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The effect of berberine on the histological changes induced by methioninemia in male albino rats

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Abstract Berberine, an active component of the plant was used in the study to investigate its possible protective effect in ameliorating methionine-induced histopathological changes on male albino rat liver. Thirty-two (32) adult male albino rats, average age 12–16 weeks and body weight ranging from 200 to 300 g were included in the current work. The study was conducted at an animal's house, Department of Biology, College of Education, University of Al-Qadisiyah. The animals were randomly divided into four groups of eight (8) rats as follows:Group 1 (G1): Eight animals as negative control group and food and water were available ad libitum throughout the experiment period.
Group 2 (G2): Consisted of 8 animals treated by gavage with methionine (100 mg/kg/body weight), comprising the positive control group.Group 3 (G3): Consisted of 8 animals that received orally methionine at a dose of 100 mg/Kg body weight and berberine at a dose of 40 mg/kg bw by the same way as group II over the entire experimental period.Group 4 (G4): Consisted of 8 animals that received berberine orally at a dose of 40 mg/kg body weight for the entire period of the experiment.
Doses were administered orally for eight weeks. After the experimental period was over, the liver and kidney tissues were excised and prepared for histological screening by hematoxylin and eosin staining. The control and berberine (?)-only groups exhibited regular hepatic and renal architecture with well-preserved cell arrangement. The methionine-treated rats, on the other hand, demonstrated severe pathologic changes including marked of macrovesicular hepatic steatosis and sinusoidal congestion fibrotic deposition as well as cystic renal dilation associated with tubular degeneration. Of particular importance, in the methionine and berberine groups, we observed only some mild hepatic steatosis and that tubular changes were limited, suggestive of strong protection.These results could indicate the capacity of berberine to alleviate methionine-induced tissue injury due to its antioxidant, anti-inflammatory, and metabolic modulatory effects. The results indicate that berberine may be a potential therapeutic compound for liver and kidney damage in hypermethioninemia.

Keywords: Berberine; Methionine; Liver; Kidney; Albino rats.

Introduction Excessive consumption of protein-rich foods, whether by ordinary individuals or athletes who rely on protein supplements to enhance performance and build muscle, can lead to serious metabolic disturbances. Proteins of animal origin, in particular, contain high levels of sulfur-containing amino acids such as methionine and cysteine, which contribute to increased blood homocysteine levels accompanied by oxidative stress and impaired liver and kidney function, thus elevating the risk of chronic diseases in the long term(1). This issue represents a key focus for research aimed at identifying preventive or therapeutic strategies based primarily on natural compounds with biological activity and minimal side effects. Methionine is an essential amino acid that cannot be synthesized by the body. It is obtained mainly from dietary animal sources such as meat, fish, poultry, and cheese, as well as from some plant sources like rice and fruits, though its amount in vegetables is relatively limited (2). . Methionine plays a central role in numerous physiological processes, most notably in protein synthesis and methylation reactions, which contribute to regulating gene activity and maintaining cellular structure stability (3).

Excessive intake of protein-rich foods, especially those of animal origin, is associated with a higher risk of developing several diseases. This is because such proteins contain sulfur-rich amino acids, namely methionine and cysteine, which serve as precursors for the synthesis of other sulfur-containing

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compounds and amino acids, including homocysteine, taurine, cystic acid, cystathionine, and keto-methionine (4, 5)

Excessive accumulation of sulfur-containing amino acids in the body adversely affects several vital functions. It is linked to increased oxidative stress, which in turn contributes to a range of health issues such as cardiovascular diseases, atherosclerosis, neurodegenerative disorders, as well as higher susceptibility to cancer, poor bone health, and kidney disorders(6, 7).

Medicinal plants represent a rich source of bioactive compounds that have been used for thousands of years in traditional medical systems such as Chinese, Indian, and Arabic medicine to treat various ailments. Interest in herbal medicine has significantly increased in recent decades, largely because many chemical drugs are associated with undesirable side effects, while medicinal plants are often considered safer when used in appropriate doses (8). . Additionally, the high cost of pharmaceutical treatments has encouraged people to turn to herbal remedies as a more economical option. The growing problem of microbial resistance to antibiotics has also motivated researchers to explore natural alternatives (9). .

Plant-derived compounds possess diverse therapeutic properties, including antioxidant, anti-inflammatory, and immunomodulatory effects, making them valuable for both prevention and treatment (10) . The global shift toward complementary and alternative medicine reflects an increasing desire among people to return to natural sources and adopt safer, more affordable therapeutic approaches. Consequently, medicinal plants have become a central focus in modern research for discovering new and more effective drugs, in addition to their long-established traditional role (11).

Among the promising plant-derived compounds, berberine stands out as a yellow, bitter-tasting isoquinoline alkaloid extracted from several medicinal plants such as Berberis vulgaris and Coptis chinensis, both known for their high berberine content. It is also found in other species such as Nandina domestica and Phellodendron amurense. Various methods are employed for berberine extraction, including solvent extraction and active compound refinement to obtain the pure substance, berberine exhibits broad pharmacological properties that make it of great medical interest. It has been used for centuries in traditional Chinese and Indian medicine to treat a wide range of health conditions due to its antioxidant, anti-inflammatory, and anticancer properties, as well as its protective effects on the liver and kidneys and its ability to improve glucose and lipid metabolism. These characteristics make it an important therapeutic candidate for counteracting the damage caused by excessive consumption of methionine-rich proteins and the associated metabolic disturbances (12, 13)

Such properties render berberine a promising therapeutic agent against hypermethioninemia-related problems, including hyperhomocysteinemia, oxidative stress, and their harmful effects on the liver, kidneys, and heart (14).The present study aims to evaluate the effect of berberine in reducing the damage resulting from excessive methionine intake and oxidative stress in adult male albino rats through histological examination of liver and kidney tissues.

Materials and Methods

Ethical approval

The project was approved (Ref. No. 35 in 15/2/2025) by the Committee for Research Ethics at the College of education, University of Al-Qadisiyah, Iraq.

Chemicals:

Methionine was obtained from HIMEDIA (India) in the form of a 25 g container of white powder and administered at a dose of 100 mg/kg body weight (15). Berberine was obtained from BIO TEST (USA) in the form of capsules containing 500 mg of yellow powder and administered at a dose of 40 mg/kg body weight (16).

Experimental Animals:

Adult male laboratory rats were obtained from the College of Veterinary Medicine, University of Al-Qadisiyah. The animals were aged between 12–16 weeks and weighed 200–300 g. They were acclimatized for two weeks to ensure their health status and kept under controlled environmental conditions at a temperature of 22 ± 5°C and a 12-hour light/dark cycle. All procedures were performed in accordance with institutional ethical guidelines for animal care (17).

Experimental Design:

•Group 1 (G1): Contained 8 rats serving as the negative control group, provided with water and feed ad libitum throughout the experimental period.

•Group 2 (G2): Contained 8 rats orally administered methionine at a dose of 100 mg/kg body weight, serving as the positive control group.

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•Group 3 (G3): Contained 8 rats orally administered methionine (100 mg/kg) and berberine (40 mg/kg) throughout the experimental period.
•Group 4 (G4): Consists of eight rats, which were treated orally with berberine (40 mg/kg) during the entire period of experiment.

Dissection and Histological Preparation:

The rats were anesthetized with chloroform by the closed-inhalation way at the end of experimental period. The animal was then placed in the dorsal position and needles fixated. The blood was withdrawn from the heart by cardiac puncture into plain tubes. Internal organs were then removed after conducting a T-shaped incision toward the sternum from groin. The livers and kidneys were carefully removed with forceps, rinsed in saline solution to eliminate the blood, and then fixed in 10% formaldehyde for histopathological examination.

Results of Histological Changes

Histological Changes in the Liver

Microscopically, the liver sections of G1 (negative control group) had normal histological architecture. The hepatic lobules had the classic pattern of hepatocytes with the central vein running centrally in each lobule (Fig 4-1) A & B

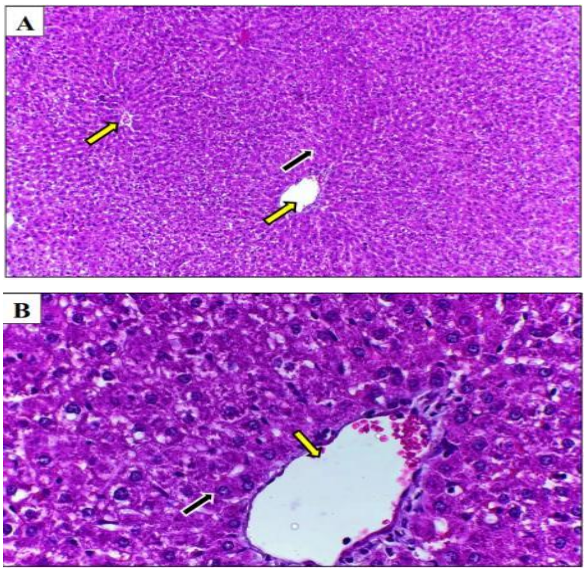
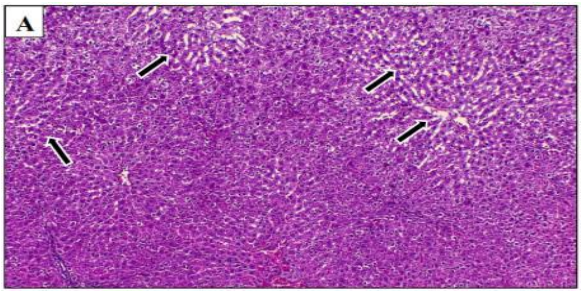
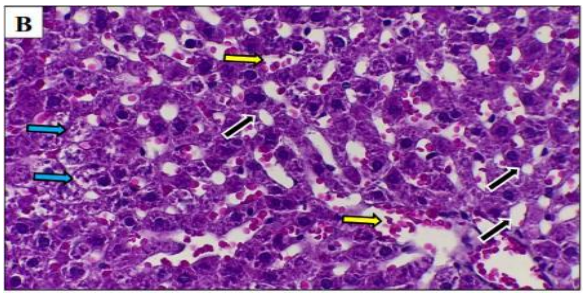


Figure (4-1): Histological section of the rat liver control group (G1): A and B representing normal histological architecture of the livers. The hepatic lobules show normal distribution of hepatocytes (black arrow) around central vein (yellow arrow) that is located in middle of the lobule. Stain: H&E. Magnification: A – 100x, B – 400x.In contrast, histological examination of the liver sections from the methionine-treated group (G2) for 60 days revealed fatty changes in the liver characterized by large vacuolar fat droplets. The diameter of the lipid vacuoles was approximately half the size of the hepatocyte radius and involved most of the affected lobular areas. The fat vacuoles were clearly visible within the hepatocytes. The steatohepatitis lesions occupied about 50% of the examined liver section area. In addition, mild sinusoidal congestion and scattered foci of red blood cell extravasation were observed, mainly concentrated in the central



lobular regions, as shown in Figure (4-2) A and



B.

Figure (4-2): Histological section of a rat liver from the methionine-treated group (G2): A and B show fatty liver changes in the form of large lipid vacuoles, with the diameter of the fat vacuoles reaching approximately half the size of the hepatocyte radius and involving most of the affected lobular regions (black arrow). In

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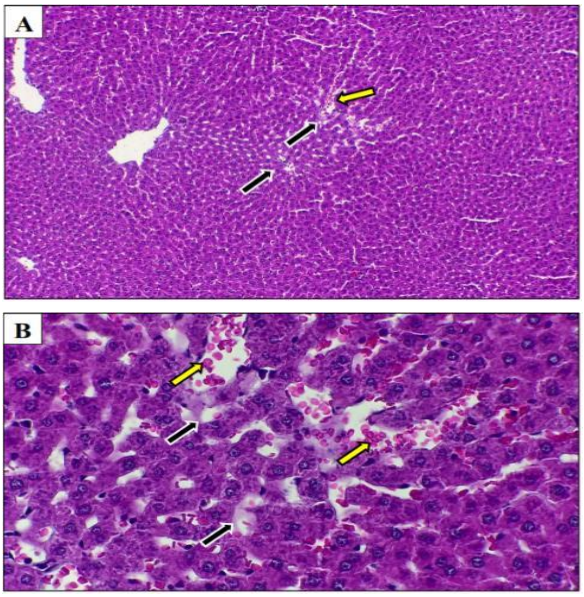
addition, mild sinusoidal congestion and scattered foci of red blood cell extravasation (yellow arrow) were clearly observed, mainly concentrated in the central lobular areas. Stain: H&E. Magnification: A – 100x, B – 400x.As for the group treated with both berberine and methionine (G3) for 60 days, the histological sections of the liver showed mild fatty changes, characterized by lipid vacuoles appearing as focal areas within the affected lobules. In addition, hepatic fibrosis was observed in one or two lobules of the liver. Mild sinusoidal



congestion was also noted, mainly concentrated in the central regions of the lobules, as shown in Figure (4-3) A and B.

Figure (4-3): Histological section of a rat liver from the group treated with both berberine and methionine (G3): A and B show mild fatty changes in the liver (black arrow), along with mild sinusoidal congestion (yellow arrow) that is clearly visible and mainly concentrated in the central regions of the lobules. Stain: H&E. Magnification: A – 100x, B – 400x.

For the berberine-only group (G4), histological examination of the liver sections revealed a normal histological structure. The hepatic lobules exhibited a typical arrangement of hepatocytes, with a clearly visible central vein located within each lobule, as shown in Figure (4-4) A and B.

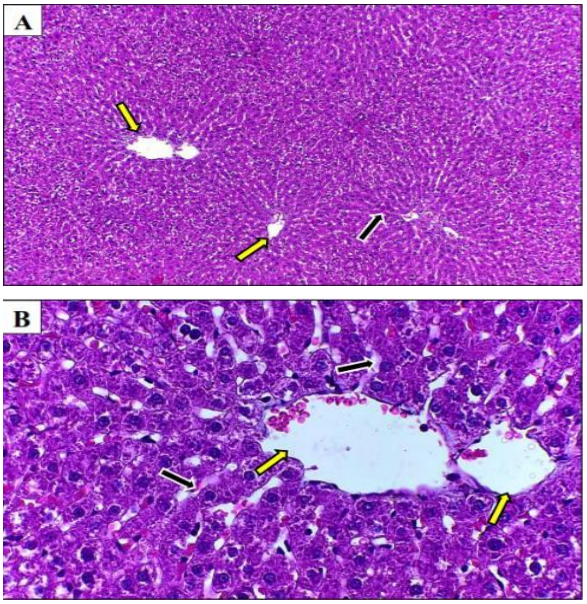


Figure (4-4): Histological section of a rat liver from the berberine-treated group (G4): A and B show the normal histological structure of the liver. The hepatic lobules display a typical arrangement of hepatocytes (black arrow), with a clearly visible central vein (yellow arrow) located within each lobule. Stain: H&E. Magnification: A – 100x, B – 400x.

Histological Changes in the Kidney

The histological section of the renal cortex from the negative control group (G1) showed a normal histological structure of the cortical region. The glomerulus was observed to be surrounded by both proximal and distal convoluted tubules, as shown in Figure (4-5) A and B.

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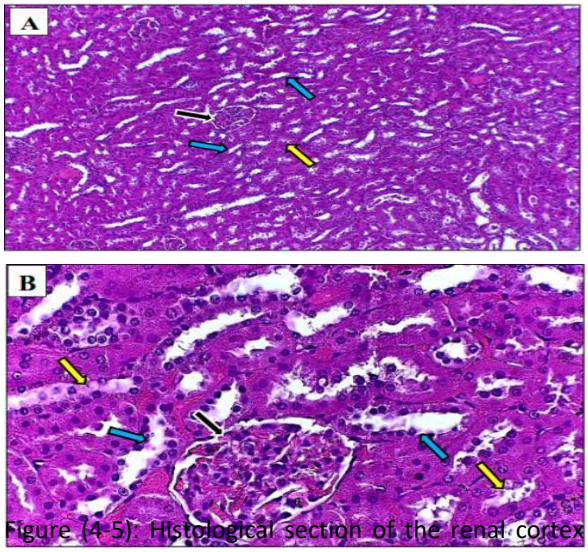

Figure (4-5): Histological section of the renal cortex from rats in the negative control group (G1): A and B show the normal histological structure of the cortical region. The glomerulus (black arrow) is surrounded by proximal convoluted tubules (yellow arrow) and distal convoluted tubules (blue arrow). Stain: H&E. Magnification: A – 100x, B – 400x.

The histological sections of the renal medulla revealed a normal structure, showing the presence of the descending and ascending thin limbs as well as the thick descending and ascending limbs of the loop of Henle, as shown in Figure (4-6) A and B.

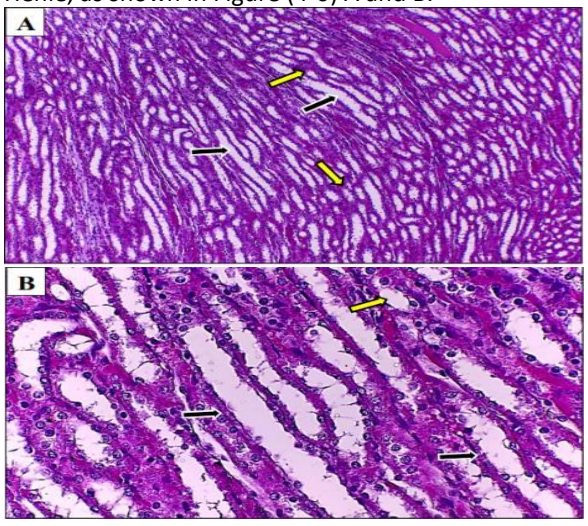

Figure (4-6): Histological section of the renal medulla from rats in the negative control group (G1): A and B show the normal histological structure of the medulla. The thin descending and ascending limbs (black arrow) and the thick descending and ascending limbs (yellow arrow) of the loop of Henle are clearly visible. Stain: H&E. Magnification: A – 100x, B – 400x.

For the methionine-treated group (G2) after 60 days, the histological sections of the renal cortex showed several large cystic lesions of varying sizes, some reaching the diameter of large blood vessels. The cystic spaces were lined with thin, flattened tubular epithelial cells. Additionally, some cyst cavities were filled with a clear to eosinophilic proteinaceous fluid, while others appeared empty. The cystic lesions indicated severe degenerative dilation of the tubules, and infiltration of inflammatory cells was observed along with distinct collagen fiber deposition in the affected cortical region, as shown in Figure (4-7) A and B.

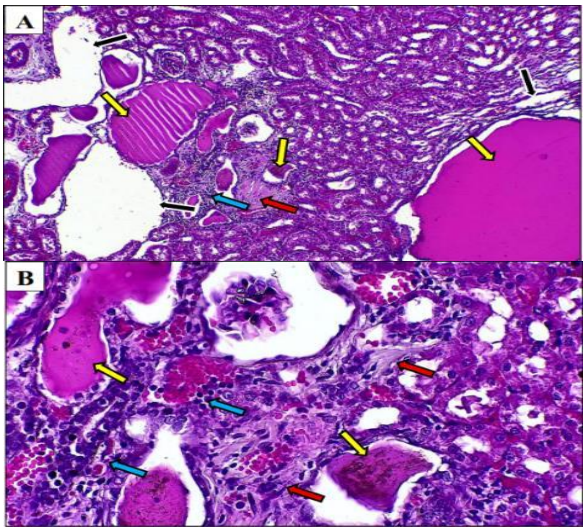

Figure (4-7): Histological section of the renal cortex from rats in the methionine-treated group (G2): A and B show several large cystic lesions (black arrow) of varying sizes, with some cysts reaching diameters comparable to large blood vessels. The cystic spaces were lined with flattened and thinned tubular epithelial cells. Some cyst cavities were filled with clear to eosinophilic proteinaceous fluid (yellow arrow), while others appeared empty. The cystic lesion indicates severe tubular dilation and degeneration. Infiltration of inflammatory cells (blue arrow) and evident collagen fiber deposition (red arrow) were observed in the affected cortical region. Stain: H&E. Magnification: A – 100x, B – 400x.

Regarding the medullary region, histological examination of the kidney medulla from the methionine-treated group showed that the loops of Henle appeared as thin-walled structures composed of delicate basement membranes. In many sections, the epithelial cells of the affected tubules were either

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absent or barely visible, indicating epithelial cell atrophy or erosion, as shown in Figure (4-8) A and B.

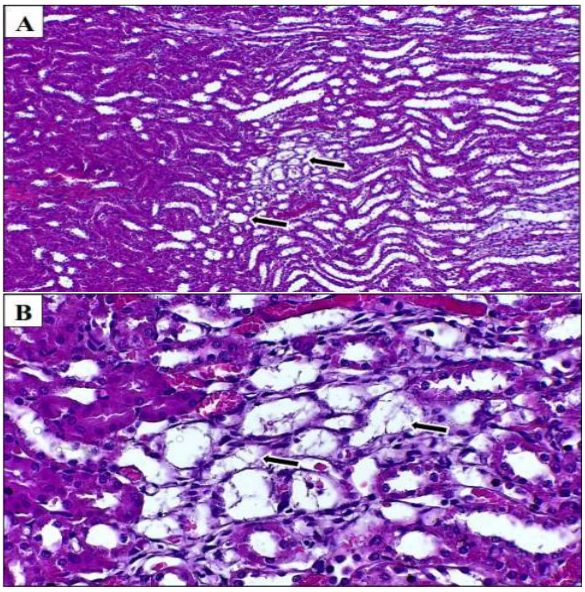
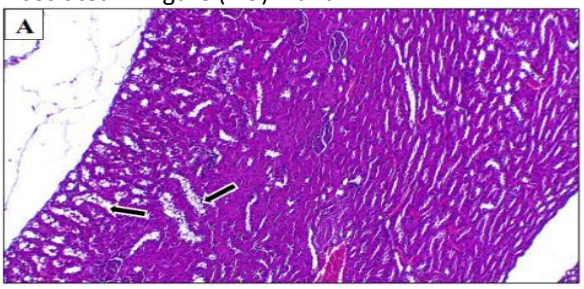


Figure (4-8): Histological section of the renal medulla from rats in the methionine-treated group (G2): A and B show the loops of Henle appearing as thin-walled structures (black arrow) composed of delicate basement membranes. In many sections, the epithelial cells of the affected tubules were either absent or barely visible, indicating epithelial cell atrophy or erosion. Stain: H&E. Magnification: A – 100x, B – 400x.

As for the group of rats treated with both methionine and berberine (G3), histological examination of the renal cortex revealed focal degeneration of epithelial cells in the convoluted renal tubules in a limited number of tubules. Moreover, no cystic lesions were observed, in contrast to the methionine-treated group, which showed prominent cystic formations, as illustrated in Figure (4-9) A and B.



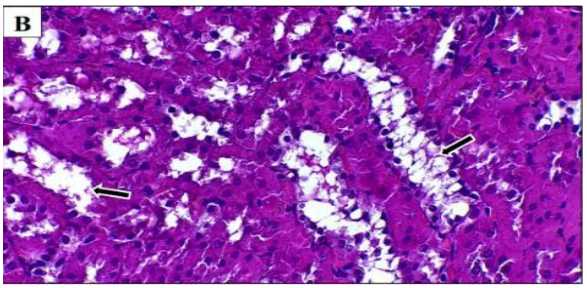


Figure (4-9): Histological section of the renal cortex from rats treated with both methionine and berberine (G3): A and B show focal degeneration of epithelial cells in the convoluted renal tubules (black arrow) in a limited number of tubules. However, no cystic lesions were observed, in contrast to the methionine-treated group, which exhibited prominent cystic formations. Stain: H&E. Magnification: A – 100x, B – 400x.

In addition, the methionine and berberine co-treated group (G3) showed the loops of Henle as thin-walled structures composed of delicate basement membranes. In many sections, the epithelial cells of the affected tubules were absent or barely visible, indicating epithelial cell atrophy or erosion. However, this lesion appeared only locally and involved relatively smaller areas of the medulla compared with the methionine-treated group, which exhibited more widespread medullary injury, as shown in Figure (4-10) A and B.

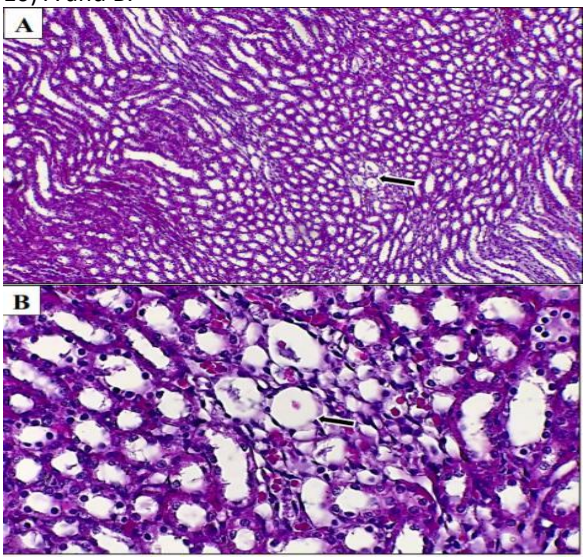


Figure (4-10): Histological section of the renal medulla from rats in the group treated with both methionine and berberine (G3): A and B show the loops of Henle as thin-walled structures (black arrow) composed of delicate basement membranes. In many sections, the epithelial cells of the affected tubules were absent or

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barely visible, indicating epithelial cell atrophy or erosion. However, this lesion appeared only locally and involved relatively smaller areas of the medulla compared with the methionine-treated group, which exhibited more extensive medullary injury. Stain: H&E. Magnification: A – 100x, B – 400x.

As for the berberine-only group (G4), the histological sections showed that the renal cortex maintained a normal structural and histological organization. The glomeruli were observed to be surrounded by both proximal and distal convoluted tubules, as shown in Figure (4-11) A and B.

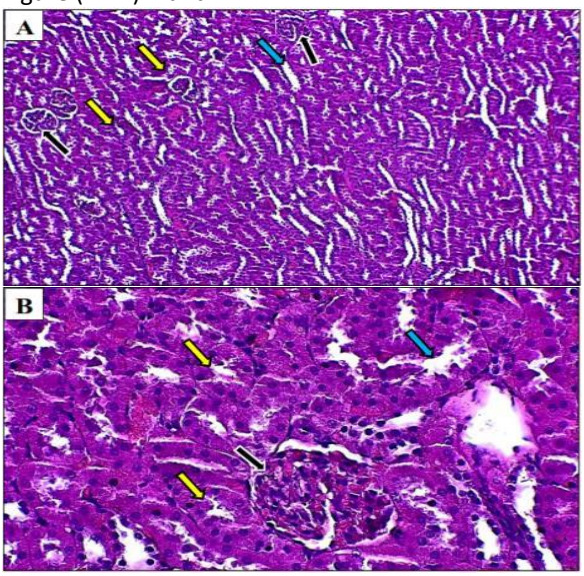
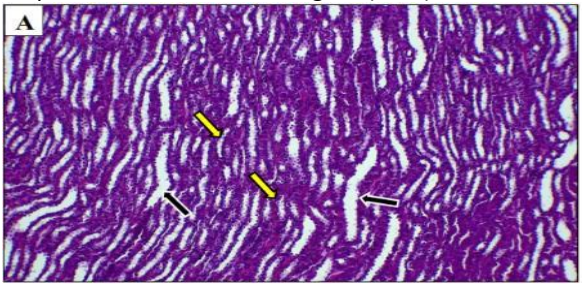


Figure (4-11): Histological section of the renal cortex from rats in the berberine-treated group (G4): A and B show the normal histological structure. The glomerulus (black arrow) is surrounded by proximal convoluted tubules (yellow arrow) and distal convoluted tubules (blue arrow). Stain: H&E. Magnification: A – 100x, B – 400x.

The histological sections of the renal medulla revealed a normal histological architecture, showing the presence of thin descending and ascending limbs as well as thick descending and ascending limbs of the loop of Henle, as shown in Figure (4-12) A and B.



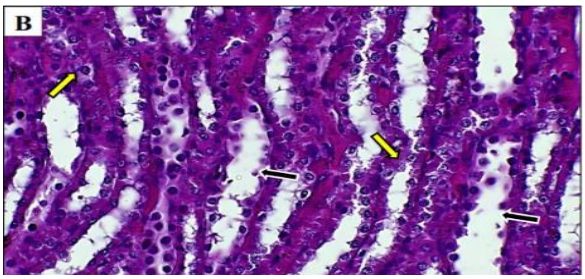


Figure (4-12): Histological section of the renal medulla from rats in the berberine-treated group (G4): A and B show the normal histological architecture of the medulla, with clearly visible thin descending or ascending limbs (black arrow) and thick descending and ascending limbs (yellow arrow) of the loop of Henle. Stain: H&E. Magnification: A – 100x, B – 400x.

Discussion

The histological results were obviously different between the four groups including control, methionine only, methionine plus berberine and berberine only. Hepatic and renal pathologic changes Clear normal hepatic and renal architecture were observed in the control group and berberinum groups, however obvious pathological lesions were observed in the methionine group, with marked macrovesicular steatosis, fibrosis of both liver (Fig. 1A-C) and cystic change of kidney (Fig. 2A-D). Notably, combined use of berberine and methionine prevented most structural damage, demonstrating its potential to attenuate methionine-induced injury.

Histological Changes in the Liver

The main hepatic changes observed due to the methionine supplementation in this study were steatosis, vacuolar degeneration and hepatic fibrosis. They are in line of our finding, in (18), they showed that disturbances of methionine metabolism results the disturbance between S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) and caused methylation disequilibrium and oxidative stress. This imbalance leads to increased lipid accumulation and hepatocyte injury, hallmark features of methionine-induced liver injury.

The occasional sinusoidal congestion and focal RBC extravasation in the methionine group also indicate vascular injury and inflammation. (19) observed that methionine- and ethanol-induced metabolic changes may contribute to oxidative stress, further facilitating inflammatory infiltration and hepatocellular degeneration.

In comparison, the liver structure of rats treated with berberine alone was intact. Protective effects of

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berberine on liver can be due to good control over lipid and glucose metabolism, improved antioxidant capacity, and decreasing inflammation. (20) demonstrated that berberine was able to modulate lipid profiles, glycemic control as well as to decrease oxidative stress, which consequently prevent hepatic steatosis and fibrosis. Likewise, berberine ameliorates liver inflammation and fibrosis by modulating gut microbiota composition (which increases Akkermansia muciniphila that reinforces the intestinal barrier and decreases endotoxin translocation) as also reported in here (21).

In this study, the combination of berberine and methionine led to mild hepatic alterations characterized by little fibrosis and focal steatosis. These results are consistent with those of (22) who proved berberine ameliorates insulin resistance and fat accumulation, protecting the whole body from metabolic disturbances. In addition, berberine formulations were reported to greatly reduce collagen deposition and improve hepatocellular structure in the models of chemically induced fibrosis (20), indicating that berberine might have anti-fibrotic effects.

Histological Changes in the Kidney

In the present study, methionine exposure led to severe renal lesions like cystic dilatation with atrophy of epithelium and deposition of collagen in cortical region and degeneration of tubules in medullar region. These results are suggestive of tubular dysfunction and interstitial fibrosis. (23) obtained similar results and observed damage in the renal methylation status, that leads to nephropathy due to oxidative stress from altered gene expression.

In contrast, berberine-treated rats exhibited normal renal structure signifying its renoprotective effect. (24) confirmed that berberine plays a protective role in kidney diseases by regulating lipid metabolism, alleviating inflammatory response and ameliorating cell function. Moreover, it was reported (25) that berberine high content extracts relieved the biochemical indices of renal and hepatic damage, suggesting the preservation of tissue architecture.

In the methionine+berberine-treated group, only focal tubular degeneration without cystic lesions was presented compared to widespreadcystic pathology that found in the methionine-only groups. This is consistent with those of (26,27), claiming that berberine represses NF-κB–mediated inflammation and conserves renal tissue from diabetic and metabolic distress. Likewise, (27) reported that berberine alleviates ischemia–reperfusion liver injury by inhibiting HMGB1/TLR4/NF-κB signaling, which could be the reason for a reduction in the observed renal injury in the combined group.

Altogether, the current findings confirm that methionine leads to hepatic and renal injury through pathways implicated in oxidative stress, methylation deficit and inflammatory signaling. In contrast, berberine has various protective mechanisms such as regulating lipid/glucose metabolism, reducing inflammation mediators level and improving the antioxidant ability of organism through different pathways including alteration of gut microbiota. These versatile actions offer an explanation of preserved liver and kidney architecture in berberine-treated groups.

Conclusion

The results from this study also show that high intake of methionine leads to liver and kidney lesions such as fatty degeneration, fibrosis and tubular cystic lesion. "However, the preservation of normal tissue architecture and reduction in extent of damage were significant when berberine was co-administered with methionine." Based on these results, it was proposed that berberine plays a protective role as an antioxidant, anti-inflammatory and metabolic regulator and should be recommended as an effective therapeutic drug in ameliorating methionine-induced hepatic and renal damage with scopes to conduct clinical trials.

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Conflict of interest

There is no conflict of interest in this study as stated by the authors.

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