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Hepato-Renal Protective Effects of Nano-Kaolin Conjugated with Amino and Organic Acids (Biocid®) in Drug-Induced Toxicity Models in Wistar Rats

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Abstract | The liver plays a fundamental role in metabolism, detoxification, and the synthesis of essential compounds, while the kidneys are crucial for regulating fluid balance, electrolytes, and waste elimination. Consequently, the integrity of these two organs is vital for overall health. Both the liver and kidneys are susceptible to various forms of chemical-induced injury, which can lead to severe systemic complications. Many conventional therapeutic agents seriously harm liver and/or kidneys, necessitating the exploration of novel protective agents. This experiment explores the hepato-renal protective effects of nano-kaolin conjugated with a synergistic blend of amino and organic acids (NK-AA-OA, proprietary name Biocid®) in a drug-induced toxicity model. Fifty Wistar rats were divided into 2 experiments. Toxicity was induced using large doses of paracetamol (experiment 1) or gentamicin (experiment 2). Treatment with NK-AA-OA (300 mg/kg body weight daily for 5 days) significantly attenuated the elevations in serum hepatocellular enzymes (ALT, AST, GGT), bilirubin, urea, and creatinine levels observed in the both drug-intoxicated groups. Furthermore, NK-AA-OA demonstrated potent antioxidant activity, evidenced by reduced serum malondialdehyde (MDA) levels and enhanced superoxide dismutase (SOD) and glutathione (GSH) activities. Histo- and immuno-histopathological examination revealed a marked reduction in cellular degeneration, inflammation, vascular congestion, and apoptotic response associated with paracetamol/gentamicin toxicity in livers and kidneys of NK-AA-OA treated animals. These findings suggest that the Biocid® possesses significant hepato-renal protective effects, likely through its antioxidant, anti-inflammatory, detoxification and/or pharmacokinetic interaction mechanisms, highlighting its potential as a novel food-supplement in preventing organ damage.

Keywords | Nano-Kaolin, Amino acids, Organic acids, Hepatoprotective, Nephroprotective

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INTRODUCTION

This study aimed to investigate the hepato-renal protective effects of nano-kaolin conjugated with a blend of amino and organic acids (pharmaceutical product under proprietary name of Biocid®) in an *in vivo* rat's model of drug-induced liver and kidney injuries. The findings from this research could pave the way for developing novel,

clay-based therapeutic strategies for organ protection. Liver and kidneys are the top vital organs responsible for metabolism, detoxification, excretion, and maintaining homeostasis within the body. Their sophisticated functions make them particularly vulnerable to be harmed from a multitude of factors, including environmental toxins, pharmaceutical agents, pathogens, and oxidative stress (Abogresha *et al*., 2016; Pisoschi *et al*., 2021). Hepatic and renal damage can be manifested as acute or chronic conditions, leading to significant morbidity and mortality if left untreated. Current therapeutic approaches often involve symptomatic management, specific antidotes, or organ transplantation in severe cases, all of which carry limitations in terms of efficacy, accessibility, and potential adverse effects. Consequently, there is an urgent need to explore novel, safe, and effective strategies for preventing and mitigating organ damage.

The assessment of natural materials, particularly minerals, in medicinal purposes has gained significant traction in recent years as evidenced by various academic works that explore their efficacy and safety. The article of Wijenayake *et al*. (2016) highlights the antimicrobial potential of traditional herbometallic drugs, emphasizing that certain metal ions possess remarkable antimicrobial properties that surpass those of organic molecules alone.

For biomedical purposes, natural mineral clays (involving kaolin) are gaining more attention year after year, that is due to their biocompatibility, adsorptive properties and high surface area (Dong *et al*., 2021). Kaolin, a hydrous aluminum phyllosilicate, has traditionally been used orally to treat diarrhea owing to its ability to adsorb toxins and pathogens within the gastrointestinal tract (Awad *et al*., 2017). Recent advancements in nanotechnology have enabled the synthesis of nano-kaolin, which exhibits enhanced physicochemical properties, including increased surface area-to-volume ratio, improved reactivity, and potential for sustained drug delivery. These characteristics make nano-kaolin an attractive candidate for various biomedical applications, including detoxification and sustained drug release (Yang *et al*., 2016; Dong *et al*., 2021).

To further augment the therapeutic potential of nano-kaolin, surface modification through conjugation with bioactive molecules offers a promising avenue. Amino acids and organic acids are ubiquitous biomolecules known for their diverse physiological roles. Amino acids like L-cysteine and L-glutamine are precursors to glutathione, a master antioxidant, and play principal roles in detoxification pathways. Furthermore, arginine, glycine and threonine are known for their anti-Inflammatory property while other amino acids are documented for their immune and cognitive support (Wu, 2009). Organic acids, such as citric acid and succinic acid, are integral components of cellular metabolism, possess antioxidant properties, exert anti-inflammatory effects, and can chelate metal ions (Drincovich *et al*., 2016). The combination of these molecules, when delivered through a nano-carrier system like kaolin, could potentially offer enhanced protective effects against organ injury.

In a recent study conducted by El-Naby *et al*. (2025) on the Biocid® product, the researchers found that it improved growth performance and final weights, increased digestive enzyme activities, promoted gut function, and enhanced antioxidant and immune responses at varying levels. The rationale behind conjugating amino and organic acids to nano-kaolin stems from several theoretical advantages: (1) Nano-kaolin can serve as a stable, biocompatible platform for sustained release or enhanced delivery of the conjugated acids; (2) The adsorptive capacity of nano-kaolin might help in binding circulating chemicals and toxins, thereby reducing their burden on the liver and kidneys; (3) The integrated antioxidant and anti-inflammatory properties of the conjugated acids could directly counteract the damage induced by reactive oxygen species and inflammatory mediators; and (4) The nano-scale size could facilitate better biodistribution and cellular uptake of the protective agents.

MATERIALS AND METHODS

Drugs, chemicals and kits

Paracetamol (Injectmol®,10mg/mL) was bought from Amriya Company (Cairo, Egypt) in the form of a sterile liquid solution. Gentamicin sulfate injection solution (Garamycin®, 80mg gentamicin base/2mL) was gained from Memphis Co. for Pharmaceutical and Chemical (Egypt). Boicide® (Nano-kaolin bearing amino and organic acids, NK-AA-OA) was generously donated by Al-Huda Company, Egypt. The NK-AA-OA average primary particle size ranging from 55 to 130 nm. Brunauer-Emmett-Teller (BET) nitrogen adsorption-desorption analysis was approximately 45 m^2/g compared to bulk kaolin. NK-AA-OA compositions are shown in Table 1.

Animals, experimental design and samples

In a clean conditioned room (temperature and humidity ~25°C and 65%, respectively), fifty apparently healthy male Wistar rats (11 weeks age and 164.4±8.3 g weight) were kept for 2 weeks for adaptation before being enrolled in this study. Rats were housed in plastic cages with galvanized wire lids (5 rats per cage,). Rats' commercial pellets and water were provided *ad libitum* throughout the experiment. Rats were acquired from Animal House, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. The animals were randomly assigned to 2 equal experiments conducted in parallel, with 25 rats

allocated to each experiment, experiment (A) was used to investigate the hepato-protective while experiment (B) was used to investigate the nephro-defensive effects. Rats in each experiment were further divided to 5 groups of five rats each. In each experiment, biocid was administered either for 5 days prior to the induction of organ injury by the intoxicant drugs (preventive scenario) or simultaneously with the intoxicant drugs to replicate real-world application scenarios, as illustrated below.

Table 1: Mineral and acids compositions of Biocid®

Mineral composition (PPT)	Amino acid (PPM)	Organic acid (PPM)
SiO_2 (465.6)	L. alanine (797.8)	Tartaric (383.4)
TiO_2 (3.5)	L. glycine (217.8)	Citric (114.4)
Al_2O_3 (86.8)	L. glutamic (5097.9)	Maleic (772.6)
Fe_2O_3 (11.3)	L. serine (1339.3)	Oxalic (1251.4)
MnO (1.0)	L. valine (364.3)	Succinic (6941.3)
MgO (16.4)	L. leucine (647.4)	Fumaric (485.8)
CaO (165.5)	L. isoleucine (751.7)	
Na_2O (8.7)	L. cysteine (214.6)	
K_2O (6.5)	L. proline (289.5)	
P_2O_5 (2.7)	L. methionine (1496.9)	
SO_3 (17.5)	L. aspartic (609.2)	
Cl (5.5)	L. phenylalanine (675.9)	
L.O.I (207.8)	L. arginine (156.7)	
	L. tyrosine (1599.9)	
	L. threonine (1213.2)	
	L. histidine (430.2)	
	L. tryptophane (25.6)	

PPT= parts per thousand, PPM= parts per million, LOI stands for Loss on Ignition (a measure used to determine the amount of volatile substances present in the sample). The first column represents the nano-kaolin mineral composition, while in the second and third columns speak for the active coating AA and OA compositions and concentrations.

Experiment (A): Group 1 (negative control) received solvent vehicle, group 2 (paracetamol intoxicated) was injected with single intra-peritoneal dose of paracetamol at 12:00 PM (750 mg/kg body weight, Hota *et al*., 2022), group 3 (prevention group) received Biocid for 5 days (at 8:00 AM) then paracetamol in previously mentioned doses, group 4 (simultaneous treated) administered with Biocid for 5 days while paracetamol was injected in the 3rd day of treatment with Biocid, group 5 (Biocid group) administered with Biocid solution via stomach gavage at a daily dose of 300 mg/kg body weight for 5 consecutive days.

Experiment (B): was grouped and conducted as experiment (A) with replacing paracetamol with gentamicin (80 mg intramuscularly/kg body weight/day for 5 days, Ali *et al*., 2005) to induce renal injury as subsequent. Group 1 (negative control) received solvent vehicle, group 2 (gentamicin intoxicated) rats were injected with intramuscular dose of gentamicin at 12:00 PM (80 mg/kg body weight/day for 5 days), group 3 (prevention group) received Biocid for 5 days then gentamicin in previously mentioned doses, group 4 (simultaneous treated) received Biocid solution via stomach gavage and then gentamicin i.m. injection for 5 days, group 5 (Biocid group) received Biocid preparation by stomach gavage in a dose of 300 mg/kg body weight daily for 5 days. In the 2 experiments, Biocid was administered 4 hours apart from both paracetamol and gentamicin.

Three days after paracetamol or last dose of gentamicin injections, rats were anaesthetized, then venous blood samples were collected (from inner canthus retro-orbital plexus) in plain centrifuge tubes via a clean capillary tube. After blood clotting and centrifugation, clear sera were achieved and stored at -20 °C to be used for biochemical investigation. Rapidly after blood collection, rats were euthanized to collect liver and kidney samples. Tissue samples were washed in cold buffered saline twice, then fixed in 10% buffered formalin for the histopathological microscopic examination. All experimental steps and conditions were executed according to the "Guide for the Care and Use of Laboratory Animals" following the Egyptian local committee of Suez Canal University, Faculty of Veterinary Medicine (Ethical statement No., 2023056).

Liver and kidney biochemical performance tests

Gamma glutamyl transferase (GGT), alanine aminotransferase (ALT) and aspartate amino transferase (AST) enzymatic activities as well as the total proteins (TP), albumin (ALB), total bilirubin (TB) and direct bilirubin (DB) quantities in serum were assayed colorimetrically consuming commercial kits following the manufacturer's (Laboratory Biodiagnostics Co., Giza, Egypt) recommendations. Regarding to kidney function, urea and creatinine serum concentrations were determined spectrophotometrically using Diamond diagnostic kits (Egypt) as specified by Young *et al*. (1975).

Antioxidant/oxidative status bio-indicators

The total antioxidant capacity (TAC) of Biocid along with serum levels of malondialdehyde (MDA, measured as lipid peroxidation indicator) and glutathione peroxidase (GPx) were assessed at optical densities of 560 nm, 532 nm, and 340 nm, respectively, using colorimetric assay Kits (Thermo Fisher Scientific®, USA) or Biodiagnostic kits from Egypt (Ohkawa *et al*., 1979). Superoxide Dismutase (SOD) and catalase (CAT) activities were measured depending on the inhibit the autoxidation of pyrogallol and decomposition of hydrogen peroxide, respectively (Marklund and Marklund, 1974; Aebi, 1984).

Cellular, histopathological and immunohistochemical inspection

The formalin fixed liver and kidney specimens were subjected to regular histological steps following Bancroft and Gamble (2008) technique. Sketchily, washed organs were dehydrated in ascending grades of alcohol, xylene cleared, embedded and blocked in paraffin, sectioned at 4-5 µm thickness and stained with hematoxylin and eosin. The tissue slides were examined under optical microscope fitted with digital camera (Olympus DP25) at department of Cytology and Histology, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Concerning the immunohistochemical investigation, deparaffinized hepatic and renal sections were processed following the manufacturer's technique. Concisely, deparaffinized organs sections were treated with 0.3% hydrogen peroxide to deactivate endogenous peroxidase, then incubated with anti-caspase-3 antibodies. After rinsing, a secondary antibody was applied, followed by diaminobenzidine for staining. The sections were counterstained, dehydrated, and prepared for microscopy. Caspase 3 staining in liver and kidney slices was analyzed using the Leica Quin 500 program, measuring staining as a percentage in several fields at 400x magnification. The sections with brown hue immunoreaction (positive) were chosen for estimate.

Statistics

Standard statistical procedures were performed on the obtained results. Initially, the equality of variances was assessed using Bartlett's test. One-way variance analysis (ANOVA) followed by Duncan's post hoc tests was employed to compare the differences among group responses at a 95% confidence level ($p \leq 0.05$) when Bartlett's test indicated equal variance. In cases where equal variance was not observed, the nonparametric Kruskal-Wallis test was utilized. By the aid of MINITAB 18.0 scientific software (State College, Pennsylvania, USA), all the statistical analyses were performed.

RESULTS

We postulated that nano-kaolin conjugated amino and organic acids (Biocid®) may prevent or ameliorate drug-induced liver and kidney damages, reduce oxidative stress, and mitigate histopathological alterations in the liver and kidneys of treated rats. For that intention, the following measurements were accomplished.

Liver and kidney biochemical performance tests

As shown in Table 2, the paracetamol-intoxicated group (Group 2) exhibited a significant increase ($p < 0.05$) in serum ALT, AST, GGT, and total and direct bilirubin levels compared to the control group (Group I), indicating acute severe hepatocellular injury. Except for blood albumin, administration of NK-AA-OA, either before or during paracetamol intoxication, significantly ($p < 0.05$) attenuated these elevated levels compared to the paracetamol-intoxicated group. Simultaneous treatment with NK-AA-OA demonstrated a more pronounced reduction than preventive treatment (NK-AA-OA administration before the paracetamol).

Regarding kidney function parameters, the gentamicin-intoxicated group (Group 2) showed a significant increase ($p < 0.05$) in serum creatinine and blood urea nitrogen (BUN) levels compared to the control group, indicating substantial renal dysfunction. Treatment with NK-AA-OA significantly ($p < 0.05$) decreased these elevated renal markers, with the most pronounced reduction observed in the group that received NK-AA-OA simultaneously with the gentamicin injection (group 4). Administration of NK-AA-OA for 5 days (group 5) didn't result in any significant alteration in liver or kidney examined parameters.

Table 2: Influences of nano-kaolin conjugated with amino and organic acids (Biocid®) oral administration on serum hepatic and kidney biochemical parameters in rats intoxicated with high doses of paracetamol or gentamicin.

Groups	ALT u/L	AST u/L	Alb g/dl	GGT u/L	Total bilirubin mg/dL	Direct bilirubin mg/dL	Creatinine mg/dL	BUN mg/dL
Group 1	53.2±4.53^{cd}	92.0±6.07^{c}	4.18±0.14^{a}	2.92±0.24^{c}	0.24±0.02^{b}	0.12±0.02^{b}	1.01±0.14^{b}	19.6±1.89^{c}
Group 2	161.8±11.15^{a}	249.2±27.3^{a}	3.08±0.21^{c}	6.34±0.41^{a}	1.06±0.14^{a}	0.58±0.07^{a}	2.19±0.17^{a}	46.8±2.91^{a}
Group 3	94.2±5.95^{b}	165.8±3.57^{b}	3.48±0.15^{bc}	4.02±0.22^{b}	0.66±0.09^{ab}	0.36±0.05^{ab}	1.19±0.13^{b}	32.6±3.39^{b}
Group 4	70.8±3.46^{c}	104.0±4.63^{c}	3.66±0.16^{bc}	3.48±0.15^{bc}	0.42±0.04^{b}	0.18±0.04^{ab}	1.10±0.08^{b}	26.0±2.07^{bc}
Group 5	50.2±3.98^{d}	106.6±5.58^{c}	4.22±0.12^{a}	2.86±0.30^{c}	0.26±0.02^{b}	0.12±0.03^{b}	0.94±0.09^{b}	18.4±1.78^{c}

Group 1 (non-intoxicated control group), group 2 (paracetamol or gentamicin intoxicated group), group 3 (prevention group) received Biocid for 5 days before high doses of paracetamol or gentamicin, group 4 (simultaneous treated) administered with paracetamol or gentamicin during Biocid treatment, group 5 (Biocid group) non-intoxicated rats administered with Biocid (300 mg/kg body weight) for 5 consecutive days. AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; GGT=Gama glutamyl transferase; Alb=albumin and BUN=blood urea nitrogen. For each group, n=5; and for each column, significant differences ($p \leq 0.05$) are represented by different superior small litters.

Antioxidant/oxidative status bio-indicators

The paracetamol/gentamicin combination significantly ($p < 0.05$) increased MDA and decreased GSH levels, SOD, and CAT activities in intoxicated rat group (group 2), indicating severe oxidative stress. Biocid ® (NK-AA-OA) treatment, especially when administered during intoxication, significantly ($p < 0.05$) reversed these changes. MDA levels were notably reduced, while GSH content and the activities of SOD and CAT were significantly elevated in NK-AA-OA treated group (group5) compared to the toxicant-nontreated group (group 2). The observed antioxidant effects were comparable to or better than those of the negative control (Table 3).

Table 3: Oxidative stress of paracetamol/gentamicin high doses in rats' sera and its counteraction by nano-kaolin conjugated with amino and organic acids (Biocid®).

Groups	MDA umol/L	GSH mg/dl	SOD (U/mL)	CAT (U/mL)
Group 1	3.68±0.23^{c}	22.6±1.44^{a}	325.8±33.7^{a}	206.8±33.6^{a}
Group 2	7.94±0.34^{a}	11.5±0.69^{c}	108.6±12.2^{c}	71.4±10.1^{c}
Group 3	6.68±0.37^{b}	16.4±1.17^{bc}	262.6±26.0^{a}	123.8±13.1^{bc}
Group 4	4.20±0.33^{c}	13.5±3.14^{c}	190.6±19.0^{b}	118.4±15.3^{c}
Group 5	3.40±0.33^{c}	21.2±1.16^{ab}	311.4±24.4^{a}	186.4±29.8^{ab}

Group 1 (non-intoxicated control group), group 2 (paracetamol/gentamicin intoxicated group), group 3 (prevention group) received Biocid for 5 days before high doses of paracetamol/gentamicin, group 4 (simultaneous treated) administered with paracetamol/gentamicin during Biocid treatment, group 5 (Biocid group) non-intoxicated rats administered with Biocid (300 mg/kg body weight) for 5 consecutive days. MDA = malondialdehyde; GSH= glutathione; SOD= superoxide dismutase; CAT = catalase. For each group, n=5; and for each column, significant differences ($p \leq 0.05$) are represented by different superior small litters.

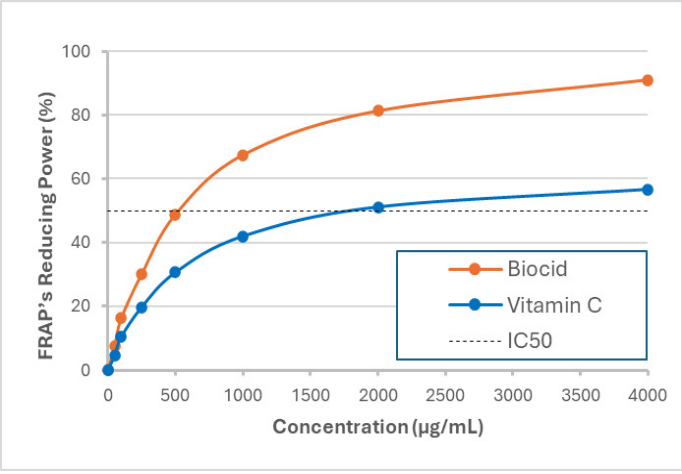

Figure 1: IC50 value of Biocid (521 µg/mL) and vitamin C (1760 µg/mL) against ferric radical solution. IC50 values were obtained from a concentration response curve that detects ferric reducing antioxidant power (FRAP) at optical densities of 560 nm.

The IC50 value of Biocid® against ferric radical, which quantifies the total antioxidant capacity, was found to be 521 µg/mL. This value was about 3.5 times lower than that of L-ascorbic acid (IC50 was 1.76 mg/mL), indicating high TAC of Biocid® compared with vitamin C, the used reference drug in our experiment (Figure 1).

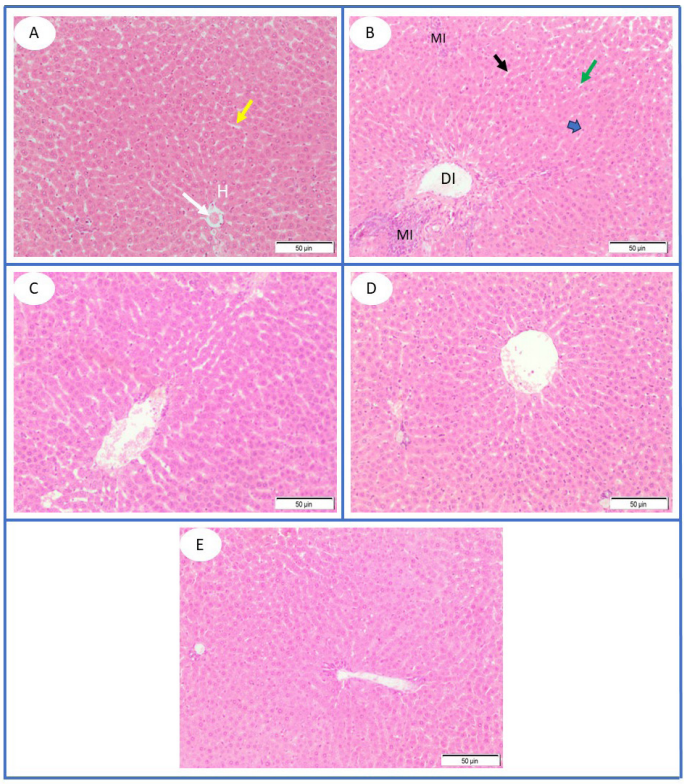

Figure 2: Representative photomicrograph of H and E-stained hepatic sections of control and all treated rats; group 1 (A), group 2 (B), group 3 (C), group 4 (D) and group 5 (E). Central vein (white arrow), hepatocyte (H), hepatic sinusoids (yellow arrow), congested and dilated central vein (DI) and hepatic nusoids (black arrow), pyknotic hepatocytes (thick blue arrow), hepatocytic vacuolization (green arrow), mononuclear cells infiltration (MI).

Histopathological results

Photomicrographs of H and E-stained hepatic (Figure 2) and renal (Figure 3) tissues, obtained from control and all treated rats. The light microscopy assessment of H and E-stained liver sections, obtained from control group, revealed regular hepatic architecture with distinct normal hepatocytes arranged in cord-like pattern, radiating from the central vein to the peripheral of the hepatic lobule, where narrow hepatic sinusoids are located in-between. Hepatocytes appeared as polyhedral cells, having eosinophilic cytoplasm and spherical vesicular nuclei with prominent nucleoli. The hepatic sinusoids were lined by Kupffer cells and endothelial cells (Figure 2A). On the opposite, the hepatic architecture in group 2 (paracetamol overdose) displayed disarrangement and disruption of

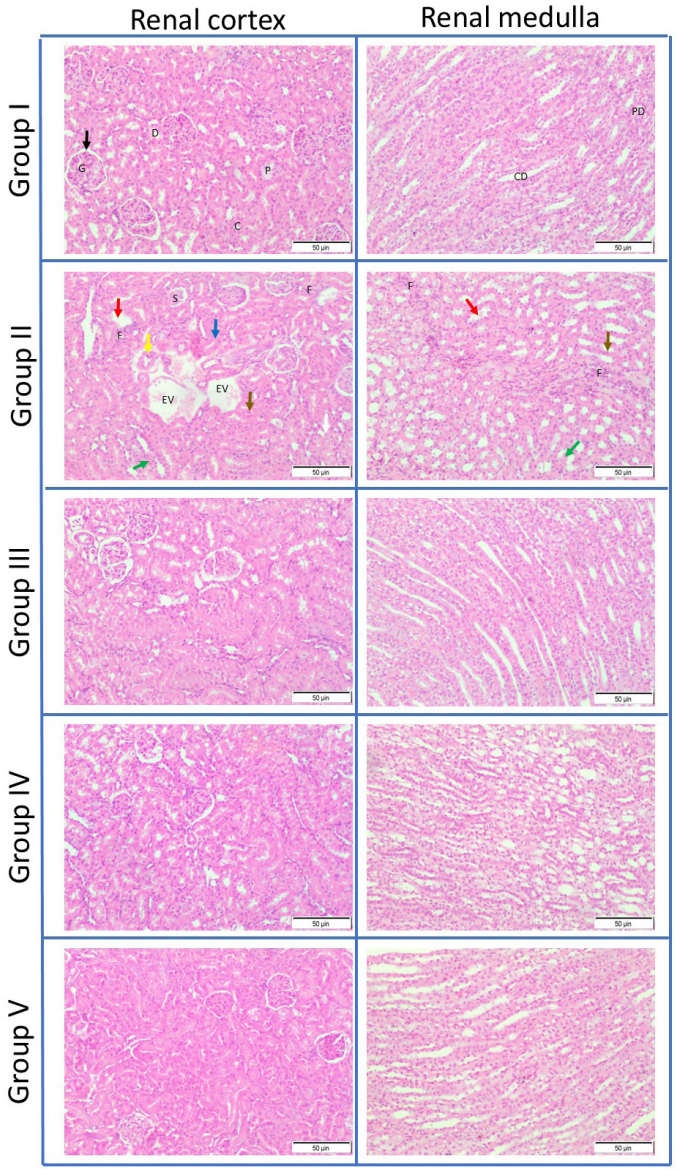

Figure 3: Representative photomicrograph of H and E- stained renal sections (cortex and medulla) of control and all treated rats; groups 1, 2, 3, 4 and 5. Glomerulus (G), Bowman' capsule (black arrow), proximal convoluted tubule (P), distal convoluted tubule (D), connecting tubules (C), collecting duct (CD), papillary duct (PD), shrunken glomerulus (S), interstitial inflammatory cells infiltration (F), extravasated red blood cells (EV), tubular cell swelling (blue arrow), dilatation of tubular lumen (red arrow), vascular congestion (yellow arrow), exfoliated and sloughed epithelial cells (green arrow), pyknotic nuclei (brown arrow).

hepatocytic cords along with Kupffer cell hyperplasia and a sign of hepatocytic degeneration. The hepatocytes lost their normal structure, and vacuolization appeared in their cytoplasm. Additionally, numerous hepatocytes appeared with shrunken darkly stained pyknotic nuclei. Moreover, the central veins were markedly congested and dilated with areas of sinusoidal dilatation and mononuclear cells infiltration (Figure 2B). Liver of group 3 demonstrated mild hepatic variations like central vein and sinusoidal congestion and dilation. Some of hepatocytes showed little cytoplasmic vacuoles, along with darkly stained pyknotic nuclei (Figure 2C). However, hepatic tissue of group 4 showed lesser damages when compared to groups 2 and 3 (Figure 2D). Meanwhile, group 5 exhibited a marked alleviation and restoration of normal appearance of hepatocyte and central vein with a mild sinusoidal congestion (Figure 2E).

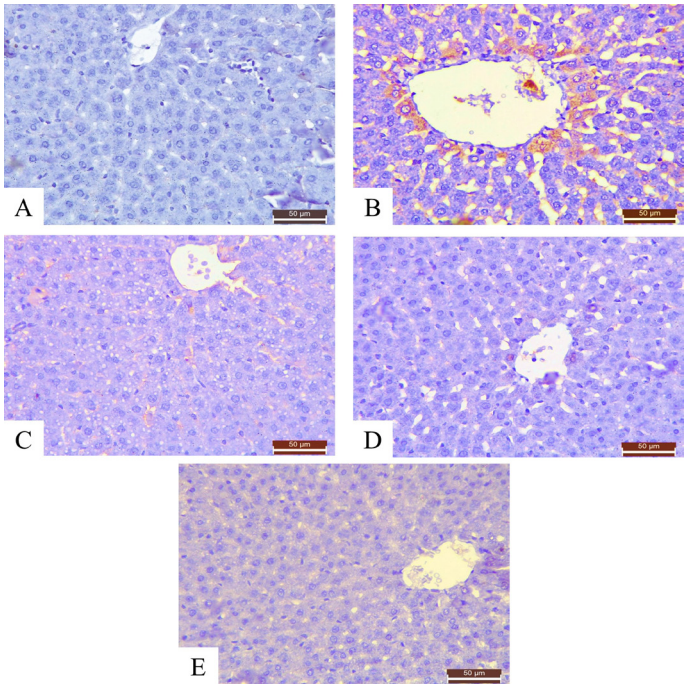

Figure 4: Representative Photomicrograph demonstrating immune expression in the caspase 3 stained liver sections (x400). (A) showing mild caspase expression in the hepatic tissue obtained from the control group (group 1), (B) remarking intense expression of a brown stain in the paracetamol-exposed group. (C) moderate expression in hepatic tissue (group3), (D and E) (simultaneous and Biocid treated groups (groups 4 and 5, respectively).

Similarly, the kidney tissue sections of the control group revealed normal histological architecture, consisting of both renal cortex and medulla. The cortical tissue contained renal corpuscles and their tubular series; proximal convoluted, distal convoluted and connecting tubules. Meanwhile the renal medulla composed mainly of Henel's loops and excretory duct system, collecting and papillary ducts. The renal corpuscle was composed of a glomerulus that formed of numerous loops of blood capillary surrounded by a Bowman' capsule. The renal tubular epithelial lining was of cuboidal to columnar types with prominent vesicular nuclei. The medullary ductal epithelium was of columnar to transitional type (Figure 3). Contrary, the animals of group 2 (gentamicin overdose) showed histopathological alterations in their kidneys, including congestion of the renal blood vessels and inter-tubular capillaries,

extravasated red blood cells and tubular degeneration. Such degenerative changes were indicated by tubular cell swelling, dilatation of their lumens, pyknotic nuclei, epithelial lining fragmentation, exfoliated and sloughed epithelial cells along with interstitial inflammatory cells infiltration around the ductal and tubular series. Glomerular structures were found to be shrunken, with wide subcapsular urinary spaces. Regarding group 3, the renal cortical and medullary degenerative changes were seen to be slightly declined when compared with that recorded in group 2. However, in group 4, kidney tissues showed a marked improvement of the degenerative changes when compared with groups 2 and 3. Less inflammatory cell infiltration and vascular congestion, as well as minor epithelial damage were recorded within this group. Additionally, renal specimens obtained from rats of group 5 exhibited normal renal architecture of the corpuscles and surrounding tubular series in the renal cortex as well as excretory duct system in the renal medulla that were of typical histological appearance.

Immunohistochemical inspection findings

The analysis of caspase-3 expression in liver and kidney sections demonstrated a significant reduction in apoptosis in the normal and NK-AA-OA treated groups (Groups 1, 3, 4, and 5) compared to the paracetamol and gentamicin intoxicated groups (Group 2, Figures 4B and 5C-D). Group 2 exhibits significantly higher area % of expression in both liver (28.31±7.89) and kidney (21.23±2.00) compared to other groups. The data suggests that Group 2 represents treatment-associated increased apoptosis, while Groups 1, 3, 4, and 5 show relatively low area of expression, indicating a less significant apoptotic activity. This reduction correlates with the significant decrease in MDA levels in these groups, indicating diminished oxidative stress. In the NK-AA-OA treated groups (Groups 3-5, Figures 4C-E and 5E-J), improved cellular antioxidant defenses were expressed as lower caspase-3 activities (less brown color). Table 4 presents quantitative scoring of Caspase 3 expression area percentages across five groups for liver and kidney tissues. These findings were further supported by elevated levels of GSH and enhanced activities of SOD and CAT in these groups.

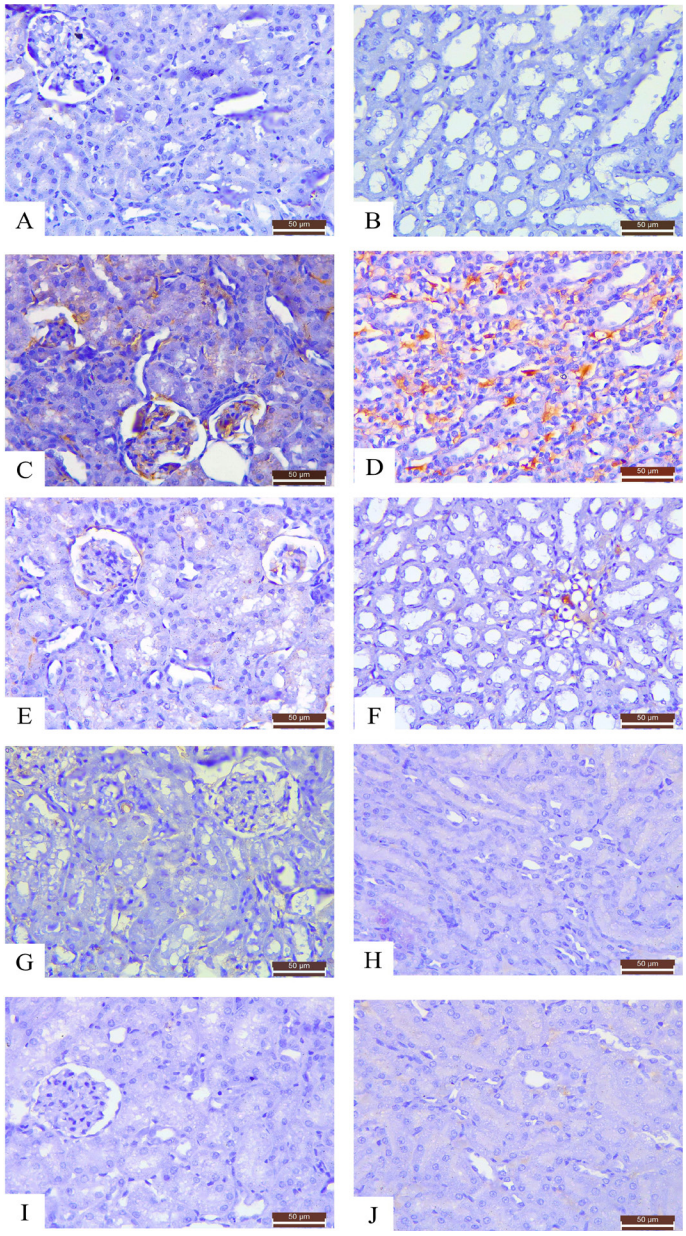

Figure 5: A photomicrograph demonstrating caspase 3 expression in the renal tissue sections (x400). (A and B) display mild positive expression in the renal tissue obtained from the control non-intoxicated group, (C and D) express an intense brown color in the gentamicin-exposed group (group2), (E and F, G and H, I and J) review mild to null expression of in groups 3, 4, and 5, respectively.

Table 4: Caspase 3 expression area percentage in normal and drug intoxicated rats' liver and kidney (paracetamol and gentamicin, correspondingly). All groups, except groups 1 and 2, received 300 mg of Biocid per kg of body weight.

Quantitative scoring	Caspase 3 expression area % ± Standard error				
	Group 1	Group 2	Group 3	Group 4	Group 5
Liver	1.02±0.40^{b}	28.31±7.89^{a}	9.95±1.01^{b}	6.31±0.87^{b}	6.07±0.57^{b}
Kidney	0.77±0.33^{c}	21.23±2.00^{a}	8.40±0.85^{b}	8.15±1.41^{b}	6.84±1.14^{b}

Group 1 (non-intoxicated control group), group 2 (paracetamol or gentamicin intoxicated group), group 3 (prevention group) received Biocid for 5 days before high doses of paracetamol or gentamicin, group 4 (simultaneous treated) administered with paracetamol or gentamicin during Biocid treatment, group 5 (Biocid group) non-intoxicated rats administered with Biocid only. In each row, the use of letters (a, b, c) denotes significant differences between groups, with 'a' indicating the highest expression levels.

DISCUSSION

This investigative study provides compelling evidence for the hepato-renal protective efficacy of nano-kaolin conjugated with a blend of amino and organic acids (NK-AA-OA, proprietary name Biocid®) in a rat model of drug-induced toxicity. High doses of paracetamol (also known as acetaminophen), a well-established hepatotoxic agent, and gentamicin (a potent nephrotoxic antibiotic) effectively induced severe liver and kidney damage, as evidenced by significant alterations in biochemical and oxidative markers besides the profound histopathological changes. Our results demonstrate that oral administration of NK-AA-OA effectively counteracted these toxic effects, restoring organ function and integrity to far extent.

The significantly elevated serum levels of ALT, AST, GGT, and total bilirubin in the paracetamol-intoxicated group are indicative of hepatocellular injury and compromised liver function (Abdou *et al*., 2025). These enzymes are typically released into the bloodstream upon hepatocyte damage. Similarly, increased BUN and creatinine levels reflect impaired renal filtration and kidney dysfunction (Abogresha *et al*., 2016; Assar *et al*., 2023). The reduction of these markers following NK-AA-OA treatment suggests that the tested preparation preserved the structural integrity of hepatocytes and renal tubular cells, thereby maintaining their functional capacity. The efficacy observed, particularly with simultaneous NK-AA-OA treatment during toxicant administration, was comparable to the normal hepatocytes and nephrons, indicating its reliable protective potential. Similar reductions, although non-significant, in ALT, AST, GGT, urea and creatinine were observed in chicken when Biocid was administered in drinking water and feed (Mansour *et al*., 2024). Likewise, when Biocid was supplemented to juvenile tilapia fish for 90 days (in feed at 0.25-2 g/Kg ration), variable reduction in ALT, AST, ALP, urea and creatinine were observed specially with the high dose (El-Naby *et al*., 2025). These reductions in liver and kidney markers were less prominent in the previously mentioned studies because they supplemented the NK-AA-OA to healthy non-intoxicated animals while, in the present study, we challenged NK-AA-OA with hepatic and renal toxic doses of drugs. The comparisons made with studies in healthy chickens and tilapia, while highlighting potential broader applications of Biocid, may not directly relate to our toxicity model. These comparisons should be approached cautiously, as the physiological responses in healthy versus intoxicated states can vary significantly.

Furthermore, the slight reductions in liver and kidney biomarkers in Biocid non-intoxicated group compared with normal group may suggest that Biocid has effects that extend beyond mere detoxification or baseline function. Biocid with its amino and organic acids contents may has nutrient, antioxidant and metabolic enhancements those could augment the overall efficiency of liver and kidney functions. While these minor reductions in liver and kidney markers in non-intoxicated groups suggest that Biocid may confer benefits beyond detoxification, claiming that these effects are universally applicable must be cautious. Such claim raises questions about the underlying mechanisms. Are these effects due to direct cytoprotection, or do they stem from an enhanced baseline function?

Oxidative stress plays a pivotal role in the pathogenesis of both paracetamol-induced hepatotoxicity and gentamicin-induced nephrotoxicity. Paracetamol (acetaminophen) is metabolized to reactive free radicals (N-acetyl-p-benzoquinone imine, NAPQI), which initiate lipid peroxidation and deplete antioxidant defense systems, mainly glutathione stores, (Mazaleuskaya *et al*., 2015). Gentamicin also generates reactive oxygen species (ROS), leading to mitochondrial dysfunction and oxidative damage in renal cells (Lopez-Novoa *et al*., 2011). Our findings clearly show that the paracetamol and gentamicin-intoxicated rats suffered from severe oxidative stress, characterized by elevated MDA levels (a marker of lipid peroxidation) and depleted GSH, SOD, and CAT activities. The impressive ability of NK-AA-OA to significantly reduce MDA and augment the endogenous antioxidant defense mechanisms (GSH, SOD, CAT) strongly suggests that its protective effects may be largely mediated through its potent antioxidant properties. A detailed analysis should incorporate the complexities of oxidative stress mechanisms. This includes distinguishing whether the protective effects are primarily antioxidant in nature or if other pathways, such as modulation of inflammatory responses or pharmacokinetic interactions, contribute to the observed protective potential.

The organic and amino acids in Biocid; including citric, succinic, L-cysteine and L-glutamine, are known for their antioxidant and cytoprotective roles. For instance, L-cysteine is the precursor for GSH synthesis, directly contributing to cellular detoxification and known for its radical scavenging. L-glutamine supports immune function and acts as an antioxidant, arginine and glycine have anti-inflammatory properties, supports blood flow and support tissue repair (Wu *et al*., 2004). While organic acids are involved in energy metabolism and can act as direct free radical scavengers (Abdel-Salam *et al*., 2014). However, the details of how these compounds interact at the cellular level remain to be fully investigated. The unique combination on the nano-kaolin platform likely provides a harmonious effect, where the sustained release or enhanced delivery of these bioactive molecules by nano-kaolin maximizes their protective potential (Yang *et al*., 2016; Awad *et al*., 2017). The NK-AA-OA showed direct total antioxidant capacity which exceeded that of vitamin

C, these tests were conducted in a cell-free system. While these values provide initial insights into the antioxidant capacity of Biocid, it does not fully account for the complexities of *in vivo* conditions, such as bioavailability and cellular uptake. Extend studies are essential to assess the relevance of these findings in a biological context, where factors such as absorption, distribution, metabolism, and excretion play a critical role.

Nano-kaolin itself, with its high surface area and adsorptive properties, could potentially contribute by binding toxins in the gastrointestinal tract, thereby reducing systemic absorption and subsequent organ damage (Yang *et al*., 2016; Dong *et al*., 2021). However, in this model, the systemic administration of paracetamol and gentamicin suggests that the primary mechanisms likely involve the direct antioxidant, anti-inflammatory and/or detoxification effects, as well as pharmacokinetic interactions between the conjugated acids with the intoxicating drugs.

Histopathological evaluations provided visual confirmation of the biochemical findings. The congested blood vessels, vacuolization, inflammation, and cellular degeneration resulted by overdoses of paracetamol and gentamicin in intoxicated-groups were considerably reduced in the NK-AA-OA treated animals. The preservation of normal cellular architecture in both liver and kidney tissues clearly supports the hepato-renal protective effects of the NK-AA-OA. This indicates that NK-AA-OA not only preserved biochemical functions but also maintained the structural integrity of the vital organs, underscoring its therapeutic promise.

The overall improvement in liver and kidney status observed in the Biocid simultaneous administration scenario (administered concurrently with the intoxicant) compared to the preventive scenario may suggest a mechanism of direct competition or detoxification, rather than solely a pre-emptive priming of antioxidant defenses.

While this study strongly supports the hepato-renal protective effects of NK-AA-OA, some limitations should be acknowledged. Firstly, the study focused on a specific acute toxicity model; future research should investigate the efficacy of NK-AA-OA in chronic toxicity models and against other types of organ injury. Secondly, while the antioxidant/anti-inflammatory properties are acceptable as the main protective mechanisms, detailed mechanistic investigations, such as gene expression analysis of inflammatory cytokines or apoptosis markers, would provide deeper insights. Lastly, while the dose of 300 mg/kg body weight was chosen based on prior pilot studies (using 3 graded doses; 75, 150, and 300 mg Biocid/kg body weight), it is important to investigate lower doses for possible efficacy, and higher doses or longer administration for better understand of NK-AA-OA *in vivo* toxicity profile.

CONCLUSIONS AND RECOMMENDATIONS

Biocid® appears to provide hepato-renal protective effects against specific drug-induced oxidative stress or injuries, as indicated by its ability to reduce MDA and maintain or enhance levels of GSH, SOD, and CAT. Additionally, the protective effects of NK-AA-OA appear to mitigate the apoptotic response associated with paracetamol/gentamicin toxicity. Overall, these findings highlight the potential of NK-AA-OA in preserving cellular integrity under oxidative stress conditions.

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NOVELTY STATEMENT

This research paper represents the first investigation into the efficacy of nano-kaolin conjugated with a blend of amino and organic acids (proprietary name Biocid®) as a hepatoprotective and renoprotective agent in a rat model of drug-induced toxicity.

AUTHOR'S CONTRIBUTION

Waleed Khalil, Sahar Ezeldien and Yousry Ibrahim developed the initial concept and rational of the experiments. All authors, except Yousry Ibrahim, conducted the experimental work including drug administration, samples collection and samples analysis. Sameh M. Farouk and Alaa A. Elhakim conducted the histopathological and immunohistochemical explorations Waleed Khalil and Sahar Ezeldien analyzed the results and created the initial manuscript. All authors engaged in discussions about the results and contributed to the final manuscript.

Generative AI and AI-assisted technology statement

The authors confirm that the creation of this manuscript did not involve the use of any Generative AI technologies. All content has been developed solely through traditional research and writing methods.

Conflict of interest

The authors declare that they neither have competing

financial interests nor personal relationships that could have influenced the results or assessments presented in this paper.

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