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F2-Isoprostanes Reflect the Protection of Black Rice Anthocyanin Nanoparticles for Kidney against Methotrexate-Induced Nephrotoxicity in Rats

Soheir N. Abd El-Rahman[a], Sheikha M. AlGhannam[b], Ferhad M. Serag Eldin[c], Raafat N. Sandak[a]

[a] Crops Technology Research Department, Food Technology Research Institute, Agricultural Research Center, Giza 12619, Egypt; [b] Chemistry Department, College of Science; Imam Abdulrahman Bin Faisal University; P.O.Box 1982, 31441; [c] Dammam; Saudi Arabia. Center Lab. of Research, General Food Security authority (GFSA), Riyadh, KSA

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KEYWORDS

Anthocyanin silver nanoparticles (An-AgNPs), Antioxidant enzymes, nephrotoxicity, Methotrexate, Oxidative Stress, F2-isoprostanes (F2-IsoPs).

ABSTRACT:

Introduction: Anthocyanins are the most antioxidant and prevalent natural colorants.

Objectives: The target of this research was to determine the preventative influence of black rice anthocyanin silver nanoparticles (An-AgNPs) as naturally occurring antioxidants against methotrexate-induced nephrotoxicity and protect F2-Isoprostanes.

Methods: Characteristics of An-AgNPs were identified by X-ray and scanning electron microscopy (SEM). Albino rats were divided into five groups (negative control (NC): fed on basel diet, positive control (PC): injected with only one dose of methotrexate (20 mg/kg.b.w) intravenously, and three other groups injected by only one dose of MTX (20 mg/kg, b.w) and taken orally separately with An-AgNPs at 10, 15, and 20 mg/kg, b.w/ day, respectively. F2-isoprostanes (F2-IsoPs) malondialdehyde (MDA), and catalase (CAT) were measured, also the histological changes of kidney tissues were studied.

Results: The results indicated that PC group have considerably lower levels of the enzymatic antioxidant CAT, as well as higher MDA and F2-IsoPs compared to NC group. The renal nephrotoxicity in groups 4 and 5 which taken orally An-AgNPs 15, and 20 mg/kg.b.w respectively revealed improvement in the kidney functions' findings. It was discovered that the renal improvement was nearly or exactly the same as that of healthy rats (NC). Furthermore, Histopathological data that demonstrated the curative impact of groups 4 and 5 keep away from kidney impairment and kidney fibrosis against nephrotoxicity caused by methotrexate provided additional support for previously these findings. An-AgNPs at 15, and 20 mg/kg, b.w improved kidney function and enzyme activity, as could be seen from the obvious findings.

Conclusions: As a result, it could be advised that black rice is a reliable source for overall wellness and improved health.

1. Introduction

The most common issue encountered worldwide is kidney illness. The kidney is the most critical organ for maintaining normal blood pressure, metabolism, homeostasis, the removal of toxic metabolites from the body, and regulating the toxicity of a variety of medications and environmental toxins **[**1]. All hazardous compounds are filtered by the kidney, and metabolites are excreted in the urine. According to **Popović *et al.* [**2] reported that substances harmful to the kidneys can directly result in acute renal failure. The kidney is especially susceptible to toxicants because it filters an enormous amount of toxins. Systemic toxicity may result from this, which can harm internal processes like maintaining fluid balance and weakening the body's capacity to eliminate waste. Apoptosis, necrosis, oxidative stress, and inflammation are the main diseases causing kidney issues **[3].** Diabetes, hypertension, and glomerulonephritis account for two-thirds of instances of chronic kidney disease. Nephrotoxicity is a side effect of acute renal failure that is brought on by nephrotoxic chemicals, medications, and hypoperfusion

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[4]. Inflammation in the renal tubules, glomerulus, and surrounding cellular matrix brought on by oxidative stress and excessive reactive oxygen species generation is frequently implicated in nephrotoxicity (ROS) **[5].**
Exposure to a variety of medications, chemicals, poisons, and microorganisms can cause damage to the liver and kidney, which can result in serious illness states **[6].** A frequent disorder that accounts for around 60% of all occurrences of acute kidney injury is drug-induced nephrotoxicity **[1].** Methotrexate (MTX) is frequently utilized in the clinical treatment of many inflammatory disorders and malignancies **[7].** The antifolate MTX prevents thymidine and purine production, DNA replication and repair, and cellular growth. Nephrotoxicity is one of the most important toxicities brought on by MTX treatment because it accounts for 90% of the drug's kidney excretion **[5].** Due to crystalline nephropathy, which is caused by high-dose MTX therapy precipitating in the renal tubules, 2-12% of patients may get abrupt renal failure. Additionally, prolonged, continuous treatment with low doses of MTX might result in serious toxicities **[8].** Increased oxidative stress, pro-inflammatory cytokine levels, and antioxidant defense system disruption are all linked to MTX-induced kidney injury, which can lead to pro-inflammatory cell death **[**9].
NPs and nanostructured materials are essential in nano-biomedical technology because of their characteristics and wide range of possible uses **[10].** Additionally, NPs have shown that they can act as antioxidants, reducing the production of ROS brought on by oxidative stress-causing chemicals **[**6]. Nanosilver possesses outstanding physical, chemical, and biological qualities. Regarding safe transport various treatments to target organs with few adverse effects. It has been demonstrated that AgNPs can reduce hepato-renal damage. In a study, superoxide dismutase (SOD) and catalase (CAT) levels were also restored while bilirubin levels were kept at normal levels by treatment with AgNPs**[**11].
Along with carotenoids, chlorophylls, and betalains, anthocyanins are the most prevalent natural colorants and are present in thousands of plants. Chemically speaking, anthocyanin compounds are 2-phenylbenzopyrilium derivatives with two benzyl rings that are glycosylated polyhydroxy or poly methoxy compounds **[**12]. Black rice is a type of pigmented rice with black bran covering the endosperm of the rice kernel. It is an aromatic and pigmented rice variety popular, whose demand and consumption are increasing day by day in the world due to its numerous health benefits. It is not consumed as a staple food but consumed as a functional food because of its anthocyanin content, which acts as a major bioactive compound. Accumulation of anthocyanin (Cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-glucoside) in the outer layers promotes the black color of rice grains. Anthocyanins are water-soluble pigment which is responsible for the anti-oxidative and anti-inflammatory properties of black rice [13]. Moreover, it is one of the phenolic compounds that enter flavonoid compounds that have a role for the plant itself and for humans as black rice consumers **[14].**

2. Objectives

This research aimed to determine the characteristic of prepared An-AgNPs, also, the nephrotoxicity of MTX on kidney biomarkers and F2-IsoPs in male rats, as well as the protective effect of black rice anthocyanin silver nanoparticles against kidney dysfunction caused by MTX.

3. Methods

3.1. Extraction of anthocyanin from black rice

From the Giza Agricultural Research Center in Egypt, black rice was obtained. The black rice anthocyanin extract was prepared by mixing 100g of black rice powder with 150 mL of methanol and stirring for 24 hours. To obtain the black rice anthocyanin extract, the finished extract underwent centrifugation **[15].**

3.2. Preparation of anthocyanin silver nanoparticles

Anthocyanin silver nanoparticles were prepared by reducing black rice anthocyanin with metallic ions. To synthesize anthocyanin nanoparticles, 0.06 M $AgNO_3$ were used: 6.6 ml methanol and 16.6 ml anthocyanin extract from black rice were added to 200 ml boiling distilled water. Notice immediate color change, which indicates the presence of anthocyanin silver nanoparticles. After that, turn off the heat and continue stirring until the solution has cool **[**16]. Then, the solution was centrifuged at 6000rpm for 20min and was placed the sample in the refrigerator until uses.

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3.3. Characterization of anthocyanin silver nanoparticles

Using a D/max r-B, Rigaku, Japan, X-ray powder diffract meter, the crystallinity was evaluated. The electron microscope was utilized to study the microstructure of anthocyanin silver nanoparticles. Silver was sputter-coated onto the samples using a vacuum evaporator with an accelerating voltage range of 5 to 15 kV and a magnification power of 750–6,000.

3.4. Biological methods

Fifty male albino rats (210-250g) were provided from The Vaccination Centre in Helwan-Giza, Egypt. They were housed in the animal house at the Ophthalmology Research Institute in Giza, Egypt.Under the recommended circumstances, they consumed a nutrition-rich meal for ten days that included 4% salt, 10% corn oil, 1% vitamin, 70% corn starch, 10% casein, and 5% cellulose. Five groups of ten animals each were formed from the experimental animals at random as follows:

G1: Negative Control (NC) group: fed on basal diet for 10 days during the experimental period.

G2: Positive Control (PC) group:injected with Methotrexate (MTX) (20 mg/kg, b.w) given only one dose administered intravenously during the experimental period (10days).

G3, 4, and 5: injected with MTX (20 mg/kg b. w.) administered intravenously and taken orally separately 10, 15, and 20 mg/kg / bw of An-AgNPs, for G3, G4 and G5, respectively during the experimental period (10days).

Anthocyanin Silver Nanoparticles (AnNPs) was dissolved in deionized water and kept under magnetic stirring for 20min and followed by another 20 min to ensure the complete dissolution for taken daily oral administration using the oral gavage technique once a day for 10 days during the experimental period **[**17].

3.5. Biochemical assays

Rats were slaughtered, and blood were collected and separated into the serum by centrifuging. Evaluation of kidney function parameters including creatinine, urea, and uric acid were evaluated by **Salem *et al.* [18].** Serum lipid peroxidation was determined colorimetrically as malondialdehyde (MDA) according to **Goudarzi *et al.* [19],** catalase enzyme (CAT) according to **Aebi, [20],** and F2-isoprostane (F2-IsoPs) according to **Elizabeth *et al.* [21].**

3.6. Histopathological examination

The renal tissues were dehydrated using a gradient series of aqueous EtOH from 70% to 100% after being fixed in 10% neutral formaldehyde. According to **Obaid *et al.* [**22] the dehydrated tissues were then embedded in paraffin, divided into 4-5 m slices, and stained with hematoxylin dye (H&E staining). Leica Microsystems IR GmbH, Switzerland's DM750 light microscope was used to carefully inspect the stained sections in order to spot any histopathological changes brought on by various treatments.

3.7. Statistical analysis

Software Jandel Sigma Stat Statistical Software, version 2.0 for Windows, was used to analyze the study's data. A one-way completely randomized design for the analysis of variance (ANOVA), in addition Fischer's test at $P \leq 0.05$ to compare means.

4. Results

4.1. X-ray of anthocyanin silver nanoparticles

Figure (1) shows the An-AgNPs' XRD patterns. The An-AgNPs diffraction peaks, in order, correspond to the diffraction levels (1 0 0), (0 0 2), (1 0 1), (102), (103), (1 1 2), and (2 0 1). The silver nanoparticles are crystalline, according to the XRD spectrum. The measured diffraction peaks at 2è = 38.140, 44.310, 64.560, 77.640, and 81.850.

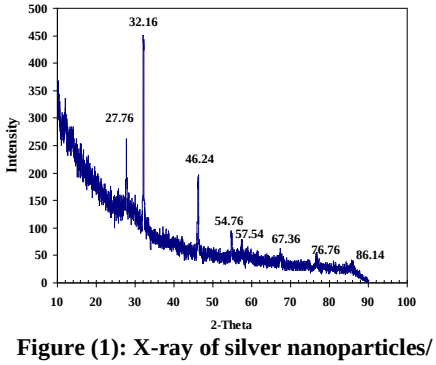

Figure (1): X-ray of silver nanoparticles/ anthocyanins nano-composite

4.2. Scan electron microscope of anthocyanins silver nanoparticles

SEM images for An-AgNPs produced from black rice anthocyanine doping were found in Figure (2). The sample of An-AgNPs in Figure 2 exhibits a tendency to aggregate, and the particle size, and diameter ranged from 0.07 to 0.13 µm (70nm - 130nm).

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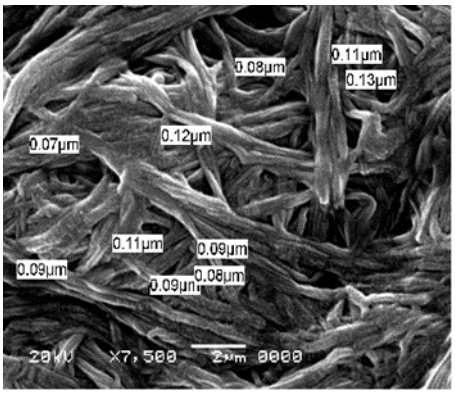

Figure (2): SEM of silver nanoparticles/ anthocyanins nanocomposite (An-AgNPs)

4.3. Effect of An-AgNPs on Kidney functions

According to Table (1), the findings observed that the blood levels of urea, uric acid, and creatinine significantly increased ($p \leq 0.05$) in positive control group which injected by one dose only of methotrexate (20 mg/kg.b.w.). These levels were 23.92, 7.67, and 3.92 mg/dl, respectively. While normal rats fed a basal diet had the lowest kidney functions of 16.96, 3.15, and 0.87 mg/dl, respectively.

The elevated decrease in urea, uric acid, and creatinine enzymes was observed in the nephrotoxicity rats in group (4) by 19.18, 5.38, and 1.51 mg/dl, respectively, whereas, the highest decrease was observed in group (5) by 16.56, 4.03, and 0.91 mg/dl, respectively.

Table (1): Effect of An-AgNPs on Kidney functions of MTX-induced nephrotoxicity in rats

Values (means ±SD) in the columns are statistically considerably various at ($P \leq 0.05$).

Groups	Urea mg/dl	Creatinine mg/dl	Uric acid mg/dl
G1 Control Negative	16.96^{d}±1.15	0.87^{d}±0.01	3.15^{d}±0.61
G2 Control Positive	23.92^{a}±2.03	3.92^{a}±0.53	7.67^{a}±0.13
G3 (An-AgNPs 10 mg/kg b.w+MTX)	21.47^{b}±2.23	2.78^{b}±0.31	6.15^{b}±0.15
G4 (An-AgNPs 15 mg/kg b.w+ MTX)	19.18^{c}±1.84	1.51^{c}±0.01	5.38^{c}±0.13
G5 (An-AgNPs 20 mg/kg b.w+ MTX)	16.56^{d}±1.76	0.91^{d}±0.02	4.03^{d}±0.11

4.4. Effect of An-AgNPs on MDA, CAT, and F2-IsoPs

In the nephrotoxicity caused by methotrexate in several rat groups, malondialdehyde (MDA), catalase (CAT), and F2-isoPs were measured and compared to the control rat group. According to the findings in Table (2), the CAT enzyme was reduced by 19.37U/L in the PC group. The results gradually improved to 31.91, 45.04, and 51.03 U/L in each rat group injected with MTX (20 mg/kg.b.w) administered intravenously and taken orally separately from An-AgNPs (10, 15, and 20 mg/kg.b.w, respectively).

MDA and F2-isoPs in the PC were greater by 17.22 nmol/ml and 17.51 pg/ml, respectively, than they were in the NC at 4.27 nmol/ml and 2.30 pg/ml. The results in the An-AgNPs-treated rats were decreased to 4.46 nmol/ml and 2.45 pg/ml, respectively, in the rat group that received An-AgNPs taken orally at 20mg/kg.b.w and injected with MTX (20 mg/kg.b.w) administered intravenously.

Table (2): Effect of An-AgNPs on MDA, CAT, and F2-IsoPsof MTX-inducednephrotoxicity in rats

Values (means ±SD) in the columns are statistically considerably various at ($P \leq 0.05$).

Groups	MDA nmol/ml	CAT U/L	F2-IsoPspg/ml
G1 (NC)	4.27^{d}±0.15	48.15^{b}±0.08	2.30^{d}±0.06
G2 (PC)	17.22^{a}±0.28	19.37^{e}±0.18	17.51^{a}±0.12
G3 (AnAgNPs 10 mg/kg b.w+ MTX)	14.94^{b}±0.23	31.91^{d}±0.07	7.35^{b}±0.04
G4 (AnAgNPs 15 mg/kg b.w+ MTX)	8.98^{c}±0.23	45.04^{c}±0.05	3.40^{c}±0.08

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G5 (AnAgNPs 20 mg/kg b.w+ MTX)	4.46^{d}±0.26	51.03^{a}±0.04	2.45^{d}±0.08

4.5. Histological experimental of Kidney

The kidney portion of the rats received an intraperitoneal injection of 20 mg/kg of MTX demonstrated infiltration of interstitial mononuclear cells, congested renal blood vessels, and cystic dilatation of the renal tubules (Fig. 3b). The renal tissue of rats injected with a single dosage of MTX (20 mg/kg b.w) i.p and administered orally An-AgNPs (15 mg/kg b.w) or An-AgNPs (20 mg/kg b.w) was fully normal, showing no histopathological alterations (Figs. 3d and 3e), and most likely identical to that section for the normal rats (Fig. 3a). However, G3 which receiving a single injection dose of MTX (20 mg/kg.b.w) intravenously with 10 mg/kg.b.w of An-AgNPs (taken orally) (Fig. 3c) showing cystic dilatation of renal tubules and thickening of the basement membrane of renal tubules and the parietal layer of bowman's capsule.

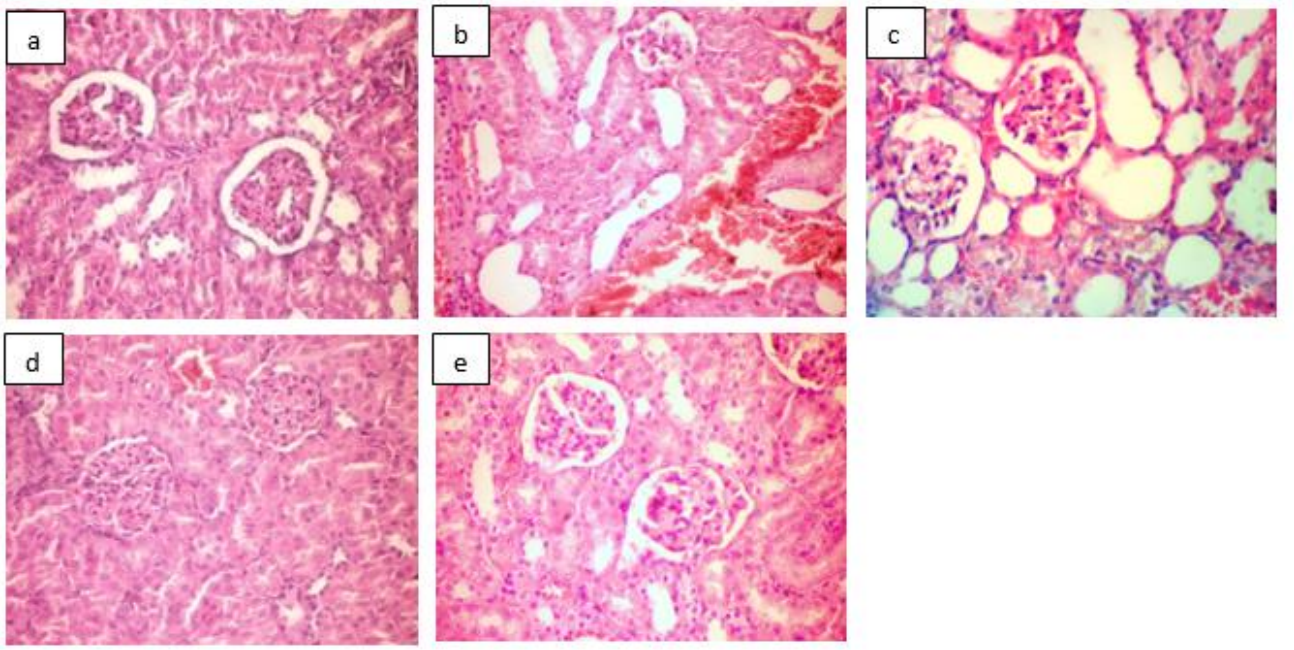

Figure (3): Histological sections of renal rat exposed to MTX (20 mg/kg.b.w) injection intravenously (Stained with H&E, 200×). (a) Normal Control (NC) group, (b) Positive Control (PC) group, (c) Group (3), (d)Group (4), (e) Group (5).

5. Discussion

The measured diffraction peaks at 2è = 38.140, 44.310, 64.560, 77.640, and 81.850 are the same as those previously reported for normal silver metal. The sizes of the following crystallites have been identified: DAgNPs-C=7.7 nm [**23].**

The findings concur with those of **[**24], who claimed that the particles are in bundles with sizes between 70 and 130 nm. AgNPs-C nanoparticles are between 9 and 82 nm in size **[23]. Dupeyrón *et al.* [**25] prepared anthocyanin nanoparticles and found that; the sample image is apparent as a huge (~10 m) irregular cluster. Similar to how other photographs demonstrate, the sample's NPs also have the propensity to aggregate. Agglomerated smaller NPs appear to be the cause of the agglomerates. Intermolecular hydrogen bonds play a major role in the agglomeration of NPs.

These results may be possible that anthocyanin silver nanoparticles, due to their potential antioxidant properties improve renal function via attenuating oxidative stress-mediated decline in renal

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hemodynamics. In addition, the anthocyanin silver nanoparticles prevented MTX-induced structural changes, suggesting the possible involvement of ROS inmediating these histological alterations.

A vital organ of the human body is the kidneys. They control the levels of water, electrolytes, and acid/base, produce some hormones, and aid in the metabolism of other substances **[**26]. Serum creatinine is the most widely used method for assessing kidney function. However, a considerable increase in urea and creatinine levels may indicate renal failure, followed by retention of them in the blood **[27].** In mammals, the main by-product of protein catabolism is urea, which serves as the body's primary transporter for removing toxic ammonia. Additionally, elevated urea levels are associated with nephritis, renal ischemia, and general obstruction of the urinary tract; thus, urea measurement is very useful for controlling kidney function **[**28].

These findings imply that rat kidneys were protected against MTX-induced nephrotoxicity in different rat groups which injected with MTX and given orally An-AgNPs, which contained anthocyanins as natural antioxidants in varying quantities. Additionally, NPs have demonstrated their ability to operate as an antioxidant, squelching the generation of ROS caused by substances that cause oxidative stress. Finally, because of their pro-oxidant properties, they are also known to increase the level of ROS, which causes malignant liver and kidney cells to undergo apoptosis. As a result, NPs can be regarded as a double-edged sword whose inherent therapeutic benefits can be refined as we work to comprehend them in terms of their toxicity **[**6].

Numerous disease processes have been linked to oxidant stress. Measuring lipid peroxidation is one technique for calculating oxidative damage. The lung damage caused by a high degree of lipid peroxidation may be due to free oxygen radicals and a loss in antioxidant factors. Higher than normal levels of malondialdehyde (MDA) were identified in rat lungs, and this led to reduced levels of both enzymatic and non-enzymatic antioxidants as well as severe DNA damage.

The results of MDA, CAT, and F2-isoPs levels gradually improved in each rat group injected with MTX (20 mg/kg.b.w) administered intravenously and taken orally separately from An-AgNPs (10, 15, and 20 mg/kg.b.w, respectively). These results support by **Chen *et al.* [**29] they observed that polyphenols increase the activity of catalase (CAT) proteins.

In line with this finding, **Shayesteh *et al.* [**30] discovered that methotrexate-treated rats had lower CAT levels. **Adeyemi and Faniyan [**31] also noticed a variable decline in the levels of catalase in comparison to the control. Additionally, the decreased CAT activity was unable to catalyze the conversion of oxy-radicals at the end. These findings suggest that the antioxidant defence was severely weakened and that reactive oxygen species (ROS) production may have increased **[32].**

According to **Afsar *et al.* [**33] an accurate assessment of oxidative stress can be made in vitro and in vivo by quantifying a class of prostaglandin F2-like substances called F2-isoprostanes [34].

F2-isoprostanes are a well-studied indicator of lipid peroxidation and redox stress and have been shown in many studies to increase with advancing CKD in humans **[**35]. In addition, redox stress appears to contribute to disease progression in animal models of CKD, making it a possible therapeutic target [36]. In MTX-induced renal damage, it can be assumed that direct damage to the glomeruli, tubular obstruction or direct tubular toxicity, are further nephrotoxic mechanisms **[**36]. It has been reported that the kidneys of MTX-treated rats result in severe glomerular and tubular nephritis. The glomeruli are necrotized and are infiltrated with fibrous tissue **[**38]. **Grönroos *et al.* [**39] demonstrated that treatment with MTX shows cell swelling and necrosis in renal tubular cells.

6. Conclusion

The black rice extract's anthocyanin silver nanoparticles have an antioxidant function property. The present results show that the groups treated with An-AgNPs were improved malonaldehyde, CAT, and F2-IsoPs levels as well as kidney functions. Moreover, the results from renal histological confirmed the earlier findings. It could be concluded that An-AgNPs act in the kidney as a potent scavenger of free radicals to prevent the nephrotoxicity effects of MTX. More research is still required to determine the precise molecular mechanism by which AnNPs act against methotrexate-Induced nephrotoxicity in rats, but no more found research for this achieved.

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