

Effects of Morphine and Genistein Injections on a Few Histological and Biochemical Liver Characteristics in White Male Laboratory Mice

Habib, J. Saheb¹, Murtadha, F. Al-Hillo², Alaa S. Kathim^{3*}

^{1 2 3} Biology Department, Qurna College of Education, University of Basrah

hibaabjasm@gmail.com, Murtadha.abdulhussian@uobasrah.edu.iq,

Alaa.kathim@uobasrah.edu.iq

*Corresponding_ author.: Alaa S. Kathim (Ph.D.) Department - of Biology, College of Education of Al Qurna, University of Basrah, Iraq, alaa.kathim@uobasrah.edu.iq

Abstract

Objectives: The therapeutic value of genistein as an antioxidant and anti-inflammatory in reducing the negative effects of morphine was examined in this study.

Methods: Four groups of 48 male laboratory rats were established. Each group had twelve mice. The control group received a 0.1 M injection of sterile saline. The morphine group received an injection of morphine at a dose of 20 mg/kg; The genistein group received an injection of genistein at a dose of 25 mg/kg; The morphine and genistein group received an injection of morphine at a dose of 20 mg/kg for 15 days, followed by 15 days of receiving an injection of genistein. Rats were separated into three periods and given a subperitoneal injection once a day (five, ten and fifteen days of injection).

Results: Morphine caused a rise in the levels of the enzymes ALP, AST, and ALT during the injection period. In ten and fifteen days, while genistein caused the concentration to drop of liver enzymes,

Keywords: Morphine, genistein, liver enzymes, histopathology, mice.

Introduction

Opioids are widely used in many areas of life, especially in the relief of acute and chronic pain (Tsuno *et al.*, 2022), the most prominent opiates used for this purpose is morphine. Studies that supported the existence of significant adverse effects of morphine abuse revealed that it can lead to addiction and withdrawal when used excessively over an extended period of time, as well as hypogonadism and a defect in hormone production, shortness of breath, vomiting, decreased intestinal secretions, weight loss, ataxia, and immune diseases (Hemati *et al.*, 2021). It may cause cytotoxicity through lipid peroxidation, reactive oxygen, free radical generation, and a decrease in the level of antioxidant enzymes, which may cause their association with lipids in cell membranes, causing oxidative stress and cancer diseases and their impact on blood parameters and activation of programmed cell death (Jia *et al.*, 2022).

As a result of the harmful effects shown by the chronic abuse of morphine, the researchers focused largely on finding appropriate strategies to reduce these effects. Among the proposals put forward is the use of materials with antioxidant properties. Genistein, which belongs to the class of isoflavones, is a natural plant estrogen extracted from soybeans, legumes, seeds, fruits, and vegetables such as alfalfa, broccoli, and cumin (Ebrahimisadr *et al.*, 2021). It is characterized by a structure and properties similar to estrogen and has been widely used in medical and academic fields due to its antioxidant properties, as it is characterized by its ability to deplete free radicals and raise the level of antioxidant enzymes, anti-inflammatory and cancerous tumors (Gan *et al.*, 2022) Endocrine disorders, non-alcoholic fatty liver, osteoporosis (Semeniuk *et al.*, 2021), liver and kidney disorders, anti-depression and obesity reduction (Zamani *et al.*, 2021).

This study aims to identify the possibility of using the hormone In order to lessen the detrimental effects that morphine usage can have on the concentration of liver enzymes and liver tissue, genistein is used as an antioxidant and anti-inflammatory.

Materials and Methods

2-1: Experimental animals

48 male *Mus musculus* L/BALB mice, aged 10-12 weeks and weighing 20-25g, were cared for in a supervised environment at the College of Education, Qurna, University of Basrah, in plastic cages lined with wood. Animals have unrestricted access to water and food.(Ali Abd *et al.*,2016: Aledani *et a.l.*,2020:Al-Sarry, and Al-Karishy ,2007)

2-2: Experimenta design

Twelve rats were included in each of the four groups, which were divided as follows: 1: The control group was injected with 0.1 ml of sterile saline; 20 mg/kg morphine was given as a group syringe for opioids in group 2. (Kuthati *et al.*, 2021); 3: Genistein group: injected at a concentration of 25 mg/kg in (Salahshoor *et al.* 2016); 4: The combination of morphine and genistein had a period of 15 days of morphine injection at a dose of 20 mg/kg, followed by 15 days of genistein injection at a dose of 25 mg/kg. Mice were separated into three periods and injected into the subperitoneal membrane once for 15 days (five, ten, and fifteen days post injection). Mice were anesthetized with chloroform at the end of each period, and blood was collected directly from the heart using a sterile 1 mL vial, with some being used for blood analysis. Regarding serum and its applications in biochemical analysis,

2-3: Measuring the ALP, AST, and ALT concentrations.

ALP, AST, and ALT concentrations were assessed. according to biological diagnostic kit (Egypt) and as described by (Reitman *et al.*, 1957).

Results

1-3: Effect of morphine and genistein on the liver enzymes level

The findings of the present investigation demonstrated that morphine injections into male rats resulted in a substantial ($p \leq 0.05$) increase in the blood serum's ALP, AST, and ALT enzyme concentrations between the control group and the genistein group during the ten and fifteen-day injection periods. The injection of genistein caused a drop significantly ($p \leq 0.05$) in ALP, AST, and ALT levels in the blood serum compared to the control group during the course of the experiment's fifteen-day injection period. During the fifteen-day injection period, the group receiving morphine and genistein injections shown a considerable improvement in the amount of enzymes in the blood serum, with no discernible differences from the control group. (table 1,2,3).

Table (1). Effect of different treatments on ALT enzyme concentration (IU) $\pm$ Se

Treatment	Day		
	5	10	15
0.1 ml of normal saline	37.33 ^b $\pm$ 2.1	36.3 ^b $\pm$ 1.5	37.67 ^b $\pm$ 1.5
Morphine 20 mg/ kg	43.67 ^b $\pm$ 4.1	54.6 ^a $\pm$ 4.6	70.33 ^a $\pm$ 2.8
Genistein 25 mg/ kg	34.97 ^b $\pm$ 2.1	32.6 ^b $\pm$ 1.5	21.1 ^c $\pm$ 1.7
Morphine and Genistein	63.1 ^a $\pm$ 7.0	50.4 ^a $\pm$ 5.6	44.32 ^b $\pm$ 4.7

The different letters denote significant changes within the same column at the probability level ($P < 0.05$)

Table (2) Effect of different treatments on AST enzyme concentration (IU) $\pm$ standard error

Treatment	Days		
	5	15	10
0.1 ml of normal saline	160.7 ^b $\pm$ 1.4	155.67 ^b $\pm$ 3.5	155.6 ^b $\pm$ 3.2
Morphine 20 mg/ kg	174.8 ^b $\pm$ 3.1	227.8 ^a $\pm$ 5.5	250.3 ^a $\pm$ 2.5
Genistein 25 mg/ kg	144.6 ^b $\pm$ 5.5	136.9 ^b $\pm$ 4.5	104.7 ^c $\pm$ 9.5
Morphine and Genistein	244.1 ^a $\pm$ 7.5	213.6 ^a $\pm$ 8.3	173.9 ^b $\pm$ 8.1

The different letters denote significant changes within the same column at the probability level ($P < 0.05$)

Table (3) Effect of treatments on ALP enzyme concentration (IU) $\pm$ standard error

Treatment	Days		
	5	10	15
0.1 ml of normal saline	24.67 ^b $\pm$ 0.6	25.5 ^b $\pm$ 1.5	29.8 ^c $\pm$ 2.1
Morphine 20 mg/ kg	33.8 ^b $\pm$ 6.1	49.7 ^a $\pm$ 3.8	58.5 ^a $\pm$ 3.2
Genistein 25 mg/ kg	22.1 ^b $\pm$ 3.6	18.9 ^b $\pm$ 2.4	12.7 ^b $\pm$ 1.5
Morphine and Genistein	47.8 ^a $\pm$ 4.9	45.3 ^a $\pm$ 4.1	31.3 ^c $\pm$ 2.5

The different letters denote significant changes within the same column at the probability level ($P < 0.05$)

2-3: Effect of morphine and genistein on liver tissue

A: control group: The liver consists histologically of a group of hexagonal lobes. There is in the center of each lobule a central vein surrounded by a group of hepatocytes arranged in the form of radial bands around the central vein. Each liver cell contains a single central nucleus. These bands are separated from each other by small spaces called semi-sinuses fig (1).

B: morphine group: Five days after the injection, the results of the histological analysis of the liver revealed that the central vein was enlarged and congested, expansion of the hepatic sinuses, the accumulation of inflammatory cells near the central vein, degeneration of the hepatocyte cytoplasm, vacuolization of the hepatocytes, thickening of the nucleus, and necrosis of the liver cells. The results revealed that the wall of the central vein had developed necrosis 10 days after receiving the morphine injection., and necrosis and edema of hepatic cells and hyperpigmentation of cells and changes in the nucleus tissue hyperplasia and enlargement and Fifteen days after the injection with morphine, dilatation and congestion of blood vessels, expansion of hepatic sinuses, necrosis, vacuolization, loss of normal features of liver tissue, and dissolution of the nuclei of liver cells were observed fig(2,3,4 ,5,6,7,8).

C: genistein group: The results showed that there were no histological changes in the liver, as the central hepatic vein, hepatic cell bands, and hepatic sinuses were observed in a normal state fig (9,10,11 ,12).

D: morphine & genistein group: The outcomes revealed a small improvement in the form throughout the course of the five-day injection period. And organization of the hepatocytes, with the presence of some pathological conditions that still appear in the liver tissue, which included the expansion and congestion of blood vessels, the expansion of hepatic sinuses, and the occurrence of swelling, necrosis, and vacuolization in the hepatocytes. and The form and structure of the hepatocytes significantly improved ten days following the injection, appearing normal despite certain pathological abnormalities that persisted in the hepatic tissue., such as the presence of expansion and congestion of blood vessels, expansion of hepatic sinuses, and simple necrosis. the exams revealed a considerable improvement in the characteristics of the liver tissue following genistein treatment, fifteen days later, by observing the emergence of a normal central vein and normal cells, with a slight expansion of the hepatic sinuses (13,14,15).

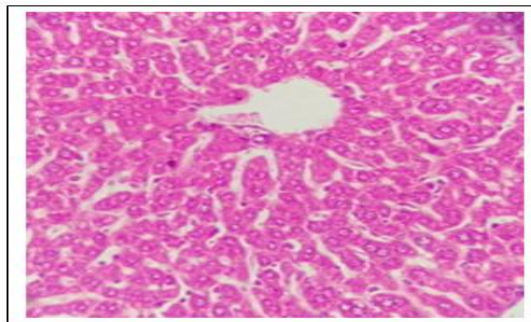


Figure (1) A cross section of the seminiferous tubule in the testes of the control group showing the normal structural of liver, H&E, 4X.

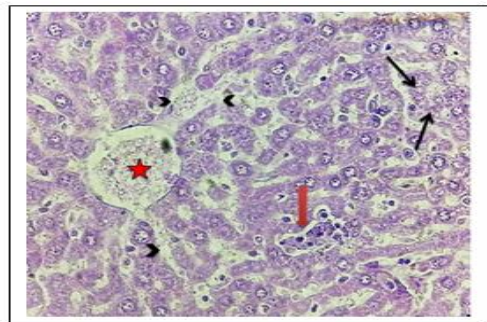


Figure (2) a) A section of the liver of morphine-injected mice during the five-day injection period dilatation and congestion of the is illustrated dilated hepatic sinusoids central vein (star) The degeneration of hepatocyte cyto (arrowhead) Hepatocytes evaporate and their plasm (arrow), nucleus thickens (red arrow), H&E, 10X.

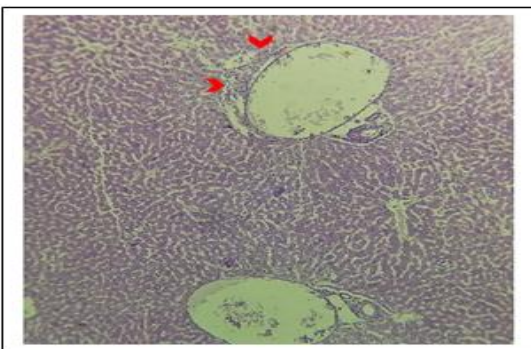


Figure (3) a portion of the animal's liver morphine-injected mice during the five-day injection period is illustrated Inflammatory cell collection near the central vein (arrowhead). H&E, 40X.

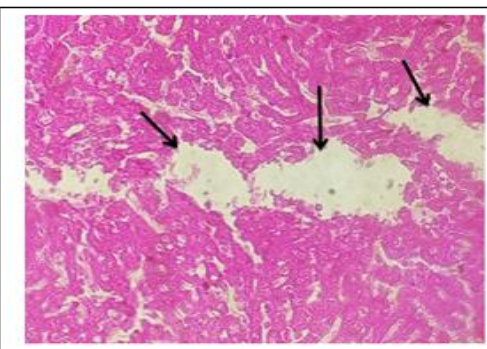


Figure (4) A section of the liver of morphine-injected mice during the five-day injection period is illustrated Hepatocyte necrosis (black arrow) H&E, 10X.

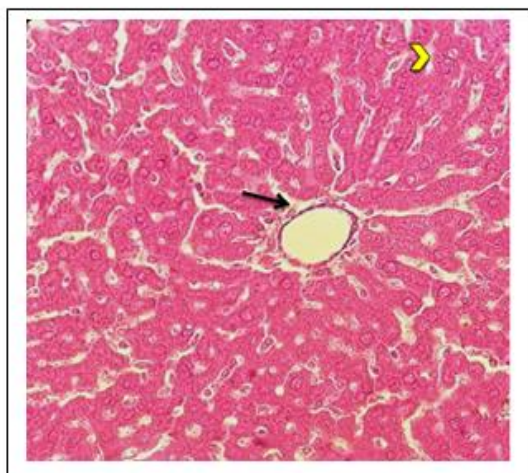


Figure (5) A section of the liver of morphine-injected mice during a ten-day injection period is illustrated Necrosis of the wall of the central vein (black arrow) Hepatocellular hyperplasia (yellow arrowhead).H&E,40X.

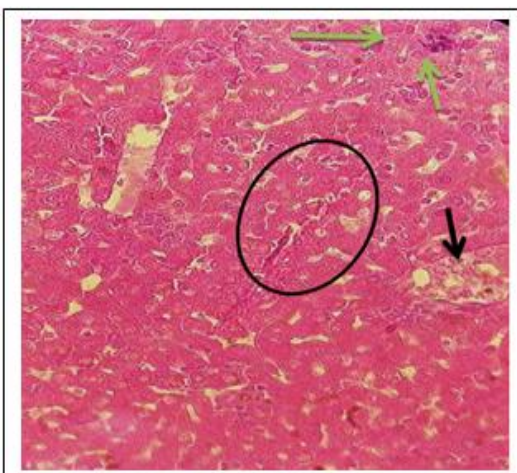


Figure (6) A section of the liver of morphine-injected mice during a ten-day injection period is illustrated Hepatocyte necrosis (black arrow) Hyperpigmentation of cells and changes in the nucleolus (green arrow). Histological hyperplasia and hypertrophy (circle) H&E,40X.

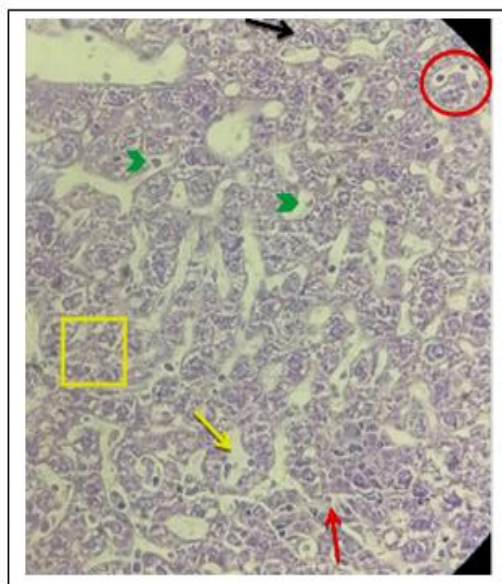


Figure (7) A slice of a liver morphine-injected rats during the fifteen-day injection period Cell necrosis (black arrow), Loss of normal cell features (red circle), Hepatocellular vacuoles (green arrowhead) Nucleolysis, (yellow square), Hepatocyte lysis (yellow arrow) H&E,40X.

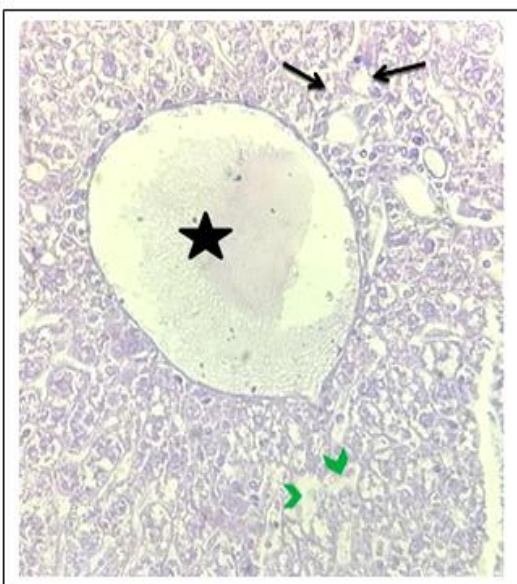


Figure (8) A section of the liver of morphine-injected rats during the fifteen-day injection period Congestion and vasodilatation (star), Hepatocellular necrosis (arrow),Cell vacuolization (green arrowhead) H&E , 40X.

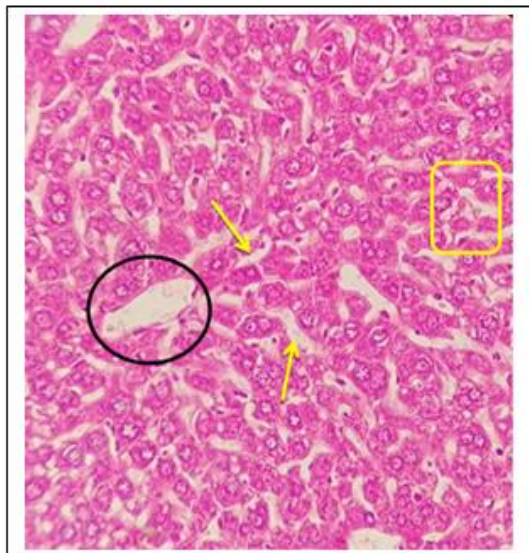


Figure (9) shows a piece of the liver from genistein-injected mice during the five-day injection period is illustrated central vein normal (circle) Bands of normal hepatocytes (yellow arrow) H&E, 4X.

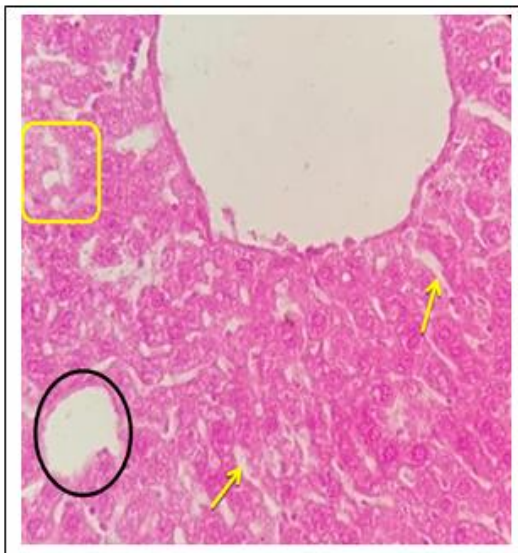


Figure (10) shows a slice of mouse liver. injected with genistein during the ten-day injection period is illustrated central vein normal (circle), Bands of normal hepatocytes (yellow arrow) Hepatic sinuses are normal (yellow square) H&E, 4X.

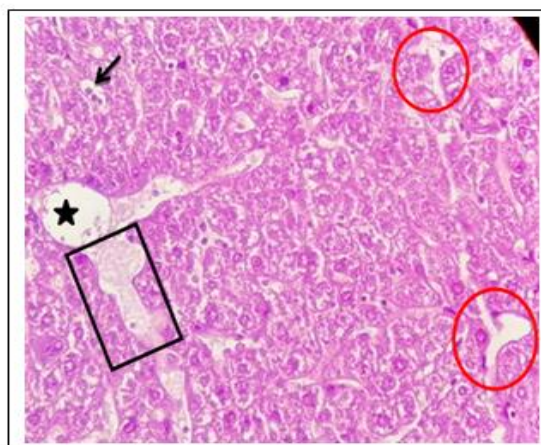


Figure (13) Section of testes of mice injected with morphine and genistein during a five-day injection period showing: dema of hepatocytes (square), Hepatic sinus enlargement (circle), dilatation and congestion of blood vessels (star) H&E, 10X.

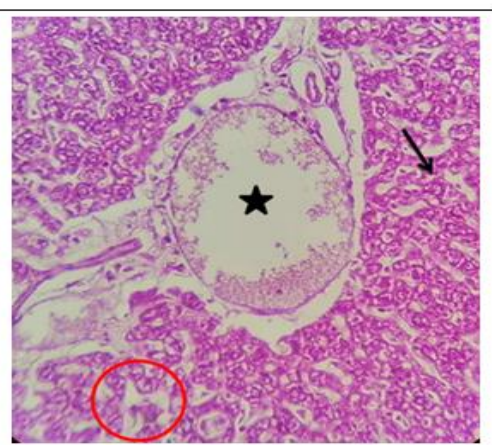


Figure (14) Section of testes of mice injected with morphine and genistein during a five-day injection period shows: Vascular congestion and hemorrhage (star), Hepatic sinus enlargement (circle), normal hepatocytes (arrow), H&E. 10X.

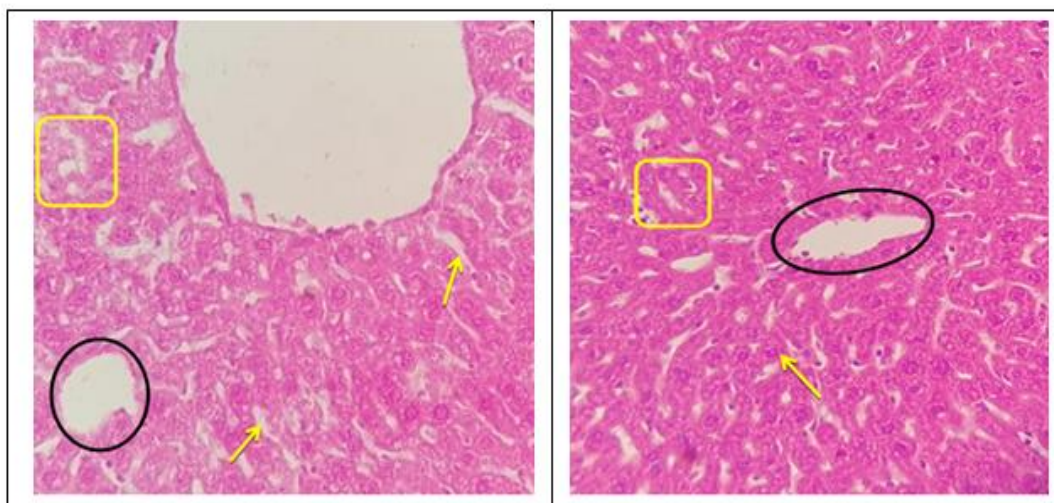


Figure (11) Section of testes of mice injected with genistein during a ten-day injection period showing: normal sperm (green arrow), normal features of seminiferous tubules (straight line), slight hyperplasia of primordial spermatozoa, H&E, 10X.

Figure (12) Section of testes of mice injected with genistein during a fifteen-day injection period shows: normal sperm (green arrow), normal features of seminiferous tubules (straight line), simple necrosis (black arrow), cytoplasmic degeneration (red circle).H&E, 40X.

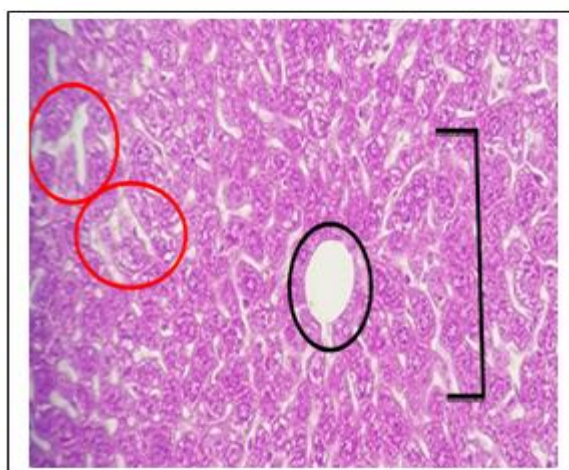


Figure (15) A section of the liver of rats injected with morphine & genistein during the fifteen-day injection period Central vein normal (black circle). Slight dilatation of the hepatic sinusoids (red circle). The shape of the hepatic tissue is normal (straight)H&E, 10X.

Discussion

According to a study's findings, there has been a considerable rise in the concentration of liver enzymes such as ALP, AST, and ALT when morphine was injected, compared with the control group. Its level exceeds the level of antioxidant enzymes, which may cause oxidative stress. Because the cell membrane contains unsaturated fatty acids, This might alter how cells exude fluid and disrupt protein receptors, leading to a defect in the permeability of necessary substances to and from cells and a defect in DNA, causing weakness or death of hepatocytes, which increases the leakage of enzymes into the bloodstream (Reitman *et al.*, 2021) Or that the metabolic process of morphine may cause the depletion of hepatic glutathione, which may cause damage to hepatocytes. and the penetration of its enzymes into the bloodstream (Chen *et al.*, 2009).

The results showed that the injection of genistein in mice caused the levels of the liver enzymes ALP, AST, and ALT to drop significantly., and the process of injection of genistein in the group injected with morphine caused a significant improvement in the level of enzymes, which may be due to the role of genistein in inhibiting the production of radicals and peroxide fats, reactive oxygen, nitric oxide, and an increase in the concentration of antioxidant enzymes in the body, which reduces the damage caused by oxidative stress on hepatocytes, thus maintaining the structure of cells and the integrity of their membranes (Mansour *et al.*, 2017).

While the outcomes of the histology investigation demonstrated that the liver had certain histological alterations that may occur as a result of the injection of morphine in mice during the different injection periods, those changes were the expansion of blood vessels and their congestion, which may be due to an increase in cell metabolism in order to remove toxins from the body during the detoxification process (Khorsandi *et al.*, 2006). Or because of the increased expansion of blood vessels that occur as a result of increased release of histamine, which may cause blood stagnation and congestion of blood vessels (Bini *et al.*, 2022) The results also indicated that the use of genistein reduced the expansion and congestion of blood vessels and hepatic sinuses, and this may be due to Genistein works to inhibit the production of nitric oxide, free radicals, and lipid peroxidation, while increasing the concentration of antioxidant enzymes, thus preventing the occurrence of oxidative stress and reducing the occurrence of vascular expansion (Duan and Ding, 2022).

The findings also demonstrated that morphine contributed to the buildup of inflammatory cells in the liver, which may be related to the drug's metabolic activities in hepatocytes, which induced the release of inflammatory cytokines like tumor necrosis factor and interleukin in response to the drug's toxic effects., as they are recognized by vascular endothelial cells. blood vessels, which causes the filtering of immune cells from the bloodstream to the affected tissues as a result of the cells lining the blood vessels shrinking due to their response to certain chemicals or as a result of

the loss of desmosomes that bind the cells lining the blood vessels, allowing those cells to enter the bloodstream and migrate to the infected tissues and collect them there. (Chadzinska *et al.*, 2009) The results also showed the role of genistein in improving the negative effects of morphine as an anti-inflammatory hormone, thus the other cells' production of inflammatory cytokines such tumor necrosis factor and interleukin is also inhibited. that cause stimulation of immune cells, which inhibits the migration of immune cells and reduces inflammation that affects the liver (Wan *et al.* al, 2017).

It was also observed that there was necrosis, edema, degeneration, thickening of the nuclei, and their dissolution in the liver of those injected with morphine, which may be because morphine increases lipid peroxidation, which promotes the formation of free radicals. This process disrupts the differentiation of germ cells and causes alterations to the fatty structures of cells, cell division, and necrosis. also, edema (Houston *et al.*, 2018). Additionally, the outcomes demonstrated a notable improvement in the histological modifications. caused by morphine, which may be due to the fact that genistein reduces oxidative stress that affects cells as a result of the use of morphine, as it reduces the level of free radicals generated and increases antioxidant enzymes. Thus, it prevents the occurrence of fatty oxidation of cells It inhibits programmed cell death, suppresses DNA damage to cells, repairs damaged cells, and treats edema and degeneration (Yoon *et al.*, 2014).

Genistein also works to reduce oxidative stress that affects cells as a result of the use of morphine, as it reduces the level of free radicals generated and increases antioxidant enzymes, thus preventing the occurrence of fatty oxidation of cells, inhibiting the occurrence of programmed cell death, suppressing DNA damage to cells, repairing damaged cells, and treating edema and degeneration (Yoon *et al.*, 2014)

Additionally, the findings revealed fatty degeneration in the liver tissue, which is a result of morphine-induced changes in lipid metabolism or an increase in the production of lipoproteins that are converted into triglycerides and deposit as fat-containing vacuoles inside hepatic cells. (Chahkandi *et al.* 2015). The results showed a noticeable improvement in the liver tissue after the injection of genistein As genistein works to decrease fat formation and increase fat oxidation, decomposition, and use as an energy source, it protects tissues from fatty degeneration that occurs due to cellular poisoning caused by the use of morphine. (Zamani *et al.*, 2021). According to (Jamshidian *et al.* (2019), the adverse effects of morphine cause hormonal imbalances that activate cell proliferation, which results in an increase in the size of the organ and may be the cause of the liver's hypertrophy. In the rats used in the study, hepatomegaly, a type of inflammation, is a condition that develops as a result of this activation of cell proliferation. It also caused the occurrence of hyperplasia in the liver, kidneys, lung, and seminal tubules, which may be due to the fact that the occurrence of hyperplasia in the studied mice is a form of inflammation that occurs due to the harmful effects of morphine, as it causes hormonal imbalances that cause activation of cell proliferation

proliferation, which causes an increase in the size of the organ (Jamshidian *et al.*, 2019).

References

1. Jamshidian, H., Amini, E., Karvar, M., Ayati, E., Ayati, M., Pishgar, F., ... Aghamiri, S. M. (2019). Effects on opium dependency on testicular tissue in a rat model: an experimental study. *Urology Journal*, 16(4)379-375
2. Chen, Y., & Sommer, C. (2009). The role of mitogen - activated protein kinase (MAPK) in morphine tolerance and dependence. *Molecular neurobiology*, 40 (2), 101-107
3. Ebrahimisadr, P., Ghaffarifar, F., Jabari, J. Horton, J., Sharifi, Z., Dalimi, A., & Dayer, M. S. (2021). Therapeutic and Preventive Effects of Morphine Against Leishmania Major and Evaluation the Expression of TLRs and Cytokines in Infected Macrophages in Vitro and in BALB / c Mice. *Research Square*, 131-157
4. Gan, M., Chen, X., Chen, Z., Chen, L., Zhang, S., Zhao, Y., ... & Zhu, L. (2022). Genistein Alleviates High - Fat Diet - Induced Obesity by Inhibiting the Process of Gluconeogenesis in Mice. *Nutrients*, 14 (8), 1551
5. Hemati, K., Pourhanifeh, M. H., Dehdashtian E., Fatemi, I., Mehrzadi, S., Reiter, R. J., & Hosseinzadeh, A. (2021). Melatonin and morphine: potential beneficial effects of co use. *Fundamental & Clinical Pharmacology*, 35 (1), 25-39
6. Jia, J., Xu, G., & Zeng, X. (2022). The Biology of Morphine and Oxidative Stress
7. Kuthati, Y., Busa, P., Tummala, S., Rao, V. N., Davuluri, V. N. G., Ho, Y. P., & Wong, C. S. (2021). Mesoporous polydopamine nanoparticles attenuate morphine tolerance in neuropathic pain rats by inhibition of oxidative stress and restoration of the endogenous antioxidant system. *Antioxidants*, 10 (2), 195.
8. Mansour, D. F., Saleh, D. O., & Mostafa, R. E. (2017). Genistein ameliorates cyclophosphamide - induced hepatotoxicity by modulation of oxidative stress and inflammatory mediators. *Macedonian Journal of Medical Sciences*, 5 (7), 836.
9. Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, 28(1), 56-63.
10. Semeniuk, M., Ceré, L. I., Ciriaci, N., Bucci Muñoz, M., Quiroga, A. D., Luquita, M. G., ... & Ruiz, M. L. (2021). Protective effect of genistein pre - treatment on paraquat hepatotoxicity in rats. *Toxicology and Applied Pharmacology*, 426, 115636.
11. Tsuno, T., Fujimiya, T., Kawaguchi, T., Yanaizumi, R., Kojima, K., Miyasato, A., ... & Hakamata, H. (2022). Psychological barriers to the use of opioid analgesics for treating pain in patients with advanced recurrent cancer (BAROC): protocol for a multicentre cohort study. *BMJ open*, 12(3), e.054914

-
12. Zamani - Garmsiri, F., Hashemnia, S. M. R., Shabani, M., Bagherieh, M., Emamgholipour, S., & Meshkani, R. (2021). Combination of metformin and genistein alleviates nonalcoholic fatty liver disease in high - fat diet fed mice. *The Journal of Nutritional Biochemistry*, 87, 108505.
 13. Aledani, A. H. E., Khudhair, N. A., & Alrafas, H. R. (2020). Effect of different methods of anesthesia on physiobiochemical parameters in laboratory male rats. *Basic Journal of Veerinary Research*, 19, 206-214.
 14. Ali Abd, Al-Latif Al, Fares, Shaker Katea, & Safa. (2016). *Mus musculus L. Biochemical and histopathological changes of dimethoate in some organs of rats. Basrah Journal of Agricultural Sciences*, 29 (2).
 15. Al-Sarry, M. H. M., & Al-Karishy, K. J. S. (2007). The physiological effects of *Graecum. trigonellfoenum* seed and *Zea. Mays* (corn fiber) that use to treatment experimental acute renal failure in laboratory rats *Rattus norvegicus*. *basrah journal of science*, 25(2B arabic).
 16. Bini , G. , Cohen , E. B. , Chiavaccini , L. , Messenger , K. M. , & Bailey , K. M. (2022) . Intravenous dexmedetomidine , morphine , or a combination can result in gallbladder wall thickening ; with no significant association with plasma histamine concentrations . *Veterinary Radiology & Ultrasound* .
 17. Chadzinska , M. , Savelkoul , H. F. , & Verburg- van Kemenade , B. L. (2009) . Morphine affects the inflammatory response in carp by impairment of leukocyte migration . *Developmental & Comparative Immunology* , 33 (1) , 88-96 .
 18. Chahkandi , M. , Askari , N. , و Asadikaram , G. (2015) . The Effect on Acute and Chronic Morphine on Some Blood Biochemical Parameters in an Inflammatory Condition in Gonadectomized Male Rats . *Addiction Health* , 7 (3-4) , 130
 19. Duan, X., Li, Y., Xu, F., & Ding, H. (2021). Study on the neuroprotective effects of Genistein on Alzheimer's disease. *Brain and Behavior*, 11(5), e02100.
 20. Houston , B. J. , Nixon , B. , Martin , J. H. , De luliis , G. N. , Trigg , N. A. , Bromfield , E. G. , ... & Aitken , R. J. (2018) . Heat exposure induces oxidative stress and DNA damage in the male germ line . *Biology of reproduction* , 98 (4) , 593-606 .
 21. Khorsandi, L. S., Taherimobarakeh, M., & Kalantari, H. (2006). The protective effect of turmeric ((*Curcuma Longa*)(CL)) extract on acetaminophen-induced liver damage in mice. *J Adv Med Biomed Res*, 14(55), 23-29.
 22. Wan, C., Jin, F., Du, Y., Yang, K., Yao, L., Mei, Z., & Huang, W. (2017). Genistein improves schistosomiasis liver granuloma and fibrosis via dampening NF-kB signaling in mice. *Parasitology research*, 116(4), 1165-1174.
 23. Yoon, G. A., & Park, S. (2014). Antioxidant action of soy isoflavones on oxidative stress and antioxidant enzyme activities in exercised rats. *Nutrition research and practice*, 8(6), 618-624.
 24. Salahshoor , M. R. , Khazaei , M. , Jalili , C. , و Keivan , M. (2016) . Crocin improves damage induced by nicotine on a number on reproductive parameters in male mice . *International journal on fertility و sterility* , 10 (1 , (