

Hesperidin Alleviates Malathion-Induced nephrotoxicity in Rats: Impact on Oxidative Damage, Inflammation, Pyroptosis and Apoptosis.

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Submit Date : 04 Nov. 2024

Revised Date : 16 Nov. 2024

Accept Date : 18 Nov. 2024

Keywords

- Injury
- Malathion
- Hesperidin
- Oxidative insult
- Inflammation
- Apoptosis
- Pyroptosis

Abstract

Abstract: Malathion is widely used organophosphorus insecticides available in various forms for controlling insect pests. However, it has a severe side effect on the body's antioxidant systems. Hesperidin, a famous flavonoid, has a beneficial effect such as antioxidant, anti-inflammatory properties, which can inhibit the harmful effects. The aim of this work is to investigate the nephroprotective effect of hesperidin against malathion-induced nephrotoxicity. Four groups of rats, six per each: Control group (Group I), Hesperidin group (Group II), Malathion group (Group III), and Malathion+Hesperidin group (Group IV). Blood samples were taken to assess renal function by measuring serum level of creatinine and Kim-1. Renal homogenates were used to measure oxidative stress markers MDA, SOD, CAT, GSH, IL-1 β , TNF- α , IL-6, apoptotic markers (caspase-3, Bcl-2, Bax), and pyroptotic markers (NLRP3, caspase-1). Renal tissues were fixed in formalin for histopathological examination. Hesperidin significantly decreased serum creatinine and Kim-1, restoring renal function affected by malathion administration. Also, hesperidin combats malathion-induced renal oxidative stress by reducing MDA, and increasing SOD, CAT, GSH levels. Hesperidin also, antagonizes malathion-induced renal inflammation by decreasing levels of TNF- α , IL-6, IL-1 β . Moreover, it decreases malathion-induced apoptosis and pyroptosis by its reducing protein levels of caspase-3, Bax, NLRP3, caspase-1, while enhancing Bcl-2 levels. Hesperidin and malathion co-treatment improved the renal pathological alternation. Hesperidin offers a promising nephroprotective effect against malathion-induced nephrotoxicity through its antioxidant, anti-inflammatory, antiapoptotic and anti-pyroptotic capabilities.

Introduction

Despite stringent control measures, the global use of OP pesticides remains prevalent in agriculture, public gardens, and households, leading to daily low-level exposure [1]. The liver and kidneys are the most affected organs due to the toxicokinetic properties of OPs. While the precise mechanisms of OP-induced renal dysfunction are not fully understood, oxidative stress is identified as the primary harmful process [2].

Extensive application of malathion leads to environmental contamination [3,4]. Excessive exposure can cause acute or chronic intoxication, especially in underdeveloped countries [5]. Due to its lipophilic nature, malathion is quickly absorbed and distributed throughout the body, leading to several illnesses [6]. Human cells exposed to malathion show excessive oxidative damage, resulting in increased production of reactive oxygen species [7-9]. This exposure disrupts both enzymatic and nonenzymatic antioxidant activities in tissues [10], potentially causing DNA damage, mitochondrial dysfunction, and apoptosis. Additionally, malathion has been found to cause liver injury [11].

Pyroptosis, a newly recognized manner of programmed cell death, primarily occurs in macrophages and dendritic cells [12]. However, it also takes place in other organs, such as the liver [13] and kidneys [14,15]. This type of cell death is characterized by plasma membrane rupture, DNA damage, and inflammatory cytokines release [16,17]. Pyroptosis is triggered by pore-forming proteins called gasdermins, which are substrates of caspase-1 and caspase-4/5/11, producing N-terminal fragments [18,19]. The NLR and ASC form the multiprotein complex known as the inflammasome, with NLRP3 being the most well-known type [20]. The assembly of NLRP3,

procaspase and, ASC activates the NLRP3 inflammasome [21]. This active inflammasome then recruits and activates caspase-1, leading to IL-1 β and IL-18 activations, ultimately resulting in pyroptosis [20].

Nutritional therapy using natural antioxidants may help reduce the toxicity caused by organophosphate pesticides (OPs). Research indicates that antioxidants can also influence signaling pathways related to inflammation and apoptosis [22,23]. HES, a polyphenolic bioflavonoid presents mainly in grapefruits, tangerines, and orange peels [24], possesses various therapeutic and biological properties. These include antioxidant, anti-allergic, antiviral, anti-inflammatory, antimicrobial, and neuroprotective effects [24]. This study aims to explore the possible protective effects of hesperidin against malathion-induced nephrotoxicity and the mechanisms behind these effects.

2. Material and Methods

2.1. Animals

This study utilized twenty-four male Wistar rats (8 weeks old), each weighing 190-220 g. The rats were housed in clean cages with 12-hour dark/light cycle, at a temperature of $22 \pm 2^\circ\text{C}$. All the time of the experiment, the animals were provided with standard food and water. The study was conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publication No. 8023, revised 1996).

2.2. Experimental Design

After a one-week acclimatization period, the rats were divided into four groups, each consisting of six rats. The groups were as follows:

- Group I (Control group): Received normal saline.
- Group II (Hesperidin group): Received 20 mg/kg of hesperidin via intraperitoneal (I.P.) administration daily thirty days [25].

- Group III (Malathion group): Rats of this group received 100mg/kg/day of malathion orally [26].
- Group IV (Hesperidin + Malathion group): Received 20 mg/kg of hesperidin via I.P. administration, followed 3 hours later by malathion with a dose of 100mg/kg/day like the dose in the group III.

After four weeks of treatment, the rats were anesthetized with ketamine and xylazine injections one day after the experiment. Blood was taken from the orbital venous plexus, and serum was separated using a centrifugation instrument to 3000 rpm for ten minutes to analyze renal functional testes. The rats were then decapitated, and their kidneys were removed, and rinsed in ice-cold saline. Renal tissues putted in 10% buffered formalin for histological analysis. Additionally, another kidney specimens were stored at -20°C to analyze oxidative stress markers.

2.3. assessment of renal function indicators

For serum separation, blood samples were centrifugated for fifteen minutes at 9000x g. The separated sera were then used to measure creatinine levels by Creatinine Assay Kit, Kim-1 levels were measured using ELISA kit (Cat# E-CL-R0409) following the manufacturer's guidelines.

2.4. Antioxidant Tissue Parameters Analysis

Lipid peroxidation (LPO) was detected by measuring MDA using spectrophotometry, following the method of Ohkawa et al [27]. GSH levels were determined using Ellman's [28] method, CAT and SOD activities were measured according to the methods of Aebi, [29] Sun et al. [30], respectively.

2.5. Histopathological Examination

Renal tissues were fixed in 10% formalin after immersed in a paraffin block following the procedures of [31]. The renal tissue in the paraffin block were sectioned into five-micron thick slices,

stained with hematoxylin and eosin (H&E), and examined under light microscope.

2.6. ELISA examination

The levels of inflammatory cytokines TNF- α , IL-6, IL-1 β , and pyroptosis pathway markers NLRP3, caspas-1, and apoptosis markers caspase-3, Bax, Bcl-2 in renal tissues of different groups were measured using available kits (cat#E-EL-R2856, E-EL-R0015, E-EL-R0012, ab277086, E-EL-R0371, MBS018987, E-EL-R0098, E-EL-R0096), respectively, accompanying the factory instructions.

2.7. Statistical Analysis and Data Interpretation

Our collected data were analyzed using GraphPad Prism version 8.0.0. Quantitative data were presented as mean \pm SD, following normality analysis with the Sharpino-Wilk test. A one-way ANOVA was used, followed with post hoc Tukey's test to compare between groups. Statistical significance was set at the 0.05 level.

3. Results

3.1. Effect of hesperidin on renal functions and renal histology

There was a significant $p < 0.05$ elevation in the sera level of Kim-1 and creatinine in malathion group compared to normal rats [Fig. 1A, B]. Creatinine and Kim-1 levels in Malathion+Hesperidin group were markedly decreased, when compared to the malathion group. This indicates that hesperidin rescues renal function in malathion-induced nephrotoxicity.

Furthermore, histological examination of control and hesperidin groups showed the normal picture of normal medullary tubules, interstitial tissues and blood vessels [Fig. A-D]. Meanwhile, oral intake of malathion showed clear tubular degeneration, including epithelial hydropic degeneration and tubular dilatation, with congested blood vessels [Fig. 2E,F]. Fortunately, renal medullary sections

of Malathion+Hesperidin group showed mild tubular dilatation and congested blood vessels [Fig. 2G,H], which was manifested by a significant $p < 0.05$ decrease in kidney lesion score [Fig. 2I].

The histological improvement by hesperidin confirms its neuroprotective effect against malathion-induced kidney injury.

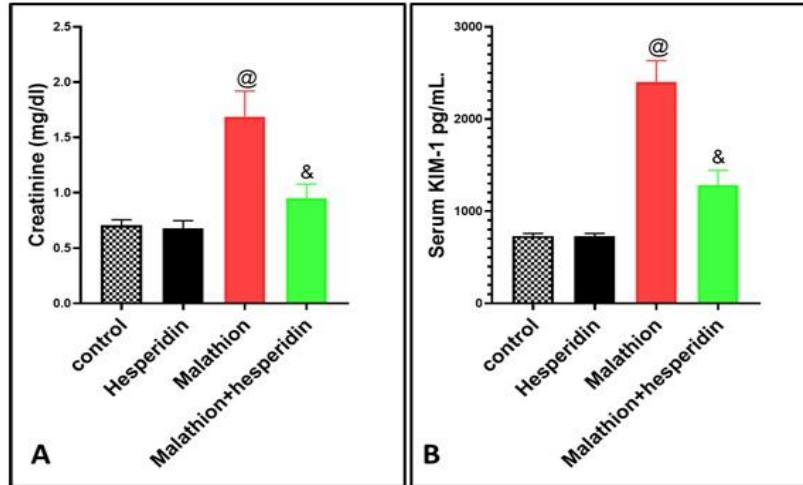


Fig. 1. Protective effect of hesperidin on serum creatinine (A), and Kim-1 (B). All data expressed as a mean \pm SD. @ $p < 0.05$ significant compared to normal groups; & $p < 0.05$ significant to malathion group

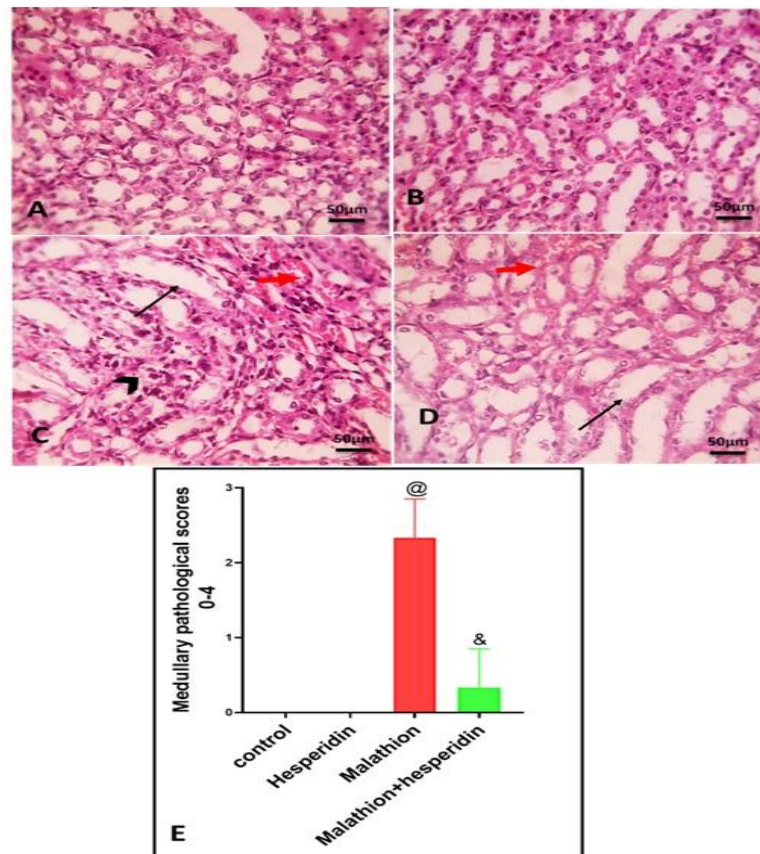


Fig. 2. Microscopic pictures of H&E-stained renal sections showing the normal medulla consisting of normal tubules, blood vessels and few interstitial tissues in the normal and Hesperidin groups (A,B). Renal medulla from Malathion group showing tubular dilatation (black arrows) with hydropic degeneration in epithelial lining of many tubules (arrowheads), congested blood vessels (red arrows) (C). Renal medulla from Malathion+Hesperidin group showing minimal tubular dilatation (black arrows) with mildly congested blood vessels (red arrows) (D). Magnifications: X400 (bar= 50 μ m). Histogram of medullary pathological score (E). @ Significantly to the control & Hesp groups ($p < 0.05$). & Significantly to the malathion group ($p < 0.05$). All results are reported as a mean \pm SD.

3.2. Protective effect of hesperidin against malathion-induced oxidative stress

Oxidative stress biomarkers levels were assayed in renal homogenates. Malathion oral intake with a dose of 100 mg/kg/day significantly $p < 0.05$ elevated MDA levels and decreased SOD, CAT, GSH levels in comparison to normal rats

[Fig. 3A-D]. Cotreatment with hesperidin significantly $p < 0.05$ scavenged the redox stat by decreasing MDA level and increasing the levels of antioxidants compared to the malathion group. Thus, the antioxidant property of hesperidin became clear on malathion-induced renal redox state.

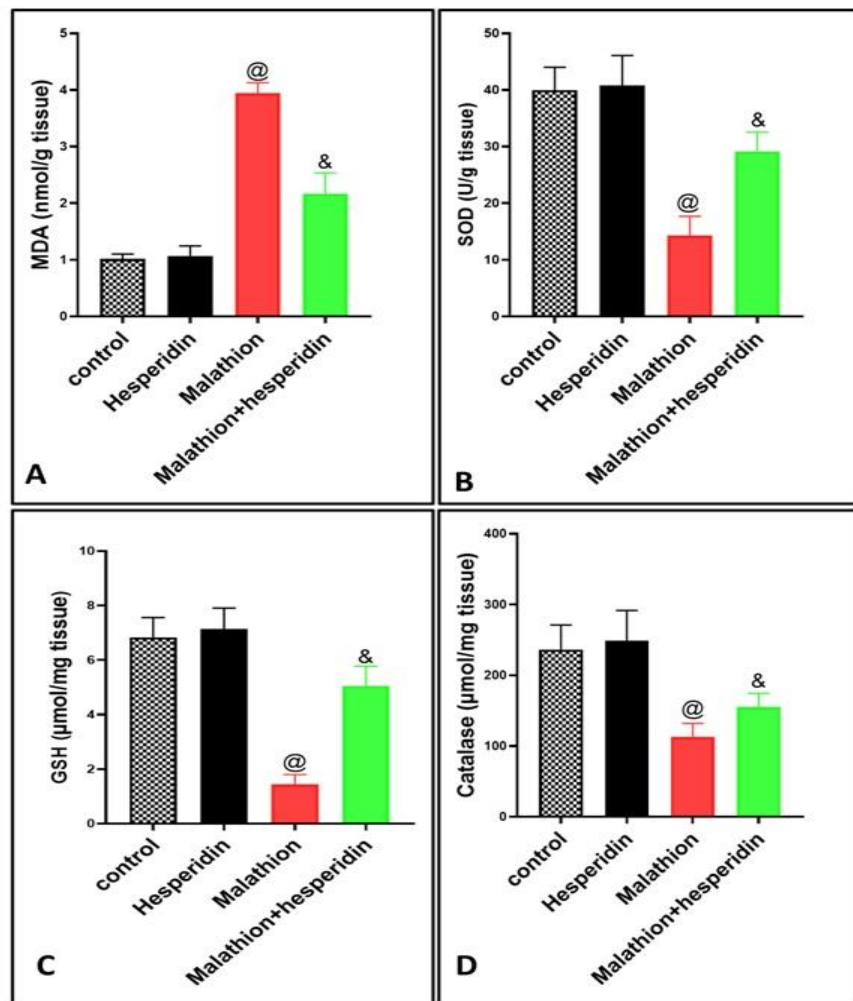


Fig. 3. Impact of malathion and hesperidin on oxidative stress markers MDA (A), SOD (B), GSH (C), and CAT (D). @ $p < 0.05$ significant to the control & Hesp groups. & $p < 0.05$ significant to the malathion group. All results are reported as a mean \pm SD.

3.4. Impact of hesperidin on renal inflammatory markers

To evaluate the renal inflammation, we measured the supernatant protein levels of inflammatory cytokines TNF- α , IL-1 β , IL-6 by ELISA assay. As shown in Figure 4, the malathion group showed a massive secretion of inflammatory mediators

compared to control group. However, cotreatment with hesperidin demonstrated a strong anti-inflammatory character by its decrease to the gush of inflammatory cytokines. Depending to these results, hesperidin offers a potent anti-inflammatory effect against malathion-induced renal inflammation.

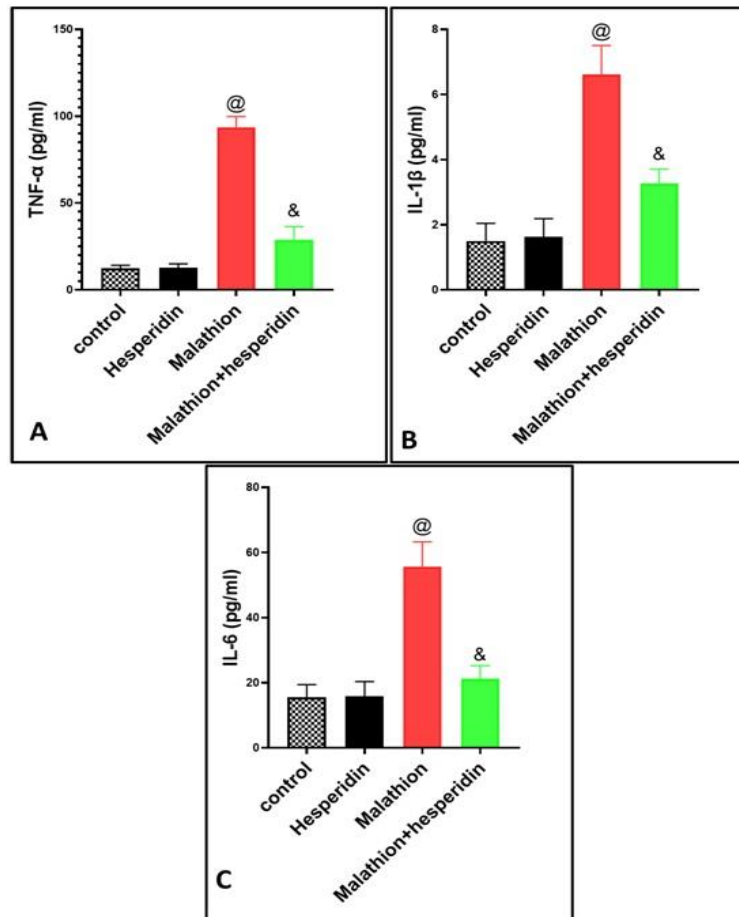


Fig. 4. Impact of malathion and hesperidin on TNF- α (A), IL-1 β (B), and IL-6 (C), [@] $p < 0.05$ vs normal groups. [&] $p < 0.05$ vs malathion group. All results reported mean \pm SD.

3.5. Effect of hesperidin on the renal tissues pyroptotic markers.

For the detection of pyroptotic inflammatory mediated cellular death, we estimated the protein level of NLRP3 inflammasomes, and Caspase-1 using ELISA. The malathion group has a significant $p < 0.05$ higher level of NLRP3, and caspase-1 when compared to control groups [Fig.

5A,B]. Meanwhile, coadministration of malathion with hesperidin significantly $p < 0.05$ reduced the level of pyroptotic markers (NLRP3, caspase-1). Collectively, these findings documented that hesperidin improved malathion-induced nephrotoxicity by diminishing the inflammatory response. There was no significant difference between control and hesperidin groups.

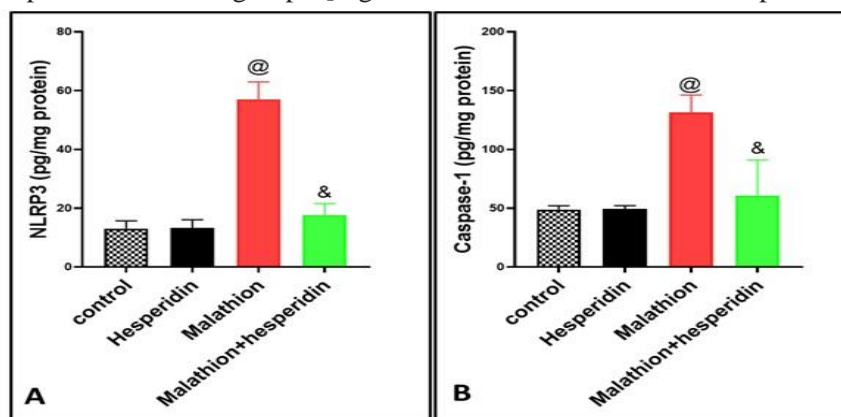


Fig. 5. Effect of hesperidin and malathion on pyroptosis markers (A) NLRP3 inflammasome, (B) caspase-1. [@] $p < 0.05$ means significance to control animals. [&] $p < 0.05$ means significance to malathion group. All data were represented as mean \pm SD.

3.6. Ameliorative effect of hesperidin on malathion-induced renal apoptosis

As shown in Figure 6, the malathion treated rats showed a significant $p < 0.05$ elevation in the protein level of proapoptotic markers caspase-3, and Bax, and decline in the protein level of Bcl-2

anti-apoptotic marker in comparison to control rats. On the contrast, hesperidin intake with the malathion showed a significant $p < 0.05$ decrease in caspase-3 and Bax and increase in bcl-2. Taken together, hesperidin implies the renal apoptosis resulted from administration of malathion.

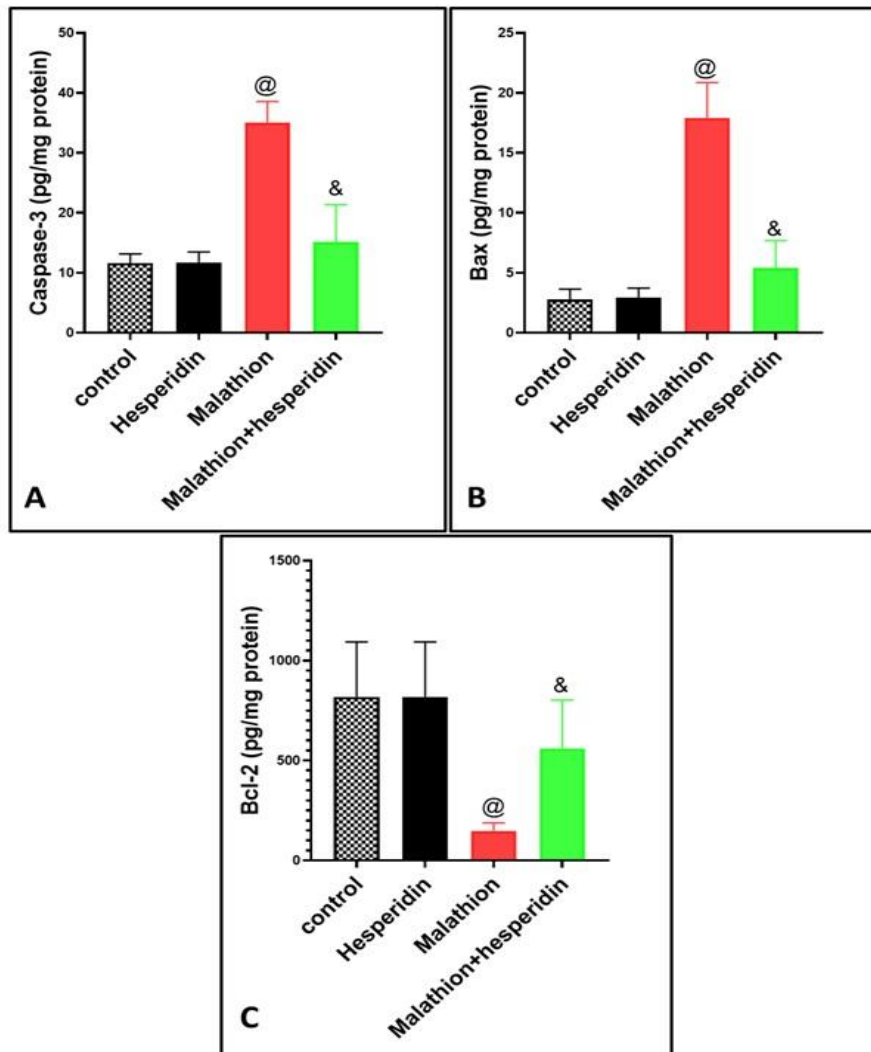


Fig. 6. Antiapoptotic effect of hesperidin against (A) Caspase-3, (B) Bax, and (C) Bcl-2. [@] $p < 0.05$ indicates control rats significance. [&] $p < 0.05$ indicates malathion group significance. All values were reported as mean \pm SD.

Discussion

Malathion is a well-known organophosphorus insecticide used to control several insects [32]. Malathion and its metabolites are involved in hepatic and renal impairment due to their ability to create oxidative stress state [33], destroying cell membranes, and leading to DNA damage through ROS overproduction. This results in cellular

oxidative damage [34,35]. Adipose tissues and the gastrointestinal tract are believed to have higher levels of malathion, as biliary excretion is considered the primary site for the excretion of Malathion metabolites. The kidneys are also thought to contain high levels of the toxic metabolites MCA and DCA [36].

It was founded that lungs, diaphragm, kidney, and liver are the more susceptible organ to malathion toxicity due to sever oxidative damage [34]. According to the previous work done by Abd-Eltawab Tammam et al. [37] hesperidin has been reported to have antioxidant effect against nickel oxide-induced hepatotoxicity and nephrotoxicity. Moreover, hesperidin possesses several pharmacological effects, such as hepatoprotective, anticarcinogenic, antioxidant, ant-inflammatory, and antidiabetics [38]. This encourages us to examine hesperidin as a powerful antioxidant in our study for the elimination of histopathological and molecular renal changes resulting from malathion intoxication.

Concerning biochemical analysis, our study results reveled that oral intake of 100 mg/kg/day of malathion causes a significant elevation in renal function tests, including creatinine and Kim-1, one of the famous indicators of renal tubular injury expressed in damaged renal tubular cells [39] compared to control rats. Furthermore, malathion administration caused some histological alternations in the renal tissues, such as tubular dilation and hydropic degeneration, which explain the elevation in renal function testes. This data concurrent with the findings of a previous study by Kata, [40], who reported that intraperitoneal administration of malathion in mice resulted in elevated serum creatinine and histological changes in renal tissues. On the other hand, intraperitoneal injection of hesperidin significantly improved renal functions testes, including creatinine, and Kim-1, and are associated with improvements in the histological appearance of renal tissues. This in line with the previous work by Hassan et. [41], who documented the ameliorative effect of

hesperidin against aluminum chloride-induced renal impairment, characterized by increased renal function markers (creatine and urea), and histological changes such as, destructed tubules, pyknotic nuclei, and interstitial infiltration. From this result, we can conclude the nephroprotective effect of hesperidin against malathion-induced renal injury, with its histological improvement.

Remarkably. It is well known that Free radical's production due to oxidative stress, which consumes intrinsic antioxidant defense systems, is the primary cause of malathion intoxication [33]. Malathion detoxification results in oxidative stress through its conjugation to glutathione, leading to ROS production and antioxidant enzymes depletion, marked by elevation in MDH, and decrease in GSH stores [42], so our study reported that, malathion intake significantly increased MDA and depleted SOD, CAT, GSH. These findings are consistent with the previous study done by Jalili et al. [43]. Our finding confirms that organophosphorus exposure is associated with several health problems due to its induction of oxidative stress [44]. In contrast, hesperidin co-treatment significantly increased the renal homogenate level of antioxidant enzymes, and decreased lipid peroxidation, parallel to the findings of the work by Anandan et al. [45], who reported the antioxidant effect of hesperidin against gentamicin-induced nephrotoxicity. The antioxidant effect of hesperidin can be attributed to its direct stimulatory effect on the production of antioxidant enzymes, and its ability to neutralize ROS production including hydroxyl radicals, superoxide anion, and nitric oxide radical [46-48]. TNF- α is a well-known proinflammatory cytokine that stimulates the inflammatory process through

its activation to innate and acquired immunity, and tissue apoptosis [49]. In case of malathion intoxication, TNF- α serum level are founded to be elevated [50]. There was a significant elevation in level of proinflammatory markers TNF- α , IL-1 β , and IL-6 in renal tissues of malathion-injected rats compared to control rats. These results are in harmony with the findings of a previous study by Ghamry et al. [51]. However, hesperidin-cotreatment markedly decreased the levels of IL-6, TNF- α , IL-1 β , compared to their levels in malathion group. This finding aligns with the results a study by Abd-Eldayem et al. [52], who reported the nephroprotective effect of hesperidin against cyclosporine-induced renal injury through its reduction of inflammatory markers NF- κ B, and TNF- α . The anti-inflammatory effect of hesperidin can explain its nonprotective action against malathion. Additionally, this effect can be attributed to its scavenging power against ROS, and its direct inhibitory effect on proinflammatory mediators.

Caspase-3 plays a leading role in extrinsic and intrinsic-mediated apoptotic pathways [53]. It has been founded that the apoptosis process was involved in malathion-intoxication [54]. The results of our study revealed that the malathion group showed a significant increase in the protein levels of proapoptotic protein caspase-3, Bax, with a noteworthy decrease in antiapoptotic marker Bcl-2 in relation to control rats. Meanwhile, co-administration of hesperidin with malathion decreased apoptotic markers (caspase-3, Bax), and increased the antiapoptotic marker (Bcl-2) compared to the malathion group. This finding supported by the results of the study by Siddiqi et al. [55], who reported the inhibitory effect of

hesperidin on apoptotic process in trichloroethylene-induced renal damage through its alternation of the expression of caspase-3, Bax, Bcl-2. The antiapoptotic action of hesperidin can be attributed to its scavenging effect on free radical, and its anti-inflammatory properties.

The NLRP3 inflammasome was considered a multiprotein complex that, upon activation, triggers the caspase-1-dependent release of IL-18 and IL-1 β , leading inflammatory form of cell death [56]. The results of our study explored the upregulatory effect of malathion on pyroptotic pathway through its significant elevation to NLRP3, caspase-1, IL-1 β a pyroptotic markers. There is a deficiency of information on the direct effect of malathion on the pyroptosis in nephrotoxicity, but there are some papers which discuss the role of NLRP3 inflammasomes in organophosphorus-induced cytotoxicity [57]. On the other hand, hesperidin intake with malathion significantly decreased pyroptotic-mediated inflammatory death by its significant decrease to ELISA level of NLRP3, caspase-1, and IL-1 β the components of pyroptosis pathway complex. This finding aligns with the previous studied done by Cao et al. [58], and Xie et al. [59], who documented the inhibitory role of hesperidin on pyroptosis pathway for the improvement of the depression symptoms in experimental study. Also, the study done by Abo El-Magd et al. [60], who reported the hepatoprotective effect of hesperidin on thioamide-induced encephalopathy through its depression to NLRP3 level.

Conclusions

The present study documented nephroprotective effect of hesperidin against malathion-induced

renal damage. This can be attributed to its antioxidant, anti-inflammatory, antiapoptotic, and anti-pyrototic effects. Hesperidin administration mitigates oxidative stress through its elevation to SOD, CAT, GSH and reduction to MDA.

Additionally, hesperidin, depressed inflammatory markers (TNF- α , IL-1 β , IL6), and modulates apoptotic and pyrototic markers (caspase-3, Bax, Bcl-2, NLRP3, caspase-1) resulting from malathion administration.

List of abbreviations	
Kim-1	Kidney injury molecule-1
MDA	Malonaldehyde
SOD	Superoxide dismutase
CAT	Catalase
GSH	Reduced glutathione
IL-1 β	Interleukin-1 beta
TNF- α	Tumor necrosis factor-alpha
IL-6	Interleukin-6
Bcl-2	B cell lymphoma-2
Bax	(Bcl-2) associated protein x
NLRP3	NOD-like receptor protein 3
OP	Organophosphate
HES	Hesperidin
LPO	Lipid peroxidation
ROS	Reactive oxygen species

References

1. **Cobilinschi C, Tincu RC, Cobilinschi CO, Neagu TP, Becheanu G, Sinescu RD, Checherit ă IA, Grintescu IM, Lasca ăr I.** Histopathological features of low-dose organophosphate exposure. *Rom J MorpholEmbryol* 2020;61(2):423–432.
2. **Cakici O, Akat E.** Effects of oral exposure to diazinon on mice liver and kidney tissues: biometric analyses of histopathologic changes. *Anal Quant Cytol Histol* 2013;35:7–16.
3. **Dos Santos AA, Naime AA, de Oliveira J, Colle D, Dos Santos DB, Hort MA, Moreira ELG, Suñol C, de Bem AF, Farina M.** Long-term and low-dose malathion exposure causes cognitive impairment in adult mice: Evidence of hippocampal mitochondrial dysfunction, astrogliosis and apoptotic events. *Arch Toxicol* 2016;90:647–660.
4. **Navarrete-Meneses M, Salas-Labadía C, Sanabrais-Jiménez M, Santana-Hernández J, Serrano-Cuevas A, Juárez-Velázquez R, Olaya-Vargas A, Pérez-Vera P.** Exposure to the insecticides permethrin and malathion induces leukemia and lymphoma associated gene aberrations in vitro. *Toxicol In Vitro* 2017;44:17–26.
5. **Varol S, Başarslan S, Fırat U, Alp H, Uzar E, Arıkano ă glu A, Evliyao ă glu O, Acar A, Yücel Y, Kıbrıřlı E.** Detection of borderline dosage of malathion intoxication in a rat's brain. *Eur Rev Med Pharm Sci* 2015;19:2318–2323.
6. **Selmi S, El-Fazaa S, Gharbi N.** Oxidative stress and cholinesterase inhibition in plasma, erythrocyte and brain of rats' pups following lactational exposure to malathion. *Environ Toxicol Pharmacol* 2012;34:753–760.

7. **Yan J, Xiang B, Wang D, Tang S, Teng M, Yan S, Zhou Z, Zhu W.** Different toxic effects of racemate, enantiomers, and metabolite of malathion on HepG2 cells using high-performance liquid chromatography–quadrupole–time-of-flight-based metabolomics. *J Agric Food Chem* 2019;67:1784–1794.
8. **Shieh P, Jan CR, Liang WZ.** The protective effects of the antioxidant N-acetylcysteine (NAC) against oxidative stress associated apoptosis evoked by the organophosphorus insecticide malathion in normal human astrocytes. *Toxicology* 2019;417:1–14.
9. **Bhardwaj JK, Saraf P, Kumari P, Mittal M, Kumar V.** N-Acetyl-cysteine mediated inhibition of spermatogonia cells apoptosis against malathion exposure in testicular tissue. *J Biochem Mol Toxicol* 2018;32:e22046.
10. **Abdel-Salam OM, Youness ER, Mohammed NA, Yassen NN, Khadrawy YA, El-Toukhy SE, Sleem AA.** Nitric oxide synthase inhibitors protect against brain and liver damage caused by acute malathion intoxication. *Asian Pac J Trop Med* 2017;10:773–786.
11. **Akbel E, Arslan-Acaroz D, Demirel HH, Kucukkurt I, Ince S.** The subchronic exposure to malathion, an organophosphate pesticide, causes lipid peroxidation, oxidative stress, and tissue damage in rats: The protective role of resveratrol. *Toxicol Res* 2018;7:503–512.
12. **Yang H, Wang J, Liu ZG.** Multi-faceted role of pyroptosis mediated by inflammasome in liver fibrosis. *J Cell Mol Med* 2022;26:2757–2765.
13. **Zhao H, Liu H, Yang Y, Wang H.** The Role of Autophagy and Pyroptosis in Liver Disorders. *Int J Mol Sci* 2022;23:6208.
14. **Wang Y, Li Y, Xu Y.** Pyroptosis in kidney disease. *J Mol Biol* 2022;434:167290.
15. **Shao F, Fitzgerald KA.** Molecular mechanisms and functions of pyroptosis. *J Mol Biol* 2022;434:167461.
16. **Li Z, Mo F, Wang Y, Li W, Chen Y, Liu J, Chen-Mayfield T, Hu Q.** Enhancing Gasdermin-induced tumor pyroptosis through preventing ESCRT-dependent cell membrane repair augments antitumor immune response. *Nat Commun* 2022;13:6321.
17. **Newton K, Wickliffe KE, Maltzman A, Dugger DL, Reja R, Zhang Y, Roose-Girma M, Modrusan Z, Sagolla MS, Webster JD.** Activity of caspase-8 determines plasticity between cell death pathways. *Nature* 2019;575:679–682.
18. **Feng S, Fox D, Man SM.** Mechanisms of gasdermin family members in inflammasome signaling and cell death. *J Mol Biol* 2018;430:3068–3080.
19. **Xia W, Li Y, Wu M, Jin Q, Wang Q, Li S, Huang S, Zhang A, Zhang Y, Jia Z.** Gasdermin E deficiency attenuates acute kidney injury by inhibiting pyroptosis and inflammation. *Cell Death Dis* 2021;12:139.
20. **Zheng D, Liwinski T, Elinav E.** Inflammasome activation and regulation: Toward a better understanding of complex mechanisms. *Cell Discov* 2020;6:36.

21. **Zeng X, Liu D, Huo X, Wu Y, Liu C, Sun Q.** Pyroptosis in NLRP3 inflammasome-related atherosclerosis. *Cell Stress* 2022;6:79–88.
22. **Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, Lamas S.** Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biol* 2015;6:183–197.
23. **Torres-Arce E, Vizmanos B, Babio N, Márquez-Sandoval F, Salas-Huetos A.** Dietary Antioxidants in the Treatment of Male Infertility: Counteracting Oxidative Stress. *Biol* 2021;10(3):241.
24. **Garg A, Garg S, Zaneveld LJ, Singla AK.** Chemistry and pharmacology of the Citrus bioflavonoid hesperidin. *Phytother Res* 2001;15(8):655–669.
25. **Zarein M, Zarban A, Shoorei H, Gharekhani M, Hassanzadeh-Taheri M.** The amelioration of ovarian dysfunction by hesperidin in malathion-treated mice through the overexpression of PCNA and FSHR proteins. *Heliyon* 2023;9(12):e22484.
26. **Gur C, Kandemir FM.** Molecular and biochemical investigation of the protective effects of rutin against liver and kidney toxicity caused by malathion administration in a rat model. *Environ Toxicol* 2022;38:555–565.
27. **Ohkawa H, Ohishi N, Yagi K.** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351–358.
28. **Ellman GL.** Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70–77.
29. **Aebi H.** Catalase in vitro. *Methods Enzymol* 1984;105:121–126.
30. **Sun Y, Oberley LW, Li Y.** A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988;34:497–500.
31. **Bancroft JD, Gamble M.** Theory and Practice of Histological Techniques. Amsterdam: Elsevier Health Sciences; 2008.
32. **Mostafalou S, Eghbal MA, Nili-Ahmadabadi A, Baeri M, Abdollahi M.** Biochemical evidence on the potential role of organophosphates in hepatic glucose metabolism toward insulin resistance through inflammatory signaling and free radical pathways. *Toxicol Ind Health* 2012;28:840–851.
33. **Lasram MM, Lamine AJ, Dhouib IB, Bouzid K, Annabi A, Belhadjmida N, Ahmed MB, El Fazaa S, Abdelmoula J, Gharbi N.** Antioxidant and anti-inflammatory effects of N-acetylcysteine against malathion-induced liver damages and immunotoxicity in rats. *Life Sci* 2014;107:50–58.
34. **Possamai F, Fortunato J, Feier G, Agostinho F, Quevedo J, Wilhelm Filho D, Dal-Pizzol F.** Oxidative stress after acute and sub-chronic malathion intoxication in Wistar rats. *Environ Toxicol Pharmacol* 2007;23:198–204.
35. **Fortunato JJ, Feier G, Vitali AM, Petronilho FC, Dal-Pizzol F, Quevedo J.** Malathion-induced oxidative stress in rat brain regions. *Neurochem Res* 2006;31:671–678.

36. **Morgade C, Barquet A.** Body distribution of malathion and its metabolites in a fatal poisoning by ingestion. *J Toxicol Environ Health Part A Curr Issues* 1982;10:321–325.
37. **Abd-Eltawab Tammam A, Khalaf AA, Zaki R, Mansour Khalifa M, Ibrahim A, Mekkawy M, Abdelrahman E, Farghali R, Noshay A.** Hesperidin protects rats' liver and kidney from oxidative damage and physiological disruption induced by nickel oxide nanoparticles. *Front Physiol* 2022;13:912625. doi: 10.3389/fphys.2022.912625.
38. **Choi EJ.** Antioxidative effects of hesperetin against 7, 12-dimethylbenz (a) anthracene-induced oxidative stress in mice. *Life Sci* 2008;82:1059–1064. doi: 10.1016/j.lfs.2008.03.002.
39. **Tian M, Tang L, Wu Y, Beddhu S, Huang Y.** Adiponectin attenuates kidney injury and fibrosis in deoxycorticosterone acetate-salt and angiotensin II-induced CKD mice. *Am J Physiol-Ren Physiol* 2018;315:F558–F571.
40. **Kata FS.** Short-time Effects of Malathion Pesticide on Functional and Histological Changes of Liver and Kidney in Female Mice. *Pak J Biol Sci* 2020;23:1103–1112.
41. **Hassan NH, Yousef DM, Alsemeh AE.** Hesperidin protects against aluminum-induced renal injury in rats via modulating MMP-9 and apoptosis: biochemical, histological, and ultrastructural study. *Environ Sci Pollut Res* 2023;30:36208–36227. doi: 10.1007/s11356-022-24800-0.
42. **Aboubakr HM, Elzohairy EA, Ali AA, Rashed LA, Elkady NK, Soliman AS.** Therapeutic effects of N-acetylcysteine against malathion-induced hepatotoxicity. *Egypt J Forensic Sci* 2019;9:1–9.
43. **Jalili C, Roshankhah S, Moradi Y, Salahshoor MR.** Resveratrol attenuates malathion-induced renal damage by declining oxidative stress in rats. *Int J Pharm Investig* 2018;8:192–199.
44. **Morgade C, Barquet A.** Body distribution of malathion and its metabolites in a fatal poisoning by ingestion. *J Toxicol Environ Health Part A Curr Issues* 1982;10:321–325.
45. **Anandan R, Subramanian P.** Renal protective effect of hesperidin on gentamicin-induced acute nephrotoxicity in male Wistar albino rats. *Redox Rep* 2012;17:219–226. doi: 10.1179/1351000212Y.0000000019.
46. **Garg A, Garg S, Zaneveld LJ, Singla AK.** Chemistry and pharmacology of the Citrus bioflavonoid hesperidin. *Phytother Res* 2001;15:655–669. doi: 10.1002/ptr.1074.
47. **Kim JY, Jung KJ, Choi JS, Chung HY.** Hesperetin: a potent antioxidant against peroxynitrite. *Free Radic Res* 2004;38:761–769. doi: 10.1080/10715760410001713844.
48. **Wilmsen PK, Spada DS, Salvador M.** Antioxidant activity of the flavonoid hesperidin in chemical and biological systems. *J Agric Food Chem* 2005;53:4757–4761. doi: 10.1021/jf0502000.

49. **Pober JS, Min W.** Endothelial cell dysfunction, injury and death. *Handb Exp Pharmacol* 2006;176 Pt 2:135–156.
50. **Severcan C, Ekremoglu M, Sen B, Pasaoglu O, Akyurek N, Severcan S, Pasaoglu H.** Acute effects of different doses of malathion on the rat liver. *Clin Exp Hepatol* 2019;5:237–243.
51. **Ghamry HI, Aboushouk AA, Soliman MM, Albogami SM, Tohamy HG, Okle OS, Althobaiti SA, Rezk S, Farrag F, Helal AI, Ghoneim HA, Shukry M.** Ginseng® Alleviates Malathion-Induced Hepatorenal Injury through Modulation of the Biochemical, Antioxidant, Anti-Apoptotic, and Anti-Inflammatory Markers in Male Rats. *Life (Basel)* 2022;12:771. doi: 10.3390/life12050771.
52. **Abd-Eldayem AM, Makram SM, Messiha BAS, Abd-Elhafeez HH, Abdel-Reheim MA.** Cyclosporine-induced kidney damage was halted by sitagliptin and hesperidin via increasing Nrf2 and suppressing TNF- α , NF- κ B, and Bax. *Sci Rep* 2024;14:7434. doi: 10.1038/s41598-024-57300-x.
53. **Tait SWG, Green DR.** Mitochondria and cell death: Outer membrane permeabilization and beyond. *Nat Rev Mol Cell Biol* 2010;11:621–632.
54. **Geng X, Shao H, Zhang Z, Ng JC, Peng C.** Malathion-induced testicular toxicity is associated with spermatogenic apoptosis and alterations in testicular enzymes and hormone levels in male Wistar rats. *Environ Toxicol Pharmacol* 2015;39:659–667.
55. **Siddiqi A, Nafees S, Rashid S, Sultana S, Saidullah B.** Hesperidin ameliorates trichloroethylene-induced nephrotoxicity by abrogation of oxidative stress and apoptosis in wistar rats. *Mol Cell Biochem* 2015;406:9–20. doi: 10.1007/s11010-015-2400-8.
56. **Zheng Z, Li G.** Mechanisms and therapeutic regulation of pyroptosis in inflammatory diseases and cancer. *Int J Mol Sci* 2020;21:1456.
57. **Wang X, Sui X, Sun Y, Cui Z, Ma N, Wang S, Yang J, Liu F, Yang W, Xiao Z, Zhu T, Luo Y, Wang Y.** Potential Common Mechanisms of Cytotoxicity Induced by Organophosphorus Pesticides via NLRP3 Inflammasome Activation. *Geohealth* 2024;8:e2023GH000888. doi: 10.1029/2023GH000888.
58. **Cao H, Yang D, Nie K, Lin R, Peng L, Zhou X, Zhang M, Zeng Y, Liu L, Huang W.** Hesperidin may improve depressive symptoms by binding NLRP3 and influencing the pyroptosis pathway in a rat model. *Eur J Pharmacol* 2023;952:175670. doi: 10.1016/j.ejphar.2023.175670.
59. **Xie L, Gu Z, Liu H, Jia B, Wang Y, Cao M, Song R, Zhang Z, Bian Y.** The Anti-Depressive Effects of Hesperidin and the Relative Mechanisms Based on the NLRP3 Inflammatory Signaling Pathway. *Front Pharmacol* 2020;11:1251. doi: 10.3389/fphar.2020.01251.
60. **Abo El-Magd NF, El-Kashef DH, El-Sherbiny M, Eraky SM.** Hepatoprotective and cognitive-enhancing effects of hesperidin against thioacetamide-induced hepatic encephalopathy in rats. *Life Sci* 2023;313:121280. doi: 10.1016/j.lfs.2022.121280.