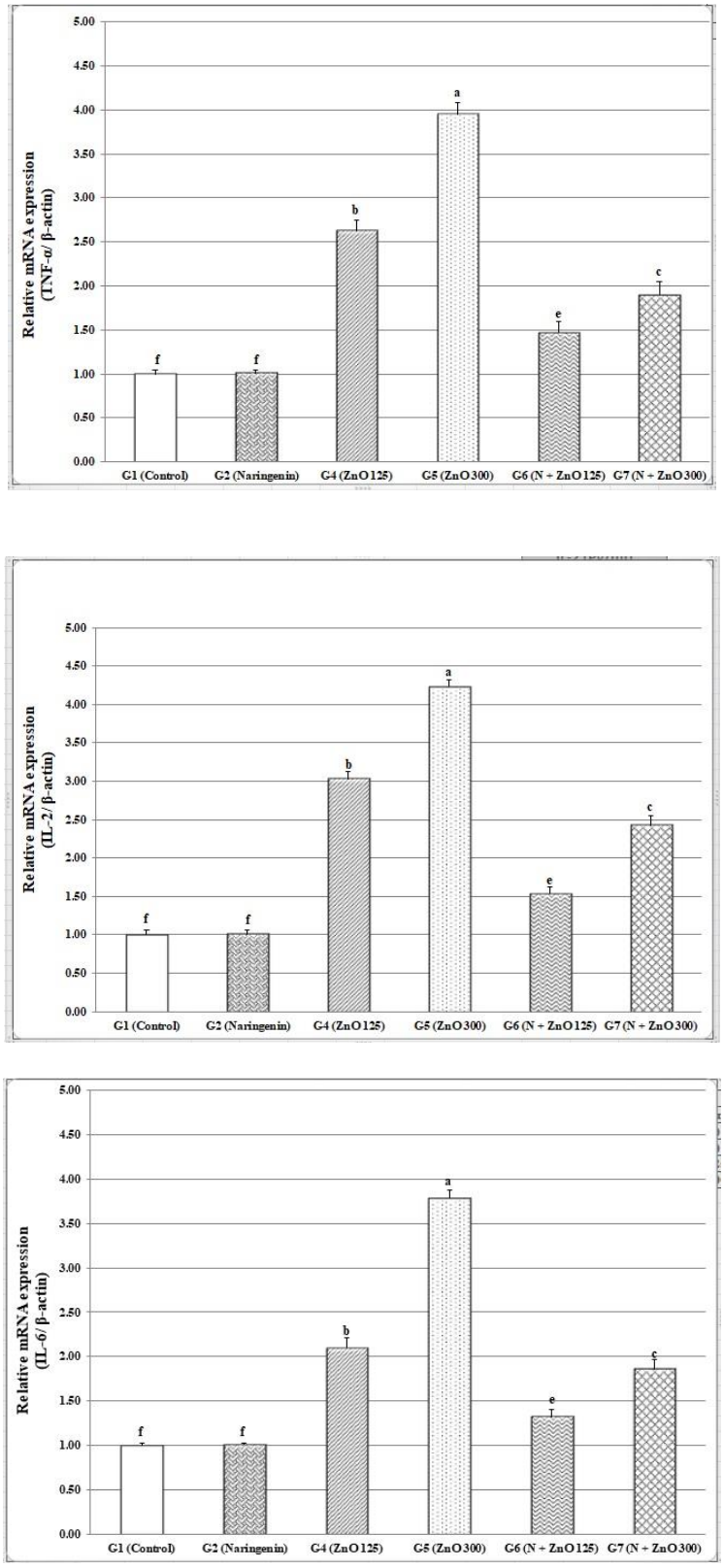

Figure 1: The relative gene expression of TNF-α, IL-2, IL-6 in the studied groups

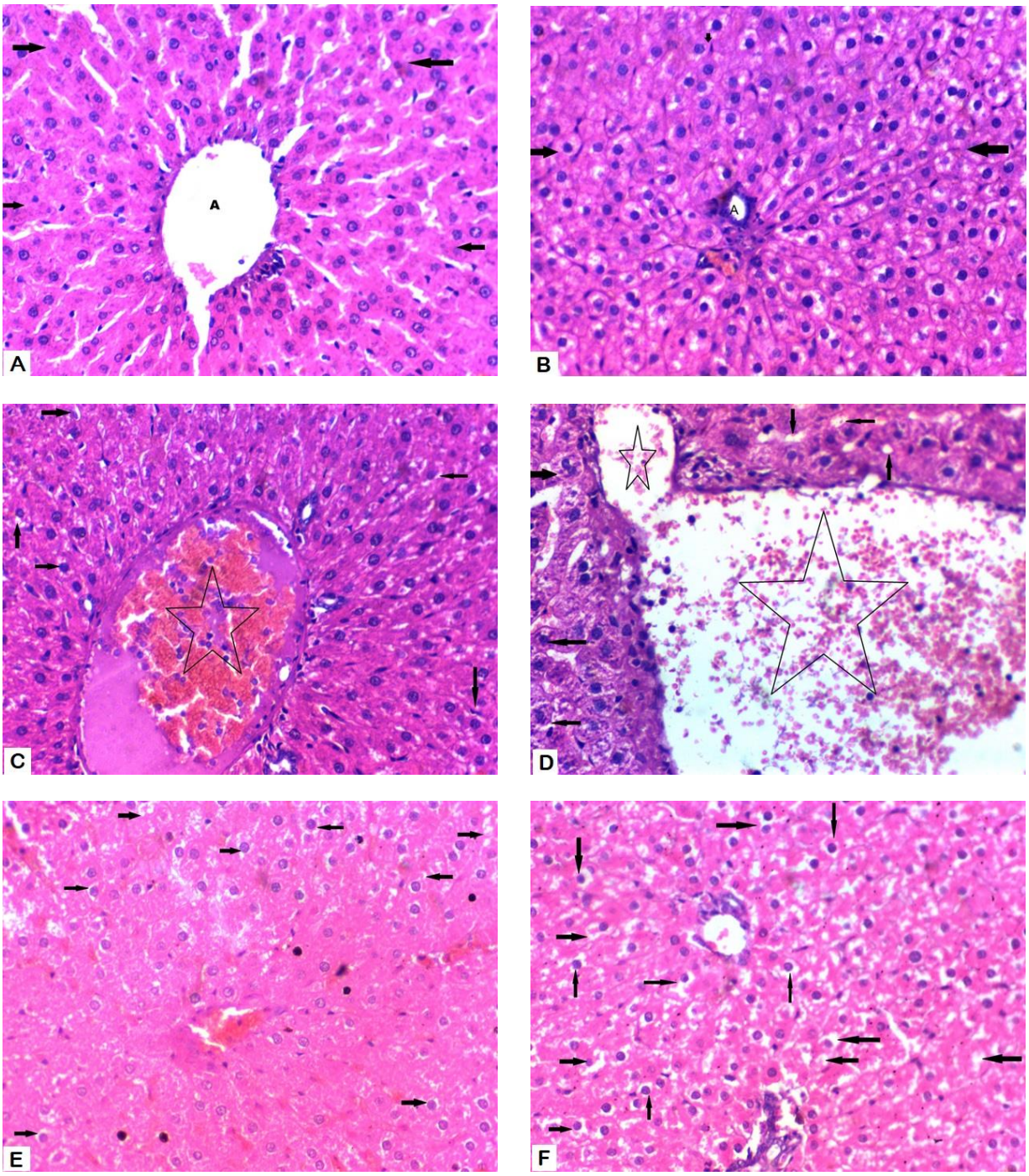

Figure 2: Histological Investigation Histopathology of liver: **(A)** Control **(B)** Naringenin **(C)** 125 mg/Kg bw of ZnO-NPs **(D)** 300 mg/Kg bw of ZnO-NPs **(E)** 125 mg/Kg bw of ZnO-NP + N. **(F)** 300 mg/Kg bw of ZnO-NP + N.