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Original Article

Protective effects of N-acetylcysteine and S-adenosyl-L-methionine against nephrotoxicity and immunotoxicity induced by ochratoxin A in rats

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ABSTRACT

Objective: The present study was designed to investigate the nephroprotective and immunoprotective effects of S-adenosyl-L-methionine (SAMe) in comparison to N-acetylcysteine (NAC) against ochratoxin A (OTA) – intoxication.

Methods: Forty-eight adult male Sprague–Dawley rats were categorized into four groups: Control; OTA intoxication (5 mg OTA/kg diet); OTA + NAC, rats received 200 mg NAC/day before feeding balanced diet contaminated with OTA; and (OTA + SAMe). Rats received 200 mg SAMe/day dissolved in distilled water orally just before feeding a balanced diet contaminated with OTA.

Results: OTA administration altered serum kidney function biomarkers. These effects were pronouncedly alleviated by treatment with NAC. Results revealed a correlation between OTA-induced immunotoxicity and the reduced white blood cell (WBC) count. Treatments with SAMe significantly improved the WBCs count and hemoglobin concentration.

Conclusion: NAC and SAMe have a protective role against nephrotoxicity and immunotoxicity induced by continuous administration of OTA. NAC was more effective in reducing OTA nephrotoxicity, whereas SAMe was more potent than NAC in reducing OTA immunotoxicity.

Keywords: Immunotoxicity, mycotoxins, N-acetyl cysteine, nephrotoxicity, ochratoxin A, S-adenosyl-L-methionine

Introduction

Mycotoxins are byproducts of fungus metabolism produced through growth and reproduction. They are often highly toxic and have a low molecular weight. The simultaneous occurrence of several mycotoxins in nature, particularly in food and feed, makes it difficult to completely eradicate and detoxify mycotoxins. The kidney's particular metabolism and high blood flow make it particularly susceptible to nephrotoxic attack.[1] One type of mycotoxin that is commonly found in many different foods and animal feeds is ochratoxin A (OTA).[2] Exposure to OTA through poisoned food has a nephrotoxic detrimental effect on human health.[3] Previous research has demonstrated that OTA exposure can cause multiorgan toxicity.[4] Numerous studies showed that OTA can cause carcinogenicity, immunotoxicity, and nephrotoxicity,[5,6] The cytotoxic mechanisms include apoptosis, DNA damage, and oxidative stress.[7]

N-acetylcysteine (NAC), one of the most important defenses against the damaging effects of oxidation, is used to create glutathione (GSH) as a free radical scavenger to protect against the dangerous effects of numerous chemicals.[8,9] Numerous studies have demonstrated that NAC increases the levels of free sulfhydryl and GSH in cells, which have been shown to have direct and indirect antioxidant capabilities. NAC may be able to lessen the harm done by reactive oxygen species (ROS).[10] NAC can lessen toxicity in numerous organs including the liver[11] and kidney[12] through enhancing energy metabolism and antioxidant capability.[13]

S-adenosyl-L-methionine (SAMe), an important biological sulfonium compound, takes part in numerous metabolic activities. SAMe is synthesized by the reaction of methionine with ATP that is catalyzed by SAMe synthetase.[14] SAMe is thought to be the primary methyl contributor compound for methylation processes that take place in all living cells, which are essential in cell division by regulating key metabolic pathways. SAMe

is a significant metabolite that can function both *in vivo* and *in vitro* as a stress sensor.[15] Homocysteine, cysteine, and reduced GSH are biosynthesized from SAMe. SAMe contributes to the folic acid cycle and has an impact on one-carbon metabolism as a crucial methionine cycle intermediary. The primary carbon source for the one-carbon metabolic pathway is serine, which might precisely control adaptive immunity by utilizing T-cell proliferative capability.[16] The purpose of this investigation is to evaluate the nephroprotective and immunoprotective effects of SAMe and NAC against OTA intoxication.

Materials and Methods

Chemicals

OTA (CAS No. 303-47-9) was bought from Sigma Chemical Co. (St. Luis, MO, USA).

SAMe (CAS No.29908-03-0) was purchased from Natural Factors Company (WA, USA). Each tablet containing 200 mg of pure SAMe was crushed into powder and dissolved in 5 mL of distilled water to make the SAMe dose used in the experiment (200 mg/5 mL/day).

NAC (CAS No. 616-91-1) was purchased in the form of tablets from Now Food company for natural food supplements (Bloomingdale, IL, USA). Each tablet containing 1000 mg of pure NAC was crushed into powder, dissolved in 25 mL of distilled water, and divided into five portions to make the NAC dose used in the experiment (200 mg/5 mL/day).

Ethical consideration

The Research Ethics Committee of Ain Shams University evaluated and approved the experimental procedures of this study (Code Sci. 1432305001).

Experimental animals and design

Adult male albino rats (Sprague-Dawley) were used in this study, initially weighing 219 ± 7 g. Rats were partitioned and housed in environmentally controlled cages (24 ± 2°C, 50 ± 5% humidity, and 12 h light/dark cycle). Rats were fed on a balanced standard diet and allowed water *ad libitum* throughout the whole experiment.

The study was conducted between January 2023 and March 2023 at Central Laboratory Unit – Ain Shams University. 48 male rats were classified into four groups (12 rats/group) as follows:

1. Control group: Rats fed a standard balanced diet
2. OTA-intoxicated group: Rats fed a standard balanced diet contaminated with OTA (5 mg OTA/kg diet) daily to induce nephrotoxicity and immunotoxicity
3. NAC treated group (OTA + NAC). Rats received 200 mg NAC/day dissolved in distilled water orally by gavage just before feeding a balanced diet contaminated with OTA
4. SAMe treated group (OTA+SAMe). Rats received 200 mg SAMe/day dissolved in distilled water orally just before feeding a balanced diet contaminated with OTA.

Analytical procedures

On the past day of the experimental period (42 days), rats were fasted overnight and then anesthetized with ether, and blood samples were drawn from the hepatic portal vein and then transported into centrifuge tubes. Tubes were centrifuged at 5000× g for 15 min at 23°C to collect serum for the biochemical examination. Serum samples were stored at −20°C until used for various biochemical analyses. To determine hematological parameters, additional blood samples (approximately 1 mL) were collected into test tubes containing EDTA.

The kits of urea, uric acid, creatinine, total proteins, albumin, Cystatin-C, neutrophil gelatinase-associated lipocalin (NGAL), sodium, potassium, superoxide dismutase (SOD), reduced GSH, nitric oxide (NO), malondialdehyde (MDA), and total antioxidant capacity (TAC) were obtained from Bio diagnostics Co. (Cairo, Egypt) whereas 8-hydroxy-2deoxyguanosine (8-OHdG) and enzyme-linked immunosorbent assay kits were utilized to measure the levels of caspase 3, nuclear factor kappa-B (NF-κB), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) according to the manufacturer Kamiya Biomedical Co. (CA, USA).

Complete blood cell count with differential count

The total and differential leukocyte counts as well as the erythrocyte and platelet counts were evaluated using an AcT 5diff Cap Pierce hematology analyzer (Beckam Coulter, USA). Hemoglobin (Hb) was measured by the colorimetric method according to the manufacturer (Biodiagnostic Co. for research reagents).

Statistical analysis

Using the GraphPad PRISM version 5.0, data were statistically evaluated. Data were displayed as averages ± standard error of the mean (SE) and a one-way ANOVA test. $P \leq 0.01$ was used to determine the significance of differences.

Results

Results in Table 1 displayed the effect of different treatments on body weight gain and food consumption of all experimental groups. Comparing results showed a significant reduction ($P \leq 0.01$) detected in the OTA-intoxicated group. The relative weights of kidneys in rats fed a diet contaminated with OA were significantly increased. NAC or SAMe effectively reversed these effects.

Nephrotoxicity is a result of OTA treatment, as demonstrated by significant changes in serum markers of renal function. In comparison to the normal control group, OTA treatment resulted

Table 1: Effects of all treatments on body weight gain, food consumption, and relative weights of kidney

Biological investigation	Control group	OTA-intoxicated group	OTA+NAC group	OTA+SAMe group
Body weight gain (g)	150.7±5.2	62.2^{a}±2.3	106.5^{ab}±5.7	111.3^{ab}±6.9
Food intake/day	18.25±1.1	10.80^{a}±0.9	14.30^{ab}±0.8	13.22^{ab}±1.3
Relative weights of kidney (g%)	0.630±0.07	0.850^{a}±0.04	0.655^{ab}±0.08	0.698^{ab}0.05

OTA: Ochratoxin A, NAC: N-acetylcysteine, SAMe: S-adenosyl-L-methionine, Values are expressed as means±SE. (n=12), ^{a}Significance against control, ^{b}Significance against OTA group at P≤0.01. There was a significant difference between means with the same alphabetic superscript (a and b) in the same row

in increased levels of urea, uric acid, cystatin C, NGAL, and creatinine and decreased levels of serum albumin, total protein, sodium, and potassium. These effects were pronouncedly alleviated by treatment with NAC (by −42.8%, −32.8%, −42%, −47.9%, −30.6% for NGAL, cystatin C, creatinine, urea, and uric acid, respectively) and (by +12.1% and +33.1% for sodium and potassium, respectively) compared with the HDF group. The nephroprotective effect was more apparent when NAC was administered as a protective agent. Moreover, treatment with SAMe also caused significant differences in kidney function markers but the levels were less observed than the NAC treated group (by −35.6%, −23.8%, −33.6%, −41.8%, −27.8% for NGAL, cystatin C, creatinine, urea, and uric acid, respectively) and (by +2.1% and +6.1% for sodium and potassium, respectively) compared with the HDF group [Figure 1].

As compared to the control group, the TAC, GSH, and SOD levels in rats given OTA were significantly lower (by 55.2%, 33.6%, and 59.2%, respectively) ($P < 0.01$). The serum levels of NO and MDA were higher ($P \leq 0.01$) in the OTA-intoxicated group (by 71.8% and 161.2% for NO and MDA, respectively, as compared with the control group (G1). NAC or SAMe effectively reversed these effects during oxidant damage. Treatment with SAMe maintained GSH levels higher than NAC [Figure 2].

The results indicated that in comparison to the control group (G1), OTA treatment (G2) significantly ($P \leq 0.01$) increased the serum levels of inflammatory and cellular death biomarkers (CRP, NF-κB, TNF-α, caspase 3, and 8-OHdG) by 258.0%, 89.9%, 141.3%, 134.2%, and 65% for CRP, NF-κB, TNF-α, caspase 3, and 8-OHdG, respectively. Treatment with NAC (G3) and SAMe (G4) caused a significant reduction in inflammatory biomarker levels when compared to OTA-intoxicated group (G2) [Figure 3]. Results revealed a correlation between OTA-induced nephrotoxicity and the elevated serum levels of caspase-3 activity thus indicating apoptosis of renal cells. Furthermore, OTA increased the level of serum 8-OHdG, a marker of oxidative damage of DNA as compared to all other treated groups. On the other hand, an improvement in these biomarker levels was detected following treatment with NAC and SAMe [Figure 3].

Results revealed a correlation between OTA-induced immunotoxicity and the reduced white blood cell count (WBCs) (by 30%), monocytes (by 49.5%), lymphocytes (by 26.6%), and total neutrophils (by 22.9%). Mycotoxin intake causes hematological abnormalities, primarily thrombocytopenia and leukopenia. Treatment with NAC (G3) caused an increase in RBCs, platelets count, and Hb levels when compared to OTA group (G2). Moreover, treatments with the SAMe (G4) significantly improved the WBCs count, RBCs, platelets, and Hb concentration [Figure 4].

Discussion

The current study's findings proved that the administration of OTA caused nephrotoxicity, as evidenced by notable alterations in the levels of serum kidney function biomarkers. In comparison to the normal control group, OTA treatment resulted in elevated levels of urea, uric acid, cystatin C, NGAL, creatinine, and lower levels of serum total protein, albumin, sodium, and potassium. Numerous investigations demonstrated that prolonged exposure to mycotoxins causes nephropathies.[17]

The lowering effect of OTA on glomerular filtration and protein catabolism rates, which reduced the renal capacity to eliminate urea, may be the cause of the elevated urea and creatinine levels. Concentrations of total proteins are widely used as indicators of humoral immunity. Vascular leakage, an inability to generate proteins, and elevated proteolysis rates could have all contributed to a drop in protein levels.[18] The mechanisms of OTA-induced nephrotoxicity imply DNA damage and inhibition of protein synthesis.[19]

Oxidative stress is a key mechanism for OTA-induced nephrotoxicity. Because OTA accumulates in the proximal tubule epithelial cells and causes oxidative stress, which in turn triggers an inflammatory response and cellular damage, it is recognized as a potent nephrotoxin. OTA causes oxidative stress in the kidneys of rats by raising MDA and NO levels and lowering GSH and SOD. In addition, OTA-induced NO synthase (NOS) expression causes nitrosative stress in kidney cells.[20] OTA increases the production of NO in many kinds of kidney cell lines.[21,22]

The results of the present study exposed that rats fed on diets contaminated with OTA demonstrated a significant reduction in TCA and elevated serum levels of caspase-3 activity indicating apoptosis of renal cells. The results also found that cystatin C and NGAL values were significantly elevated after kidney injury. When kidney damage occurs, NGAL is expressed in the tubular epithelium and is increased in the affected cells.[23]

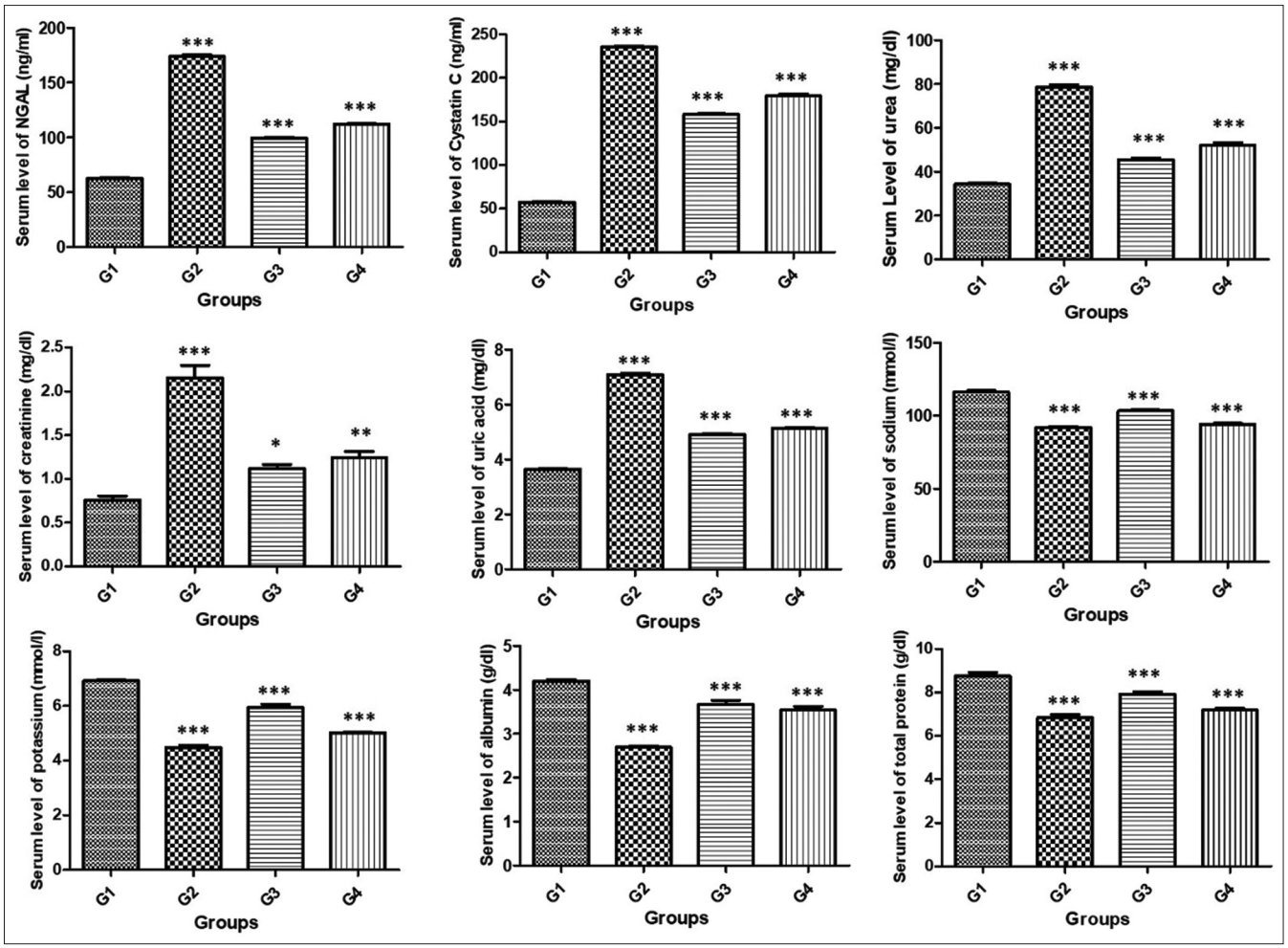

Figure 1: The nephrotoxic effect of different treatments on kidney function biomarkers. G1: Control group; G2: OTA intoxicated group; G3: OTA + NAC group, and G4: OTA + SAMe group. The *P*-values were calculated using one-way ANOVA test. OTA: Ochratoxin A, NAC: N-acetylcysteine, SAMe: S-adenosyl-L-methionine.

N-acetyl cysteine has remarkable antioxidant potential as a significant therapeutic representative, reducing cell damage induced by oxidative stress.[24] NAC might be used as an accessory treatment aspect in urinary tract infections to reduce nephrotoxic adverse reactions of antibiotic drugs. These results supported that N-acetyl cysteine has serious experimental consequences as a renal protective agent of OTA.[25] In the present study, administration of NAC decreased OTA-induced nephrotoxicity and restored kidney functioning. This was demonstrated by the restoration of serum creatinine, urea, uric acid, albumin, sodium, potassium, cystatin C, NGAL, and total proteins. Furthermore, the serum MDA level was significantly lowered, and the primary enzymatic antioxidants' activity was significantly increased. The findings of this investigation are consistent with previous studies which observed that NAC can decrease serum creatinine and reduce the level of cystatin C.[26] Furthermore, NAC might improve GFR in hypertensive rats.[27] In addition, NAC has demonstrated the ability to attenuate various inflammatory pathways mediated by NF-κB as well as to restore the intracellular redox imbalance, indicating potential anti-inflammatory, antiviral, and antioxidant effects.[28]

The convinced effects of NAC are linked to its potent free radical scavenger activity. Due to its free sulfhydryl group, it can immediately react with substances that are electrophilic, such as free radicals. In addition, it increases the production of cellular GSH, which strengthens the natural antioxidant system. These could help to explain how NAC was able to increase antioxidant enzyme activity in our study. NAC has been used to prevent the condition of nephropathy.[29] NAC is utilized to prevent the development of contrast-induced nephropathy because of its antioxidant and vasorelaxant properties, which lower ROS and kidney tissue damage, decrease vasoconstriction, and stabilize renal hemodynamics.[30] Through the direct and indirect antioxidant effects of GSH, NAC may dismiss renal damage, improve renal flow, decrease vasoconstriction, decrease renal cell death, promote cell repair, and enhance the expression of NOS.[31] According to the above-mentioned findings, NAC was crucial in protecting the kidneys against OTA intoxication, and its protective effects were moderately attributed to the control of oxidative stress.

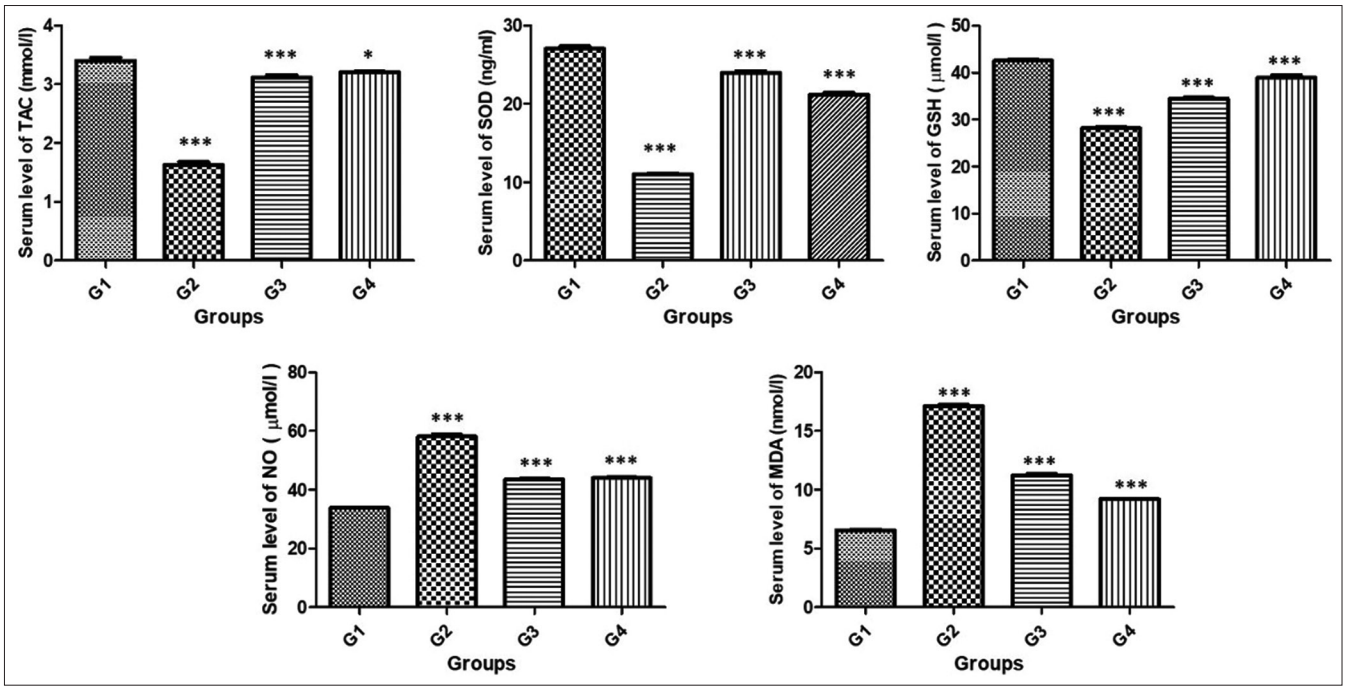

Figure 2: The effect of all treatments on serum levels of nitrosative and oxidative stress biomarkers. G1: Control group; G2: OTA intoxicated group; G3: OTA + NAC group, and G4: OTA + SAMe group. The *P*-values were calculated using one-way ANOVA test. TAC: Total antioxidant capacity, SOD: Superoxide dismutase, GSH: Reduced glutathione, NO: Nitric oxide, MDA: Malondialdehyde, OTA: Ochratoxin A, NAC: N-acetylcysteine, SAMe: S-adenosyl-L-methionine

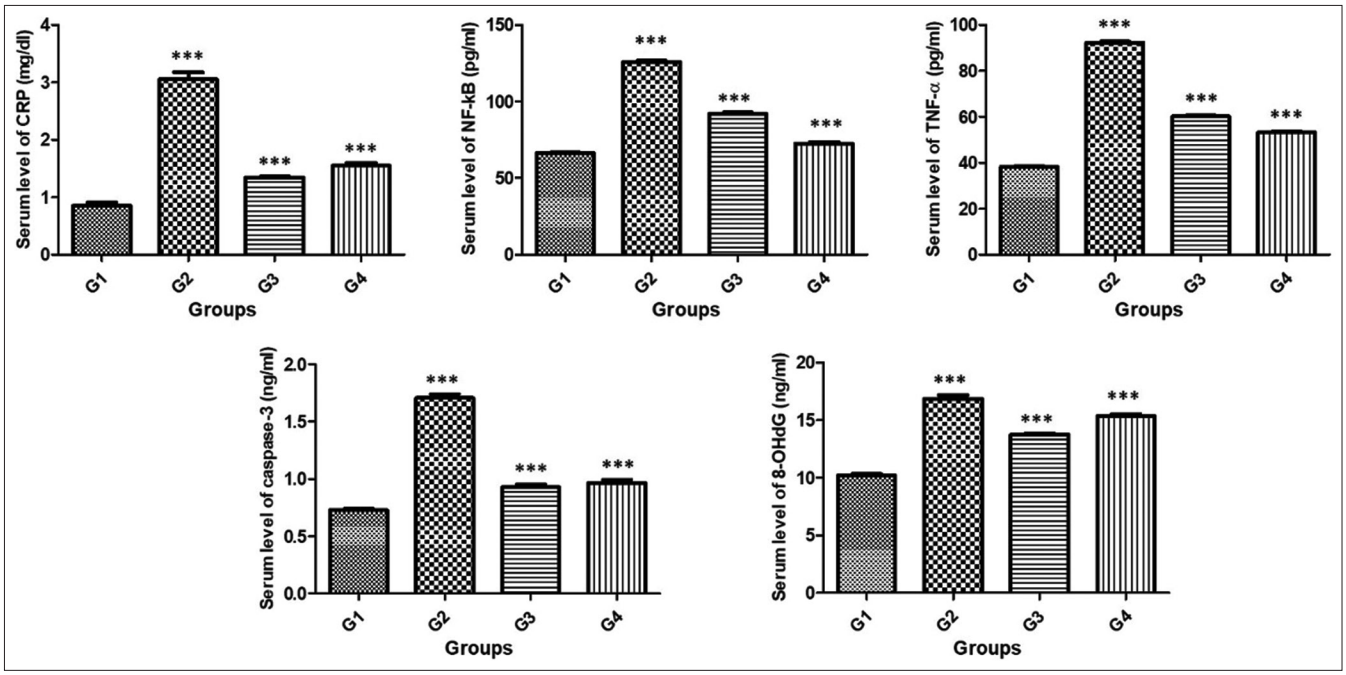

Figure 3: Effect of all treatments on serum level of inflammatory and cellular death biomarkers. G1: Control group; G2: OTA intoxicated group; G3: OTA + NAC group, and G4: OTA + SAMe group. The *P*-values were calculated using one-way ANOVA test. CRP: C-reactive protein, NF-κB: Nuclear factor kappa B, TNF-α: Tumor necrosis factor-alpha, 8-OHdG: 8-hydroxy-2deoxyguanosine, OTA: Ochratoxin A, NAC: N-acetylcysteine, SAMe: S-adenosyl-L-methionine

SAMe works as a cosubstrate in the anabolic processes of transmethylation, transsulfuration, and aminopropylation. It is an active intermediate metabolite of methionine and a precursor of choline, cysteine, and GSH.[32] The activity of SAMe is specified by the existence of an active sulfur atom and methyl group in its structure. The transmethylation reaction boosts phosphatidylcholine synthesis and keeps the phospholipid bilayer of the cell membrane.[33] SAMe may be thought of as

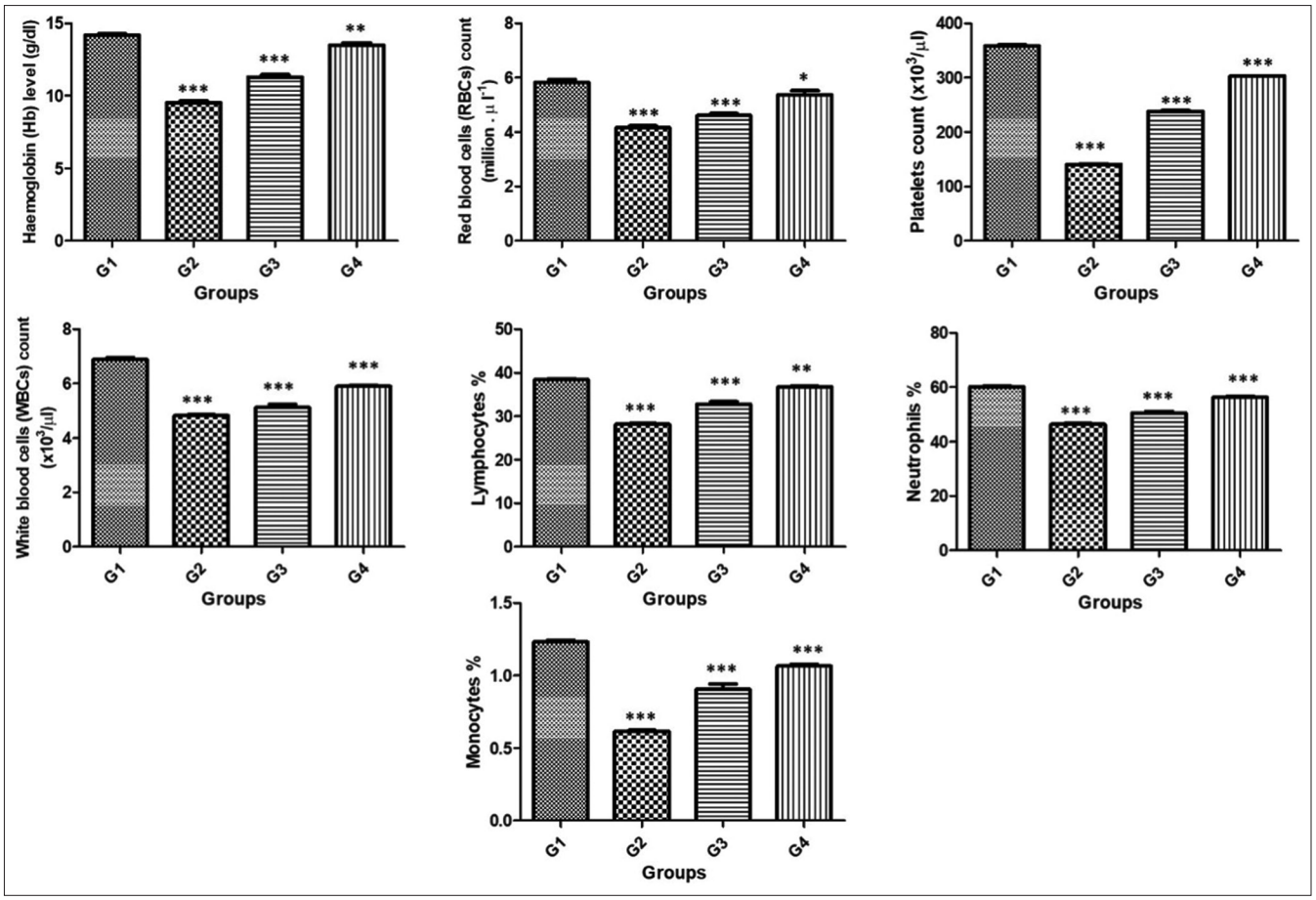

Figure 4: Hematological effect of all treatments on the levels of hemoglobin, red blood cells, platelets, and white blood cell count. G1: Control group; G2: OTA intoxicated group; G3: OTA + NAC group, and G4: OTA + SAMe group. The *P*-values were calculated using one-way ANOVA test. OTA: Ochratoxin A, NAC: N-acetylcysteine, SAMe: S-adenosyl-L-methionine

a cytoprotective agent because numerous pathogenic causes predominantly damage cell membranes, which results in disruptions in cellular metabolism.[34] The body's detoxification system includes GSH and sulfates which are produced during the transsulfuration reaction. The aminopropyl groups from SAMe are transferred to polyamines necessary for protein synthesis, which stimulates cell growth and promotes cell regeneration.[35]

The result of the present study showed that SAMe prevented severe damage to cells. A considerable rise in serum albumin and a reduction in creatinine, urea, and uric acid when compared to OTA-intoxicated rats showed that excretory kidney function has been preserved. In addition to its cytoprotective abilities, SAMe has the potential to increase the production of neurotransmitters during transmethylation reactions. The protective role of SAMe facilitated the prevention of the major ions from loss which is evidenced by an increase in the reabsorption of sodium and potassium. SAMe may have a direct protective impact against OTA-induced oxidative stress by scavenging free radicals and preventing lipid peroxidation by lowering MDA and NO levels, or it may have an indirect protective effect by boosting the antioxidant activity of SOD and TAC.[36] SAMe contributes to the synthesis of endogenous GSH, an antioxidant, and cofactor of antioxidant enzymes and replenishes its pool. The antioxidant action promotes the recovery of renal tubular cell membrane function and strengthens nephrocytes' resistance to ROS damage.[37]

Inflammatory processes have been hypothesized to be crucial in the development of kidney disease.[38] Increasing suggestions have proposed that some inflammatory markers including WBC count and TNF may be linked with kidney function and could expect the risk of renal function loss.[39] Results of the present study revealed a correlation between OTA-induced immunotoxicity and the reduced WBC count, monocytes, lymphocytes, and total neutrophils. The OTA-intoxicated group displayed also a decrease in RBCs count, platelets, and hemoglobin level. These results supported the findings of Sawale *et al*.,[40] who revealed a significant decrease in the erythrocytes count in broiler chickens fed an OTA-contaminated diet. Inhibiting protein synthesis and causing lipoperoxidation are potential causes of OTA immunotoxicity as well as its known nephrotoxicity.[41] Because OTA binds firmly to albumin, so its removal by glomerular filtration is minimal. As an alternative, the primary excretion route in the kidney is tubular secretion, and tubular reabsorption could

be comparatively responsible for intracellular accumulation of OTA.[42] OTA depletes antioxidant reserves and impairs immunological function, which makes animals more vulnerable to metabolic disorders and tissue damage.[43]

Due to the immune system's complexity, the immunotoxicity of mycotoxins cannot be fully identified and evaluated by a single parameter. In general, the immunotoxicity of mycotoxins is identified as the adverse effects on the functioning of both local and systemic immune systems. Mycotoxins were immunotoxic, according to earlier studies, because of their immunosuppressive properties.[44] As a strong inhibitor of protein synthesis, OTA suppresses the immune system by delaying the cell division of the immune system.[45] In the current study, NAC enhanced the suppressed immune response induced by OTA intoxication. The increased numbers of total leukocytes, monocytes, and lymphocytes suggested a healthier immunological response. Furthermore, simultaneous induction with SAMe possibly counteracts the reduction in total RBCs and Hb concentration induced by OTA. Recently, it was discovered that SAMe elevated DNA methylation and then reduced the ability of regulatory T cells (Treg cells) to suppress immune responses by lowering protein levels. Treg cells suppress the immune system and counteract the antitumor effects of cytotoxic T cells.[46] This finding highlights SAMe's therapeutic potential for forthcoming uses in immunotherapy. SAMe also participates in the folic acid cycle, affects one-carbon metabolism, and is an essential methionine cycle intermediate.[47]

Conclusion

Our study is the first to compare SAMe with NAC as an effective remedy for OTA toxicity. The administration of SAMe or NAC daily before feeding diets contaminated with OTA showed that NAC was more effective than SAMe in reducing OTA renal toxicity, The nephroprotective effect manifests by the maintenance of the kidney function and restoration of the prooxidant-antioxidant in kidneys of animals. The present study demonstrated that SAMe modulated CRP, NF-κB, and TNF-α production by different mechanisms.

Ethics Approval and Consent to Participate

The Research Ethics Committee of Ain Shams University evaluated and approved the experimental procedures of this study (Code Sci. 1432305001).

Availability of Data and Materials

The data sets used in this study are available with the corresponding author and will be provided on a reasonable request.

Competing Interest

The authors have no conflict of interest to declare.

Authors' Contributions

Prof. Fares Khalifa and Prof. Huda Al Doghaither supervised the project. Dr. Nesrin Tarbiah and Dr. Nuha Alkhattabi performed and analyzed the experiments, Prof. Ayat Al-Ghafari and Dr. Aliaa Sabban reviewed and validated the analysis, statistics, and grammar checking at the final stages and all the authors had written the manuscript, reviewed the manuscript, and agreed to submit it.

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