European Scholar Journal (ESJ)
Available Online at: https://www.scholarzest.com
Vol. 4 No.01, January 2023
ISSN: 2660-5562

EFFECTS OF MORPHINE AND GENISTEIN INJECTIONS ON THE HISTOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE KIDNEYS IN WHITE MALE LABORATORY MICE.

Habib, J. Saheb[1], Murtadha, F. Al-Hillo[2], 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

Article history:

Received: 24th November 2022
Accepted: 26th December 2022
Published: 30th January 2023

Abstract:

Objectives: This study looked at the antioxidant and anti-inflammatory benefits of genistein in minimizing the side effects of morphine.
Methods: A total of 48 male laboratory rats were divided into four groups. There were twelve mice per group. A sterile saline injection at 0.1 M was administered to the control group. The morphine group received an injection of 20 mg/kg of morphine. The Genistein group received a 25 mg/kg injection; The morphine and genistein group received 15 days of 20 mg/kg morphine injection, followed by 15 days of 25 mg/kg genistein injection. Once daily subperitoneal injections are given to the mice divided into three periods (five, ten and fifteen days of injection).
Results: Morphine caused an increase in the levels of urea and creatine during the injection period. ten and fifteen days, while genistein caused a decrease in the level of urea and creatine in blood serum during a period of fifteen days of injection. An improvement was observed in the level of urea and creatine in the group treated with morphine and genistein, with an improvement in the kidney tissue.

Keywords: Morphine, genistein, urea, creatine, kidney tissue changes, rats

INTRODUCTION

The most notable opiate used for this purpose is morphine. Opioids are widely employed in many aspects of life, notably in the treatment of acute and chronic pain (Tsuno *et al.*, 2022). Studies that supported the existence of significant adverse effects of morphine abuse revealed that it can cause hypogonadism and a defect in hormone production, shortness of breath, vomiting, decreased intestinal secretions, weight loss, ataxia, and immune diseases when used excessively over an extended period of time (Hemati *et al.,* 2021). It may result in cytotoxicity through lipid peroxidation, reactive oxygen, the production of free radicals, and a drop in the level of antioxidant enzymes. This association with the lipids in cell membranes may result in oxidative stress, cancer, and its effects on blood parameters and the activation of programmed cell death (Jia *et al.*,2022).

Because prolonged morphine usage has negative repercussions, researchers have mostly concentrated on developing techniques to mitigate these effects. One of the suggestions is to employ substances having antioxidant qualities. Genistein, an isoflavone that belongs to the family of plant estrogens, is obtained from fruits, vegetables, seeds, legumes, and legume products, including alfalfa, broccoli, and cumin (Ebrahimisadr *et al.,* 2021).It is characterized by a

structure and properties similar to estrogen and has been widely employed in academic and medical settings for its antioxidant qualities, which include its capacity to reduce the levels of free radicals and increase those of antioxidant enzymes, anti-inflammatory compounds, and malignant tumors (Gan *et al.*, 2022) Osteoporosis, non-alcoholic fatty liver disease, liver and renal problems, anti-depression, and obesity reduction are among the conditions that affect the endocrine system (Zamani *et al.*, 2021).

As genistein is used as an antioxidant and anti-inflammatory, the goal of this study is to determine whether it is possible to utilize it to lessen the negative effects that morphine usage may have on urea, creatine, and renal tissue.

MATERIALS AND METHODS.

2-1: Experimental animals.

At the College of Education, Qurna, University of Basrah, 48 male Mus musculus L/BALB mice, aged 10–12 weeks and weighing 20–25g, were cared for in plastic cages coated with wood in a monitored environment. Animals may get food and water without any restrictions. (Essa and Hassan, 2013; Al-Ghareebawi, and ALkalby, 2012)

2-2: Experimenta design

The mice were divided into four groups, each containing 12 mice, as follows: The control group received an injection of 0.1 mL of sterile saline. The second group was also injected with 20 mg/kg of morphine (Salahshoor *et al,.*2016); the third group was also injected with genistein at a dose of 25 mg/kg (Kuthati *et al.*, 2021). The fourth group is the morphine and genistein group, as they were injected with morphine at a dose of 20 mg/kg for a period of 15 days, and then they were injected with genistein at a dose of 25 mg/kg. The rats were divided into three periods (five, ten, and fifteen days after injection) and injected into the subperitoneal membrane. At the conclusion of each session, mice were given chloroform anesthesia. Blood was then drawn straight from the heart using a sterile 1 mL vial, part of which was taken for blood analysis. In relation to serum and its use in biochemical analysis,

2-3: Measuring the level of urea and creatine.

The urea concentration was measured using a test kit prepared by the French company Biolabo, according to the method (Wills and Savory, 1981), and the level of creatinine in the serum of the tested rats was measured using a kit prepared by the French company Biolabo, according to the method of (Tietz, 1999),

2-4: Perform tissue sections

The method of (Humason, 1972) was adopted in the preparation of histological sections of the kidneys of laboratory rats

RESULTS

1-3: Effect of morphine and genistein on urea and creatine levels

The results of our current study showed that over ten to fifteen days post-injection, male rats given morphine injections had significantly higher blood levels of urea and creatine compared to controls and groups given genistein ($p \leq 0.05$) . During the fifteen-day injection period, administration of genistein resulted in a significant decrease ($p \leq 0.05$) in serum urea and creatine levels compared to the control group. The group that received injections of morphine and genistein had a significant improvement in blood urea and creatine levels during the fifteen-day injection period, with no significant changes compared to the control group. (Tabie,1,2).

(Table 1). Effect of different treatments on urea level (mg/dl) ± standard error

Treatment	Day		
	5	10	15
0.1 ml of normal saline	± 2.429.9^{b}	±1.730.1^{c}	± 3.331.1^{b}
Morphine 20 mg/ kg	±2.535.6^{b}	±2.546.3^{a}	±3.151.1^{a}
Genistein 25 mg/ kg	± 1.531.3^{b}	±2.127.6^{c}	± 1.722.2^{c}
Morphine and Genistein	±1.542.6^{a}	±1.137.1^{b}	±3.135.8^{b}

Different letters indicate significant changes within the same column at the probability level (P < 0.05).

Table (2). Effect of different treatments on creatine level (mg/dL) ± C ± standard error

Treatment	Days		
	5	10	15
0.1 ml of normal saline	± 0.120.71^{b}	±0.250.53^{c}	± 0.610.66^{b}
Morphine 20 mg/ kg	±0.140.97^{b}	±0.141.73^{a}	± 0.152.23^{a}
Genistein 25 mg/ kg	± 0.50.71^{b}	±0.110.20^{c}	± 0.150.16^{c}
Morphine and Genistein	±0.31.61^{a}	±0.91.11^{b}	± 0.21.03^{b}

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 kidney tissue

A: control group: Histologically, in the mouse kidney, its structures are intact, and it is represented by the glomerulus, which is composed of a network of blood vessels overlapping with mutated epithelial cells. The glomerulus is surrounded by a capsule called Bowman's capsule. It is lined from the inside with capillaries that form the glomerulus. The outer layer forms the outer surface of the capsule. The two layers are separated by Bowman's capsule space, as well. The kidney contains tubules lined with simple epithelial tissue that varies with the type of tubule.(Fig 1)

B: morphine group: The results of the histological examination of the kidneys showed, five days after the injection, the appearance of histological changes represented in hyperplasia of the cells lining the glomeruli, loss of the normal shape of the tissues lining the renal tubules, death of the renal glomeruli, hyperpigmentation, and loss of histological features (Fig2,3), and after ten days of injection, there was bleeding. Acute blood vessels and their expansion, loss of normal histological features of the renal tubules, fusion of adjacent tubules, obstruction of these tubules, tubular separation and divergence, hypertrophy (Fig.4). Fifteen days later, the results showed hemorrhage, expansion of blood vessels, loss of normal tissue features, death of the glomerulus, Kidney enlargement, with necrosis and swelling of some renal cells (Fig5.6,7,8)

C: genistein group: When compared to the control group, there were no degenerative abnormalities in the rats' kidneys after all rounds of injection, according to the results of a histological investigation (Fig.9,10).

D: morphine & genistein group: The results after five days of injection showed an improvement in the structure of the renal glomeruli, with the presence of some pathological conditions that still appeared in the renal tissue, which included hemorrhage, expansion of blood vessels, aggregation of inflammatory cells, hyperinflation of cells lining the tubules, and loss of glomeruli ((Fig11,12,13). Ten days after the injection, the results showed a significant improvement in the histological structure of the renal glomeruli, as they appeared normally, with the presence of some pathological conditions that still appeared in the renal tissue, which included hemorrhage in the blood vessels, hyperinflation of the cells lining the renal tubules, hyperpigmentation, and necrosis. Cells lining the renal tubules and their degenerative (Fig 14,15).While the results of the tests demonstrated a considerable improvement in the renal histology parameters following treatment with genistein, fifteen days after the injection, renal tissue and renal tubules were noted to appear properly, with the detection of a minor blood vessel congestion (Fig16,17).

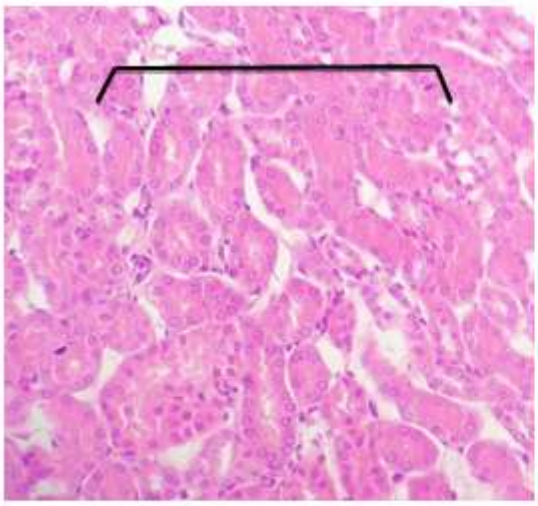


Figure (1) A cross-section of the tests of the control group showing the normal structure of the kidneys tubules, H&E, 1X

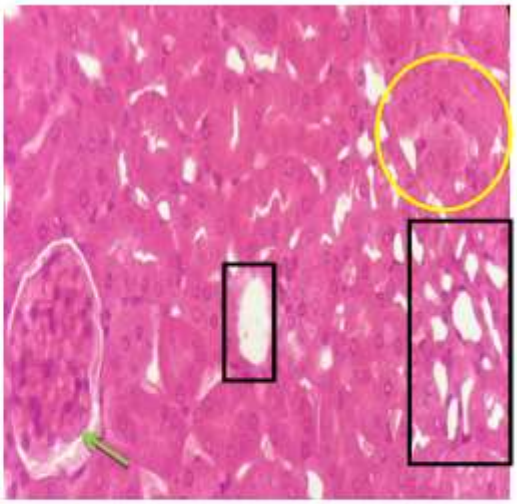


Figure (2): A slice through the kidneys of mice given morphine injections over a five-day period reveals hyperplasia of the glomeruli's lining cells (yellow circle), as well as a loss of the glomeruli's typical shape (rectangle). H&E,

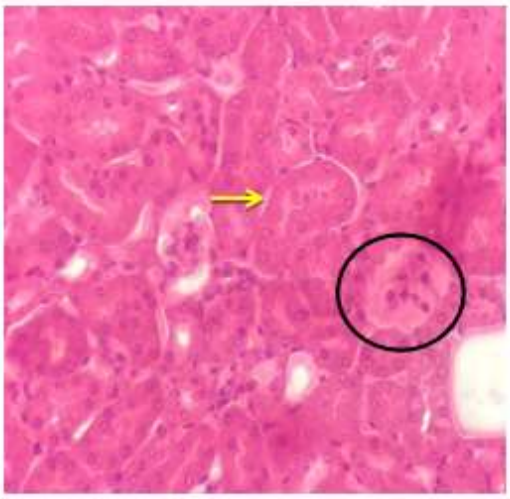


Figure (3) depicts a section of the kidneys of morphine-injected mice over a five-day period.Death of the glomeruli (circle), hyperpigmentation, and loss of histological features (yellow arrow) H&E, 4X

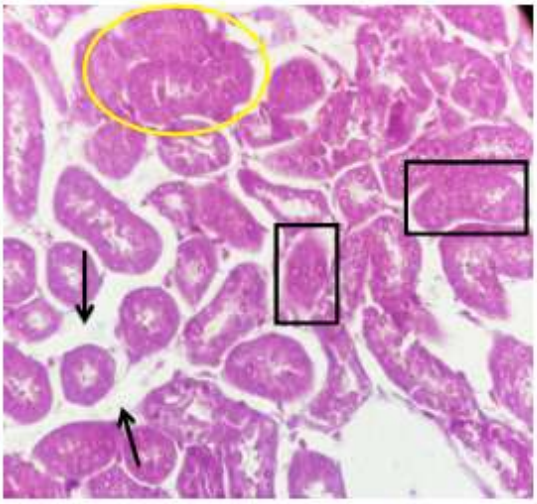


Figure (4) shows a section of kidneys of morphine-injected mice over the ten-day injection period, glomeruli dead (circle), hyperpigmentation, and loss of histological features (yellow arrow) H&E, 4X

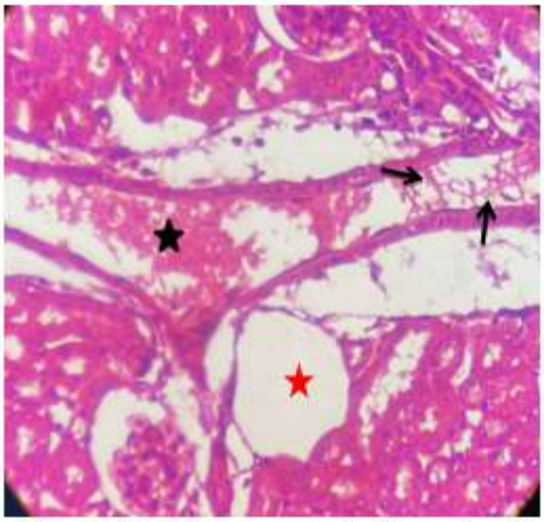


Figure (5) Mice were administered with morphine over a fifteen-day period, and a part of their kidneys exhibited serious hemorrhage (black star). loss of the glomerulus and separation of the cells and connective fibers (black arrow, red star) H&E, 4X

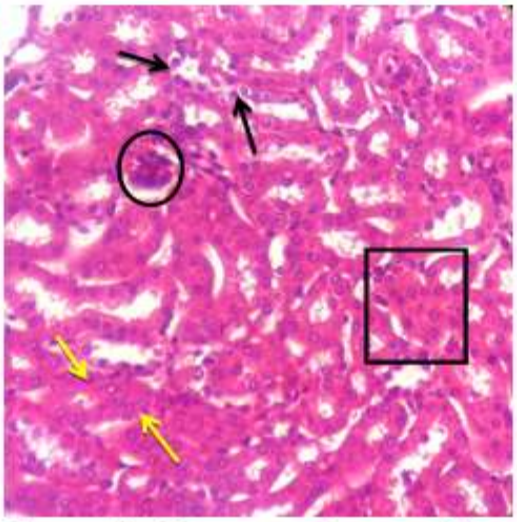


Figure (6) Mice were given morphine injections over a 15-day period, and a portion of their kidneys revealed glomerular cell necrosis (black arrow). Tuberular blockage, hyperpigmented glomeruli (yellow arrow), and glomerular death (black circle) (black square) H&E, 4X

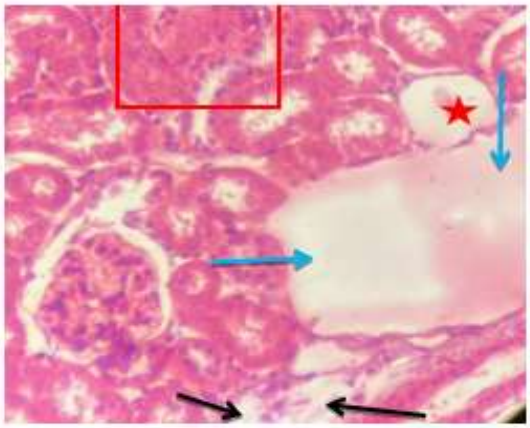
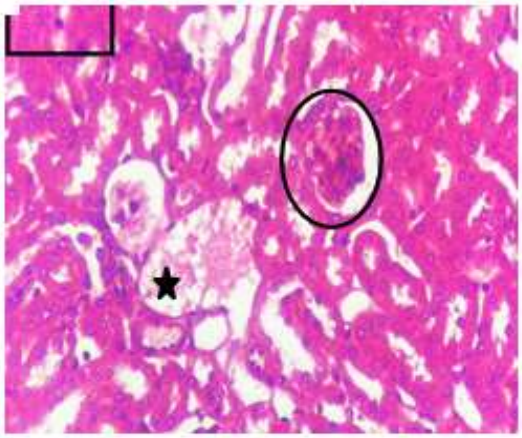


Figure (7) A section of the kidneys of mice injected with morphine during the fifteen-day injection period shows hyperplasia of renal tissue (red square). glomerulus loss (red star) edema (blue arrow) renal tissue necrosis (black arrow) H&E, 4X



Figure(8): A section of the kidneys of mice injected with morphine during the fifteen-day injection period, showing the loss of normal tissue features (black square), death of the glomerulus (black circle), and severe hemorrhage (black star). H&E, 4X

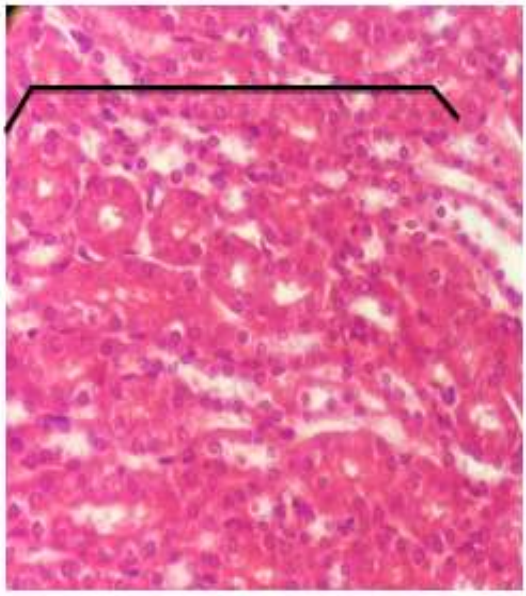


Figure (9) A section of the kidneys of mice injected with genistein during the five-day injection period shows the normal appearance of the kidneys (straight) H&E, 4X

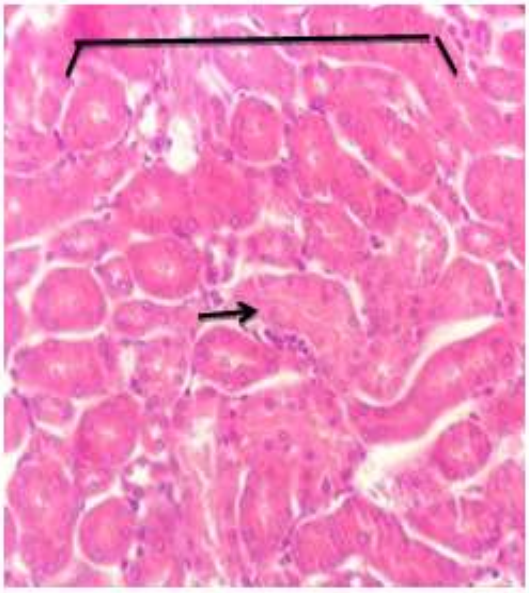


Figure (10) A section of the kidneys of mice injected with genistein during a ten-day injection period shows the normal appearance of the kidneys (straight) and cell hyperplasia (arrow) H&E, 4X

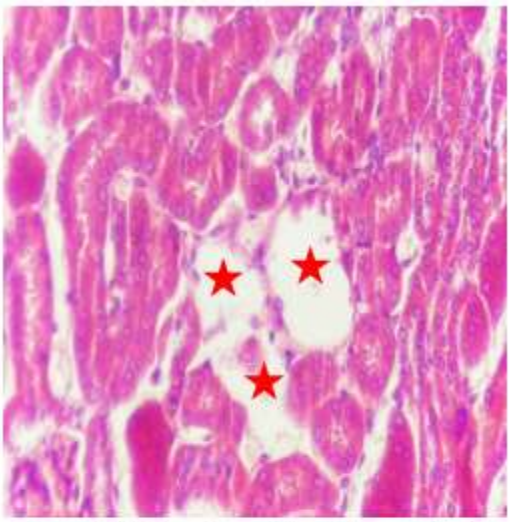


Figure (11) A section of the kidneys of mice injected with morphine & genistein during a five-day injection period, showing the loss of glomeruli (red star)H&E, 4X

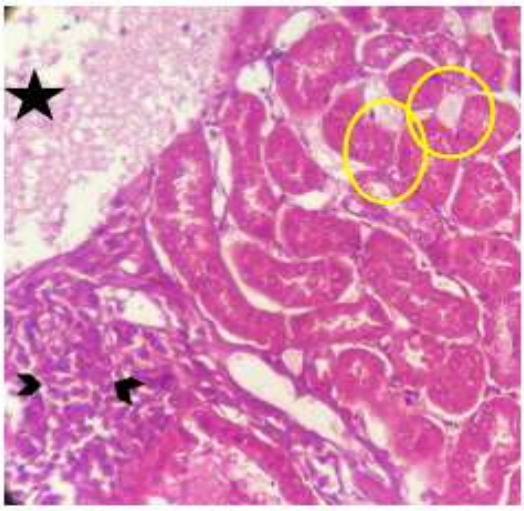


Figure (12) A section of the kidneys of mice injected with morphine & genistein during a five-day injection period, showing vascular hemorrhage (black star), inflammatory cell aggregation (arrowhead), hyperinflation of the cells lining the tubules (yellow circle) H&E, 4X

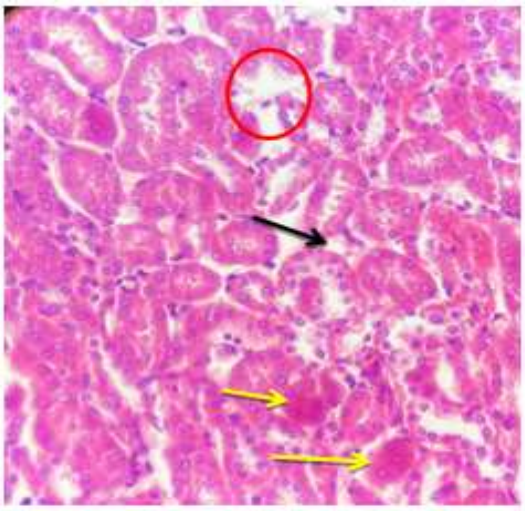


Figure (13) shows a section of the kidneys of mice injected with morphine and genistein during the five-day injection period.Renal cell necrosis (black arrow)Cell degeneration (red circle) H&E, 4X

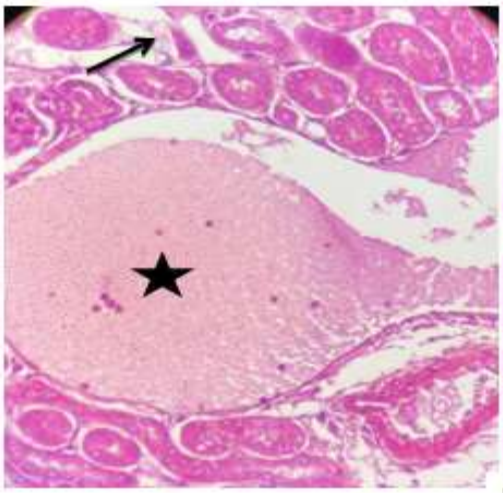


Figure (14) A section of the kidneys of mice injected with morphine & genistein during a ten-day injection period, showing cell degeneration and necrosis (black arrow) H&E, 4X

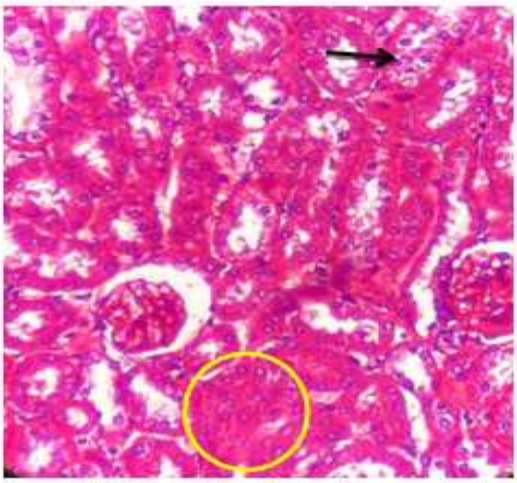


Figure (15) A section of the kidneys of mice injected with morphine & genistein during a ten-day injection period shows hyperinflation of the cells lining the renal tubules (yellow circle) and necrosis of the cells lining the renal tubules (black arrow) H&E, 4X

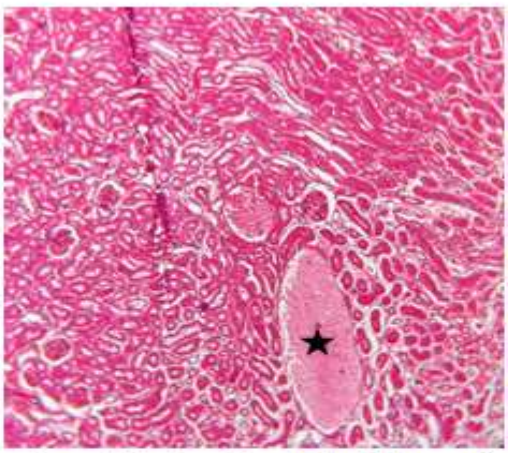


Figure (16) A section of the kidneys of mice injected with morphine & genistein during a fifteen-day injection period, showing congestion of blood vessels (star) H&E, 4X

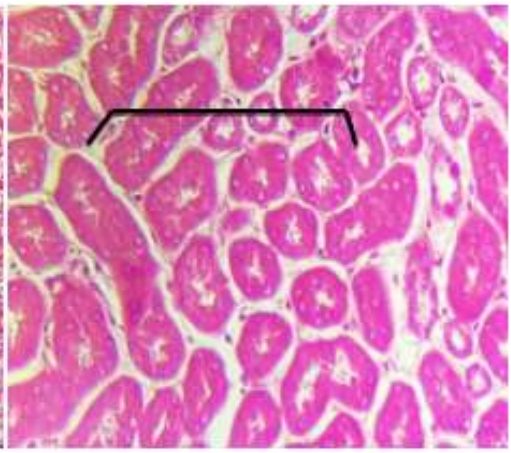


Figure (17) shows a section of the kidneys of mice injected with morphine & genistein during the fifteen-day injection period H&E, 4X (Normal renal tubules (straight)

DISCUSSION.

The results revealed that the rats given morphine injections had significantly higher levels of urea and creatine. The oxidative stress that results from morphine metabolism in the liver and kidneys increases the risk of oxidative stress and the accompanying severe damage to the renal tubules that form the glomeruli and their necrosis, which may impair the function of the glomeruli. in filtering urea and creatine and pumping them out of the body, potentially leading to a variety of health problems (Esfandiari *et al*., 2014).

This may be because genistein, an anti-oxidant and anti-inflammatory estrogenic hormone, causes an increase in the concentration of antioxidant enzymes within the body. This safeguards the body and renal cells from oxidative stress that developed as a result of the increased concentration of free radicals brought on by morphine abuse, which protects the glomeruli and repairs damage. increased urea and creatine filtration (Kim *et al*., 2012). According to some studies, the use of genistein inhibits the activity of enzymes involved in the synthesis of urea, such as nucleotide deaminases, glutamate dehydrogenases, and AMP deaminases, which break down proteins and turn them into urea. This, in turn, results in a decrease in the body's production of urea (Choi *et al.,* 2015).

While the findings of the tissue inspection revealed that the kidney suffers from blood vessel congestion and expansion, this may be because of increased histamine production, which may result in blood stagnation and vessel congestion brought on by the use of morphine (Bini *et al*., 2022). Morphine also causes bleeding in the kidneys, which may be caused by toxins activating the phosphorylation of cellular proteins that damage the process of establishing cellular communications between the epithelial cells lining the blood vessels, which may allow blood to filter through openings outside the blood vessels (Ohnishi *et al*., 1997).

The outcomes also demonstrated that genistein use decreased blood vessel growth and congestation. This may be because genistein prevents oxidative stress and lessens the incidence of vascular expansion by working to decrease the generation of nitric oxide, free radicals, and lipid peroxidation while increasing the concentration of antioxidant enzymes (Duan *et al*., 2021). Or that genistein inhibits the function of the hormone protaglandins, which stops blood vessels from dilation (Venza *et al*., 2018).

Necrosis was also seen in the kidneys of morphine-injected mice. This could be because the intracellular enzymes of the affected cells activate the cell demolition process or because of morphine's direct impact on a process that could damage DNA's structure and integrity and prevent the production of proteins required for cell growth and maturation (Trivedi *et al*., 2014). Another possibility is that morphine harms the nucleus by stimulating it to the point where oxidative stress results in apoptosis (Barrow *et al*., 2017). It was also noted that edema developed in kidney cells as a result of increased blood vessel perfusion with low-protein fluids caused by a drop in albumin concentration, which then accumulated in the intercellular spaces and produced edema (Hayashi *et al*., 2014), as well as that morphine abuse results in

inflammatory conditions that are accompanied by the occurrence of blood vessel expansion, increased nutrition, swelling, and deformation of the aorta (Spaide, 2016). The findings also demonstrated that morphine was responsible for the buildup of inflammatory cells in the kidneys because it causes infected hepatocytes to release prostaglandin E1, which can draw neutrophil immune cells to the affected area. Once there, the neutrophils release chemicals that draw other immune cells, leading to their aggregation and the development of an inflammatory condition (Lindberg *et al*., 1997).

The role of genistein in improving the negative effects of morphine is through genistein activating AMPK, which plays a major role in suppressing inflammation in affected rat organs as it indirectly suppresses NF-KB signaling that enables it to suppress the inflammatory response (Garcia *et al.,* 2019). As it lowers the level of free radicals produced and raises antioxidant enzymes, genistein also helps to reduce the oxidative stress caused by the use of morphine on cells. This prevents the occurrence of fatty oxidation of cells, inhibits the occurrence of programmed cell death, suppresses DNA damage to cells, repairs damaged cells, and treats edema and degeneration (Yoon *et al.,* 2014)

REFERENCES

1. 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
2. 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
3. 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
4. Jia, J., Xu, G., & Zeng, X. (2022). The Biology of Morphine and Oxidative Stress
5. 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.
6. 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
7. 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.
8. 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 .
9. 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.
10. 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.
11. Essa, Z. A. S. A. H., & Hassan, A. M. (2013). THE EFFECT OF BISMUTH CHLORIDE ON SOME BLOOD AND BIOCHEMICAL PARAMETERS IN MALE LABORATORY RATS (RATTUS-RATTUS). Basrah Journal of Veterinary Research, 12(1).
12. Wills, M. R. and Savory, J. (1981). Biochemistry on renal failure. Ann. Clin. Lab. Sci. 11:292-299.
13. Kim, J. S., Heo, K., Yi, J. M., Gong, E. J., Yang, K., Moon, C., و Kim, S. H. (2012). Genistein mitigates radiation-induced testicular injury. Phytotherapy research, 26(8), 1119-1125.

14. Venza, I., Visalli, M., Oteri, R., Beninati, C., Teti, D., و Venza, M. (2018). Genistein reduces proliferation on EP3-expressing melanoma cells through inhibition on PGE2-induced IL-8 expression. International immunopharmacology, 62, 86-95.
15. Al-Ghareebawi, A. M. A., & ALkalby, J. M. (2012). EFFECT OF MERCURIC CHLORIDE ON REPRODUCTIVE EFFICIENCY IN LABORATORY MALE RATS (RATTUS NORVEGICUS). Basrah journal of veterinary research, 11, 4.
16. Tietz, N.W. (1999). Text book on clinical chemistry. (ed). C.A. Burtis, E.R. Ashwood, W.B. Saunders. P .477-530 to 1241-1245.
17. Humason, G.L. (1972). Animal tissue techniques. Freeman, W.H. (3th ed.). San Francisco press. UAS.PP.641.
18. Esfandiari , E. , Roshankhah , S. , Mardani , M. , Hashemibeni , B. , Naghsh , E. , Kazemi , M. , و Salahshoor , M. (2014) . The effect of high frequency electric field on enhancement of chondrogenesis in human adipose - derived stem cells . Iranian journal of basic medical sciences , 17 (8) , 571
19. Choi , Y. J. , Lee , C. H. , Lee , K. Y. , Jung , S. H. , و Lee , B. H. (2015) . Increased hepatic fatty acid uptake and esterification contribute to tetracycline - induced steatosis in mice . Toxicological Sciences , 145 (2) , 273-282 .
20. Ohnishi, M., Kirkman, E., Marshall, H. W., و Little, R. A. (1997). Morphine blocks the bradycardia associated with severe hemorrhage in the anesthetized rat. Brain research, 763(1), 39-46.
21. Trivedi , M. , Shah , J. , Hodgson , N. , Byun , H. M. , و Deth , R. (2014) . Morphine induces redox - based changes in global DNA methylation and retrotransposon transcription by inhibition on excitatory amino acid transporter type 3 - mediated cysteine uptake . Molecular pharmacology , 85 (5) , 747-757
22. Hayashi, T., Ikehata, S., Matsuzaki, H., Yasuda, K., Makihara, T., Futamura, A., ... و Yamada, S. (2014). Influence on serum albumin levels during opioid rotation from morphine or oxycodone to fentanyl for cancer pain. Biological and Pharmaceutical Bulletin, b14-00119
23. Spaide, R. F. (2016). Retinal vascular cystoid macular edema: review and new theory. Retina, 36(10), 1823-1842.
24. Garcia, D., Hellberg, K., Chaix, A., Wallace, M., Herzig, S., Badur, M. G., Shaw, R. J. (2019). Genetic liver-specific AMPK activation protects against diet-induced obesity and NAFLD. Cell reports, 26(1), 192-208
25. Barrow, T. M., Byun, H. M., Li, X., Smart, C., Wang, Y. X., Zhang, Y., ... & Guo, L. (2017). The effect of morphine upon DNA methylation in ten regions of the rat brain. Epigenetics, 12(12), 1038-1047.
26. 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).