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Simultaneous Treatment with Benzoic Acid and Salicylic Acid Prevented Ochratoxin A Toxicity in Female Mice

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Abstract

MYCOTOXINS are biologically active, toxic secondary metabolites characterize by adverse health effects in both humans and animals. The present study aims to evaluate the protective effects of benzoic and salicylic acids against Ochratoxin A (OTA) that used to experimentally inducing of toxicity in female. A total of 12 mice were purchased, acclimated and divided equally and randomly into 4 groups as following; G1 (received only OTA), G2 (received OTA and benzoic acid), G3 (received OTA and salicylic acid), and G4 (not received). The findings revealed that mice exposed exclusively to OTA showed severe clinical symptoms such as poor general condition, reduced body weight gain, increased organ weights, as well as increased biochemical markers indicating liver damage, renal dysfunction and cardiac damage. Histopathological examination revealed significant tissue damage in liver, kidney, and heart tissues, confirming the severity of OTA-induced ochratoxicosis. Conversely, mice treated simultaneously with OTA and either benzoic or salicylic acids were showed substantial improvements in health status, including restored body and organ weights, normalization of biochemical parameters, and reduced histopathological damage, comparable to control animals. The findings clearly demonstrate that benzoic and salicylic acids can potentially ameliorate the health status of animals exposed to OTA toxicity.

Keywords: Ochratoxin A (OTA), Ochratoxicosis, Benzoic acid, Salicylic acid, Mycotoxins, Mice, Detoxification.

Introduction

Worldwide, there are more than 400 types of mycotoxins which are being heat-stable, persisting even after food processing, and consequently posing significant health risks [1]. Mycotoxins are biologically active, toxic secondary metabolites produced by fungi, primarily belonging to the genera *Aspergillus*, *Fusarium*, and *Penicillium*. Exposure to these toxins results in mycotoxicosis, a condition characterized by adverse health effects in both humans and animals. Among mycotoxins, aflatoxin (AF) and OTA are considered particularly hazardous to domesticated animals [2]. The harmful effects of mycotoxins depend on several factors, including the type of toxin, concentration, duration of exposure, and the overall health status of the exposed organism [3, 4]. According to the Food and Agricultural Organization (FAO), 25-50% of global agricultural crops are contaminated with mycotoxins [5, 6]. Human exposure occurs either directly through consumption of contaminated food or indirectly by the animal products derived from livestock previously exposed to mycotoxins [7]. OTA is one of the most toxic and prevalent mycotoxins, is primarily produced by fungi such as *Aspergillus nigri*, *A. circumdati*, *Penicillium verrucosum*, and *P. nordicum*. OTA contamination in agricultural commodities poses a serious risk to human and animal health globally [8- 10]. Compared to ochratoxins B and C, OTA is considered the most relevant due to its widespread occurrence and toxicity. OTA exposure has severe health implications, including acute symptoms such as liver

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and kidney dysfunction, allergic reactions, immunosuppression, and, in severe cases, death. Chronic exposure can lead to mutagenic, teratogenic, and carcinogenic effects [11]. OTA has also been detected in animal feed, human food, and even in up to 80% of human blood samples from various Western countries. OTA exposure in animal studies has demonstrated effects like reduced heart rate, hypotension, altered blood pressure, and impaired cardiac function [12]. The International Agency for Research on Cancer (IARC) has classified OTA as a possible human carcinogen (Group 2B), based on sufficient evidence from animal studies [13]. Various organic acids, including lactic, acetic, and propionic acids, have demonstrated antifungal activities by disrupting fungal cellular functions and amino acid absorption [1, 14]. Additionally, certain organic acids could exhibit antioxidant, anticancer, anti-inflammatory, hypoglycemic, and immunological properties [15]. The present study aims to evaluate the efficacy of benzoic and salicylic acids against OTA- toxicity in female albino mice under *invivo* conditions, considering their availability, low cost, low toxicity, and environmental safety.

Material and Methods

Preparation of OTA

The extract of *OTA* was produced by the isolate *Penicillium verrucosum* No. M/16, originally isolated from fresh apricot fruits, at a concentration of 46.1 ng/ml. The process of production was included the inoculation of yeast extract broth medium (containing 2% yeast extract and 15% sucrose per liter distilled water), as described by Ronald [16], with 1 ml fungal suspension containing 10^5 colony-forming units (CFU)/ml of *Penicillium verrucosum* isolate No. M/16. The inoculated medium was incubated at 28 ± 2°C for 15 days. The resulting mycelial mat was disrupted using a sterile glass rod and filtered through filter paper. The filtrate, containing OTA (referred to as the mother solution), was stored at 4°C for subsequent use. The OTA concentration was verified by High-Performance Liquid Chromatography (HPLC) according to AOAC [17].

Experimental Design

Female albino mice (6 weeks old) were obtained from the Animal House Laboratory, National Research Centre (Dokki, Cairo, Egypt). Mice were housed under controlled environmental conditions (temperature: 25°C, with a 12-hour light/dark cycle).

Experimental Protocol and Animal Groups

Mice were randomly divided into four groups (3 mice per group), (Table 1). OTA was administered orally at a dose of 2 ml per mouse daily for four weeks, and animals were monitored daily for any clinical signs of toxicity.

Blood Collection

After four weeks (day 28), blood samples were collected into anticoagulant tubes and stored at −20°C for biochemical analysis of liver and kidney functions, following methods described by Mangano et al. [18].

Biochemical Evaluations

Biochemical parameters were assessed using commercial kits according to the manufacturers' instructions, as follows:

- Transaminase Activity (ALT and AST): Measured using kits from Bio-Mérieux SA [19].
- Uric Acid Levels: Determined using an enzymatic colorimetric kit (Point Scientific Inc., USA [20].
- Creatinine Levels: Measured using commercial kits (San Antonio, Texas, USA [21].
- CK Enzyme Activity: Determined by Boehringer Mannheim kits (Germany), according to methods described by Stoev [22].

Histopathological Studies

Following blood collection, mice were euthanized humanely under anaesthesia (ether), and then sacrificed by cervical decapitation. Liver, kidney, and heart tissues were collected for histopathological analysis.

Tissue Fixation and Sectioning

Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5µm of thickness, and processed in the Histopathology Department, National Research Centre (Dokki, Egypt).

Staining Procedure

Sections were stained using Hematoxylin and Eosin (H&E), allowing clear differentiation of cytoplasm and nuclei and visualization of pathological changes [23].

Examination and Imaging

Slides were examined using a light microscope, and representative images were captured and processed using Adobe Photoshop version 8.0.

Statistical Analysis

Data analysis included the Kruskal-Wallis and Mann-Whitney tests for nonparametric comparisons and One-Way ANOVA for parametric data. Multiple comparisons were conducted using the Least Significant Difference (LSD) test. A p-value at < 0.05 was considered statistically significant. Data were visualized using Microsoft Excel (version 2019).

Results

OTA Production

Analysis of OTA production revealed that the fungal isolate *Penicillium verrucosum* No. M/16 produced OTA at a concentration of 46.1 ng/ml, as indicated by the chromatogram (Figure 1A, B).

Pathological Studies

Effects of Benzoic and Salicylic Acid on General Health Symptoms in OTA-exposed Mice

The bioassay was conducted to assess the protective efficacy of benzoic and salicylic acids against OTA- toxicity in mice. Throughout the study, no mortality was observed in any treatment group. In Group 4 that not exposed to OTA, maintained good health and exhibited normal behaviour. In contrast, mice exposed solely to OTA (Group 1) demonstrated clear signs of toxicity and general deterioration in health compared to the control group. Conversely, mice receiving OTA combined with benzoic acid (Group 2) or salicylic acid (Group 3) displayed significantly improved health status, with fewer signs of toxicity indicating the protective role of these organic acids against OTA toxicity as shown in Table (2). This demonstrates that both benzoic and salicylic acids effectively mitigate the toxic effects of OTA, reducing the severity of ochratoxicosis symptoms in mice.

Effects of Benzoic and Salicylic Acid on Body Weight Gain in OTA-exposed Mice

The protective effects of benzoic and salicylic acids on body weight gain in OTA-exposed mice are summarized in Table (3). Mice treated exclusively with OTA (Group 1) showed reductions in body weight gain over the four-week experimental period. Their mean body weight declined from an initial value of 25.3g at day 0 to 24g by day 28, representing an overall weight loss of approximately 28%. In contrast, mice co-treated with OTA and benzoic acid (Group 2) demonstrated significant improvements in body weight, increasing from an initial average of 21.3g to 28g by day 28, representing a weight gain of approximately 16%. Similarly, mice treated with OTA and salicylic acid (Group 3) exhibited a significant increase in body weights, improving from 22.7g initially to 29g at the end of the study, corresponding to a 13% increase in weight. These observations confirm that treatment with either benzoic or salicylic acid effectively counteracts the negative effects of OTA on body weight gain in mice.

Protective Effects of Benzoic and Salicylic Acid on Liver Function in OTA-Exposed Mice

The protective effects of benzoic and salicylic acids against OTA-induced liver toxicity were evaluated based on serum biochemical markers of liver function (ALT and AST), as shown in Table (4). Mice exposed only to OTA (Group 1) exhibited a significant increase in serum ALT and AST activities, with values reaching 108.7U/I (91.0% increase) and 185 U/I (99.1% increase), respectively, compared to the control group. However, mice co-treated with OTA and benzoic acid (Group 2) demonstrated significant reductions in ALT and AST enzyme activities, with levels of 77.8U/I (36.7% reduction) and 158.3U/I (70.4% reduction), respectively, compared to the OTA-only group. Similarly, mice treated with OTA plus salicylic acid (Group 3) showed further significant improvements, with ALT and AST levels decreasing to 65.3U/I (14.8% above control) and 124.3U/I (33.8% above control), closely approximating the values observed in the control group. These findings indicate that treatment with benzoic or salicylic acid effectively mitigates OTA-induced hepatic injury, significantly restoring liver enzyme activities towards normal levels.

Protective Effects of Benzoic and Salicylic Acid on Kidney Functions in OTA-Exposed Mice

The protective effects of benzoic and salicylic acids against Ochratoxin A (OTA)-induced kidney damage were evaluated based on serum urea and creatinine levels, as presented in Table (5). Mice exposed solely to OTA (Group 1) exhibited significant elevations in serum urea and creatinine concentrations, recording values of 70.5mg/dl (49.4% increase) and 1.0mg/dl (150% increase), respectively, compared to the untreated control group. In contrast, co-treatment with OTA and benzoic acid (Group 2) significantly reduced urea and creatinine levels to 63.1mg/dl (33.7% reduction) and 0.7mg/dl (75% reduction), respectively. Similarly, co-treatment with OTA and salicylic acid (Group 3) further significantly improved these parameters, reducing serum urea and creatinine concentrations to 59.0mg/dl (25% above control) and 0.6mg/dl (50% above control), respectively. These improvements closely approached the normal values observed in the control group (47.2mg/dl for urea, 0.4mg/dl for creatinine). These findings clearly indicate that benzoic and salicylic acids effectively protect against OTA-induced renal dysfunction, as evidenced by normalized serum biochemical markers of kidney function.

Protective Effects of Benzoic and Salicylic Acids on CK Enzyme Levels and Heart Function in OTA-Exposed Mice

The protective effects of benzoic and salicylic acids against OTA- cardiotoxicity were evaluated by assessing serum creatine kinase (CK) enzyme levels, as summarized in Table (6). Mice exposed to OTA alone (Group 1) demonstrated significantly elevated serum CK levels, recording 331.3U/L, representing a 55% increase compared to the control group. Increased CK activity is indicative of cardiac muscle injury and dysfunction associated with OTA

exposure. Mice co-treated with OTA and benzoic acid (Group 2) showed a significant reduction in CK levels, measuring 291.3U/L, representing a reduction to a 36.3% elevation above control levels. More pronounced improvements were observed in the group receiving OTA with salicylic acid (Group 3), where CK levels were further significantly reduced to 247.7U/L, approximately 16% above control values.

Histopathological Studies

Effects of OTA on Liver Tissue in Exposed Mice

Histopathological examination confirmed significant alterations in liver tissues following exposure to OTA. Liver sections from the control group (G4) exhibited normal hepatic architecture with no detectable pathological changes. Conversely, liver tissues from mice exposed to OTA alone (Group 1) demonstrated marked histopathological damage, indicative of ochratoxicosis. OTA exposure resulted in multifocal, randomly distributed areas of coagulative necrosis affecting approximately 35% of hepatic parenchyma. The necrotic lesions were characterized by retained cellular architecture but displayed loss of differential staining, hyper-eosinophilic cytoplasm, and nuclei exhibiting pyknosis, karyorrhexis, or complete absence (Fig. 2A). In several necrotic areas, severe disruption of hepatic cord structure occurred, accompanied by hepatocyte dissociation, sinusoidal disorganization, and replacement by hemorrhage and necrotic debris (Fig. 2B). Additionally, hepatocytes adjacent to necrotic zones displayed hydropic degeneration, characterized by swollen, pale, vacuolated cytoplasm and vesicular nuclei (Fig. 2C). Diffuse central vein congestion was also observed, frequently accompanied by moderate lymphoplasmacytic infiltration in perivascular and portal areas (Fig. 2D). Occasionally, fibrin thrombi were present within portal veins.

Protective Effects of Benzoic and Salicylic Acids on OTA-Induced Liver Tissue Damage

Histopathological analysis clearly demonstrated the protective effects of benzoic and salicylic acids against liver injury. Liver sections from mice treated with OTA combined with benzoic acid (Group 2) predominantly retained normal hepatic architecture. Only occasional, small foci (approximately 5% of tissue) showed mild coagulated necrosis with minor degeneration of hepatocytes, characterized by loss of differential staining and swollen, pale, microvacuolated cytoplasm (Fig. 2E). Mild congestion and dilation of portal veins, with slight lymphoplasmacytic infiltration in portal and periductal areas, were also observed (Fig. 2F). Similarly, mice receiving OTA combined with salicylic acid (Group 3) exhibited remarkable hepatic protection, maintaining normal liver architecture across more than 95% of examined tissues. Minor degenerative changes were occasionally seen, characterized by mild hepatocyte degeneration and pale microvacuolation (Fig. 2G). Rare, mild edema and lymphoplasmacytic infiltrates around bile ducts in portal areas were noted (Fig. 2H). These findings underscore the potent hepatoprotective properties of benzoic and salicylic acids against OTA-induced hepatotoxicity.

Effects of OTA on Kidney Tissue in Exposed Mice

Histopathological examination revealed significant pathological changes in kidney tissues following OTA exposure. Kidney sections from control mice (Group 4) exhibited normal histological structures without detectable abnormalities. In contrast, severe histopathological lesions were observed in mice exposed to OTA alone (Group 1), consistent with ochratoxicosis-induced renal injury. Approximately 50% of cortical renal tubules exhibited coagulative necrosis characterized by retained tubular architecture but with indistinct cell boundaries, loss of differential staining, hyper-eosinophilic cytoplasm, and nuclear pyknosis (Fig. 3A). In severely affected areas, disruption of tubular architecture, dissociation of tubules, capillary disorganization, and replacement by hemorrhage and necrotic debris were noted (Fig. 3B). Tubular epithelial cells in the renal cortex also showed hydropic degeneration with swollen, pale, vacuolated cytoplasm (Fig. 3C). Additional observations included extensive tubular degeneration, hemorrhagic infiltration, and marked necrosis (Fig. 3D). Focal glomerular changes included moderate hypercellularity, mild thickening of Bowman's capsule, hypertrophied parietal epithelial cells, congested vessels, and mild inflammatory edema in cortical interstitium (Fig. 3E). Tubules within the renal medulla were ectatic, containing proteinaceous eosinophilic (hyaline) and cellular casts, accompanied by interstitial edema and lymphocytic infiltration (Fig. 3F).

Protective Effects of Benzoic and Salicylic Acids on OTA-Induced Kidney Tissue Damage

Histopathological assessment confirmed protective effects of benzoic and salicylic acids on OTA-induced renal damage. Kidney sections from mice co-treated with OTA and benzoic acid (Group 2) displayed predominantly normal renal architecture. Mild tubular degeneration and limited coagulative necrosis affected approximately 10% of the cortex (Fig. 3 G&H). Scattered interstitial lymphoplasmacytic infiltration was occasionally observed. Similarly, kidney tissues from mice treated with OTA and salicylic acid (Group 3) showed excellent preservation of renal architecture (>95%). Only minor pathological changes, including mild degeneration or occasional tubular necrosis, were noted (Fig. 3 I&J). Mild interstitial lymphocytic infiltration was also present. These observations

demonstrate that benzoic and salicylic acids significantly ameliorate OTA-induced renal damage.

Effects of OTA on Heart Tissue in Exposed Mice

Histopathological examination showed no alterations in heart tissues of control mice (Group 4) (Fig. 4A). However, mice exposed exclusively to OTA (Group 1) exhibited multifocal coagulative necrosis of cardiomyocytes (Fig. 4B), necrotic cardiac myocytes with fragmented, hyper eosinophilic sarcoplasm, loss of cross striations, and pyknotic nuclei (arrowhead) (Fig. 4C), severe cardiotoxic effects characterized by multifocal areas of coagulative necrosis affecting approximately 30% of myocardium. Necrotic cardiomyocytes retained structural outlines but demonstrated hyper-eosinophilic sarcoplasm, loss of cross striations, and nuclear pyknosis (Fig. 4E).

Protective Effects of Benzoic and Salicylic Acids on OTA-Induced Heart Tissue Damage

Histopathological analysis confirmed that benzoic and salicylic acids significantly reduced OTA-induced myocardial damage. Hearts from mice treated with OTA and either benzoic or salicylic acid showed largely normal myocardial structure (~95%), with minimal evidence of mild coagulative necrosis affecting few cardiomyocytes (Fig. 4 D&F). These results highlight benzoic and salicylic acids as effective protective agents against OTA cardiotoxicity.

Discussion

Analysis of OTA production demonstrated that the *P. verrucosum* is capable of producing OTA at a concentration of 46.1ng/ml. These findings align with the results reported by Fredj et al. [24], who found that 3% of *Penicillium* isolates obtained from grapes were capable of producing OTA. Specifically, OTA concentrations produced by *Aspergillus* species ranged from 6.53 × 10^-3 to 6.82µg/ml, whereas *Penicillium* species produced 0.24- 1.53µg/ml. Moreover, Olsen et al. [25] indicated that fungal infections can result in significant contamination of agricultural products with various mycotoxins, including aflatoxin, patulin, citrinin, fumonisin, and OTA. Similarly, Rahimi and Shakerian [26] reported detectable OTA contamination in dried fruit samples (apricots, figs, raisins, dates), with contamination rates reaching approximately 10.4%, and OTA concentrations ranging from 2.3 to 14.2ng/g.

We conducted a bioassay to determine the protective efficacy of benzoic and salicylic acids against OTA-induced toxicity in mice. Our findings reveal that both benzoic and salicylic acids are effective in mitigating the harmful effects of OTA and reducing the severity of ochratoxicosis symptoms observed in the mice. These results align with findings by Stoev [22], who reported that rats exposed to OTA showed neurological disturbances, including tremors, after two weeks of treatment, especially at higher doses. Mehtab et al. [27] similarly noted OTA toxicity in poultry, with affected animals displaying stunted growth, general weakness, and higher mortality rates, emphasizing that the severity of OTA effects is dose- and duration-dependent. Additionally, previous research highlights the known antifungal and antimicrobial properties of organic acids, supporting their widespread use as food additives and agricultural preservatives [28, 29]. For instance, salicylic acid effectively inhibited germination of *P.expansum* spores and caused irreversible damage to fungal cells within 30 minutes [30, 31]. Huo et al. [32] also reported that salicylic acid exhibited broad-spectrum antifungal activity against pathogens like *A. flavus*, *B. cinerea*, and *F. avenaceum*. Collectively, these findings, including our current results, suggest that benzoic and salicylic acids may be promising compounds for reducing mycotoxin-induced toxicity. Also, treating OTA-exposed mice with benzoic acid or salicylic acid led to a significant restoration of body weight gain, effectively counteracting the toxin's negative effects. These findings are consistent with previous studies by Damiano et al. [33,34], who reported that OTA significantly reduced body weight gain in rats compared to controls after 7 and 14 days of exposure. Similarly, Li et al. [9] observed significant reductions in body weight and organ indices in OTA-treated groups, alongside clinical symptoms such as decreased appetite, weight loss, dehydration, and polyuria. Mehtab et al. [27] highlighted the dose-dependent immunosuppressive and growth-retardation effects of OTA in poultry, leading to decreased body weight and feed intake. Further, Ou et al. [35] reported significantly lower average weights in OTA-treated mice compared to controls, confirming OTA's detrimental effects on growth. Consistent with these reports, the European Food Safety Authority EFSA, [36] documented lower body weight and reduced daily weight gains in pigs and rabbits exposed to OTA at dietary concentrations as low as 0.025mg/kg feed, underscoring the substantial impact of OTA on animal performance and growth.

Benzoic and salicylic acid treatments effectively mitigated OTA-induced hepatic damage in mice, leading to a significant recovery of liver enzyme activities. These results align well with earlier findings by Aydin et al. [37], who reported severe liver and kidney damage as major pathological outcomes of OTA exposure, even at low doses. Similarly, Stoev et al. [38], Biro et al. [39], and O'Brien and Dietrich [40] correlated increased liver enzyme activities (e.g., γ-GT, AST, ALT, ALD, and alkaline phosphatase) with degenerative hepatic and renal changes following OTA exposure in pigs. Hassan et al. [41] and Bharathi et al. [42] also reported severe liver and kidney dysfunction in chickens fed diets contaminated with OTA at

concentrations as low as 100 ppb. Further, recent studies by Stoev [22] demonstrated significantly elevated serum AST and ALT activities in rats exposed to high OTA doses, highlighting OTA's severe hepatotoxicity. Similarly, Mehtab et al. [27] reported marked increases in liver enzymes alongside hepatic enlargement in poultry exposed to OTA at 400-800µg/kg feed. Moreover, Raja et al. [43] and the EFSA [36] documented significant increases in serum ALT, AST, ALP, creatinine, and urea, alongside metastatic calcifications in internal organs of pigs exposed to OTA. On the other hand, administration of benzoic and salicylic acids provided effective protection against OTA-induced renal dysfunction in mice, evidenced by the restoration of serum biochemical markers of kidney function to normal levels. Previous studies support these findings, demonstrating OTA's significant toxicity toward kidney tissues. Stoev et al. [38], Biro et al. [39], and O'Brien and Dietrich [40] reported increased serum enzymes associated with kidney and liver damage (e.g., γ-GT, AST, ALT, and ALP) following OTA exposure. Li et al. (2020) observed significant increases in serum uric acid (UA) and blood urea nitrogen (BUN) in OTA-treated mice, confirming severe renal toxicity. These findings were further supported histologically through observed renal tissue damage using H&E staining. Similarly, Mehtab et al. [27] and Rhee et al. [44] documented increased serum creatinine and uric acid levels alongside renal enlargement in poultry exposed to OTA, indicating severe renal impairment. Additionally, OTA toxicity is known to disrupt liver and colon health by altering intestinal microbiota, contributing to systemic inflammation and exacerbating renal dysfunction. The EFSA [36] further confirmed that OTA exposure significantly increases serum creatinine, urea, potassium, and alkaline phosphatase, while reducing total protein and glucose levels in exposed animals. High doses (2.5mg/kg feed) administered to piglets resulted in renal fibrosis, elevated serum creatinine, and urea, demonstrating OTA's severe nephrotoxic potential. Benzoic and salicylic acids also significantly protected against OTA-induced myocardial injury, effectively normalizing CK activity. These results agree with the findings of Wilson [45] and Hussein and Arbid [12], who demonstrated that OTA significantly impacts cardiovascular function, including reductions in heart rate, hypotension, and impaired myocardial performance in rats. Such cardiovascular alterations were associated with electrolyte disturbances, decreased serum calcium and potassium levels, and elevated plasma uric acid and creatinine levels following OTA exposure. Additionally, Hussein and Arbid [12] described significant cardiac abnormalities resulting from OTA intoxication, including abnormal sinus rhythms, ectopic beats, and atrioventricular block. They also reported that OTA could disrupt cardiac function through mitochondrial respiration inhibition, highlighting OTA's potent cardiovascular toxicity. Further supporting these observations, Stoev [22] found that OTA exposure significantly elevated serum CK and lactate dehydrogenase (LDH) enzyme activities in exposed animals, confirming myocardial and muscular damage. Overall, these findings reinforce our results, demonstrating the efficacy of benzoic and salicylic acids in alleviating OTA-induced cardiotoxic effects.

Histopathological examination of liver tissues in OTA-treated mice revealed significant alterations and substantial damage, confirming the presence of ochratoxicosis. These findings highlight OTA's significant hepatotoxic potential. Similar hepatic lesions were reported by Aydin et al. [37], who found extensive granular degeneration, central vein dilation, bile duct proliferation, periportal inflammatory infiltration, and parenchymal necrosis in OTA-exposed rats. Stoev [46] further confirmed OTA-induced severe degenerative changes in liver and kidneys, depletion of lymphoid organs, brain edema, muscle hemorrhages, and bone marrow alterations. Additionally, multi-organ damage following OTA exposure had been demonstrated including granular degeneration and fatty changes in hepatocytes [9, 22]. A number of authors also highlighted OTA-induced histopathological damage across various organs, confirming the extensive systemic toxicity associated with ochratoxicosis [27, 33, 44]. In contrast, histological examination of liver sections from mice treated with OTA alongside benzoic acid or salicylic acid revealed that the normal hepatic architecture was largely preserved, with over 95% of examined tissues exhibiting a typical structure. The presence of only mild coagulative necrosis and minor hepatocyte degeneration indicates significant hepatic protection, underscoring the potent hepatoprotective properties of benzoic and salicylic acids against OTA-induced liver damage. These results correlate with findings by Mehtab et al. [27], who documented similar protective outcomes against ochratoxin-induced histopathological damage in poultry. Collectively, these studies support the therapeutic potential of organic acids in reducing OTA toxicity. On the other hand, severe histopathological lesions, indicative of ochratoxicosis-induced renal injury, were evident in the kidney tissues of mice exposed to OTA. These results correlate closely with previous studies [9, 37, 47] which reported similar OTA-induced pathological changes in kidney tissues, including severe tubular necrosis, degeneration, and inflammatory infiltration. Other studies were further support these findings, confirming that OTA exposure consistently causes severe renal lesions [27, 36, 44, 46]. In contrast to the OTA-only group, kidney tissue examination from mice co-administered OTA and benzoic acid showed primarily normal renal architecture with minimal

tubular degeneration. Notably, mice treated with OTA and salicylic acid exhibited exceptional preservation of renal architecture, with over 95% of the tissue appearing normal. These observations demonstrate that benzoic and salicylic acids significantly ameliorate OTA-induced renal damage, aligning with histopathological outcomes previously reported by Mehtab et al. [27], who noted substantial organ protection by organic acids against ochratoxin toxicity. Additionally, histological analysis of heart tissues from mice exposed solely to OTA revealed significant and severe cardio toxic effects. These pathological findings support prior researches that documented OTA-induced cardiotoxicity, including myocardial necrosis, cardiac dysfunction, abnormal sinus rhythms, decreased heart rate, and significant blood pressure alterations [9, 12, 27]. OTA's toxicity mechanism, notably through mitochondrial ATP inhibition and increased oxidative stress, underpins these cardiac changes. While, treatment with benzoic or salicylic acid in mice exposed to OTA resulted in a significant reduction in OTA-induced myocardial damage, with only sparse indications of mild coagulative necrosis in a limited number of cardiomyocytes observed histologically. These results highlight benzoic and salicylic acids as effective protective agents against OTA cardiotoxicity, corroborating similar protective outcomes documented by prior studies [12, 27].

Conclusion

The present study demonstrated that benzoic and salicylic acids effectively reduced the toxic effects of Ochratoxin A (OTA) in female albino mice. Mice treated with OTA alone exhibited significant adverse effects, including reduced body weight gain, organ damage, elevated biochemical markers of liver (ALT, AST, and ALP), kidney (urea, and creatinine), and heart function (CK), as well as severe histopathological damage in liver, kidney, and heart tissues. In contrast, mice co-treated with OTA and either benzoic acid or salicylic acid showed marked improvements in body weight gain, normalization of organ weights, and significant restoration of biochemical parameters toward normal levels. Furthermore, microscopic examinations confirmed substantial histological recovery in liver, kidney, and heart tissues, closely resembling those of control animals. These findings suggest that benzoic and salicylic acids possess strong protective effects against OTA-induced toxicity and can effectively mitigate ochratoxicosis symptoms.

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Declarations

Ethical approval

This study was approved by the Medical Research Ethics Committee at the National Research Centre, Egypt. (No. 0842).

Consent to participate

Not applicable

Consent for publication

Not applicable

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding statement

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TABLE 1. Experimental groups

Groups (3 mice per group)	Treatments
Group 1 (positive control)	Exposed to 46.1 ng/ml OTA alone (28 days' orally)
Group 2	Exposed to 46.1 ng/ml OTA + benzoic acid (40 mg/kg BW.) (28 days' orally)
Group 3	Exposed to 46.1 ng/ml OTA + salicylic acid (40 mg/kg BW.) (28 days' orally)
Group 4 (negative control)	Control (no OTA exposure; fed a basal diet only)

BW: body weight. OTA: Ochratoxin A

TABLE 2. Weekly progression of clinical symptoms of Ochratoxin A toxicity in mice

Groups	Week 1	Week 2	Week 3	Week 4
Group 1	Subtle changes in activity, slight decrease in food intake,very minor slowdown in weight gain. Appearance mostly normal.	Noticeable lethargy, reduced food intake, definite slowed weight gain. signs of polyuria appeared.	Pronounced lethargy, significant weight loss, rough coat,appearance of tremors Diarrhea appeared.	Severe lethargy, marked weight loss. Prominent signs of illness(e.g., severe piloerection, dull eyes).
Group 2	Minimal to no noticeable changes; very slight reduction in activity or food intake.	Significantly reduced lethargy, food intake closer to control, better weight gain. Piloerection may be very mild.	Moderately reduced lethargy, better food intake and weight stability. Posture and coat improved. Diarrhea less frequent.	Slightly reduced weight. Appearance largely normal.
Group 3	Minimal to no noticeable changes; very slight reduction in activity or food intake.	Improved activity/food intake/weight gain. Less severe piloerection.	Improved lethargy, better food/weight. Posture and coat improved. Diarrhea less frequent	Generally active, stable or improved weight. Appearance largely normal.
Group 4	Normal activity, consistent food intake, healthy weight gain. Normal appearance.	Sustained normal activity,consistent food intake,continued healthy weight gain. Normal appearance.	Normal activity and posture, healthy food intake and weight gain. Clean, well-kept fur.	Continued robust health, improved weight optimal activity. No signs of illness

TABLE 3. Effect of benzoic and salicylic acid treatments on body weight gain (g) in OTA-exposed mice.

Group	Day 0	Day 7	Day 14	Day 21	Day 28	% Change (Day 28 vs Day 0)
Group 1	25.3 ± 0.15 [a]	23.7 ± 0.32 [a]	23.7 ± 0.91 [a]	24.0 ± 0.27 [c]	24.0 ± 0.34 [c]	-5.1%
Group 2	21.3 ± 0.27 [a]	24.7 ± 0.41 [a]	24.7 ± 0.68 [a]	26.0 ± 0.14 [bc]	28.0 ± 0.64 [b]	+31.5%
Group 3	22.7 ± 0.38 [a]	25.0 ± 0.28 [a]	25.9 ± 0.37 [a]	28.0 ± 0.19 [b]	29.0 ± 0.79 [b]	+27.8%
Group 4	23.7 ± 0.10 [a]	26.0 ± 0.13 [a]	27.0 ± 0.68 [a]	32.7 ± 0.27 [a]	33.3 ± 0.28 [a]	+40.5%

Results are mean values of three replicates ± standard deviation. Differences considered significant at $p \leq 0.05$.

TABLE 4. Concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes after 28 days in mice exposed to ochratoxin A and simultaneously treated with benzoic acid or salicylic acid.

Group	ALT (U/I)	Change (%)	AST (U/I)	Change (%)
Group 1	108.7± 0.55 [a]	+91.0	185.0± 0.52 [a]	+99.1
Group 2	77.8± 0.61 [b]	+36.7	158.3± 0.46 [b]	+70.4
Group 3	65.3± 0.34 [b]	+14.8	124.3± 0.39 [c]	+33.8
Group 4	56.9± 0.27 [c]	–	92.9± 0.27 [d]	–

ALT: Alanine Transaminase, AST: Aspartate Transaminase **.**Results are mean values of three replicates ± standard deviation. Statistical significance determined by One-Way ANOVA ($P \leq 0.05$)

TABLE 5. Concentrations of urea and creatinine after 28 days in mice exposed to ochratoxin A and simultaneously treated with benzoic acid or salicylic acid.

Group	Urea (mg/dl)	Change (%)	Creatinine (mg/dl)	Change (%)
Group 1	70.5± 0.81[a]	+49.4	1.0± 0.22 [a]	+150
Group 2	63.1± 0.58 [b]	+33.7	0.7± 0.39 [b]	+75
Group 3	59.0± 0.32 [b]	+25.0	0.6± 0.74 [b]	+50
Group 4	47.2± 0.82 [c]	–	0.4± 0.34 [c]	–

Results are mean values of three replicates ± standard deviation. Statistical significance was assessed using One-Way ANOVA ($P \leq 0.05$).

TABLE 6. Concentrations of creatin kinase (CK) after 28 days in mice exposed to ochratoxin A and simultaneously treated with benzoic acid or salicylic acid.

Group	CK Enzyme (U/L)	Change (%)
Group 1	331.3± 0.15 [a]	+55.0
Group 2	291.3± 0.34 [b]	+36.3
Group 3	247.7 ± 0.91 [c]	+16.0
Group 4	213.7± 0.19 [d]	–

CK Enzyme: Creatine Kinase Enzyme. Results are mean values of three replicates ± standard deviation. Statistical significance assessed using One-Way ANOVA ($P \leq 0.05$).

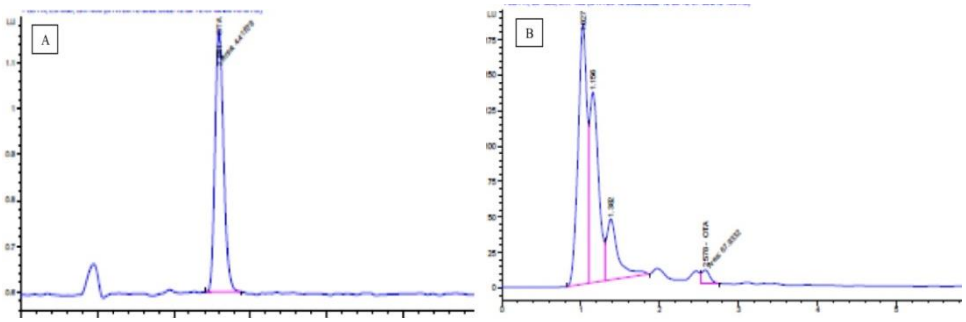

Fig. 1. HPLC chromatogram (A) Standard Ochratoxin A (OTA). (B) OTA produced by *Penicillium verrucosum* isolate No. M/16.

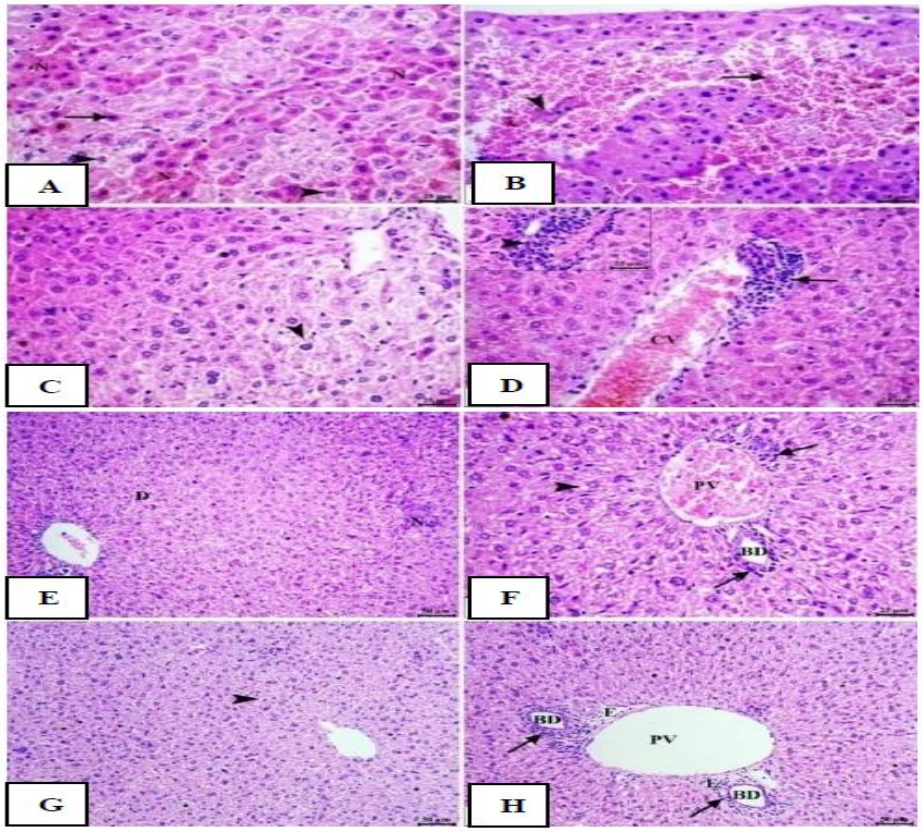

Fig. 2. Liver sections of mice from different experimental groups.

1- (A - D) Ochratoxin A (OTA) group, where (A) Multifocal areas of coagulative necrosis of the hepatic parenchyma characterized by retention of hepatocytes architecture with hypereosinophilic cytoplasm and pyknotic (arrow), karyorrhectic (arrowhead), or absent nuclei. (B) Disruption of hepatic cord architecture with dissociation of hepatocytes and replacement with hemorrhage (arrow) and necrotic debris (arrowhead). (C) Hydropic degeneration of the hepatocytes characterized by swollen pale vacuolated cytoplasm (arrowhead) and vesiculate nuclei. (D) Marked congestion of central vein with perivascular lymphoplasmacytic infiltrate (arrow). Inset: similar inflammatory reaction (arrowhead) in the portal area. N: necrosis, CV: central vein. 2- (E & F) Ochratoxin A (OTA) plus Benzoic acid-treated group, where (E) Maintained normal architecture in the majority of hepatic parenchyma with small foci of coagulative necrosis and mild degeneration of hepatocytes. (F) Congestion of the portal vein with mild perivascular and periductal lymphoplasmacytic infiltrate (arrow) in the portal area; note also high magnification of degenerated hepatocytes with pale eosinophilic micro vacuolated cytoplasm (arrowhead). 3- (G & H) Ochratoxin A (OTA) plus Salicylic acid-treated group, where (G) Maintained normal hepatic architecture with mild degeneration (arrowhead) of the few hepatocytes. (H) Mild portal edema admixed with lymphoplasmacytic infiltrate (arrow) surrounding the bile ducts. D: degeneration, N: necrosis, PV: portal vein, BD: bile duct and E: edema.

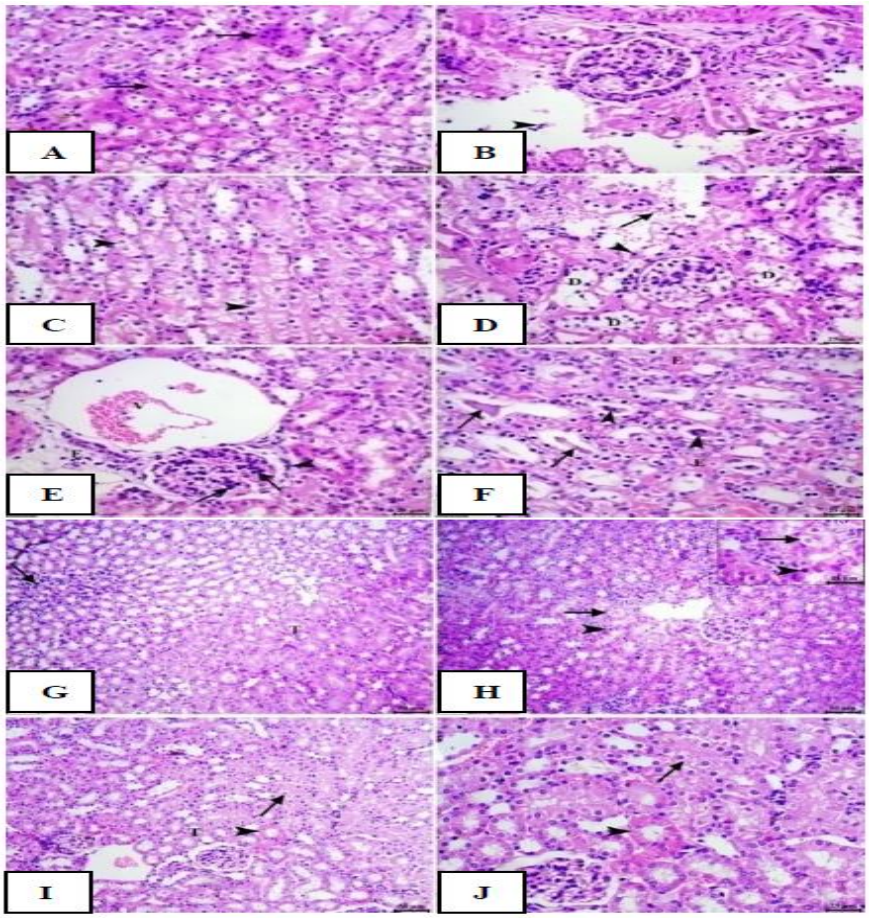

Fig. 3. Kidney sections of mice from different experimental groups.

1- (A - F) Ochratoxin A (OTA) group, where (A) Coagulative necrosis (arrow) of tubular epithelium characterized by retention of cellular architecture, indistinct cell borders, hyper eosinophilic cytoplasm, and nuclear pyknosis. (B) Severe disruption of tubular architecture in the necrotic area with dissociation of tubules, disorganization of capillaries, and replacement with hemorrhage (arrow) and necrotic debris (arrowhead). (C) Hydropic degeneration of tubular epithelium of renal cortex characterized by swollen pale vacuolated cytoplasm (arrowhead). (D) Extensive degeneration, severe disruption of tubular architecture and replacement with hemorrhage (arrow) and necrotic debris (arrowhead). (E) Moderate hypercellularity of glomerular tuft (arrow), with mild thickening of Bowman's capsule and hypertrophied parietal epithelial cells (arrowhead); note also congested blood vessels and perivascular inflammatory edema. (F) Renal medulla exhibits interstitial inflammatory edema and ectatic tubules contain protein aceous eosinophilic casts (arrow) and cellular casts (arrowhead). N: necrosis, D: degeneration, E: edema and V: blood vessel. 2- (G & H) Ochratoxin A (OTA) plus Benzoic acid-treated group, where (G) Normal renal architecture in the majority of renal tubules note small interstitial aggregates of inflammatory cells (arrow). (H) Mild degeneration (arrow) and coagulative necrosis (arrowhead) of tubular epithelial cells. Inset: higher magnification. 3- (I & J) Ochratoxin A (OTA) plus Salicylic acid-treated group, where (I) Normal renal architecture in the majority of renal tubules with mild degeneration (arrow) and coagulative necrosis (arrowhead) of a few tubular epithelial cells. (J) Higher magnification of degeneration of tubular epithelium with microvacuolated cytoplasm (arrow), and coagulative necrosis with hypereosinophilic cytoplasm and nuclear karyolysis (arrowhead). T: renal tubule.

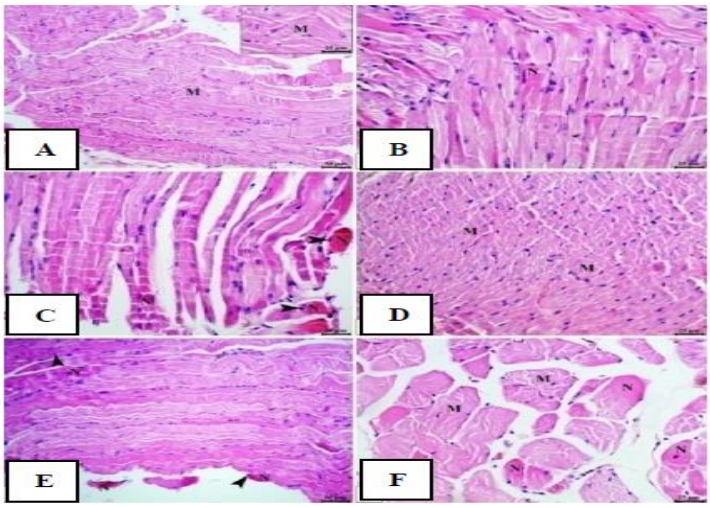

Fig. 4. Heart sections of mice from different experimental groups.
(A) Control group, normal histological features of cardiomyocytes with eosinophilic sarcoplasm, cross striations, and centrally located nuclei. Inset: higher magnification. (B) Ochratoxin A (OTA) group, multifocal coagulative necrosis of cardiomyocytes. (C) Ochratoxin A (OTA) group, necrotic cardiac myocytes with fragmented, hyper eosinophilic sarcoplasm, loss of cross striations, and pyknotic nuclei (arrowhead). (D) Ochratoxin A (OTA) plus Benzoic acid-treated group, normal architecture of cardiomyocytes in the majority of the myocardium with mild coagulative necrosis. (E) Ochratoxin A (OTA) group, coagulative necrosis of cardiomyocytes exhibits retention of architecture, with hyper eosinophilic sarcoplasm, loss of cross striations, and pyknotic nuclei (arrowhead). (F) Ochratoxin A (OTA) plus Salicylic acid-treated group, the normal architecture of the myocardium, with occasional mild coagulative necrosis of few cardiomyocytes. M: myocytes and N: necrosis.

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العلاج المتزامن بحمض البنزويك وحمض الساليسيليك يمنع سمية الأوكراتوكسين أ في إناث الفئران

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الملخص

تم إكتشاف أكثر من 400 نوع من السموم الفطرية، والتي يظهر العديد منها تأثيرات سمية حادة تعتمد على نوع السم، ومستويات التعرض، ومدته، والحالة الصحية للكائنات الحية المتأثرة. هدفت الدراسة الحالية إلى تقييم التأثيرات الوقائية لحمضي البنزويك والساليسيليك ضد السمية الناتجة عن التسمم بالأوكراتوكسين أ (OTA) في إناث الفئران (*In vivo*). تم إنتاج الأوكراتوكسين أ بتركيز 46.1 نانوغرام/مل بواسطة عزلات *Penicillium verrucosum* رقمM/16 ، المعزولة من ثمار المشمش الطازجة. أظهرت الفئران المعرضة للأوكراتوكسين أ أعراضًا حادة شملت تدهورًا في الصحة العامة، وانخفاضًا في زيادة وزن الجسم، وزيادة في أوزان الأعضاء، وارتفاعًا في القياسات البيوكيميائية الدالة على تلف الكبد (ALT، AST)، واختلال وظائف الكلى (اليوريا، الكرياتينين)، وإنزيم القلب (CK). على الجانب الأخر كشف الفحص الهستوباثولوجي عن تلف نسيجي كبير في أنسجة الكبد والكلى والقلب، مما يؤكد شدة التسمم بالأوكراتوكسين أ المستحث. وعلى النقيض من ذلك، أظهرت الفئران التي تم معاملتها بحمض البنزويك أو حمض الساليسيليك تحسينات كبيرة في الحالة الصحية، بما في ذلك استعادة وزن الجسم والأعضاء، وتحسن في كل من القياسات البيوكيميائية، والفحص الهستوباثولوجي، بشكل مماثل للحيوانات في مجموعة الكونترول. لذا تشير النتائج بوضوح إلى أن كل من حمضي البنزويك والساليسيليك يمتلكان خصائص وقائية ومضادة للسموم ، مما يخفف بشكل فعال من السمية المستحثة بالأوكراتوكسين أ وأعراض التسمم به.

الكلمات الدالة: أوكراتوكسين أ، تسمم الأوكراتوكسين، حمض البنزويك، حمض الساليسيليك، السموم الفطرية، الفئران، إزالة السمية.