Ameliorative Effect of Spirulina Against Gentamicin Toxicity in Liver and kidney Tissues of Male Rat

Original Article

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ABSTRACT

Introduction: Natural extracts are a suitable alternative in reducing the harmful effects of drugs and chemicals.
Aim of the Work: evaluated the role of the spirulina in reducing pathological tissue lesions in the liver and kidneys of gentamicin-treated rats, as well as the relationship between these effects and antioxidant levels and other biochemical factors.
Materials and Methods: In this study, 24 rats were evenly distributed into 4 groups, each group comprising 6 rats. Group 1 cocedered control, Group 2 administered 1000 mg/kg of spirulina, Group 3 was administered 100 mg/kg of gentamicin I M. Group 4 was subjected to the same dosages and treatment methods as G2 and G3, with a 5-day course of Gentamicin and a 12-day course of spirulina.
Results: Gentamicin group recorded significantly increase in Alanine aminotransferase, Aspartate transaminase, Creatinine, and Urea concentrations. The group given spirulina following gentamicin treatment had lower levels of liver and kidney enzymes than the group given gentamicin alone and there was also an increase in the glutathione, as well as a drop in malondialdehyde levels. In the histopathological analysis, the Gentamicin-treated group exhibited coagulative necrosis also vacuolar degeneration in liver cells, along with central vein congestion. In the kidneys, this group displayed glomeruli atrophy, dilatation of the Bowman's space, the presence of hyaline casts in tubules of renal, with coagulative necrosis of epithelial cells lining renal tubule, when Gentamicin combined with Spirulina, the liver section revealed intact hepatocytes. In the kidney section of the same group, glomeruli remained intact, with only mild cell swelling observed in the epithelial cells lining the tubules of renal.
Conclusion: spirulina has beneficial effects in the liver and kidneys of rats given gentamicin.

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Key Words: Gentamycin, kidney, liver, rat, spirulina.

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INTRODUCTION

Spirulina is a one source of protein, vitamin, amino acids, mineral, essential fatty acid, antioxidants, and more. Its versatile applications encompass human and animal nutrition, serving as both food and a dietary supplement. This remarkable alga has a history of traditional consumption spanning centuries and has even found use in cosmetics. Furthermore, in both in vitro and in vivo studies, Spirulina has efficacy in addressing various health concerns, including certain types of cancer, anemia,, viral infections, vascular diseases, radiation prevention[1,2,3].

Since the 1980s, spirulina has been employed in healthy food, fodder, and biochemical products. Spirulina is, in fact, has no side effects and is naturally nontoxic[4,5].

Antibiotics known as aminoglycosides are a type of antibiotic that is used to treat serious illnesses all over the world. However, its side effects, such as ototoxicity, nephrotoxicity, neuromuscular inhibition, and allergic skin reactions, remain a point of contention[6,7,8].

Gentamicin, an antibiotic in this class, is more toxic to the kidneys than other antibiotics in this class. Increased creatinine and urea, and proximal acute renal necrosis, are all symptoms of gentamicin-induced nephrotoxicity[9,10]. Although the specific mechanism of gentamicin-induced renal damage is unknown, free radical has a role in kidney injury progression[11,12].

Protein synthesis and enzyme processing, such as ALP, AST, and ALT release, are two of the liver's most important tasks. Leakaging of liver enzymes into blood occurs due to liver damge[13,14]. Previous research has shown that gentamicin administration increased activity of the liver enzymes ALT, AST, and ALP in serum when compared to a control. Higher hepatic cell membrane fluidity was linked to increased liver enzyme levels, resulting in enzyme release into the bloodstream[15,16].

Spirulina platensis, a type of bluegreen alga, is commonly employed as a protein and vitamin supplement[17]. It is rich in proteins (60-70%), vitamins (4%), minerals, amino acids, essential fatty acids, and antioxidants[18].

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The aim of our study was to see if spirulina might protect rats from the sub-acute toxicity of gentamicin, as well as the effect of gentamicin on the liver and kidney tissue, as well as the relationship between biochemical and oxidative stress indicators.

MATERIALS AND METHODS

Medicines

In this investigation, gentamicin ampoule from the (DMS CHEMICAL PHARMACEUTICAL INC LIMITED) company used a dose of 100 mg\kg. Spirulina, sourced from DXN Company, was used in the study. Dosages prepared by the dissolving 1000 mg per kilogram of body weight in 2 ml per kilogram of distilled water. These dosages were administered using an oral dosing syringe with a Gavage needle.

Experience design

Animals

This investigation involved a total of 24 male rats a weight range of 150-200 grams. These rats divided into four groups, of 6 animals.

With the treatment lasting five days for Gentamicin and 12 days for spirulina. The groups were divided into the following categories:

- G1:Control group given distal water only.
- G2:Spirulina group 1000 mg/kg oral dosage[19].
- G3:Gentamicin group intramuscular injection, 100 mg/kg[20].
- G4: Identical doses and methods of administration were used in the gentamicin and spirulina groups, spirulina first, then gentamicin.

Ethical approval

All the methodologies employed in this experiment received approval from University of Mosul's College of Vet- Medicine. Animal Ethics Committee under reference UM.VET.2021.35 (Iraq).

Biochemical measurements

At the finish of the experiment, rats were anesthetized using ether. Sampls of blood then obtained from retroorbital venous plexus and subsequently centrifuged for 15 minutes at 3000 rpm in non-heparinized tubes. The resulting serum collected then stored at -20°C until measurements of alanine aminotransferase, aspartate aminotransaminase, urea, and creatinine were performed. The Alman method was employed for the assessment of glutathione levels, while malondialdehyde levels, indicating oxidative stress, were determined using a specific method[21,22].

Histological preparations

The kidney and liver specimens were surgically removed and meticulously cleansed with water. They were subsequently preserved in 10% formalin until histological preparations were conducted. Standard protocols were followed, which involved cutting 4 mm thick paraffin sections of each tissue, followed by staining with hematoxylin stain and eosin. slides were then examining using a light-microscope to discern any pathological alterations.

Statistical analysis

Data were assessed for mean and standard error using SPSS software for statistical-analysis. A one-way analysis of variance test was then used, followed by the LSD test, with a significance at p less than 0.05.

RESULT

Biochemical measurements

Findings of biochemical tests examining the levels of liver and kidney function enzymes revealed that the ALT and AST concentrations in the group of gentamicin were significantly change from control and the other groups. The group given spirulina following gentamicin treatment had lower levels of liver enzymes than the group given gentamicin alone (Table 1).

The group OF gentamicin alone, recorded a significant difference observed in the creatinine and urea levels, signifying a substantial increase in their concentrations.

When gentamicin was combined with spirulina, the levels of creatinine and urea were significantly lower than when gentamicin was used alone (Table 1).

Spirulina group had a much higher significant level of glutathione than the other groups, according to the findings. In comparison to the other groups, the glutathione concentration in gentamicin group that significantly lower. (Table 2).

Malondialdehyde in the gentamicin group was higher when compared to the control and the rest groups (Table 2).

Result of histopathological investigation

The histopathological examination of liver and kidney tissues from different experimental groups revealed distinctive architectural features. In the control group (G1), the liver exhibited a typical configuration characterized by well-preserved hepatocytes, sinusoidal structures, and central veins (Figures 1,2). Likewise, the kidney cortex displayed the customary arrangement of the glomeruli, also the proximal renal tubules, and lastly the distal renal tubules (Figures 3,4).

In contrast, the liver sections from the Spirulina-treated group (G2) revealed a normal hepatic architecture, featuring intact hepatocytes, central veins, and portal areas (Figures 5,6). Furthermore, the kidney cortex in this group exhibited an unaltered configuration of the glomeruli, also the proximal renal tubules, and the distal renal tubules (Figures 7,8).

Conversely, the liver sections from the Gentamycin-treated group (G3) displayed pronounced pathological changes, characterized by coagulative necrosis marked by eosinophilic cytoplasm and the absence of nuclei, accompanied by vacuolar degeneration of hepatocytes surrounding central veins and central vein congestion (Figures 9,10,11). The kidney in this group exhibited an atrophy of the glomeruli, and dilatation of Bowman's space, hyaline casts within the renal tubules, and coagulative necrosis of the epithelial cells that lining the renal tubules, also infiltration of the inflammatory cell (Figures 12,13,14).

In the group treated with both Gentamycin and Spirulina (G4), the liver sections displayed intact hepatocytes and portal areas with central vein congestion (Figures 15,16). Similarly, the kidney sections in this group exhibited intact glomeruli with mild swelling of the epithelial cells that lining renal tubules (Figures 17,18). These findings suggest that Spirulina treatment appears to mitigate the adverse effects induced by Gentamycin in both liver and kidney tissues, preserving the structural integrity observed in the control group.

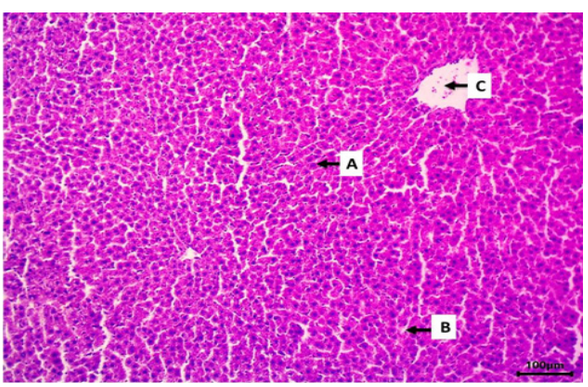

Fig. 1: Histological image of a rat liver from the Control group, displaying a typical architectural arrangement characterized by hepatocytes (labeled as A), sinusoids (labeled as B), and the central vein (labeled as C). (H&E staining), (scale bar 100µm).

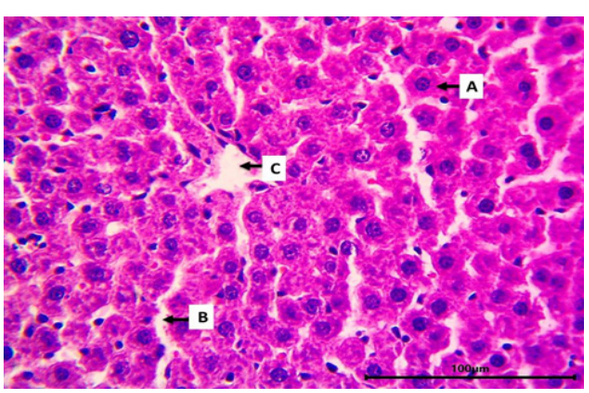

Fig. 2: Histological image of the rat liver within the Control group, illustrating the typical structural elements of hepatocytes (labeled as A), sinusoids (labeled as B), and the central vein (labeled as C). (H&E staining), (scale bar 100µm).

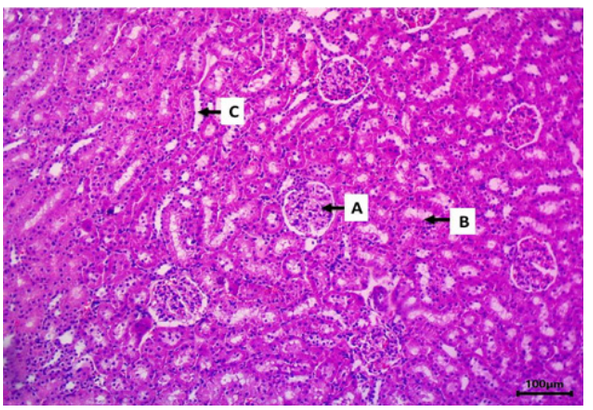

Fig. 3: Histological image of the rat kidney from the Control, showcasing the typical structural arrangement: glomeruli (labeled as A), proximal renal tubules (labeled as B), and distal renal tubules (labeled as C). (H&E staining), (scale bar 100µm).

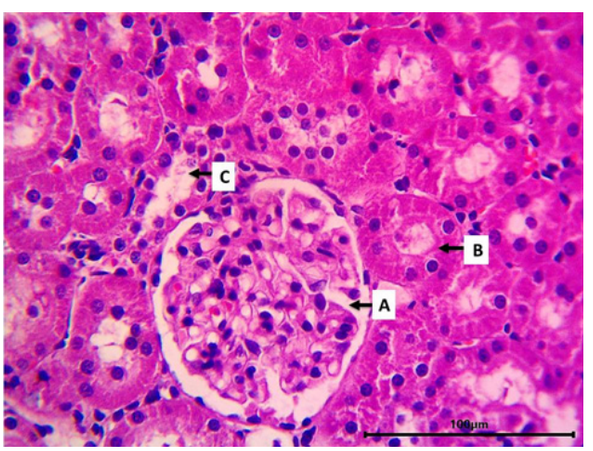

Fig. 4: Histological image of the rat kidney within the Control, revealing the customary structural configuration : glomeruli (labeled as A), the proximal renal tubule (labeled as B), and the distal renal tubule (labeled as C). (H&E staining), (scale bar 100µm).

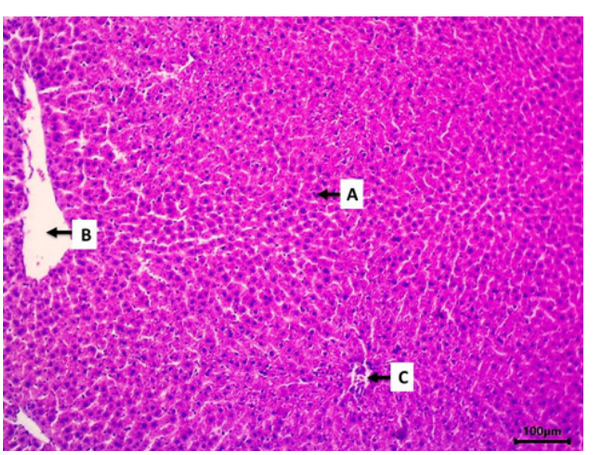

Fig. 5: Histological image of the rat liver in the Spirulina-treated group, showcasing a preserved architectural structure characterized by hepatocytes (labeled as A), central vein (labeled as B), and portal area (labeled as C). (H&E staining), (scale bar 100µm).

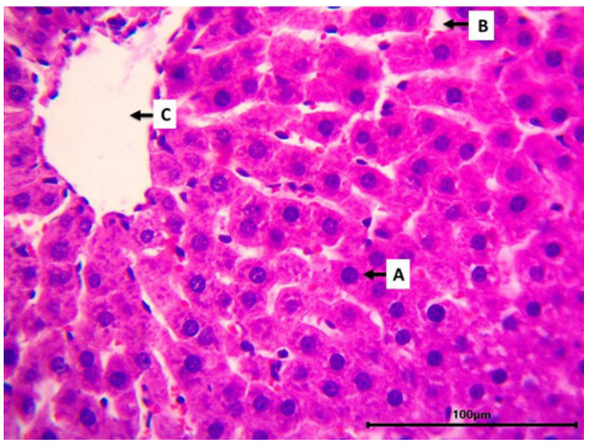

Fig. 6: Histological image of the rat liver in the Spirulina-treated group, highlighting the conserved structural arrangement characterized by hepatocytes (labeled as A), sinusoids (labeled as B), and the central vein (labeled as C). (H&E staining), (scale bar 100µm).

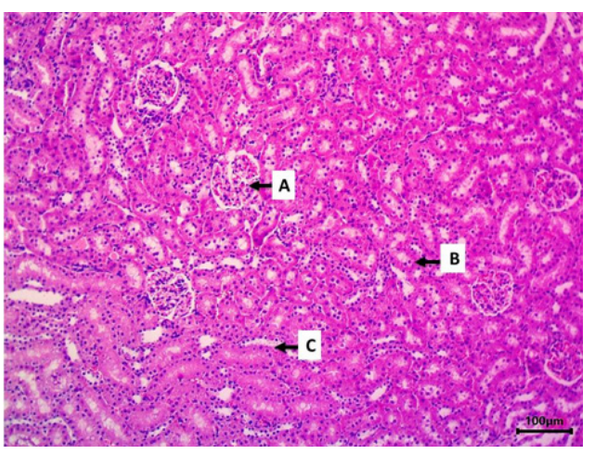

Fig. 7: Histological image of the rat kidney in the Spirulina-treated group, demonstrating the well-preserved structural configuration: glomeruli (labeled as A), the proximal renal tubule (labeled as B), the distal renal tubule (labeled as C). (H&E staining), (scale bar 100µm).

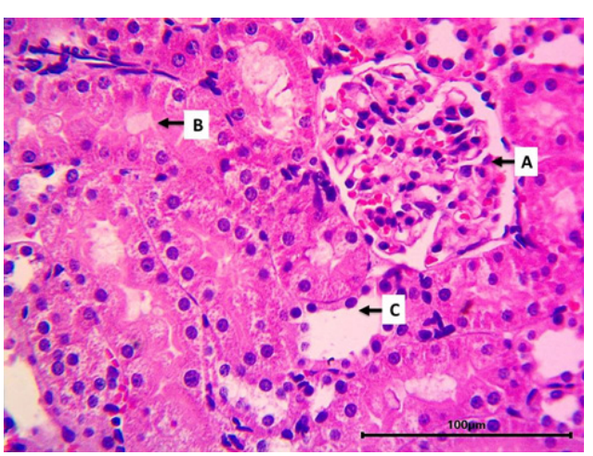

Fig. 8: Histological image of the rat kidney within the Spirulina-treated group, highlighting the preserved structural organization: (labeled as A), the proximal renal tubule (labeled as B), the distal renal tubules (labeled as C). (H&E staining), (scale bar 100µm).

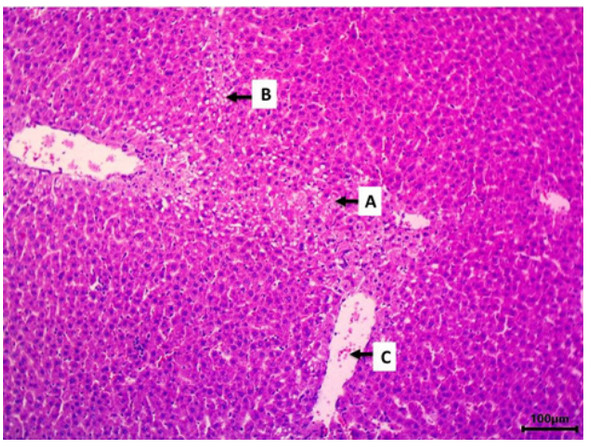

Fig. 9: Histological image of the rat liver in the Gentamycin-treated group, revealing coagulative necrosis (labeled as A) and vacuolar degeneration (labeled as B) in hepatocytes surrounding the central vein, alongside congestion of the central vein (labeled as C). (H&E staining), (scale bar 100µm).

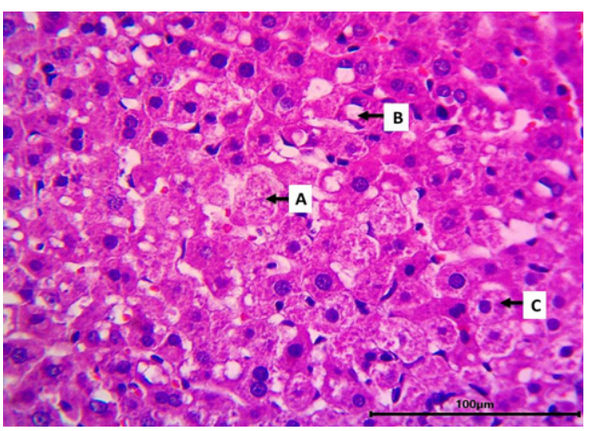

Fig. 10: Histological image of the rat liver in the Gentamycin-treated group, highlighting oncotic coagulative necrosis (labeled as A), vacuolar degeneration (labeled as B), and cloudy swelling (labeled as C) of hepatocytes surrounding the central vein. (H&E staining), (scale bar 100µm).

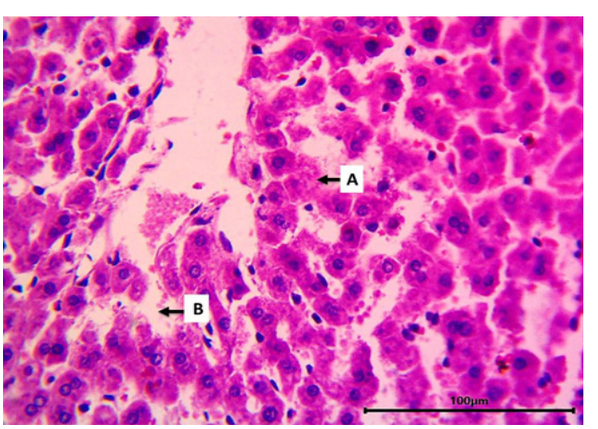

Fig. 11: Histological image of the rat liver in the Gentamycin-treated group, featuring coagulative necrosis of hepatocytes surrounding the central vein (labeled as A) and sinusoidal dilatation (labeled as B). (H&E staining), (scale bar 100µm).

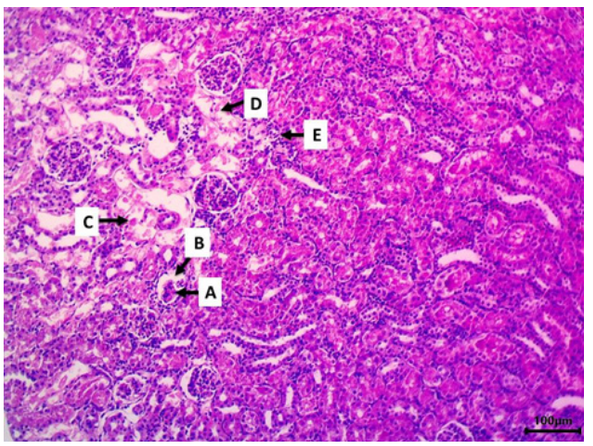

Fig. 12: Histological image of a rat kidney from a Gentamicin-treated include A) glomerular atrophy, B) Bowman's space dilation, C) the presence of hyaline casts in the renal tubules, D) the coagulative necrosis affecting epithelial cells lining renal tubule, and E) and infiltration of inflammatory cells. (H&E staining), (scale bar 100µm).

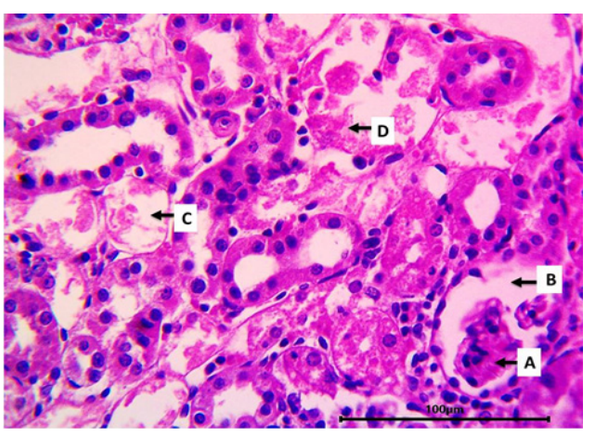

Fig. 13: Histological image of a rat kidney from a Gentamicin-treated group is presented, revealing notable pathological changes, including A) glomerular atrophy, B) Bowman's space dilation, C) the loss of renal tubules, and D) and coagulative necrosis affecting epithelial cells lining the renal tubule. (H&E) staining), (scale bar 100µm).

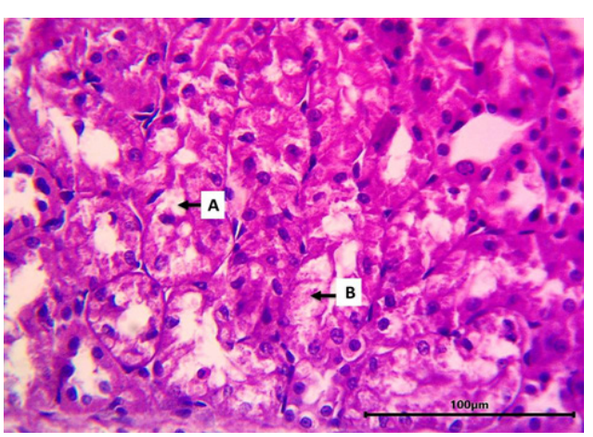

Fig. 14: Histological image of a rat kidney from a Gentamicin-treated group is presented, revealing A) coagulative necrosis and B) vacuolar degeneration of epithelial cell lining the renal tubule. (H&E staining), (scale bar 100µm).

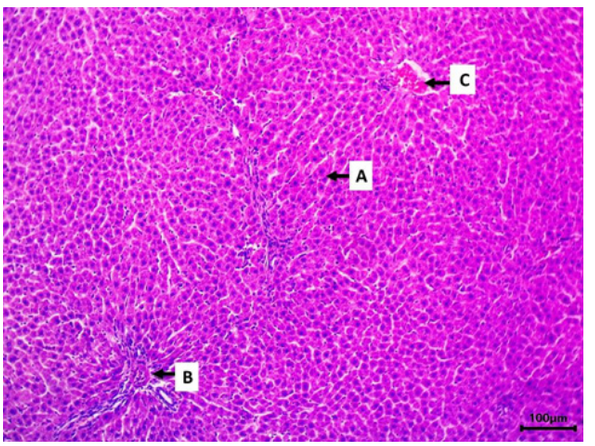

Fig. 15: Histological image of a rat liver from a group that received treatment with Gentamicin combined with Spirulina. The image exhibits healthy hepatocytes (A) and an intact portal area (B), accompanied by central vein congestion (C). (H&E staining), (scale bar 100µm).

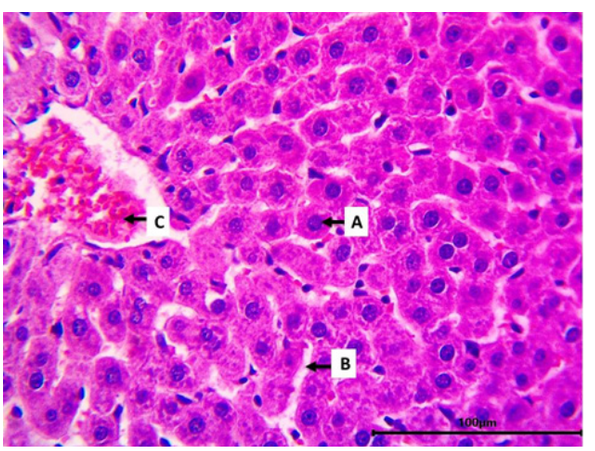

Fig. 16: Histological image of a rat liver from a group subjected to treatment with Gentamicin in combination with Spirulina. Within the image, healthy hepatocytes (labeled as A) and well-preserved sinusoids (marked as B) are observed, alongside central vein congestion (designated as C). (H&E staining), (scale bar 100µm).

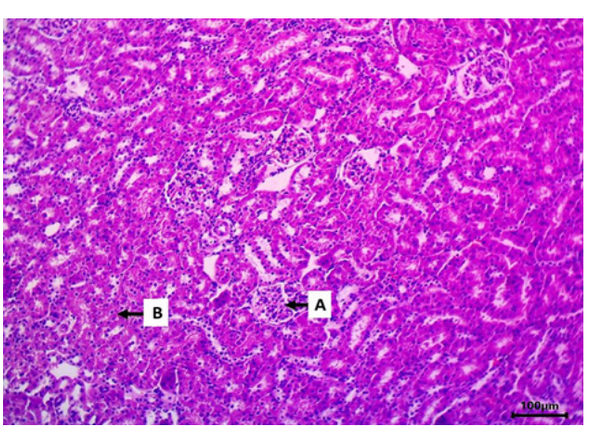

Fig. 17: Histological image of a rat kidney from a group treated with Gentamicin in combination with Spirulina. In this image, intact glomeruli (labeled as A) are observed, alongside mild cell swelling of epithelial cell lining renal tubule (indicated as B). (H&E staining), (scale bar 100µm).

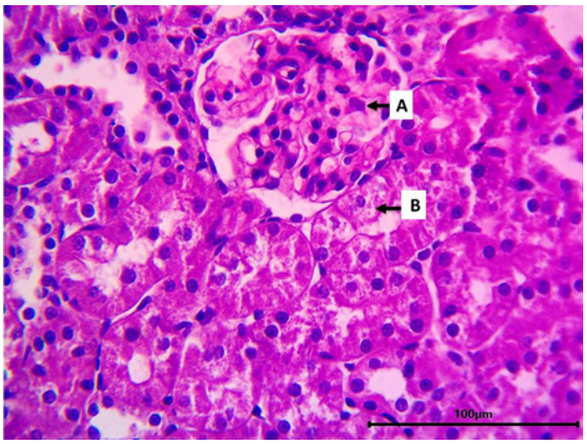

Fig. 18: Histological image of a rat kidney from a group treated with both Gentamicin and Spirulina revealing presence of intact glomeruli (labeled as A), along with a mild cloudy swelling of epithelial cell lining the renal tubule (identified as B). (H&E staining), (scale bar 100µm).

Table 1: Represents the level of some biochemical markers in serum of rats

Groups &Treatment	AL-Conc. IU/L	AST-Conc. IU/L	Creat- conc.mg/dl	Urea-Conc.mg/dl
Control	5.01 ±0.61	30.6 ±2.05	0.5 ±0.05	69.8 ±6.05
Gentamycin 100 mg/kg	13.2±0.5 *bc	47.7±6.05*bc	1.4±0.03* bc	106±9.03*bc
Spirulina 1000 mg/kg	6.04±0. 03	33.3±1.05	0.7±0.03	70.0±0.03
Gentamicin100mg/kg & Spirulina-1000 mg/kg	8.01±0.02	34.5±4.0	0.8±0.04	73.8±4.04

Each group consisted of six animals, and data as mean values with standard errors (mean ± SE). Notations were used to signify statistical significance: an asterisk (*) a significant difference compared to the control, 'b' indicated significance compared to Spirulina 1000mg/kg, and 'c' marked significant differences in groups treated with both Gentamicin and Spirulina.

Table 2: Represents oxidative stress markers in the rats of the experiment

Groups &Treatment	GSH Nmol\ml	MDA Nmol\ml
Control Group	0.01 ±0.005	4.6 ±1.05
Gentamycin 100mg/kg	0.005 ±0.001*bc	8.7±2.05* bc
Spirulina 1000mg/kg	0.04±0.003	4.9±1.05
Gentamicin100mg/kg & Spirulina1000mg/kg	0.01±0.002	4.5±1.0

Each group consisted of six animals, and data as mean values with standard errors (mean ± SE). Notations were used to signify statistical significance: an asterisk (*) represented a difference compared to control, 'b' indicated significance compared to Spirulina 1000mg/kg, and 'c' marked significant differences in groups treated with both Gentamicin and Spirulina.

DISCUSSION

Certain medications can induce oxidative stress by producing drug-derived radicals that deplete the body's antioxidant defenses and interact directly with biomolecules, potentially leading to adverse effects[23,24]. Gentamicins have side effects that are linked to oxidative stress. Long-term clinical use is limited stress in addition kidney and liver impairment[25,26].

The present of aminoglycosides in proximal convoluted renal tubule causes a variety of morphologies, including swelling, the appearance of vacuoles and necrosis in epithelial cells[27,28]. The accumulation of the drug in tubular cells and its interactions with membranes and other organelles lead to disruption of their functions with metabolic and functional changes[29,30].

Gentamicin promotes renal cortical phospholipid diseases and impairs the activities of many organelles[31,32].

The study confirmed that a 100 mg/kg dose of gentamicin resulted in moderate hepatotoxicity and renal toxicity, evident from elevated levels of AST, ALT, urea, and creatinine, along with observable hepatic and renal damage in histopathological examinations. These findings suggest that gentamicin induces oxidative stress, characterized by increased lipid peroxidation and reduced glutathione levels. Interestingly, when rats received spirulina, there was an increase in antioxidant levels, particularly glutathione, and a decrease in malondialdehyde levels.

The capacity of the biological and antioxidant characteristics of spirulina is due to its components; sulfur, polysaccharides, omega-3a, and unsaturated fatty acids, which repair the DNA damaging and it protect against

oxidative / nitrite stress enhancing the histopathological condition of the kidneys and liver[33,34]. Spirulina has the ability to lower interleukin levels in the blood, especially IL-8, and the tumor necrosis factor and inhibit the effects of the enzyme of nitric oxide synthase and enzyme of cyclooxygenase-2 also synthesis the pro-inflammatory cytokines[35,36]. Spirulina also promotes the growth of small intestinal bacteria and has probiotic effects, as well as the fact that sulfolipids inhibit the activity of DNA polymerase, inhibiting the production of superoxide anion[37]. All potentiate the protective action of the liver through this mechanism.

The findings of the curent study highlight the antioxidant, anti-inflammatory, immunomodulatory, and chemopreventive properties of spirulina, as underscored by numerous previous studies[38]. spirulina extract suppresses the induced apoptosis without affecting cell survival by significantly inhibiting the increase in free radical levels, decreasing cytochrome c release, increasing mitochondrial membrane potential, and decreasing overexpression of proapoptotic protein Bax[39,40].

CONCLUSION

Spirulina possesses antioxidant and anti-inflammatory capabilities, as well as other biological qualities, according to this study. These qualities have been used to counteract gentamicin's harmful effects on the liver and kidneys.

RECOMMENDATION

Spirulina's antioxidant activities can be utilized by combining it with some chemicals that cause oxidative stress since spirulina has beneficial effects in the hepatorenal toxicity of gentamicin.

ACKNOWLEDGMENT

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CONFLICT INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير المحسن للسبيرولينا في سمية الجينتامايسين في أنسجة الكبد والكلى في ذكور الجرذان

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مقدمة: تعد المستخلصات الطبيعية بديلا مناسبا في الحد من التأثيرات الضارة للادوية والمواد الكيميائية.
هدف البحث: هدفت هذه الدراسة تقييم دور السبيرولينا في الحد من الآفات النسجية المرضية في الكبد والكلى للجرذان المعاملة بالجنتاميسين والعلاقة بين هذه التأثيرات ومستويات مضادات الأكسدة والعوامل البيوكيميائية الأخرى.
مواد واساليب العلاج: قسمت ٢٤ جرذ الى اربع مجاميع كل مجموعة بها ٦ حيوانات. اعتبرت المجموعة الأولى مجموعة سيطرة، وتم إعطاء المجموعة الثانية ١٠٠ملغم/كغم من الجنتاميسين حقنا بالعضلة، والمجموعة الثالثة ١٠٠٠ ملغم/كغم من السبيرولينا، واعطيت المجموعة الرابعة كلا من الجنتامايسين والسيبرولينا وبنفس الجرع وطرق الإعطاء، استمر العلاج ٥ أيام للجنتامايسين.
النتائج: كانت تراكيز ناقلة الالنين امينALT وناقلة الالنين اسبارتيت AST والكرياتينين واليوريا في مجموعة الجنتاميسين أعلى بكثير مما كانت عليه في مجموعة السيطرة والمجاميع الأخرى. كان في المجموعة التي عولجت بالسبيرولينا بعد المعاملة بالجنتاميسين مستويات أقل من إنزيمات الكبد والكلى مقارنة بالمجموعة التي أعطيت الجنتاميسين وحده، كما كانت هناك زيادة في الجلوتاثيون، بالإضافة إلى انخفاض في مستويات مالونديالديهيد في المجموعة نفسها. أظهر الفحص النسجي المرضي لمجموعة الجنتامايسين نخرًا تجلطيا وتنكسًا فجويًا لخلايا الكبد المحيطة بالوريد المركزي واحتقان الوريد المركزي وفي الكلية ضمور الكبيبات وتوسع حيز بومان والقوالب الزجاجية في النبيبات الكلوية ونخر تجلطي للخلايا الظهارية المبطنة للنبيبات الكلوية. اظهرت مجموعة الجنتاميسين مع السبيرولينا خلايا كبدية سليمة مع احتقان في الوريد المركزي بالكبد، وكانت الكلى من نفس المجموعة الكبيبات الكلوية سليمة مع تورم خلوي طفيف للخلايا الظهارية المبطنة للنبيبات الكلوية.
الاستنتاج: السبيرولينا لها آثار مفيدة في كبد وكلية الجرذان البيضاء المعاملة بجرع عالية من الجنتاميسين