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Iron complexes: green and sustainable synthesis and evaluation of their potency as antioxidants and inhibitors of DNA damage in rats

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ABSTRACT. Three iron complexes were synthesized through green and sustainable procedures via chelation with salicylic acid, 3-methyl-1-phenyl-pyrazol-5-one, and ethylacetoactate under solvent-free conditions at room temperature. The synthesis assured the concept of both the twelve principles of Green Chemistry and Sustainability Commands. 48 male Wistar albino rats were administered gentamicin (GM) at a dose of 80 mg kg^{-1} day^{-1}, with or without (I-SA), (I-MPP), (I-EAA) (120 mg kg^{-1} day^{-1}) to evaluate the complexes' potential protective effects against GM-induced hepatotoxicity and nephrotoxicity. Oxidative stress markers and DNA fragmentation increased significantly, while the activity of the antioxidant enzymes SOD and CAT notably decreased. The study findings suggest that the three complexes serve as novel natural antioxidants, mitigating oxygen free radicals, lipid peroxidation, and DNA damage in gentamicin-treated rats. These compounds hold promise as potential natural drugs in future applications.

Keywords: green synthesis; sustainability; antioxidants markers; DNA damage; iron complexes.

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Introduction

Currently, research is focused on designing alternative strategies for benign, safe, and green chemical practices that address the drawbacks of chemical processes (Caldeira et al., 2022; Eissa, 2018). Consequently, there is a growing emphasis on the principles of green chemistry and sustainability in the formulation of chemical processes. This involves considerations such as pollution prevention, atom economy, less hazardous chemical synthesis, safer chemical design, the use of environmentally friendly solvents and auxiliaries, energy efficiency, utilization of renewable feedstocks, minimized derivatives, catalysis, degradation design, real-time analysis for pollution prevention, and inherently safer chemistry to prevent accidents (Eissa & Abdel Hameed, 2016; Eissa, Selim, Sayed, & Sharkawi, 2019; Eissa & Mohamed, 2018).

Iron complexes, owing to their color and chemical coordination, are extensively prevalent in bioorganic compounds and play a central role in organic and biochemical processes. Despite this, the bioactivity of iron complexes is diverse, offering numerous possibilities across various fields, particularly those pertinent to environmental and biological chemistry. The richness of bioactivity in iron complexes provides a multitude of opportunities in different areas, with a specific emphasis on aspects significant for environmental and biological chemistry (Chem & Browne, 2018).

Aminoglycosides, a class of antibiotics with heterocyclic structures derived from natural or semisynthetic sources, consist of amino sugars linked by glycoside bonds to an aminocyclitol ring. Gentamicin, a widely used medication in this category, is recognized for its broad-spectrum effectiveness against both gram-negative and gram-positive bacteria. Gentamicin induces an increase in mitochondrial reactive oxygen species (ROS), impacting macromolecules such as proteins, lipids, and nucleic acids adversely (Lopez-Novoa, Quiros, Vicente, Morales, & Lopez-Hernandez, 2011). This ROS overproduction by gentamicin leads to inflammation promotion and the inhibition of the endogenous antioxidant system (Cao, Zhi, Han, Sah, & Xie, 2019). In response, recent research efforts over the past decade have focused on antioxidants and medications with potential anti-oxidative, anti-inflammatory, and Reno protective properties (Medic et al., 2019; Elfaky et al., 2019; Cui et al., 2019). The liver, vital for detoxification, is frequently exposed to toxins and medications (Sakr, Samei, & Soliman, 2004).

Numerous studies have explored the detrimental impacts of gentamicin (GM) on the livers of animal models. These inquiries have revealed that GM can induce hepatotoxicity in rats directly, leading to oxidative stress, apoptosis, and an elevation in the serum activity of transaminases (ALT, AST, & ALP). The involvement of reactive oxygen species (ROS) is crucial in GM-mediated nephrotoxicity, contributing to a decline in renal function in rats (Heibashy, El-Nahla, Ibrahim, Saleh, 2009). Various investigations have associated the generation of reactive oxygen species (ROS) with GM-induced nephrotoxicity (Banday, Farooq, Priyamvada, Yusufi, & Khan, 2008). To counteract GM-induced nephrotoxicity, several compounds with antioxidant properties have been effectively administered for prevention or alleviation (Stojiljkovic, Stoiljkovic, Randjelovic, Veljkovic, & Mihailovic, 2012; Ali, 2003).

This article focuses on detailing a benign, green, and sustainable method employed for the synthesis of valuable iron complexes. The chemical structure of the synthesized products was verified through spectroscopic chemical analysis. The article also delves into assessing the effectiveness of these compounds as antioxidants and their capability to inhibit DNA damage, particularly in animal models.

Materials and methods

Experimental synthesis of iron complexes

At room temperature, anhydrous Iron (III) chloride (0.01 mole) and a triple molar ratio of SA, MPP, and/or EAA were ground with an agate mortar and pestle for 15 minutes or more until the deep reddish violet color of the paste remained and possibly reached its deepest color. The resulting paste was further dried and collected. The resulting complexes, I-SA, I-MPP, and I-EAA, could be recrystallized from ethanol. All the synthesized compounds were characterized and confirmed via 1H NMR spectroscopy.

I-SA: 2-carboxyphenolate; Iron (III):

Yield: 94%; Chemical Formula: $C_{21}H_{15}FeO_9$; Molecular Weight: 467.18, (m/z): 467.01; ^{1}HNMR (δ ppm)**:** 7.60-7.67, m, 6H-Ar; 7.81, t, 3H-Ar; 8.31, d, 3H-Ar; 8.31, d, 3H-Ar. SA: Salicylic acid, 6.85-7.01, d, 1H-Ar; 7.51-8.11, m, 3H-Ar; 7.86-7.91, s, 1H-OH; 8.31-8.54, s, 1H-COOH.

I-MPP: Methylphenylpyrazolone; Iron (III):

Yield: 90%; Chemical Formula: $C_{30}H_{27}FeN_6O_3$; Molecular Weight: 575.42, (m/z): 575.15; ^{1}HNMR (δ ppm)**:** 2.21, s, 9H-3CH_3; 6.48, s, 3H-CH= (pyrazole ring); 7.21-7.61, m, 12H-Ar; 9.61, s, 1H-OH. MPP, methylphenylpyrazolone 2.30, s, 3H-CH_3; 6.58, s, 1H-CH= (pyrazole); 7.45-7.81, m, 5H-Ar; 11.21, s, 1H-OH.

I-EAA: Ethylacetoacetate; Iron (III):

Yield: 89%; Chemical Formula: $C_{18}H_{33}FeO_9$; Molecular Weight: 449.29, (m/z): 449.15; ^{1}HNMR (δ ppm)**:** 1.01-1.18, d, 9H-CH_3; 1.29-1.37, d, 9H-CH_3; 1.71-1.94, q, 4H-CH_2-; 2.54-3.41, m, 9H-CHOH, CH_2-O. EAA: ethylacetate, 0.91-1.08, t, 3H-CH_3; 1.19-1.21, d, 3H-CH_3; 2.11-2.54, m, 2H-CH_2-; 3.62-4.01, m,3H-CHOH, CH_2-O.

Chemicals

All reagents were procured from Merck Serono in Cairo, Egypt, and were utilized without additional purification. Analytical-grade solvents were employed in the experiments. Using DMSO-d6 as the solvent and tetramethylsilane as the internal standard, the Varian Mercury 300 MHz spectrometer was used to record the 1H- and 13C-NMR spectra. Chem. Draw Ultra 12.0.2.1076 was employed to renew all substances.

Gentamicin (GM), an aminoglycoside antibiotic at a concentration of 80 mg 2 mL^{-1} AMP, was procured from the Memphis Company for Pharmaceutical Chemical Industries in Cairo, Egypt. Sigma Chemical Co., based in St. Louis, MO, U.S.A., supplied epinephrine, ethylene diamine tetraacetic acid (EDTA), dimethyl sulfoxide, sodium dodecyl sulfate (SDS), and thiobarbituric acid (TBA). The highest purity commercially available substances were employed for all other compounds.

Animal experimental design

Forty-eight adult male wistar rats, with weights ranging from 130 to 150 g, were obtained from the veterinary medicine animal facility at Qena University. The experimental environment maintained a consistent room temperature of 22 ± 3 °C, with the rats exposed to 12-hour cycles of light and darkness. The rats were provided with a standard pellet diet and unrestricted access to water. Throughout the trial period,

regular weighing of the animals was conducted to monitor changes, and adjustments to the chemical composition and GM dosages were made as needed. After a week of acclimation, the rats were divided into eight groups, each consisting of six individuals. Prior to the injection of gentamicin, the animals received oral pretreatment with I-SA, I-MPP, or I-EAA for one week in groups where GM was administered.

1-CONT group: served as the control and received 1 mL of saline administered orally once.

2-GM group received an intraperitoneal (i.p.) injection of 80 mg kg^{-1} of the drug every day.

3-I-SA group, a daily dose of I-SA (120 mg kg^{-1}) was administered orally every day.

4-GM+I-SA group received a daily dose of 80 mg kg^{-1} GM via the i.p.) injection and after one hour of oral administration of a daily dose of I-SA (120 mg kg^{-1} day^{-1}).

5-MPP group was orally administered a daily dose of I-MPP (120 mg kg^{-1} day^{-1}).

6-GM+I-MPP group received a daily dose of 80 mg kg^{-1} GM by (i.p.) injection after one hour of oral administration of a daily dose of I-MPP (120 mg kg^{-1} day^{-1}).

7-I-EAA group was orally administered a daily dose of I-EAA (120 mg kg^{-1} day^{-1}).

8 –GM+ I-EAA group received a daily dose of 80 mg kg^{-1} GM by (i.p.) injection after one hour of oral administration of a daily dose of I-EAA (120 mg kg^{-1} day^{-1}).

Biochemical analysis of liver enzymes, lipid profile and kidney functions

After allowing blood samples to coagulate and serum being separated was kept at -20 °C for a later biochemical examination, involving centrifugation at 5,000 rpm for 20 minutes. The separated serum samples were analyzed to determine the presence of liver enzymes, including serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), serum albumin, total protein, total serum bilirubin, triglycerides (TG), (LDL) low density lipoprotein, (HDL) high density lipoprotein, total cholesterol (TC), and kidney functions (serum creatinine, serum.urea, and uric acid). Commercially available estimation kits from Biomed Diagnostic Company were utilized following the manufacturer's instructions.

Oxidative stress markers

Liver and kidney tissues were cut into small fragments and homogenized in ice-cold buffer using a homogenizer. The resulting homogenates (10% w/v) underwent centrifugation at 5,000 rpm for 10 minutes at 4 °C. The assessment of lipid peroxidation levels in liver and kidney tissues was conducted spectrophotometrically at 532 nm, relying on the thiobarbituric (TBA) reaction products, as described by Ohkawa, Ohishi, and Yagi (1979). Nitrite levels were measured to estimate nitric oxide (NO) levels in liver and kidney tissues, following the methods outlined by Montgomery and Dymock (1961). For determining the extent of DNA fragmentation in the serum, the technique introduced by Kurita-Ochiai, Fukushima, and Ochiai (1999) was employed.

Antioxidant enzymes activity

Superoxide dismutase (SOD) activity in liver and kidney homogenates was assessed following the method developed by Misra and Fridovich (1972). This method relies on measuring the inhibitory effect of SOD on epinephrine oxidation. The Beers Jr and Sizer (1952) technique was employed to determine catalase levels in liver and kidney homogenates.

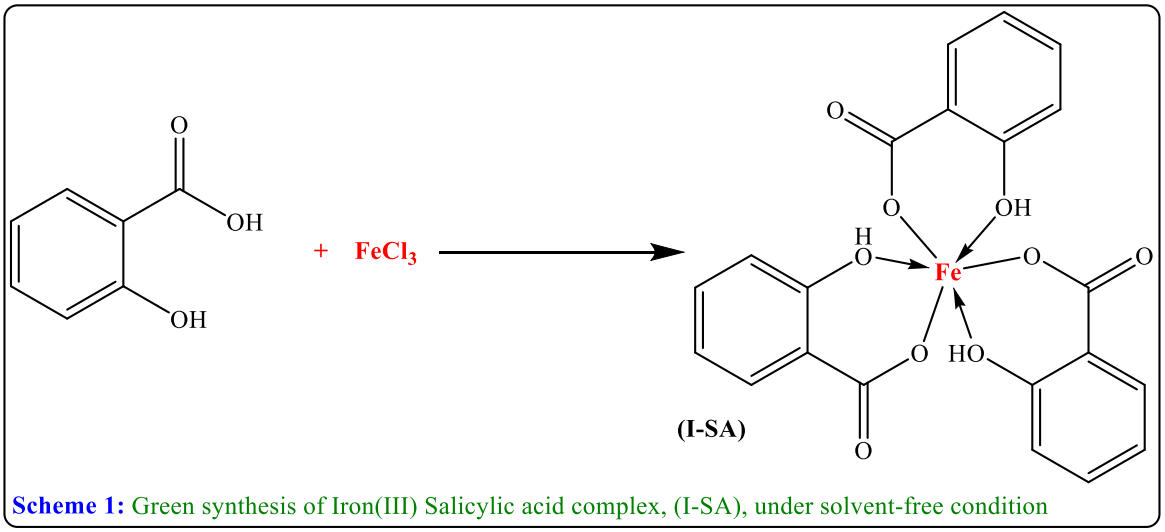
Statistical analysis

Quantitative biochemical assay data are presented as means ± SEMs. Statistical analysis of mean differences was conducted using one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls T test. This approach was employed with Minitab 19 software for comparing and statistically evaluating the obtained data.

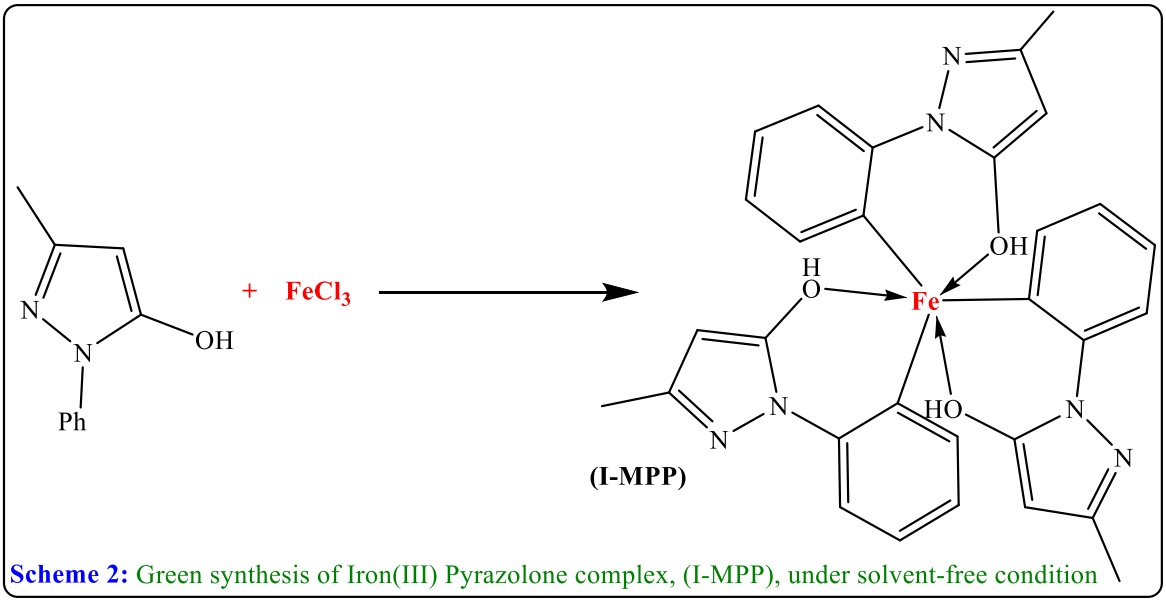
Results and discussion

Iron (III) chloride was ground at room temperature via a mortar and pestle and agate under solventless conditions for a period of a few minutes with salicylic acid (SA), 3-methyl-1-phenyl-pyrazol-5-one (MPP), and ethylacetoacetate (EAA). An increase in the violet color indicated ambient conditions conducive to chelation. Figure 1 show three iron complexes separated and characterized (I-SA, I-MPP, and I-EAA) (Schemes 1, 2, and 3, respectively). The green context of the current synthesis is highly marked by its high

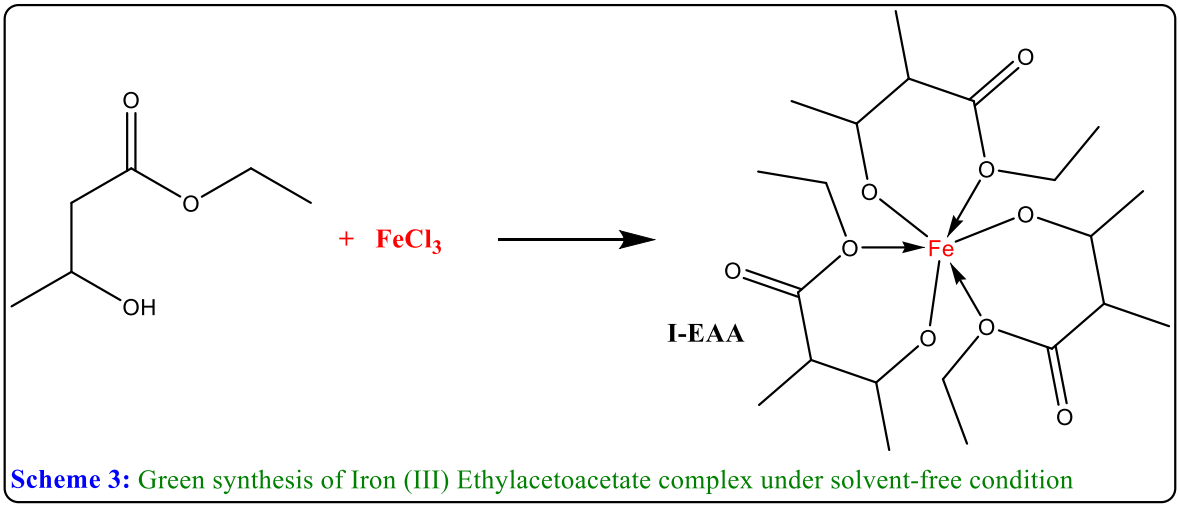
yield, excellent conversion, high atom economy, solvent-less nature, simplicity, least hazard and lowest risk, Eco-friendliness, and integrally safer chemistry practices. Moreover, these green and sustainable procedures occurred thru grinding reactants together, (i.e. without solvation energy), due to the gentle activation friction energy arising from grinding which was adequate enough to achieve the whole synthesis.



Scheme 1: Green synthesis of Iron(III) Salicylic acid complex, (I-SA), under solvent-free condition



Scheme 2: Green synthesis of Iron(III) Pyrazolone complex, (I-MPP), under solvent-free condition



Scheme 3: Green synthesis of Iron (III) Ethylacetoacetate complex under solvent-free condition

Figure 1. Iron complexes I-SA, I-MPP, and I-EAA (Schemes 1, 2, and 3, respectively).

Conventionally, these complexes could be generated by stirring in ethanol for a longer time, after which the products could be separated in lower yields and in more time than the green procedure. Here, the non-green component of the synthesis was strongly marked with more hazards and risks, smaller reaction conversions, lower yields and atom economy, higher Eco-scale ratings, hence, this process is not an eco-friendly practice.

Biochemical study

Kidney functions

The data obtained and displayed in Table 1 show that treatment with GM for 21 days resulted in highly significant increases in the serum creatinine, urea and uric acid levels compared to those in control rats. However, elevations in the serum creatinine, blood urea and uric acid levels were strongly significantly ($p < 0.001$) attenuated by I-SA, I-MPP, and I-EAA pretreatment, indicating a reduction in GM-induced nephrotoxicity. Additionally, compared with the control treatment, I-SA, I-MPP, and I-EAA treatment alone for 28 days changed the renal function test results.

Table 1. Comparison of kidney function between the control and treated groups.

	CONT.	GM	I-SA	GM+I-SA	I-MPP	GM+I-MPP	I-EAA	GM+I-EAA
Urea	34.6 ± 5.1	95 ± 13.3**	28.5 ± 1.9$^{\#\#}$	36.6 ± 4.0$^{\#\#}$	39.8 ± 1.0$^{\#\#}$	41.5 ± 1.8$^{\#\#}$	35.5 ± 2.8$^{\#\#}$	30.5 ± 3.3$^{\#\#}$
Creatinine	0.83 ± 0.03	3.1 ± 0.25**	0.58 ± 0.03$^{\#\#}$	1.5 ± 0.04*$^{\#\#}$	0.63 ± 0.0$^{\#\#}$	1.6 ± 0.1**$^{\#\#}$	0.7 ± 0.06$^{\#\#}$	1.4 ± 0.04*$^{\#\#}$
Uric acid	3.4 ± 0.1	8.2 ± 0.6**	4.2 ± 0.2$^{\#\#}$	3.8 ± 0.1$^{\#\#}$	3.9 ± 0.3$^{\#\#}$	3.7 ± 0.2$^{\#\#}$	3.8 ± 0.2$^{\#\#}$	3.9 ± 0.3$^{\#\#}$

The values are expressed in mg dL^{-1} as the mean (±SEM). The number of rats = 6 for all groups. *high significance with CONT, **very high significance with CONT, $^{\#}$high significance with GM, $^{\#\#}$very high significance with GM.

Liver enzymes and lipid profiles

Compared with those in the healthy control group, the serum activity levels of SGOT and SGPT in GM-treated rats were highly significantly different ($p < 0.001$). Compared to those in the GM group, treatment with I-SA, I-MPP, or I-EAA for 28 days considerably decreased the serum activity of SGOT and SGPT in the treated groups. Compared to those in the control group, the levels of TG, cholesterol, and albumin were elevated in the GM treatment group ($p < 0.001$), but there were no significant changes in HDL, LDL, total bilirubin, and protein. Compared to those in the GM group, cholesterol levels were significantly lower in the groups treated with I-SA, I-MPP, or I-EAA alone or in combination with GM (120 mg kg^{-1}) ($p < 0.001$). Treatment with I-SA (120 mg kg^{-1}) resulted in a highly significant reduction in TG levels ($p < 0.001$), while treatment with GM+I-EAA (120 mg kg^{-1}) resulted in a substantial reduction in TG levels ($p < 0.01$). The levels of HDL were significantly decreased following treatment with 120 mg kg^{-1} I-MPP and I-EAA ($p < 0.001$), while the levels of HDL were significantly decreased following treatment with Genta I-MPP and Genta I-EAA ($p < 0.01$). The 120 mg kg^{-1} I-SA, I-MPP, or I-EAA treatment had no discernible effects on total bilirubin or LDL levels. Additionally, treatment with GM caused high significant increase in Albumin compared with control, treatment with 120 mg kg^{-1} I-MPP, and I-EAA caused a high significant increase in the levels of albumin ($p < 0.001$), and with I-SA caused significant increase in the levels of albumin ($p < 0.01$) compared with GM group. In addition, highly significant ($p < 0.01$) decreases in total protein after treatment with I-MPP, I-SA and I-EAA were highly significant ($p < 0.001$), and highly significant ($p < 0.01$) decreases in total protein were observed in the treated groups compared with those in the GM group (Table 2).

Table 2. Comparison of liver enzymes and lipid profiles between the control and treated groups.

	CONT	GM	I-SA	GM+I-SA	I-MPP	GM +I-MPP	I-EAA	GM +I-EAA
GOT (U L^{-1})	8.8 ± 1.1	29.0 ± 2.1**	10.5 ± 0.7 $^{\#\#}$	12.1 ± 0.7$^{\#\#}$	12.1 ± 1.7$^{\#\#}$	14.6 ± 0.6$^{\#\#}$	10.5 ± 1.4$^{\#\#}$	10.0 ± 2.0$^{\#\#}$
GPT (U L^{-1})	7.0 ± 0.5	52.3 ± 7.7**	7.8 ± 0.4$^{\#\#}$	7.0 ± 0.2$^{\#\#}$	9.6 ± 0.6$^{\#\#}$	7.3 ± 0.8$^{\#\#}$	8.8 ± 1.1$^{\#\#}$	7.8 ± 0.4$^{\#\#}$
Triglyceride	92.3 ± 5.2	173.8 ± 23.3*	60 ± 5.3$^{\#\#}$	127.2 ± 17.2	118.5 ± 8.7	111.2 ± 14.6	117 ± 15.6	95.3 ± 5.8$^{\#}$
Cholesterol	126.6 ± .6	211.0 ± 19.9**	123.3 ± 5.5$^{\#\#}$	120.0 ± 4.4$^{\#\#}$	138.3 ± 6.5$^{\#\#}$	130.8 ± 5.8$^{\#\#}$	128.3 ± 0.7$^{\#\#}$	123.3 ± 4.2$^{\#\#}$
HDL	70.1 ± 4.5	91.3 ± 16	69.6 ± 8.8	61.6 ± 8.8	46 ± 1.9$^{\#\#}$	54.1 ± 4.9$^{\#}$	48.5 ± 6.5 $^{\#\#}$	57.6 ± 4.2$^{\#}$
LDL	51.5 ± 4.7	54.8 ± 4.5	64.2 ± 21.2	42.1 ± 4.8	68.5 ± 5.3	52.1 ± 7.0	48.5 ± 6.5	48 .0 ± 6.3
T. Bilirubin	0.9 ± 0.03	0.8 ± 0.03	0.8 ± 0.04	0.9 ± 0.04	0.9 ± 0.03	0.8 ± 0.07	0.8 ± 0.03	0.8 ± 0.04
Albumin	4.6 ± 0.3	2.8 ± 0.1**	3.6 ± 0.2	3.1 ± 0.1*	4.0± 0.2$^{\#\#}$	3.6 ± 0.3	3.5 ± 0.1	4.0 ± 0.2$^{\#\#}$
T. protein	5.7 ± 0.4	7.2 ± 0.42	4.3 ± 0.3$^{\#\#}$	5.0 ± 0.4$^{\#}$	4.2 ± 0.4$^{\#\#}$	4.9 ± 0.2$^{\#\#}$	5.2 ± 0.4$^{\#}$	4.4 ± 0.5$^{\#\#}$

The values are expressed as the means (±SEMs). Number of rats = 6 for all groups. *High significance with CONT, **very high significance, $^{\#}$high significance with GM group, $^{\#\#}$very high significance.

Measurement of oxidative stress biomarkers

Compared to those in the controls, the MDA concentrations in the liver and kidney homogenates of the GM-treated rats increased markedly ($p < 0.001$) (Figure 2A and B). With the exception of liver tissues in the GM, liver MDA concentrations were significantly lower in the I-SA, I-MPP, and I-EAA treatment groups ($p < 0.001$) than in the GM group. The I-MPP and GM+ I-EAA groups did not substantially differ from the GM group ($p > 0.05$). Treatment with I-SA, I-MPP, or I-EAA significantly reduced renal MDA concentrations ($p < 0.001$) in the treated group compared with those in the GM group. Compared with those in the control group, nitric oxide levels in hepatic and renal tissues increased significantly ($p < 0.001$) after GM therapy (Figure 2C and D). When applied to liver tissue homogenates, I-SA, I-MPP, and I-EAA therapy resulted in a highly significant decrease ($p < 0.001$) in the (NO) content compared to that in the GM group; however, GM+ I-SA and GM+ I-MPP treatment had no effect on the NO content. Among the renal homogenates, the I-SA, I-MPP, and I-EAA treatments caused a highly significant decrease ($p < 0.001$), but the GM+I-SA treatment significantly reduced ($P<0.01$) but not significantly ($p > 0.05$) the I-MPP group compared with the GM group.

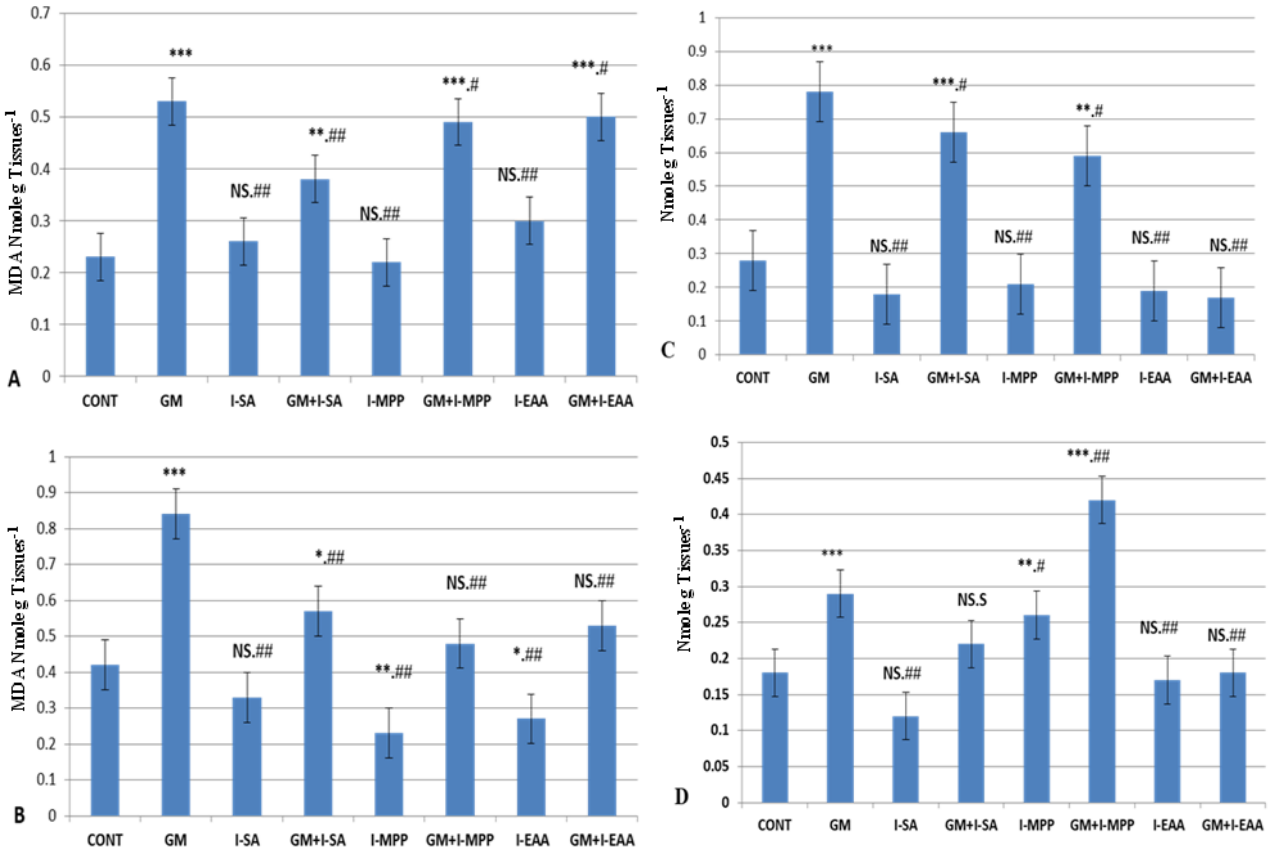


Figure 2. Effect of supplementation with (I-SA), (I-MPP), or (I-EAA) on lipid peroxidation (MDA) and nitric oxide (NO) production induced by GM in the liver and kidney of control and experimental animals. Mean values ± SEsSEs of A, B (MDA), C, and D (NO) in liver and kidney homogenates from the control group and different treatment groups of rats. NS, Nonsignificant difference from CONT, **highly significant, ***very highly significant. #no significant difference from the GM group, ##very high significance, S, significant.

Antioxidant enzyme activity

Liver and kidney SOD activity was determined by biochemical investigation, as shown in Figure 3A and B. There was a statistically significant decrease in the GM group compared to the control group ($p < 0.001$). After receiving therapy for 28 days with I-SA, I-MPP, or I-EAA, the hepatic SOD activity nearly returned to control levels, with very high significant increases in all treated groups ($p < 0.001$) and only a marginally significant increase in SOD in the I-SA group ($p < 0.01$). Pretreatment with I-SA or IP caused a very high increase ($p < 0.001$) in renal SOD activity, whereas the other groups did not significantly increase SOD activity ($p > 0.05$). In the GM group, liver CAT activity was considerably greater than that in the control group ($p < 0.001$). Hepatic CAT activity significantly recovered after receiving I-MPP and I-EAA treatment for 21 days, returning to the levels observed in the controls.

Although I-SA therapy did not result in a significant increase in CAT activity ($p > 0.05$), CAT kidney activity was significantly greater in the GM group than in the control group ($p < 0.001$). However, in the renal tissue, CAT activity did not significantly increase except in the I-EAA group. (Figure 3C and D.)

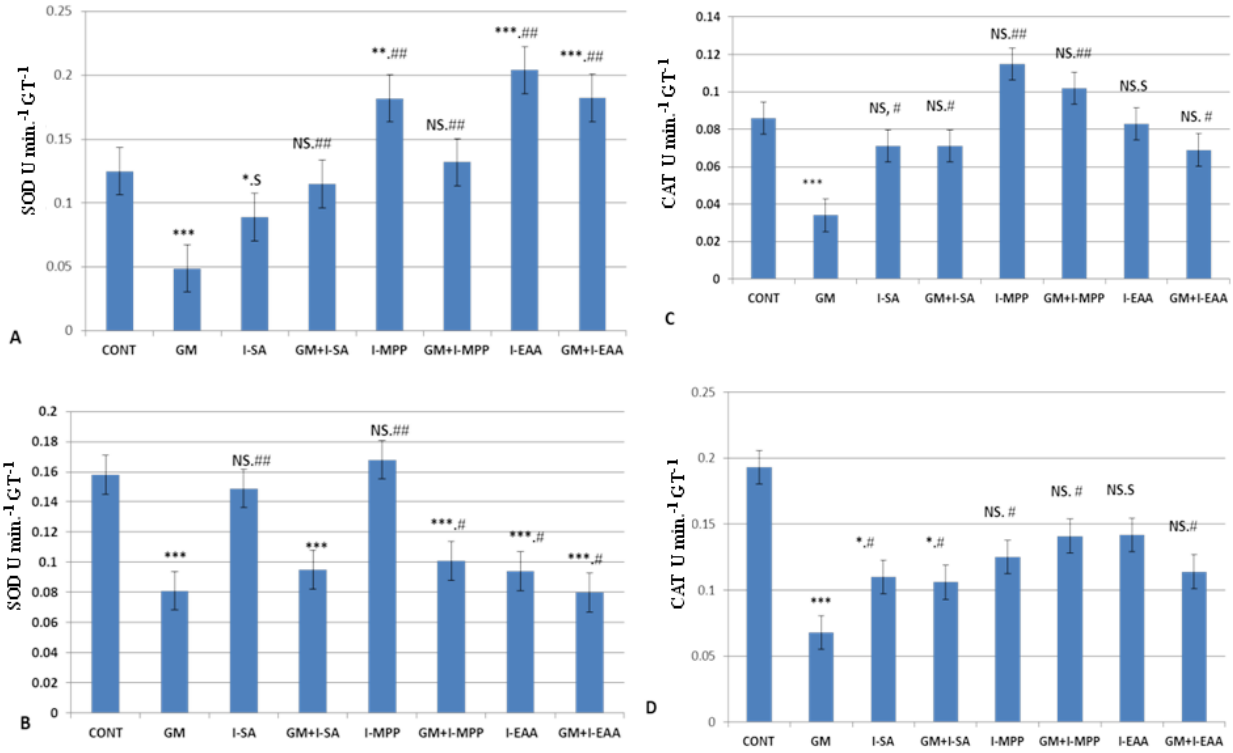


Figure 3. Effect of supplementation with (I-SA), (I-MPP), or (I-EAA) on superoxide dismutase (SOD) and catalase (CAT) in liver and kidney homogenates from control and different treatment groups of rats. Mean values ± S.E.ss of A, B (SOD) activity and C, D (CAT) activity in liver and kidney homogenates from the control and different treatment groups of rats. *Significant difference compared with CONT, ***very high significance, **highly significant, NS, not significant. #No significant difference compared with the GM-treated group; ##very high significance, S, significant.

Detection of DNA fragmentation in the serum

The alterations in DNA fragmentation found in the serum showed that the level of DNA fragmentation was markedly and significantly increased ($p < 0.001$) by GM treatment.

Nevertheless, pretreatment with I-SA, I-MPP, or I-EAA before the mice were given GM therapy resulted in a highly significant decrease ($p < 0.001$). However, compared to those in the GM group, the GM+I-SA, I-MPP, and GM+I-MPP groups exhibited significantly lower ($p < 0.01$) DNA fragmentation (Figure 4).

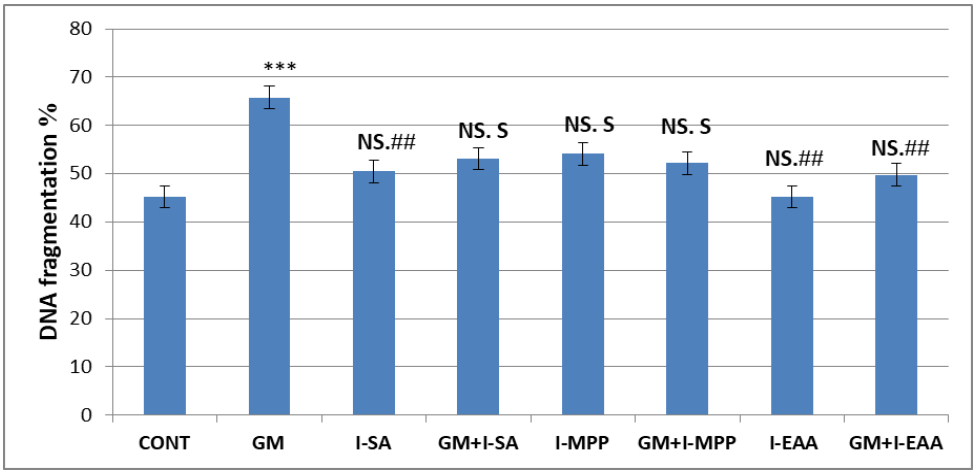


Figure 4. Mean values of DNA fragmentation (DNA) in the serum of control rats and rats in different treatment groups. Mean values ± S.E of (DNA) in the serum of the control and different treatment groups of rats. NS, not significantly different from CONT; ***very high significance; ## very high significance compared with the GM-treated group; S, significant.

Gentamicin (GM) has been identified as a catalyst for detrimental impacts on tissue structure and function by instigating the generation of free radicals, observable at the cellular level (Battin & Brumaghim, 2009). Free radical generation, observed after gentamicin usage, affects various biomolecules such as membrane lipids, proteins, and nucleic acids, particularly in renal tissues' mitochondria and lysosomes (Cuzzocrea et al., 2002). This process, mediated by reactive oxygen species (ROS), significantly contributes to hepatic and renal damage progression. The increased propagation of ROS leads to the peroxidation of polyunsaturated fatty acids on biological membranes. While low or moderate ROS production plays a physiological role in several redox-responsive signaling pathways, excessive ROS generation can result in various pathological conditions (Valko et al., 2007). The study highlights a distinctive nephrotoxic pattern induced by gentamicin administration in rats, characterized by elevated levels of creatinine, urea, and uric acid in the serum (Ataman, Mert, Yildirim, & Mert, 2018; Yilmaz, Mert, Irak, Erten, & Mert, 2018). These findings align with previous research suggesting that gentamicin may lead to a decrease in renal function (El-Ashmawy, El-Nahas, & Salama, 2006). Specifically, the study demonstrates that animals treated with I-SA, I-MPP, or I-EAA for 21 days show significant improvements in blood urea, creatinine, and uric acid levels compared to the GM group. This supports the observations of Hughes, Stricklett, Padilla, and Kohan (1996), who reported that salicylic acid treatment significantly reduced serum levels of urea, uric acid, and creatinine.

The liver, essential for protein synthesis, detoxification, and metabolism, is susceptible to drug-induced hepatotoxicity, a significant global cause of mortality. Patients using gentamicin often experience liver inefficiency issues, limiting the use of these drugs due to the associated risk of hepatotoxicity. Hepatotoxicity induced by gentamicin involves oxidative stress and free radical production. Administration of gentamicin resulted in a substantial and statistically significant ($p < 0.001$) elevation in levels of SGOT, SGPT, triglycerides, cholesterol, LDL, HDL, albumin, and total serum bilirubin. These outcomes align with previous studies (Galaly, Ahmed, & Mahmoud, 2014; Aboubakr & Abdelazem, 2016). Phenolic compounds in food plants, known for their reactive oxygen species scavenging abilities, have been extensively studied for their antioxidant properties. Salicylic acid (SA), a phenolic compound, has demonstrated antioxidant effects in various research studies (Baltazar, Dinis-Oliveir, Duarte, Bastos, & Carvalho, 2011). Additionally, salicylate has been shown to protect guinea pigs from gentamicin-induced hearing loss (Sha & Schacht, 1999). Importantly, pretreatment with I-SA, I-MPP, or I-EAA restored SGPT and SGOT levels to normal compared to the control group, indicating the protective effects of these interventions.

Lipid oxidation products are a common outcome of oxidative stress, where an imbalance between increased reactive oxygen species (ROS) generation and reduced antioxidant production leads to cellular damage (Kaneko, Emoto, & Emoto, 2014; Mohamed, Abd El-Kader, Awadalla, & El-Baga, 2023). Gentamicin poisoning results in a noticeable increase in lipid peroxidation (LPO) in the liver and kidney tissues, indicating oxidative damage. Consistent with previous studies (Galaly et al., 2014), we observed a highly significant ($p < 0.001$) rise in the level of the lipid peroxidation product (TBARS) in the GM group. However, rats treated with I-SA, I-MPP, or I-EAA displayed significantly reduced TBARS levels in the liver, suggesting a protective effect against lipid peroxidation. Levels of malondialdehyde (MDA) and nitric oxide (NO) were notably lower in the I-SA, I-MPP, and I-EAA groups compared to the GM-exposed group. Lipid peroxidation, a process linked to DNA damage and cell death, was evident, with gentamicin-induced DNA fragmentation. Notably, I-SA, I-MPP, and I-EAA therapy exhibited a protective effect, mitigating the increased DNA fragmentation induced by gentamicin.

Oxidative stress, resulting from an imbalance between reactive oxygen species formation and antioxidant defenses, leads to liver cell damage and renal toxicity. Superoxide dismutase (SOD), a crucial intracellular antioxidant enzyme, exerts a protective effect against superoxide anions. Catalase (CAT), found in all major organs with the highest concentration in the liver, catalyzes the conversion of H_2O_2 into water and oxygen (Mohamed & Abdel-Motaal, 2023). Gentamicin-treated rats exhibited lower levels of SOD and CAT in liver and kidney tissues compared to normal rats. However, the groups treated with I-SA, I-MPP, or I-EAA showed significantly higher SOD and CAT levels, returning to control levels when compared to gentamicin-exposed rats.

Conclusion

The synthesis of the targeted iron complexes was conducted using a green and sustainable approach, highlighting its green nature compared to traditional non-green methods through assuring the Concept of the 12 principles of Green Chemistry, in practice, and employing the commands of sustainability. The

biochemical study observed a substantial increase in oxidative stress markers and DNA fragmentation. Additionally, there was a significant decrease in the activity of the antioxidant enzymes SOD and CAT. These collective findings suggest that I-SA, I-MPP, and I-EAA may have potential as efficient protective agents against the nephrotoxic and hepatotoxic effects prompted by gentamicin. This affords valuable insights for potential therapeutic interventions aimed at mitigating drug-induced organ damage. Further research is necessary to uncover the underlying mechanisms and assess the clinical applicability of these defensive effects.

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