Effect of Garlic Oil on Sulfasalazine Induced Injury of Renal Tubules

Original Article

Fouad Sabry Heikal[1], Wafaa Abdel Rahman Ahmed[1], Hoda Mahmoud Khalifa[2], Rasha Mohamed Eldokmak[1] and El Sayed Aly Metwally[1]

[1]Department of Anatomy and Embryology, [2]Department of Histology and Cell Biology, Faculty of Medicine, Alexandria University

ABSTRACT

Introduction: Sulfasalazine is a disease modifying anti-rheumatic medication, used in the treatment of idiopathic arthritis. Kidney damage is one of its side effects.

Objective: To investigate the protective role of garlic oil against sulfasalazine-induced renal injury.

Materials and Methods: Thirty adult albino rats; with average weight of 150-200 gm were randomly divided into three groups. They received the medications by orogastric tube for 14 days.

Group I (control group): included 12 rats further subdivided into 2 equal groups: I a received 200 ml phosphate buffer saline (vehicle of drug). I b: received 100 mg/kg/day garlic oil. Group II (experimental group): included 18 rats, further subdivided into 3 equal groups II a (treated group): received sulfasalazine 600 mg/kg /day. II b (low garlic oil group): received sulfasalazine 600 mg/kg/day and 100 mg/kg/day of garlic oil. II c (high garlic oil group): received sulfasalazine 600 mg/kg/day and 200 mg/kg/day of garlic oil.

Results: Serum creatinine and BUN were significantly high in sulfasalazine treated group) II a(.Sulfasalazine caused wide range of proximal tubular damage; defective brush border, distorted outline, vacuolated lining cells and pyknotic nuclei. Ultrastructural changes were; perinuclear cisternal dilatation, mitochondrial abnormalities, disturbed lateral & basal infoldings and thickened basal lamina. In GII b (low garlic oil group) a significant level of protection was found in the form of restoration of normal proximal tubular structure while some tubules showed wide lumina with defective brush border and vacuolated lining cells. Ultrastructural changes were; limited tubular affection with degenerated mitochondria, partial loss of brush border. In group GII c (high garlic oil group) high degree of protection was found with few vacuolated cells and ultrastructural changes.

Conclusion: Sulfasalazine has marked renal tubular degenerative effects. Garlic oil has a dose-dependent protective effect.

Received: 07 October 2021, **Accepted:** 02 November 2021

Key Words: Garlic oil, renal tubules, serum creatinine, sulfasalazine.

Corresponding Author: Rasha Mohamed Mohamed Eldokmak, PhD, Department of Anatomy and Embryology, Faculty of Medicine, Alexandria University, Egypt, **Tel.**: +20 10 0043 2769, **E-mail:** drmahrash@Gmail.com

ISSN: 1110-0559, Vol. 46, No.1

INTRODUCTION

Sulfasalazine is a medication that is often used to treat rheumatoid arthritis and inflammatory bowel disease. 5-aminosalicylic acid and sulphapyridine make up sulphasalazine. The active chemical responsible for the drug's therapeutic success in ulcerative colitis is 5-aminosalicylic acid (mesalazine), with the sulphapyridine component (working simply as a carrier for 5-aminosalicylic acid) obviously being responsible for the bulk of side effects[1,2].

The bacterial azoreductase enzyme in the colon converts sulfasalazine to sulfapyridine and mesalazine. Around 10–30% of sulfasalazine is estimated to be absorbed unaltered into the systemic circulation. Sulfapyridine is fully absorbed. Only 30% of the mesalazine generated reaches circulation, with the rest being removed in faeces[3].

Although the exact mechanism of action is unknown, 5-aminosalicylic acid's anti-inflammatory actions appear to be linked to its effects on inflamed colonic mucosa. The therapeutic actions of 5-aminosalicylic acid may be explained in part by the inhibition of numerous mediators involved in the pathophysiology of the inflammatory response[1].

Sulfasalazine is disease-modifying antirheumatic drugs (DMARDs). In the treatment of juvenile idiopathic arthritis (JIA), it is utilized as a second-line therapy following nonsteroidal anti-inflammatory medications[4]

Sulfasalazine has frequent side effects as diarrhea, abdominal pain, nausea, vomiting, bloating, headache and allergic skin rash. In addition, it can result in kidney damage, which represents a critical side effect[5]

 DOI: 10.21608/ejh.2021.98665.1572

Although no exact mechanism for sulfasalazine-induced renal injury has been identified, some studies have shown that reactive metabolites, reactive oxygen species (ROS), and oxidative stress represent other sulfasalazine complications that may share in renal impairment[5,6]

Because oxidative stress is thought to be responsible for renal insults caused by sulfasalazine, antioxidant treatment could be a therapeutic option. As a result, substances with multifactorial protective effect, such as antioxidant capabilities, may have pharmacological benefit in the treatment of sulfasalazine-induced kidney injury[5,7]

Renal damage caused by sulfasalazine could be permanent. This includes glomerulonephritis and acute renal failure. Until today, there is no particular cure for kidney insults induced by sulfasalazine[8]

Garlic and its organo-sulfur components, primarily S-Allylcysteine and allicin, have been shown to have anticarcinogenic, antiatherosclerotic, antidiabetic, renoprotective, antioxidant, immunological modulation, antibacterial, antihypertensive, and a variety of other biological effects. Garlic's organo-sulfur compounds work as antioxidants via scavenging reactive oxygen species (ROS), boosting cellular antioxidant enzymes, and raising glutathione levels[9,10]

Garlic oil consists of the diallyl (57%), allyl methyl (37%) and dimethyl (6%) mono to hexasulphides. Garlic oil and its components help tissues to produce more endogenous antioxidants[11]

The biochemical and cellular effects of sulfasalazine on the kidney have been investigated extensively, but the subcellular effect has received little attention[5,7] So, the current study was designed to investigate the potential protective role of garlic oil supplementation against sulfasalazine-induced renal injury.

MATERIAL AND METHODS

Materials

Chemicals

Sulfasalazine and garlic oil were purchased from Sigma-Aldrich.

Experimental Animals and treatment

Thirty laboratory Swiss adult male albino rats were brought from the animal house of Medical Research Institute. Each was of average weight ranging from 150-200 gm and aged from 6 to 8 weeks. After approval of Ethics Committee, Faculty of Medicine, Alexandria University, the animals were maintained under standard temperature and humidity standard laboratory conditions in 12 hours light/dark cycle[12] After acclimatization for 2 weeks, the thirty rats were randomly subdivided into three groups as follows:

Group I (control group): It included twelve rats divide into 2 equal subgroups (each 6 rats):

- I a: The rats received 0.2 mg/ml phosphate buffer saline (PBS) (pH 7.2) (vehicle of drug) for 14 days[13]
- I b: The rats received 100 mg/kg of body weight garlic oil for 14 days by orogastric tube.

Group II (experimental group): divide into 3 equal subgroups (each 6 rats):

- II a (treated group): the rats 6 rats treated with 600 mg/Kg Sulfasalazine through orogastric tube for 14 days.[3,5,7]
- II b (low garlic oil group): The rats received sulfasalazine 600 mg/kg/day and Garlic oil at a concentration of 100 mg/kg using orogastric tube for 14 days.[14]
- II c (high garlic oil group): The rats received sulfasalazine 600 mg/kg/day and Garlic oil at a concentration of 200 mg/kg using orogastric tube for 14 days.[14]

Methods

1. Biochemical study: Blood urea and serum creatinine were done to assess kidney function from blood taken from the rats' tail.[7]
2. Histological study: The rats were sacrificed, and the kidney was excised, and specimens were divided into two portions and processed for the following:

Light microscopic study

For handling paraffin sections, a segment was fixed in formalin 10%. The mounted paraffin sections were inserted on 37°C oven on clean slides. The sections were deparaffinized in xylene then descending grades of alcohol to distilled water were used for rehydration. Staining with Hematoxyline, washing under tap water, staining with Eosin were executed. Then ascending grades of alcohol were used for dehydration and the sections were cleared in xylene. Light microscopic examination was performed at images obtained by using 20 & 40X Objective lens magnifications.[15]

Ultrastructural study

The other portion was cut immediately into small pieces (1x1mm3) and was subjected to the following procedures, fixation, washing, dehydration and filtration. The specimens were fixed in 3% glutaraldehyde, rinsed in buffer, fixed in osmium tetraoxide, dehydrated with graded ethanol, infiltrated and embedded in pre-labeled plastic capsules. The tissue was sectioned, stained with lead citrate and uranyl acetate, and examined by transmission electron microscope in Electron microscope unit, Faculty of science Alexandria University.[16]

Statistical analysis

IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) was used for data analysis. In order to

verify the normality of distribution quantitative data, the Kolmogorov-Smirnov test was used. Quantitative data were represented range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR) was done. The level of Significance of the collected data was evaluated at 5% level.

F-test (ANOVA) was used for normally distributed quantitative variables, to compare between more than two groups, while for pairwise comparisons Post Hoc test (Tukey) for was used.[17]

RESULTS

Biochemical results

Serum creatinine and blood urea nitrogen (BUN) levels were significantly increased in sulfasalazine treated group (G IIa) compared to the control group (GI a, b) and garlic treated groups (G II b & G II C), (Tables 1,2, Figure 1).

Histological results H&E stain

Control rats' renal cortices (G1a, b) showed the classic structure of proximal convoluted tubules with narrow lumina obliterated by a well-organized brush border. Tubules were lined with high cuboidal pyramidal cells with deeply stained eosinophilic cytoplasm and basal vesicular nuclei. Renal corpuscles and distal tubules were also visible. (Figure 2)

In the renal cortices of sulfasalazine-treated group (GII a), variable grades of tubular affection were found. Some proximal convoluted tubules had narrow lumina with a well-developed brush border and vesicular nuclei. Others showed degenerative changes such as wider lumina with a defective brush border and vacuolated pyramidal cells. Extruded cells and eosinophilic casts were found in some lumina. Others had distorted outline, ballooned lining cells with pyknotic nuclei. Some areas were filled with indistinct tubular structures with homogeneous hyper eosinophilic areas in between (Figure 3a). Congestion of blood capillaries and extravasated RBCs was occasionally observed. (Figures 3,4)

The kidneys of rats given sulfasalazine and a low dose of garlic oil (100 mg/kg) (GII b) showed a considerable degree of protection. Most of the proximal convoluted tubules showed classical histological structure. Some tubules, on the other hand, had wide lumina with a defective brush border and intraluminal degenerated cells. Some of the lining cells showed variable degree of vacuolation. (Figure 4).

The kidneys of rats given sulfasalazine and a high dose of garlic oil 200 mg/kg (GII c) showed a high degree of protection, with most proximal tubules having a nearly classical structure. Few tubules with slightly wider lumina, some slightly vacuolated cells were occasionally detected. (Figure 5)

Electron Microscopic results

Examination of control rats' renal cortical samples (GI a,b) revealed the classical structure of proximal convoluted tubules resting on thin regular basal lamina. Each tubule depicted a narrow central lumen and lined by pyramidal cells. Each cell revealed long closely packed microvilli representing the well-organized brush border. The apical cytoplasm showed numerous canaliculi and pinocytotic vesicles. An elaborate system of lateral and basal infoldings was noticed along the entire height of the cells. Many tubular mitochondria with lamellar cristae were seen within the basal folding. In contrast the distal tubule reflected electronlucent cytoplasm with less mitochondria, apical nuclei and few luminal microvilli (Figure 6).

Examination of the ultrastructure of renal cortices' samples of rats treated with sulfasalazine (GII a) revealed various degree of proximal tubular degenerative changes. Some proximal tubules revealed well organized luminal brush border. Its lining cells revealed large number of pleomorphic mitochondria, euchromatic nuclei, apical canaliculi and excess endocytotic vesicles. Some of these cells showed electronlucent cytoplasm, small mitochondria, and excess vacuoles. Other tubules showed disorganized lining cells with excessive vacuolation, irregular dark shrunken nuclei with perinuclear cisternal dilatation and intra luminal dark cells with pyknotic nuclei. Mitochondrial affections were variable; excess bizarre-shaped mitochondria, irregular arrangement, swollen mitochondria with widened cristae and electronlucent matrix, and others with disturbed cristae and intramitochondrial deposits. However considerable number of tubules showed severe degenerative effect. It showed necrotic lining cells as well as apoptotic cells. The necrotic cells appeared with defective brush border, dark cytoplasm, and shrunken dark irregular nuclei. Complete destruction of the lining cells and presence of intraluminal apoptotic bodies was also seen. Some of the tubules were totally destructed and revealing just apoptotic bodies. The intracellular spaces were dilated with affection of the lateral interdigitation of these cells. Many of proximal tubules showed irregularly thickened basal lamina. The distal convoluted tubules adjacent to these tubules were classical in their structure (Figures 7,8).

Ultrastructural examination of renal cortices of rats received sulfasalazine and low dose of garlic oil (GII b) revealed a considerable degree of protection. Many tubules appeared with nearly classical structure. However, they showed. mitochondrial random distribution &pleomorphism. Some swollen mitochondria with disintegrated cristae and dense intramitochondrial inclusions were seen. Partial or complete loss of tubular brush border and degenerated tubules were occasionally depicted. Few widened inter cellular spaces and disturbed lateral interdigitation were also encountered. However, intraluminal extruded cells, cellular debris and apoptotic bodies were still seen. (Figure 9).

Examination of renal cortices' samples of rats that received sulfasalazine and high dose garlic oil (GII c)

revealed nearly classical structure of most proximal convoluted tubules. However, few tubules showed widened basal infoldings pushing the mitochondria and the nuclei to a higher position. Many of these nuclei exhibited an irregular outline. Some degenerated tubules were rarely encountered. (Figure 10).

Histo-morphometric study

The ultrastructural mean values of the proximal tubules' basement membrane thickness of different groups were recorded. In sulfasalazine treated group (IIa) the thickness was increased significantly. Also there was significant reduction in the thickness in both garlic-treated groups (IIb&c) in comparison with sulfasalazine treated group (IIa). (Table 3, Figure 11)

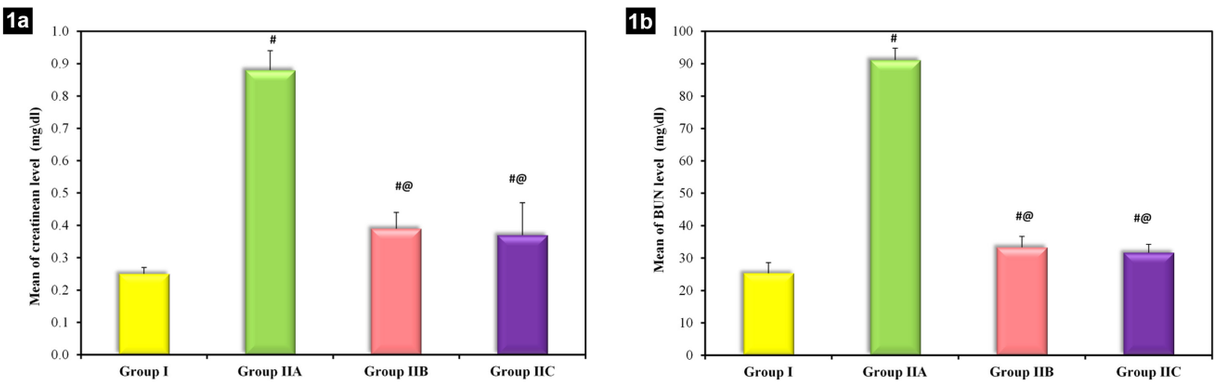

Fig. 1: Bar chart showing comparison between the different studied groups according to serum creatinine (A) and BUN (B) levels.

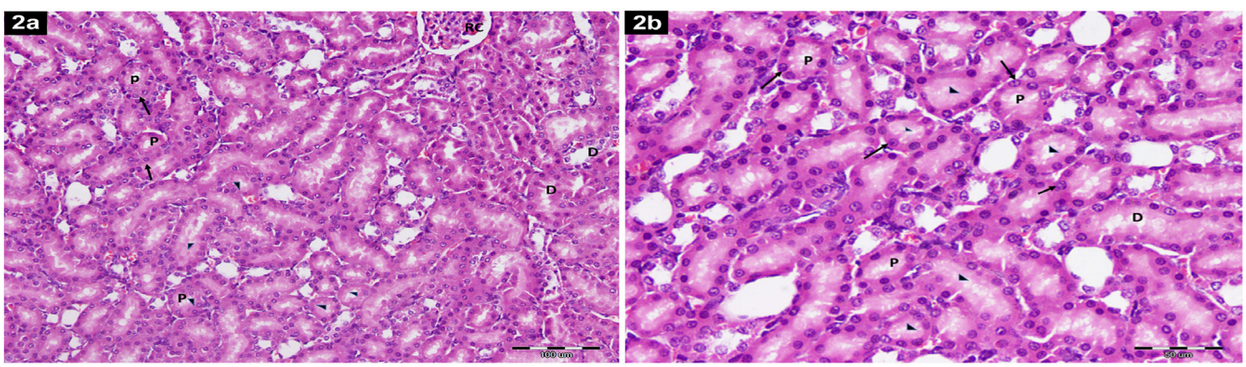

Fig. 2: Photomicrographs of renal cortices of control rats (GIa) showing classical appearance of renal cortex. Many proximal convoluted tubules (p) depicting narrow lumina occluded by well-organized brush border (▲).They are lined by pyramidal cells with deeply stained eosinophilic cytoplasm and basal vesicular nuclei (↑). Renal corpuscles (RC) and distal convoluted tubules (D) are also seen. (H&E stain Mic Mag A*200, B*400)

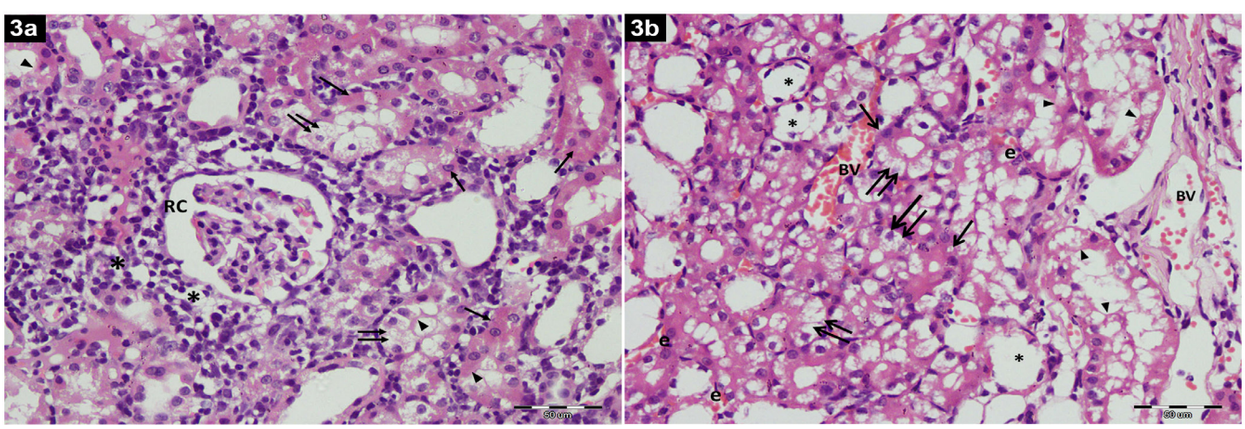

Fig. 3: Photomicrographs of renal cortices of (GII a) showing different grades of tubular affection with distorted architecture. Most tubules depict wide lumina with defective brush border (arrowhead). Some lining cells appear eosinophilic (↑) while most were pale, highly convoluted or ballooned with dark shrunken nuclei (↑↑). Completely degenerated tubules with ghost like outline are also seen (*). Congested blood vessels (BV) and extravasated RBCs (e) are present all around. RC; renal corpuscles. (H&E stain Mic Mag A*200, B*400)

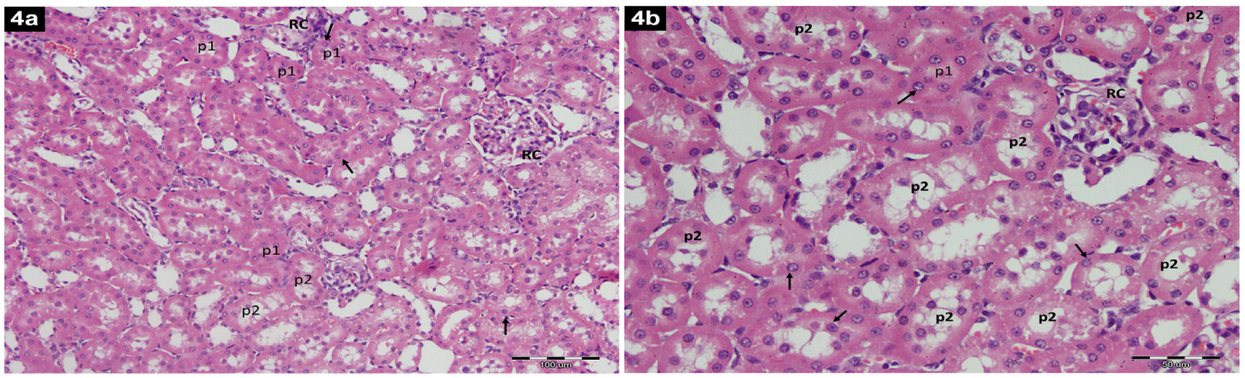

Fig. 4: Photomicrographs of renal cortices of rats received sulfasalazine and garlic oil (100 mg /kg) (GII b) showing mild affection of renal tubules. Many tubules show narrow lumina with preserved brush border and eosinophilic pyramidal cells (p1). Other depict wider lumina with defective brush border (p2) and intraluminal degenerated cells. Most of the lining cells show vesicular basal nuclei (↑). RC; renal corpuscles. (H&E stain Mic Mag A*200, B*400)

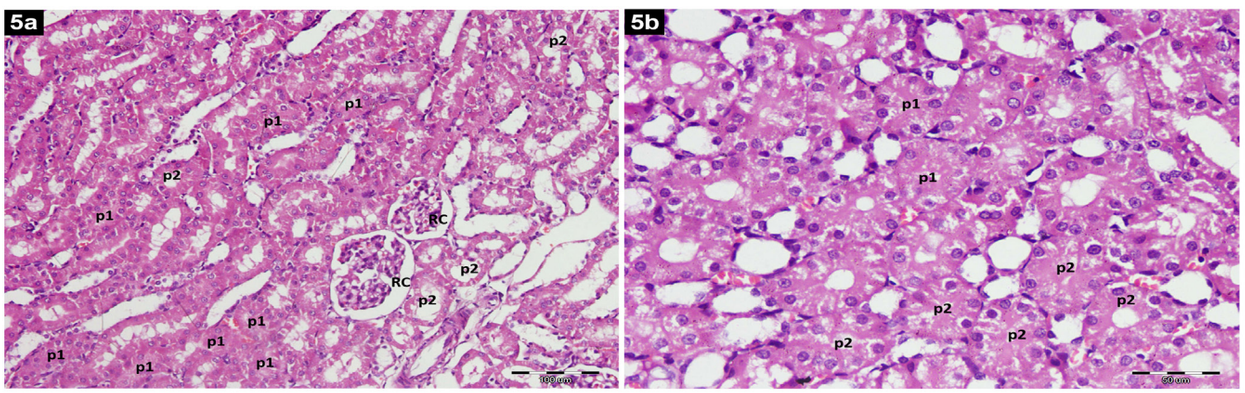

Fig. 5: Photomicrographs of kidneys of rats received sulfasalazine and high dose of garlic oil (200mg/kg) (GII c) revealing high degree of protection of renal cortices. Most of proximal convoluted tubules show narrow lumina with preserved brush border and eosinophilic pyramidal cells with vesicular nuclei (p1). Few tubules depict slightly vacuolated cells (p2) with wider lumina. RC; renal corpuscles.

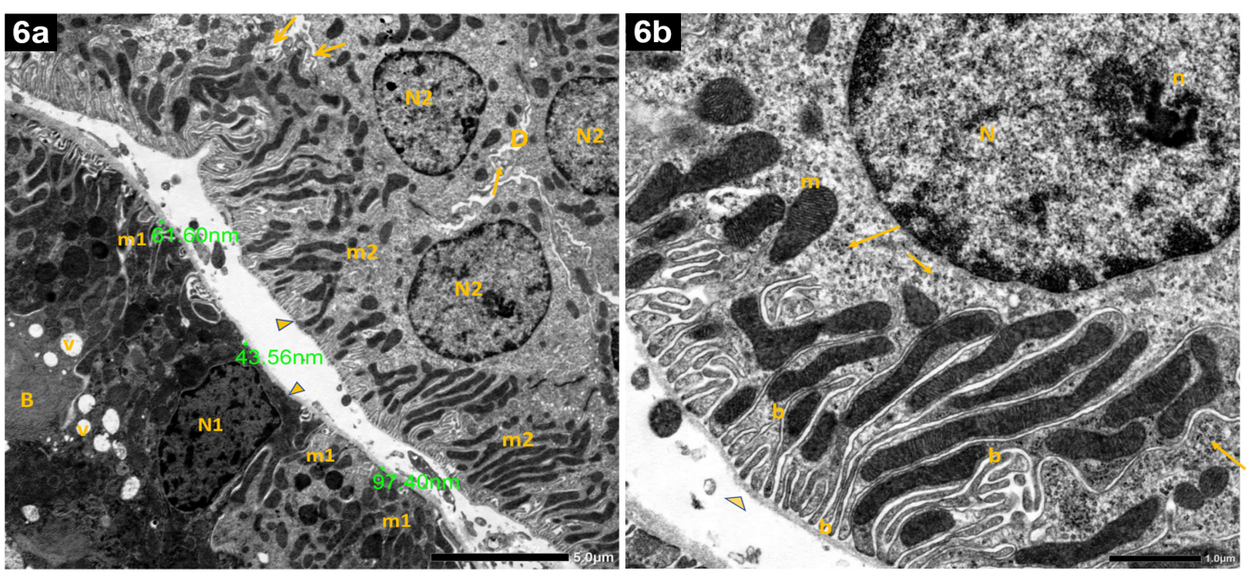

Fig. 6: An electron photomicrograph of convoluted tubules of control rat (GI a,b) showing: A: adjacent parts proximal and distal convoluted tubules. The proximal convoluted tubule rests on well-defined basal lamina (arrow head) with obliterated lumen and well-developed brush border (B). Many large endocytotic vesicles (v) are seen in the apical cytoplasm, basal mitochondria (m1) and basal nuclei (N1). The distal convoluted tubule (D) shows less basal infoldings, less mitochondria (m2) and central euchromatic nuclei (N2). Its lumen depicts few microvilli (arrow). B: proximal tubular cell with an euchromatic nucleus (N) with prominent nucleoli (n) resting on well-defined basal lamina. An elaborate system of basal infoldings (b) is noticed accommodating parallel tubular mitochondria (m) with lamellar cristae. Many polysomes are noticed scattered in cytoplasm (arrow). (Urinyl acetate lead citrate stain Mic Mag A* 1500, B* 5000)

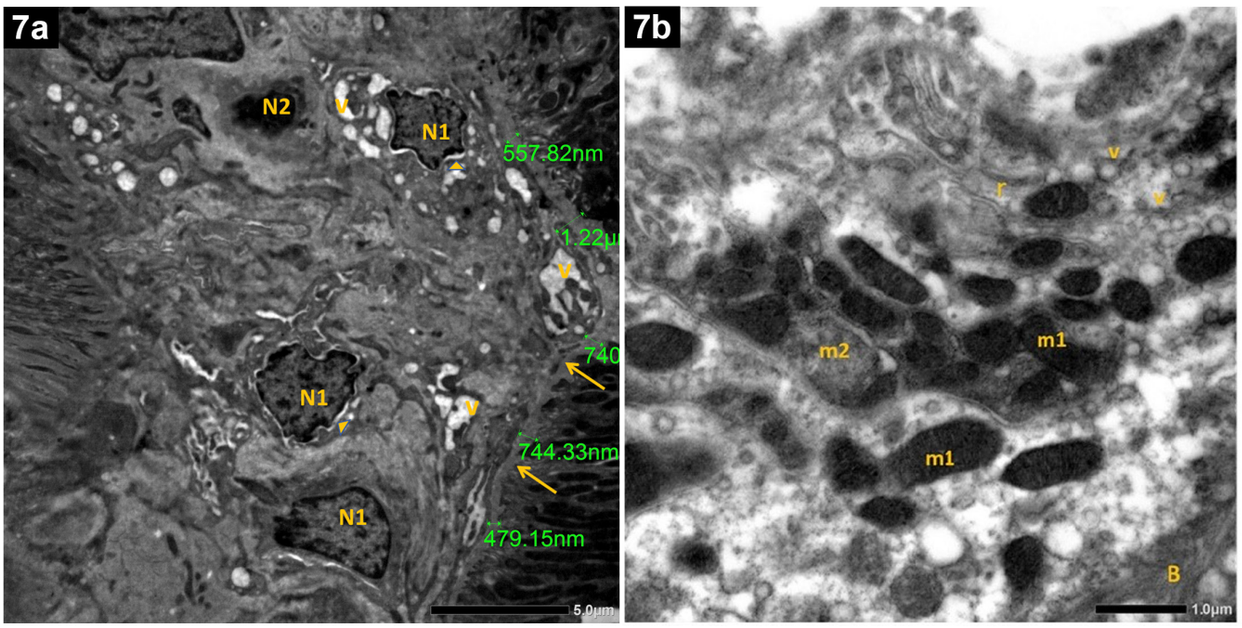

Fig. 7: An electron photomicrograph of a rat kidney (GII a) showing: A: parts of adjacent proximal convoluted tubules. One of them reveals disorganized lining cells with excessive vacuolation (v), irregular dark nuclei (N1) with perinuclear cisternal dilatation (arrowhead). Intraluminal extruded cells with shrunken dark small sized nucleus (N2) are also seen. Note the irregularly thickened basal lamina (↑). B: A high magnification of the apical part of a proximal tubular cell showing bizarre shaped mitochondria (m1) dispersed in electrolucent excessively vacuolated cytoplasm. Some of these mitochondria appear swollen with widened cristae (m2). Many pinocytotic vesicles (v) and rough endoplasmic reticulum tubules (r) are also seen. B; brush border. (Urinyl acetate lead citrate stain Mic Mag A * 1500, B * 5000)

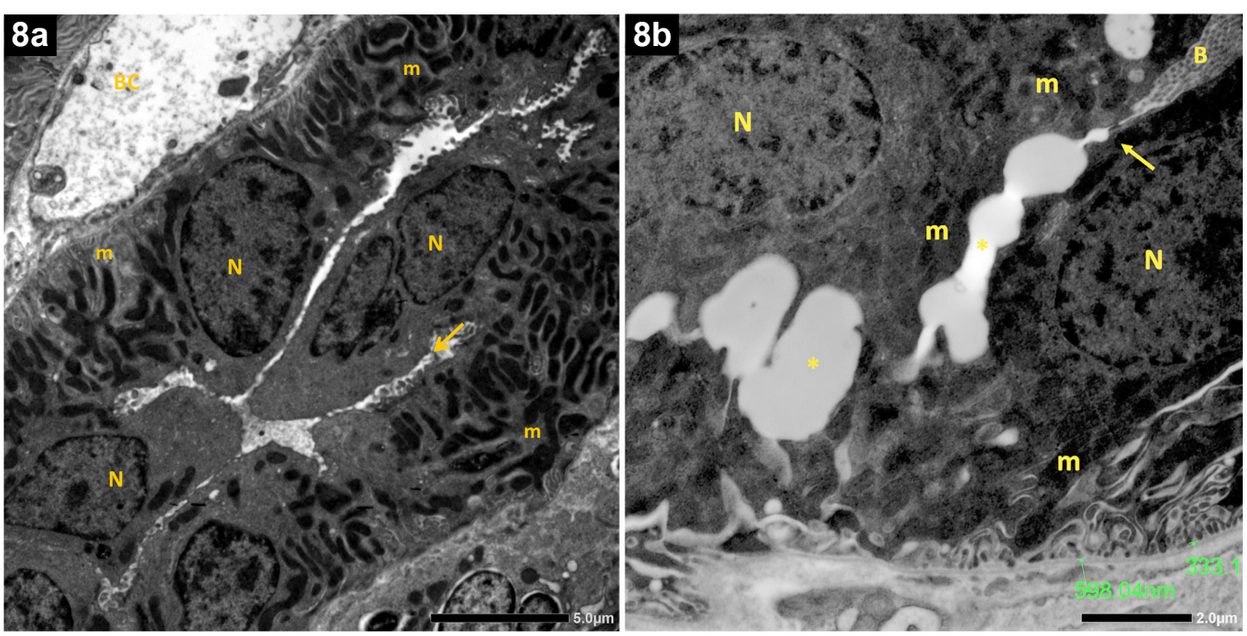

Fig. 8: An electron photomicrograph of a rat kidney (GII a) showing: A: a distal convoluted tubule lined by cuboidal cells with well-organized basal infoldings loaded by mitochondria (m). The nuclei (N) are central euchromatic and the luminal surface depicts few microvilli (↑). BC; blood capillary. B: High magnification showing proximal convoluted tubule with extensive dilatation of inter cellular spaces (*) and intact inter cellular junction (↑). N; nucleus, m; mitochondria, B; brush border. (Urinyl acetate lead citrate stain Mic Mag A * 1500, B * 3000)

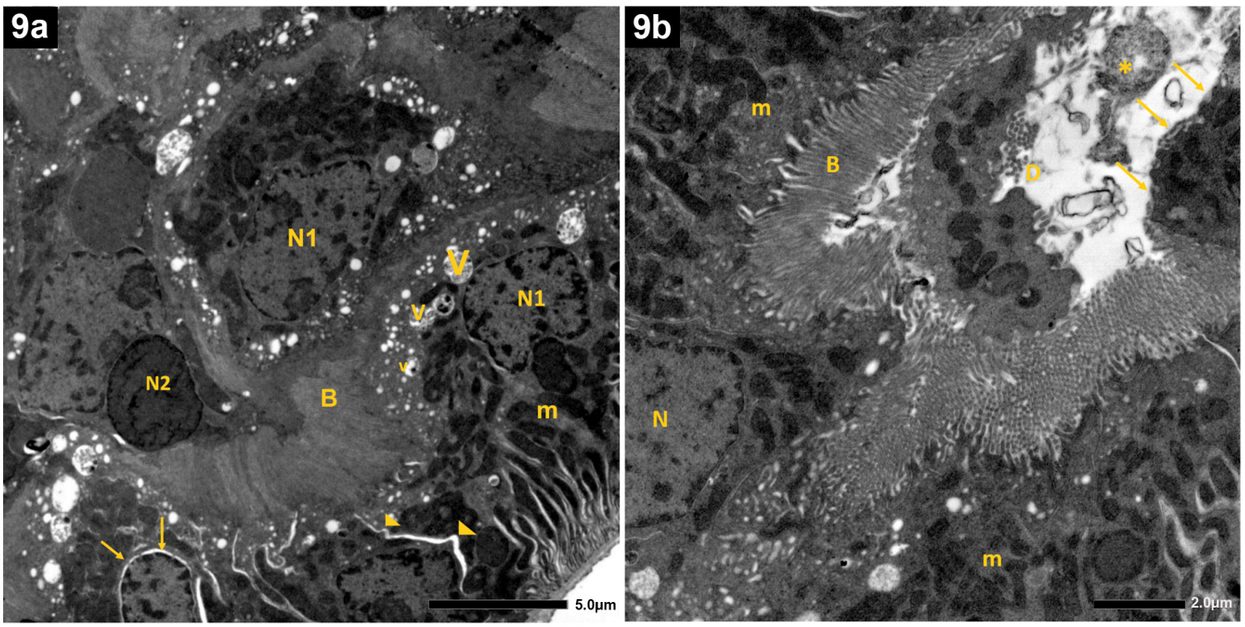

Fig. 9: An electron photomicrograph of a rat kidney (GII b) showing: A: a proximal convoluted tubule of rat showing disorganized tubular cells. Some cells are intact with many regularly oriented mitochondria (m) and euchromatic nuclei (N1). The apical cytoplasm shows excessive endocytotic vesicles (v) and vacuoles (V). Intact brush border (B) is evident. Few cells show dark irregular heterochromatic nuclei nearly to be extruded to the lumen (N2). Others show perinuclear cisternal dilatation (↑). Note; wide intercellular spaces (arrowhead) is also seen. B: showing a proximal convoluted tubule. Some of its lining cells reveal intact well-organized brush border (B), euchromatic nuclei (N) and a lot of mitochondria (m). Other cells show defective brush border (↑). Intraluminal degenerated cells (D) and cellular debris (*) are also seen. (Urinyl acetate lead citrate stain Mic Mag A * 1500, B * 2500)

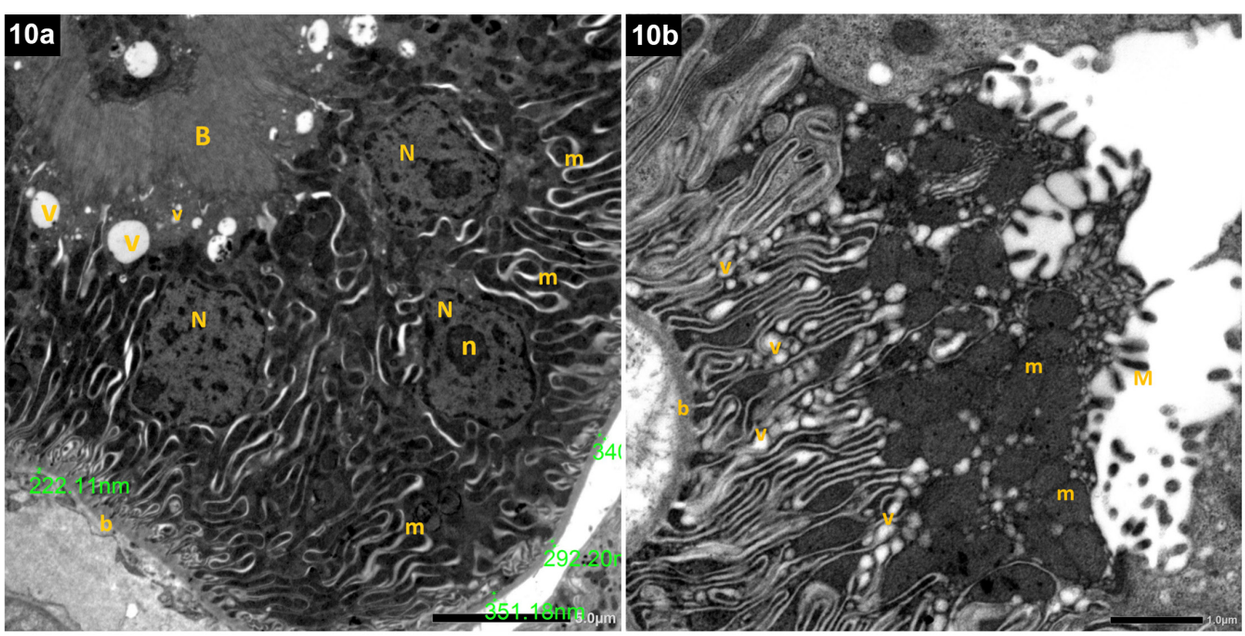

Fig. 10: An electron photomicrograph of a rat kidney (GII c) showing A: a proximal convoluted tubule showing slightly widened basal infoldings loaded with bizarre shaped mitochondria (m) pushing the nuclei up. Most nuclei (N) appear euchromatic with prominent nucleoli (n) and slightly irregular outline. The apical cytoplasm reveals many endocytotic vesicles (v) and large vacuoles (V). The cells exhibit regular brush border (B) obliterating the lumen and rest on regular basal lamina (b). B: showing distal tubular cells with slightly widened basal infoldings and excessively vacuolated cytoplasm (v) pushing the mitochondria (m) up. The luminal border shows few microvilli (M). The cells rest on regular basal lamina (b). (Urinyl acetate lead citrate stain Mic Mag A * 1500, B * 5000)

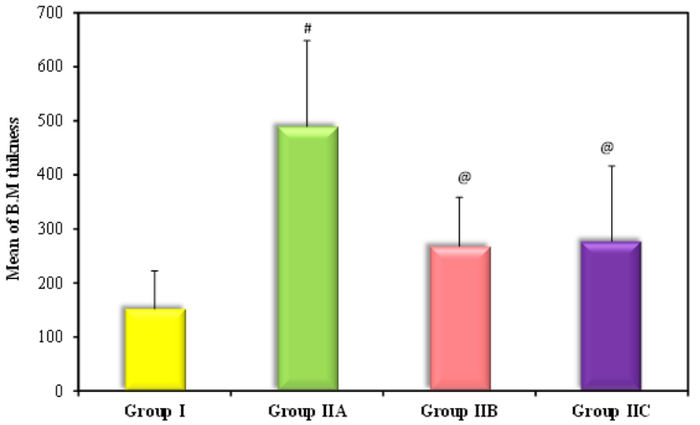

Fig. 11: Bar chart showing distribution of proximal tubules' basement membrane thickness among different study groups

Table 1: Comparison between the different studied groups according to serum creatinine level

Serum creatinine level (mg\dl)	Group I (n = 6)	Group IIA (n = 6)	Group IIB (n = 6)	Group IIC (n = 6)	F	*p*
Min. – Max.	0.22 – 0.28	0.80 – 0.95	0.33 – 0.47	0.29 – 0.50		
Mean ± SD.	0.25 ± 0.02	0.88$^{\#}$ ± 0.06	0.39$^{\#@}$ ± 0.05	0.37$^{\#@}$ ± 0.10	117.101*	<0.001*
Median (IQR)	0.25(0.23 – 0.27)	0.89(0.83 – 0.93)	0.39(0.35 – 0.43)	0.33(0.30 – 0.49)		
*p*1		<0.001*	0.004*	0.014*		
Sig. bet. grps.			*p*2<0.001*, *p*3<0.001*, *p*4=0.932			

SD: Standard deviation
IQR: Inter quartile range
p: *p value* for comparing between the studied groups
*p*1: *p value* for comparing between Group I and each other groups
*p*2: *p value* for comparing between Group IIA and group IIB
*p*3: *p value* for comparing between Group IIA and group IIC
*p*4: *p value* for comparing between Group IIB and group IIC
*: Statistically significant at $p \leq 0.05$

Table 2: Comparison between the different studied groups according to BUN level

BUN level (mg\dl)	Group I (n = 6)	Group IIA (n = 6)	Group IIB (n = 6)	Group IIC (n = 6)	F	*p*
Min. – Max.	21.0 – 29.0	87.0 – 96.0	28.0 – 37.0	28.0 – 35.0		
Mean ± SD.	25.33 ± 3.27	91.17$^{\#}$ ± 3.66	33.33$^{\#@}$ ± 3.39	31.67$^{\#@}$ ± 2.58	537.195*	<0.001*
Median (IQR)	26.0(22.0 – 28.0)	90.50(88.0 – 95.0)	34.0(31.0 – 36.0)	31.50(30.0 – 34.0)		
*p*1		<0.001*	0.002*	0.015*		
Sig. bet. grps.			*p*2<0.001*, *p*3<0.001*, *p*4=0.811			

SD: Standard deviation
IQR: Inter quartile range
p: *p value* for comparing between the studied groups
*p*1: *p value* for comparing between Group I and each other groups
*p*2: *p value* for comparing between Group IIA and group IIB
*p*3: *p value* for comparing between Group IIA and group IIC
*p*4: *p value* for comparing between Group IIB and group IIC
*: Statistically significant at $p \leq 0.05$

Table 3: Comparison between the different studied groups according to BM thickness (nm)

B M thickness	Group I (n = 6)	Group IIA (n = 6)	Group IIB (n = 6)	Group IIC (n = 6)	F	*p*
Min. – Max.	43.56 ± 261.4	275.5 – 744.3	130.7 – 401.6	137.7 – 606.7		
Mean ± SD.	152.2 ± 69.90	489.7$^{\#}$ ± 158.8	267.8$^{@}$ ± 90.85	277.1$^{@}$ ± 139.4	12.923*	<0.001*
Median (IQR)	174.2(97.4 – 194.8)	479.2(351.1–598.0)	254.0(217.8–331.7)	257.2(179.6–340.1)		
*p*1		<0.001*	0.173	0.110		
Sig. bet. grps.			*p*2=0.002*,*p*3=0.003*, *p*4=0.998			

SD: Standard deviation
IQR: Inter quartile range
p: *p value* for comparing between the studied groups
*p*1: *p value* for comparing between Group I and each other groups
*p*2: *p value* for comparing between Group IIA and group IIB
*p*3: *p value* for comparing between Group IIA and group IIC
*p*4: *p value* for comparing between Group IIB and group IIC
*: Statistically significant at $p \leq 0.05$

DISCUSSION

Sulfasalazine is usually prescribed as a remedy for multiple inflammatory diseases. Nevertheless, recent research has reported sulfasalazine-induced renal impairment which can result in lifelong kidney failure, transplantation, and perhaps even death.[3]

Bacterial azoreductase enzymes convert sulfasalazine to mesalazine and sulfapyridine in the human gut. The role of sulfasalazine entir molecule and/ or its intestinal metabolic byproducts in kidney damage and oxidateive stress is unspecified.[18]

Garlic (Allium sativum) has been proven to have antioxidant, anti-mutagenic, anti-proliferative, and chemopreventive properties.[19]

Linares *et al*[6] reported the effects of sulfasalazine on cellular defense mechanisms in sulfasalazine-treated animals. Sulfasalazine-induced changes in cellular defense mechanisms could lead to an increase in ROS production and, eventually, organ failure.

Some studies looked into the involvement of mesalazine in the nephrotoxic responses caused by sulfasalazine.[20]

In this study, serum creatinine and blood urea nitrogen (BUN) levels in the sulfasalazine-treated group (G IIa) were considerably higher than in the control (GI a,b) and garlic-treated groups (G II b & G II C). This demonstrates the sulfasalazine-induced kidney damage and the preventive impact of garlic oil.

In current study degeneration of the brush borders, perinuclear cisternal dilatation, mitochondrial affection, and widened lateral and basal dilatations with excessive cellular vacuolations were all detected in the sulfasalazine-treated group (GII a). All of this could be attributed to the ROS induced lipid peroxidation in cell membrane and membranous orgenelles. Oxygen-derived free radicals disrupt the double bonds in membrane polyunsaturated lipids. Peroxides are formed as a result of the lipid-radical interactions, which are unstable and reactive, resulting in an autocatalytic chain reaction. Sulfhydryl-mediated protein cross-linking is promoted by free radicals, resulting in increased degradation or enzymatic function loss. DNA fragmentation and Polypeptide fragmentation can also be caused by free radical reactions. Free-radical reactions with thymine in nuclear and mitochondrial DNA produce single-strand breaks. Such DNA damage has been linked to cell death, aging, and malignant transformation of cells. This could explain the current nuclear damage, which is accompanied by necrosis and apoptosis.[21]

In sulfasalazine-treated animals, it has been discovered that the dramatic rise in the production of reactive oxygen species in the kidney proved presence of oxidative stress, which has an impact on a variety of intracellular targets, including proteins, lipids, and nucleotides.[22]

In current study light microscopic examination of the renal cortices of rats given 600 mg/kg sulfasalazine for 14 days revealed obvious histological abnormalities, with the proximal renal convoluted tubules being more impacted than the distal ones. The affection was patchy, affecting some tubules while others exhibited almost normal histological pattern, and ranged from cytoplasmic vacuolation of their lining cells up to ballooning. Such findings were in agreement with Heidari, *et al*.[7]

Ultrastructurally, some proximal convoluted tubules had interrupted brush border. There was also a lot of cytoplasmic vacuoles, secondary lysosomes, vesicular bodies, and lipid droplets.

Mitochondrial affection was also variable, elongated mitochondria with lamellar Cristea seen in certain tubular cells. Others, on the other hand, were shrunken and had a homogeneous electron dense matrix. Swollen mitochondria with strange shapes were also discovered. Nuclear shrinkage (attenuation) and chromatin marginalization were the histological and ultrastructural alterations seen in the nuclei of proximal tubular cells. Some nuclei, on the other hand, had a normal chromatin arrangement.

Corica and Romano[23] reported similar rates of nephrotoxicity in a wide group of adult inflammatory bowel syndrome patients treated with mesalazine and sulphasalazine.

Examination of the groups that received concomitant sulfasalazine and garlic oil (protected group) illustrated almost normal architecture of the renal cortex. However, the lining epithelium illustrated cytoplasmic vacuolation in scattered foci, and some others showed dilation of their lumina. This protection revealed a dose dependent.

Hassan *et al*.[19] discovered that administration of Garlic oil dramatically improved kidney function, possibly attributable to garlic's antioxidant properties in scavenging free radicals.

According to Abdel-Daim *et al*.[24] garlic oil administered at a dose of 100 mg/kg effectively reversed kidney's and liver's biochemical markers alterations and antioxidant status caused by cisplatin. Garlic Oil's positive effects were due to inhibition of malondialdehyde (MDA), nitric oxide (NO), scavenging of free radicals, and an increase in glutathione (GSH) and antioxidant enzyme expression, according to the researchers.

As described by lawrence & Lawrence[25] 2.2-diphenyl-1-picrylhydrazyl, a NO scavenging agent, and β - carotene bleaching assays were used to evaluate the antioxidant potential of GO.

Anusuya *et al* [26] stated that administration of ethanolic extract of garlic can minimize nephrotoxicity in cisplatin-treated rats by lowering kidney biomarkers' serum levels like as well as raising antioxidant enzyme activity.

In our research we found that GO counteracted the bad impacts of sulfasalazine on the the function of the kidney. In addition, garlic oil (GO) resulted in reduction of reactive oxygen species (ROS) formation and improvement of histopathological lesions.

Garlic (Allium sativum L.) has been used as a traditional medicine due to its perceived effects in preventing and curing certain diseases.[27]

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Rubin DT, Ananthakrishnan AN, Siegel CA, Sauer BG and Long MD. ACG Clinical Guideline: Ulcerative Colitis in Adults. Am J Gastroenterol. (2019) 114(3):384-413.
2. Seishima R, Okabayashi K, Nagano O, Hasegawa H, Tsuruta M, Shimoda M, Kameyama K, Saya H and Kitagawa Y: Sulfasalazine, a therapeutic agent for ulcerative colitis, inhibits the growth of CD44v9(+) cancer stem cells in ulcerative colitis-related cancer. Clin Res Hepatol Gastroenterol. (2016);40(4):487-493.
3. Niknahad H, Heidari R, Mohammadzadeh R, Ommati MM, Khodaei F, Azarpira N, Abdoli N, Zarei M, Asadi B, Rasti M, Shirazi Yeganeh B, Taheri V, Saeedi A and Najibi A: Sulfasalazine induces mitochondrial dysfunction and renal injury. Ren Fail. (2017) 39(1):745-753.
4. Marsia S, Mahmood S, Raza M, Nusrat K and Saleem A: Sulfasalazine-induced Pancytopenia Indicating Bone Marrow Suppression: A Rare Pediatric Case Report from Pakistan. Cureus. (2019) 11(4):e4462.
5. Heidari R, Rasti M, Shirazi Yeganeh B, Niknahad H, Saeedi A and Najibi A: Sulfasalazine-induced renal and hepatic injury in rats and the protective role of taurine. Bioimpacts. (2016) 6(1):3-8.
6. Linares V, Alonso V and Domingo JL: Oxidative stress as a mechanism underlying sulfasalazine-induced toxicity. Expert opinion on drug safety. (2011)10(2):253-263.
7. Heidari R, Taheri V, Rahimi HR, Shirazi Yeganeh B, Niknahad H and Najibi A: Sulfasalazine-induced renal injury in rats and the protective role of thiol-reductants. Renal failure. (2016) 38(1):137-141.
8. Molnár T, Farkas K, Nagy F, Iványi B and Wittmann T: Sulfasalazine-induced nephrotic syndrome in a patient with ulcerative colitis. Inflamm Bowel Dis. (2010) 16(4):552-3.
9. Sary Ae, Fiedan IO, Ahmed E and Omar H: Acrylamide Induced Testicular Toxicity in Rats: Protective Effect of Garlic Oil. Biomark J. (2015) 1:1-8.
10. Mikaili P, Maadirad S, Moloudizargari M, Aghajanshakeri S and Sarahroodi S: Therapeutic uses and pharmacological properties of garlic, shallot, and their biologically active compounds. Iran J Basic Med Sci. (2013) 16(10):1031-1048.
11. Banerjee SK, Mukherjee PK and Maulik SK: Garlic as an antioxidant: the good, the bad and the ugly. Phytother Res. (2003) 17(2):97-106.
12. Alexandria University: Regulatory framework in Ethics of animal research: Guidelines and Sop on the Regulation of Scientific Experiments on Animals. 2018 [updated 21 April 201829/5/2020]. Available from: http://www.alexu.edu.eg/index.php/en/research/4401-regulatory-framework-in-ethics-of-animal-research.
13. Jiang G-L, Im WB, Donde Y, Wheeler LA. Comparison of prostaglandin E2 receptor subtype 4 agonist and sulfasalazine in mouse colitis prevention and treatment. Journal of Pharmacology and Experimental Therapeutics. 2010;335(3):546-52.
14. Salem NA and Salem EA: Protective antioxidant efficiency of garlic against lead-induced renal and testicular toxicity in adult male rats. J Heavy Met Toxicity Dis. (2016) 1(3):15.
15. Suvarna SK, Layton C and Bancroft JD: Bancroft's theory and practice of histological techniques. Churchill Livingstone Elsevier, Oxford. (2013).
16. Bozzola JJ: Conventional specimen preparation techniques for transmission electron microscopy of cultured cells. Methods Mol Biol. (2007) 369:1-18.
17. Kirkpatrick LA and Feeney BC: A simple guide to IBM SPSS statistics for version 20.0. Wadsworth, Cengage Learning, Belmont, Calif. (2013).
18. Birketvedt GS, Berg KJ, Fausa O and Florholmen J: Glomerular and tubular renal functions after long-term medication of sulphasalazine, olsalazine, and mesalazine in patients with ulcerative colitis. Inflamm Bowel Dis. (2000) 6(4):275-279.
19. Hassan HA, El-Agmy SM, Gaur RL, Fernando A, Raj MH and Ouhtit A: In *vivo* evidence of hepato- and reno-protective effect of garlic oil against sodium nitrite-induced oxidative stress. Int J Biol Sci. (2009) 5(3):249-255.
20. Gisbert JP, González-Lama Y and Maté J: 5-Aminosalicylates and renal function in inflammatory bowel disease: a systematic review. Inflamm Bowel Dis. (2007) 13(5):629-638.
21. Kumar V, Abbas AK and Aster JC: Robbins basic pathology e-book. Elsevier Health Sciences, London. (2017).
22. Avery SV: Molecular targets of oxidative stress. Biochem J. (2011) 434(2):201-210.
23. Corica D and Romano CL: Renal Involvement in Inflammatory Bowel Diseases. J Crohns Colitis. (2016) 10(2):226-235.

24. Abdel-Daim MM, Abdel-Rahman HG, Dessouki AA, El-Far AH, Khodeer DM, Bin-Jumah M, Alhader MS, Alkahtani S and Aleya L: Impact of garlic (Allium sativum) oil on cisplatin-induced hepatorenal biochemical and histopathological alterations in rats. Sci Total Environ. (2020) 710:136338.

25. Lawrence R and lawrence K: Antioxidant activity of garlic essential oil (Allium Sativum) grown in north Indian plains. Asian Pac J Trop Biomed. (2011) 1(1, Supplement):S51-S4.

26. Anusuya N, Durgadevi P, Dhinek A and Mythily S: Nephroprotective effect of ethanolic extract of garlic (Allium sativum L.) on cisplatin induced nephrotoxicity in male wister rats. Asian J Pharm Clin Res. (2013) 6:4.

27. Nicastro HL, Ross SA and Milner JA: Garlic and onions: their cancer prevention properties. Cancer Prev Res. (2015) 8(3):181-189.

الملخص العربى

دراسة هستولوجية للتاثير الوقائي المحتمل لزيت الثوم على إصابه الأنابيب الكلوية الناجم عن إستخدام مادة السلفاسلازين في ذكور الجرذان البيضاء البالغه

فؤاد صبري علي هيكل[١]، وفاء عبد الرحمن احمد[١]، هدي محمود احمد خليفه[٢]، رشا محمد محمد الدقماق[١]، السيد علي محمد متولي[١]

[١]قسم التشريح الآدمي وعلم الأجنة، [٢]قسم علم الأنسجة وبيولوجيا الخلية، كلية الطب، جامعة الإسكندرية

الخلفية: سلفاسالازين هو دواء معدّل لمرض الروماتيزم ، ويستخدم في علاج التهاب المفاصل مجهول السبب. يعد تلف الكلى أحد آثاره الجانبية.

الهدف: دراسة الدور الوقائي لزيت الثوم ضد الإصابة الكلوية التي يسببها السلفاسلازين.

المواد وطرق البحث: ثلاثون جرذًا بالغًا من الجرذان البيضاء ؛ بمتوسط وزن ١٥٠-٢٠٠ جم قسمت عشوائياً إلى ثلاث مجموعات. تلقوا الأدوية عن طريق أنبوب المعدة لمدة ١٤ يومًا.

المجموعة الأولى (المجموعة التحكم): تضمنت ١٢ جرذًا مقسمة إلى مجموعتين متساويتين: I أ: تلقت ٢٠٠ مجم من محلول ملح الفوسفات المعادل (مذيب الدواء). I ب: تلقت ١٠٠ مجم / كجم / يوم من زيت الثوم. المجموعة الثانية (المجموعة التجريبية): تضمنت ١٨ جرذًا ، تم تقسيمها إلى ثلاثة مجموعات متساوية: II أ (المجموعة المعالجة): تلقت سلفاسالازين ٦٠٠ مجم / كجم / يوم. II ب (مجموعة زيت الثوم المنخفض): تلقت سلفاسالازين ٦٠٠ مجم / كجم / يوم و ١٠٠ مجم / كجم / يوم من زيت الثوم. II ج (مجموعة زيت الثوم عالية): تلقت سلفاسالازين ٦٠٠ مجم / كجم / يوم و ٢٠٠ مجم / كجم / يوم من زيت الثوم.

النتائج: في المجموعه المعالجه بالسلفاسلازين(II أ) ارتفع معدل الكرياتنين وBUN بشكل ملحوظ. تسبب سلفاسالازين في العديد من الاضرار في الانابيب الكلويه القريبه كالاتي : تشوه في حدود الفرشاه ، خلايا البطانة المفرغة والنواة المتجمعة. وكانت التغييرات علي مستوي الخلية كالاتي ؛ توسع صفيحي حول النواة ، تشوهات الميتوكوندريا ، اضطرابات جانبية وقاعدية ، صفيحة قاعدية سميكة. اظهرت مجموعة زيت الثوم المنخفض(GII b) مستوى كبير من الحماية في شكل استعادة البنية الأنبوبية الطبيعية. بينما أظهرت بعض الأنابيب اتساع في تجويفها مع تشوه في حدود الفرشاه وخلايا البطانة المفرغة. وكانت التغيرات علي مستوي الخليه كالاتي ؛ تأثيرات انبوبيه محدوده مع الميتوكوندريا المتحللة ، وفقدان جزئي لحدود الفرشاة. بينما في مجموعة زيت الثوم عالية(GII c) تم العثور على درجة عالية من الحماية مع عدد قليل من الخلايا المفرغة والتغيرات في البنية التحتية.

الاستنتاج: إن سلفاسالازين له تأثيرات تدميريه أنبوبيه كلوية ولزيت الثوم تأثير وقائي يعتمد على الجرعة.