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Article

Synthesis, Characterization, and Subacute Toxicity of Hydrazide-Iron Complex in Rats

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Abstract: In this study, a hydrazide derivative and its iron (III) complex were synthesized and characterized using IR, MS, NMR, and elemental analysis. Subacute oral toxicity was assessed in male Wistar rats over 28 days. Biochemical and hematological parameters indicated dose-dependent effects on the liver and kidneys, including elevated ALT and creatinine levels. The iron complex group showed reduced RBC and hemoglobin with mild leukocytosis. These results suggest potential systemic toxicity at higher doses, highlighting the need for further investigation.

Keywords: Hydrazide, Iron(III) complex, Subacute toxicity, Liver function, Hematology

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1. Introduction

In the Schiff-base family, hydrazones are very special organic compounds and it is more substantial reagents in different organic reactions such as hydrazone iodination, Shapiro, and Bamford-Stevens' reaction [1]with vinyl compounds. Hydrazones are intermediates in the Wolff-Kishner reduction and are used as a good spectrophotometric reagent for the determination of metal ions in spectrophotometricstudies[2]. Generally, Aldehyde or ketone and hydrazide are condensed in appropriate solvents to create hydrazones, which have the general structure R1R2C=N - NH2[3]. In pharmacology, toxicology, pharmaceutical science, and other subjects, hydrazones are helpful both analytically and catalytically. They are also found in bioactive heterocyclic compounds and offer a variety of uses in the biological and pharmaceutical domains [4]. They also have antitubercular [5], antitumor [6], antimicrobial [7], antimalarial, analgesic, anti-inflammatory and antiplatelet, antidepressant [8], antimycobacterial, antiviral, anticonvulsant [9], antifungal, anticancer [10], and antioxidant [11] properties in the biological area.

The compound under investigation in this study, 4-hydroxy-N-(1-(4 hydroxyphenyl)ethylidene)benzohydrazide, is a newly synthesized hydrazide Schiff base bearing hydroxyl substituents on both aromatic rings. These structural features are known to contribute to antioxidant and potential estrogenic activity [12]. However, to our knowledge, comprehensive subacute toxicity data on this compound and its iron complex are lacking. Therefore, the present study aimed to evaluate their toxicological effects in male Wistar rats through the assessment of biochemical markers of liver and kidney function, as well as hematological parameters, following 28 days of oral exposure. Establishing a clear toxicological profile is a critical prerequisite for any biologically active compound, particularly those with structural motifs suggestive of pharmacological potential. Consequently, understanding the safety of this hydrazide derivative and its iron(III) complex serves as a foundation for future studies focused on exploring their

biological efficacy, including antioxidant or hormone-modulating properties, and guiding their potential development as therapeutic agents.

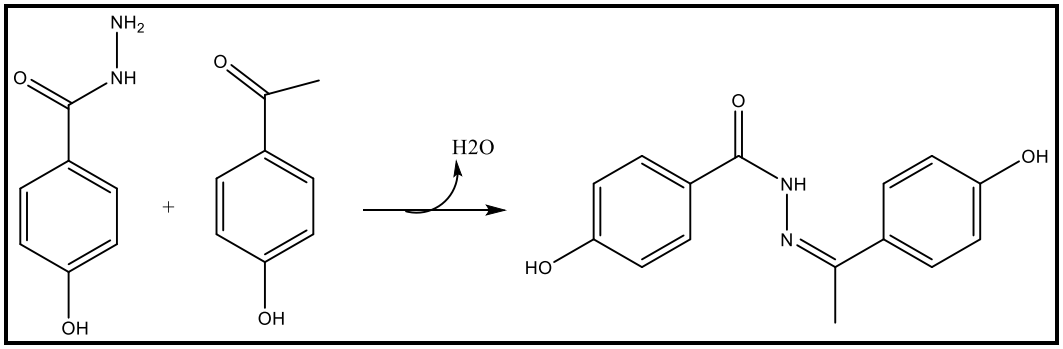
2. Materials and Methods

1- Chemistry

All chemicals were supplied by Fluka and B.D.H. Chemicals. The Shimadzu FTIR spectrometer was utilized to record the infrared spectra using the CsI disc for the metal ion complexes and the KBr disc for the generated ligands. Elements were analyzed using Thermofinigan Flash. The chemistry department of the College of Education of Pure Science, University of Basra, Iraq, employed a Bruker Vance 400 MHz spectrometer to record ^{1}H NMR and ^{13}C NMR spectral data using a Shimadzu, A-A-500 AFG, Japan, S/N 23-0932-21-0015 power150, voltage-AC110/220V, 50-60 Hz) flame. Utilizing the (HP)/MS Model 5973 Network Mass Selective Detector, the mass spectra of the ligand and complexes were recorded.

Synthesis of (z)- 4-hydroxy-*N*-(1-(4-hydroxyphenyl) ethylidene) benzo hydrazide

A solution of 4-hydroxy acetophenone (3.41 g, 25 mmol) in 40 ml of ethanol and 4-hydroxy benzo hydrazide (3.80 g, 25 mmol) in 40 mL of ethanol was prepared. These two solutions were mixed in a 250 ml round-bottom flask, and add 2ml of G.A.A to mixture as a catalyst. Reaction refluxed for 4.5 hrs. in a water bath, and a pale-yellow solid formed after refluxing. The TLC technique was then used to monitor the reaction's progress. After cooling to room temperature, the resultant reaction mixture was transferred into ice. The precipitates were shiny and pale yellow. After filtering and washing with cold ethanol, the precipitates were dried in an oven set at 95°C for an hour. It was determined that the yield percentage was 92.69%. The product had a melting point of 250-252^{o} and was recrystallized in ethanol all data as show in **Table 1**.



Equation 1: chemical reaction of the prepared compound

Preparation of the complex

General procedure for the preparation of a complex of a ligand

A solution of 5 mmol of the ligand (1:2) dissolved in absolute ethanol (20 ml) was combined with 30 ml of the transition metal salt FeCl3 dissolved in hot absolute ethanol. For 2.5 hrs. the mixture was refluxed. After the mixture was cooled using vacuum evaporation, then removed solvent. The precipitate was filtered and recrystallized by using mixture of DMF-ethanol (30–70 v/v) [11].

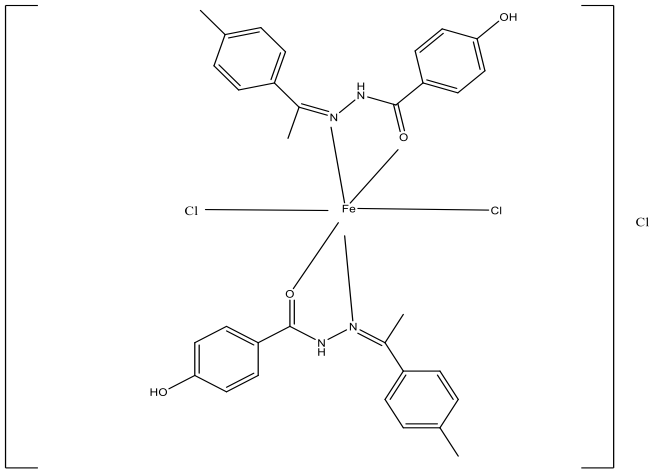


Table 1: The physical properties of synthesized compounds

Comp.	Molecular weight	Malting point	Color	% of yield
Ligand	270	250-252	yellow	92.69
L-Fe	702.3	310-312	Brawn	80.56

Toxicity study

Experimental Design and Animal Grouping

This study employed a subacute oral toxicity model to evaluate the potential systemic effects of a synthesized hydrazide derivative and its iron(III) complex. A total of **24 healthy adult male Wistar rats** (weight range: 180–220 g) were procured from a certified animal facility. The animals were housed in cages under standardized laboratory conditions (12-hour light/dark cycle, ambient temperature of 22 ± 2°C, and 50–60% humidity), with unrestricted access to a nutritionally balanced standard pellet diet and potable water throughout the study period.

the period 7 day acclimate then classification the animals to 4 group ant each group contain 6 rats .

1. **Group 1; [Control]:** Received 1 mL of vehicle (0.5% carboxymethylcellulose, CMC) via oral gavage.
2. **Group 2; (Low-dose hydrazide):** Administered the hydrazide derivative at a dose of (10 mg/kg)day.
3. **Group 3; (High- dose hydrazide):** Received 50 mg/kg/day of the hydrazide derivative.
4. **Group 4; (Iron(III) complex):** Treated with the synthesized iron complex at a with dose (20 mg/kg) day.

-Doses and Dose Calculation:

In this study, the doses of the compound were determined based on previous studies involving similar hydrazide derivatives, ensuring safe and effective doses as used in prior animal toxicity studies [13,14].

The compounds (hydrazide derivative and iron(III) complex) were accurately weighed and freshly prepared each day prior to administration. Due to their low aqueous solubility, each compound was initially dissolved in a minimal volume of dimethyl sulfoxide (DMSO, <5% v/v), then diluted with a 0.5% carboxymethylcellulose (CMC) suspension in distilled water to reach the desired final concentrations. To prepare a 10 mg/mL suspension, for example, 100 mg of the compound was dissolved in 0.2 mL of DMSO, followed by the addition of 9.8 mL of 0.5% CMC to achieve a final volume of 10 mL. The suspension was homogenized using vortex mixing and kept under continuous stirring throughout administration to ensure uniform distribution of the compound. The

use of DMSO and CMC as solubilizing and suspending agents is well-established in preclinical formulations [15].

Dosing was calculated individually based on each animal's body weight and the suspension concentration. For instance, to administer a dose of 10 mg/kg to a 200 g rat (0.2 kg), 0.2 mL of the 10 mg/mL suspension (equivalent to 2 mg of compound) was administered orally. This approach aligns with standard procedures in toxicological research [16].

- Blood Collection and Sample Preparation

On Day 29, following the 28-day dosing period, all animals were fasted overnight and anesthetized using a light ether protocol to facilitate blood collection. Sterile capillary tubes were used to collect blood samples from the retro-orbital sinus. 2-3ml of blood were drawn into plain tubes for biochemical analysis, and about 1 ml was drawn into tubes coated with EDTA for hematological examination. To separate the serum, the plain tubes were centrifuged for 10 minutes at 3,000 rpm after being allowed to clot at room temperature. All serum samples were carefully divided and keep at (−20°C)until further characterized to analyzed.

Biochemical and hematological analyses were conducted to assess potential systemic toxicity induced by the tested compounds. For biochemical assessments, serum samples were analyzed using commercially available diagnostic kits, in accordance with the manufacturers' instructions. Serum levels of alanine aminotransferase (A.L.T), aspartate aminotransferase (A.S.T), alkaline phosphatase (A.L.P), total bilirubin, total protein, and albumin were measured in order to assess hepatic function. Serum creatinine, blood urea nitrogen (B.U.N), and uric acid were indicators of renal function. An automated hematology analyzer was used to perform complete blood counts, measuring parameters like the total the blood cell with white color number (W.B.C), hemoglobin concentration (H.b); hematocrit (H.C.T); mean corpuscular volume (M.C.V); mean corpuscular hemoglobin (M.C.H); neutrophil, lymphocyte, monocyte, and eosinophil counts, and the red blood cell count (R.B.C).

Statistical study

Analyzed data by standard deviation (SD) , variance (ANOVA) after compared the result consider (< 0.05) that statistically significant.

3. Results and Discussion

Physicochemical Characterization of the Synthesized Compounds

1- IR Spectra:

The data of FTIR spectrum to prepared compound show in table 2. confirmed the Schiff base by the following band frequencies of the particular functional group,3305 band belong to OH group [17], the band at 3238 cm^{-1} related to the -NH group [18, 19], when carbonyl of amide appeared at 1643 cm^{-1} . imine group (C=N) belong at bands 1606 and 1595 cm^{-1} [17, 20], and this indicates the absorption of ($-NH_2$) in the structure of the molecule. the (-N-N) group indicated by band 1033 cm^{-1} [20, 21]. A weak peak at 3057 cm^{-1} is due to aromatic (-C‑H) bonds. In infrared (IR) spectroscopy, noticeable shifts occur in the bands due to coordination in the complexes of iron (Fe^{3+}), as the functional groups in the compound interact with the metal ions. For instance, the C=N band, representing the imine group, typically appears in the range of 1510cm^1, but it may shift to a lower range upon coordination with the metal. Similarly, the C=O band, associated with the carbonyl group, generally appears in the range of 1550cm^1, yet it can shift to lower wavelengths due to the influence of coordination. Moreover, new bands emerge, such as the ones representing metal-nitrogen (M-N) interactions in the range of 494 cm^{-1}, metal-oxygen (M-O) interactions in the range of 576 cm^{-1}, Table 1,

Table 2: FTIR data of hydrazone ligand

compound	ν-O-H (cm^{-1})	ν-N-H (cm^{-1})	ν -C=O (cm^{-1})	ν- (C=N) (cm^{-1})	ν- N-N (cm^{-1})	(ν-C-H) arom. (cm^{-1})	ν- M-N (cm^{-1})	ν M-O (cm^{-1})
ligand	3305	3238	1643	1606,1595	1033	3057	-	-
Complex	3305	3238	1550	1510	1033	3057	494	576

3- NMR spectra :

Proton and carbon chemical shifts in ppm were used to confirm the synthesized molecule in ^{1}HNMR and ^{13}CNMR data. The NH group is indicated by the signal at 11.12, the OH group at 10.06, and the CH_3 group at 2.56.

The ^{13}CNMR spectrum indicated the following values in ppm: 161.08 to carbonyl amide,149.89 to imine group, and 22.50 to CH3 group, which signals confirmed the synthesized compound[22]. Other data of ^{1}HNMR and ^{13}CNMR are shown in Table 3 and Figures 2 and 3.

Table 3: ^{1}HNMR and ^{13}CNMR data of ligand

	[^{1}H- NMR 400 MHz; DMSO δ]	[^{13}C- NMR 101 MHz; DMSO δ]
ligand	11.12 (1H,s,HN-C=O), 10.06 (11H,s,OH), 7.99- 6.90 (8H, m, ar-H), 2.50 (3H ,s,CH_3).	161.80 (NH-C=O), 158.91(C-OH), 149.83(C=N), 141.00 - 114.86 (Ar-C), 22.50(CH_3).

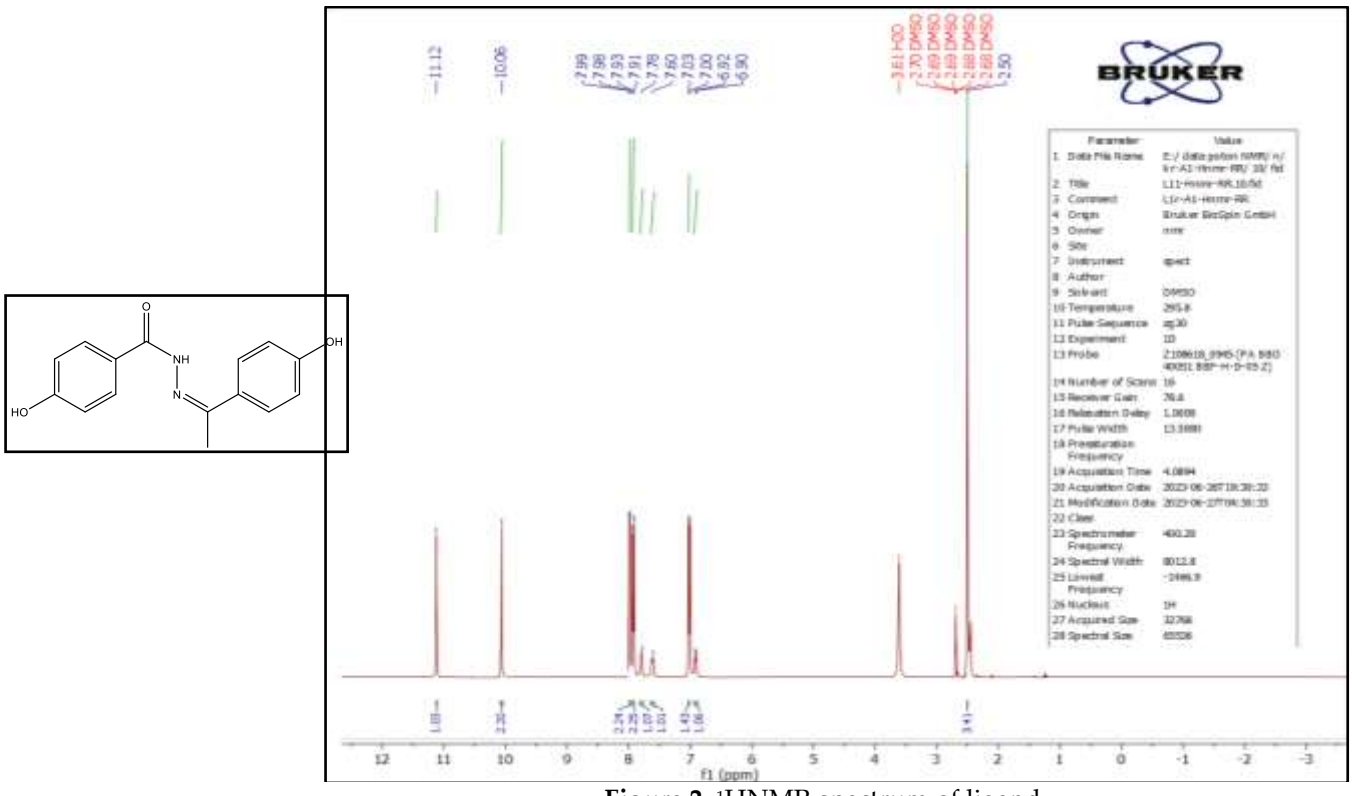


Figure 2: ^{1}HNMR spectrum of ligand

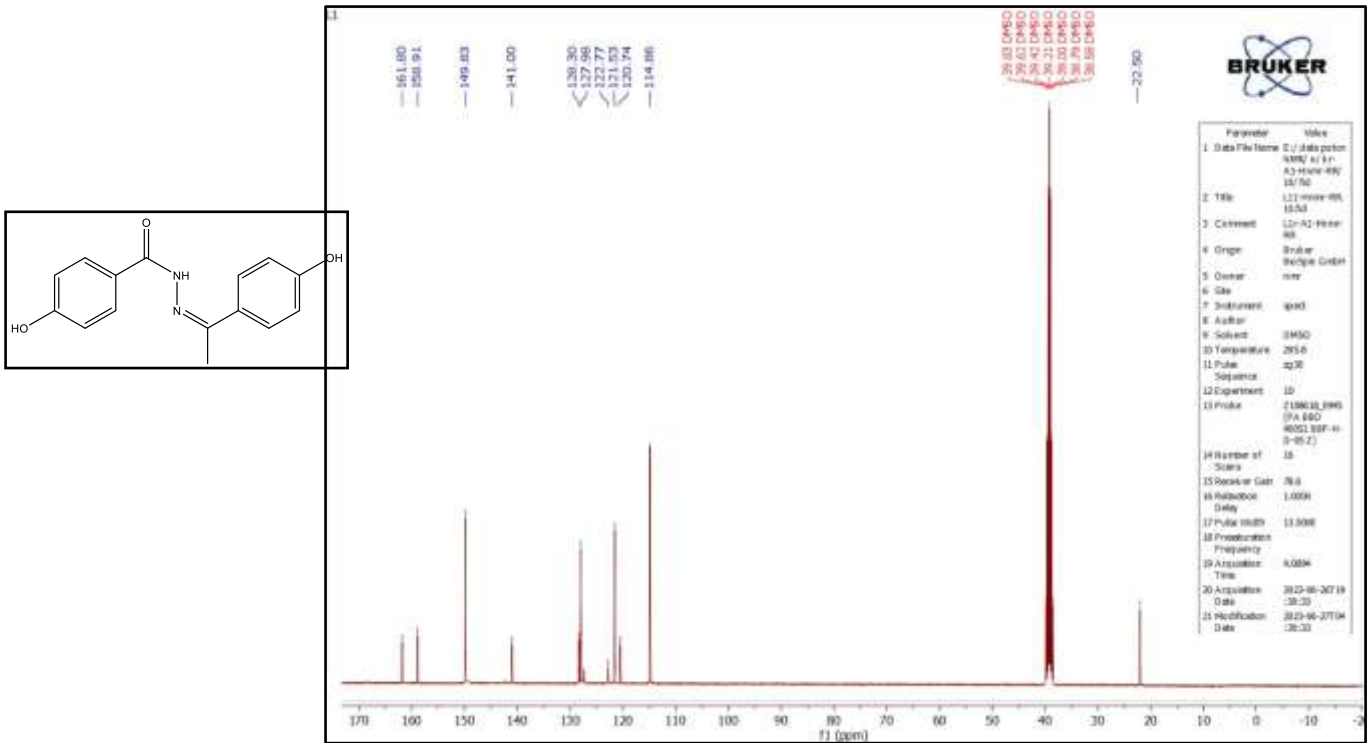


Figure 3: ^{13}CNMR spectrum of ligand

3- Mass Spectra for ligand and complex

The Figure 4 illustrated the mass spectra fragments of compound . The molecule has the molecular ion peak m/z value at 271-1=270. This is consistent with it's the M.F (formula of compound), $C_{15}H_{14}N_2O_3$ [Molecular Mass,Ms = 270].

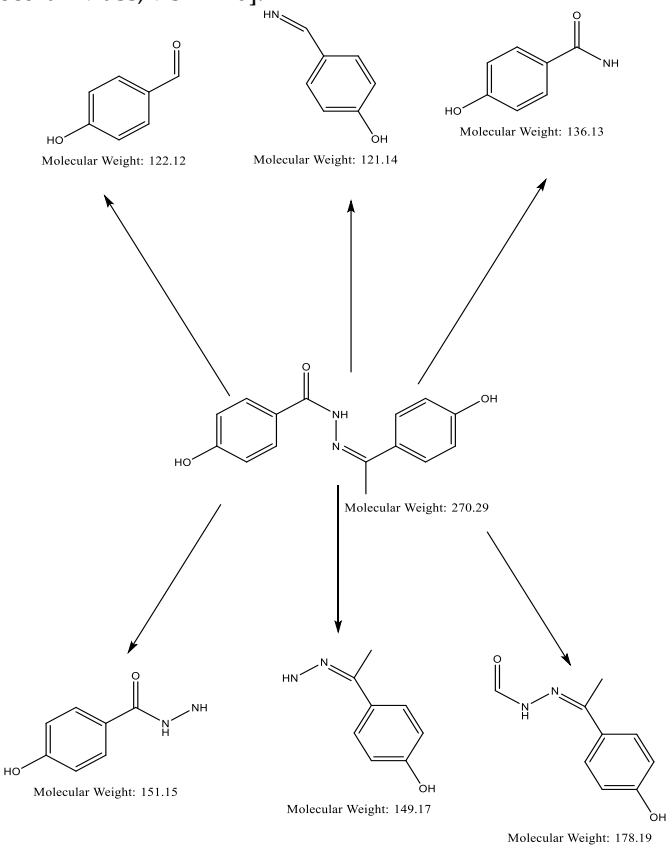


Figure 4: The mass spectrum of ligand

Table 4: The Mass spectrum fragments of ligand

No.	Molecular ion	m /z
1	$[C_{15}H_{14}N_2O_3]^{+\cdot}$	270
2	$[C_9H_{10}N_2O_2]^{+\cdot}$	178
3	$[C_8H_9NN_2O]^{+\cdot}$	151
4	$[C_7H_7N_2O_2]^{+\cdot}$	149
5	$[C_7H_6NO_2]^{+\cdot}$	136
6	$[C_7H_9O_2]^{+\cdot}$	122
7	$[C_7N_7NO]^{+\cdot}$	121

Table 5: The mass spectrum fragments of complex

No.	Molecular Ione	m/z
1	$[Fe(L)_2Cl_2]Cl$	702
2	$[Fe(L)_2Cl_2]$	666
3	$[Fe(L)_2Cl]$	631
4	$[Fe(L)_2]$	595
5	$[C_7H_6NO_2]^{+\cdot}$	136
6	$[C_7H_9O_2]^{+\cdot}$	122
7	$[C_7N_7NO]^{+\cdot}$	121

4-Elemental analysis

The element analysis is shown in Table 4.

Table 6: Elemental analysis

ligand	**% Found by analysis (from calculation)**		
	C 66.59 (66.66)	H 5.19 (5.22)	N 10.29 (10.36)

- Biochemical and Hematological Findings of Subacute Toxicity Study

1-Interpretation of Biochemical Parameters

After 28 days of oral administration, both the hydrazide derivative and its iron(III) complex induced notable alterations in liver and kidney function markers in Wistar rats. Serum levels of alanine aminotransferase (ALT) increased significantly from 35.4 ± 4.3 U/L in the control group to 51.2 ± 5.6 U/L in the hydrazide 50 mg/kg group and 57.8 ± 6.1 U/L in the iron complex group. Similarly, aspartate aminotransferase (AST) rose from 78.5 ± 6.1 U/L to 96.1 ± 7.2 U/L and 103.7 ± 8.0 U/L in the respective groups, indicating hepatic stress. Alkaline phosphatase (ALP) also increased significantly (control: 110.2 ± 10.4 U/L; hydrazide 50 mg/kg: 135.5 ± 12.3 U/L; iron complex: 140.8 ± 13.0 U/L). Total bilirubin levels were higher in treated groups (control: 0.62 ± 0.07 mg/dL vs. iron complex: 0.85 ± 0.10 mg/dL), while total protein and albumin levels declined, suggesting a reduction in hepatic synthetic capacity. Notably, blood urea nitrogen (BUN) increased from 19.3 ± 1.5 mg/dL to 26.2 ± 1.9 mg /dl and 28.5 ± 2.0 mg/dl, and serum creatinine rose from 0.52 ± 0.04 mg/dL to 0.71 ± 0.06 mg/dl and 0.76 ± 0.07 mg/dL in the hydrazide 50 mg/kg and iron complex groups, respectively. These changes point to mild renal dysfunction at higher doses.

Table 6: Biochemical Parameters in Wistar Rats Following Subacute Treatment

Parameter		**Control Group**	**Hydrazide 25 mg/kg**	**Hydrazide 50 mg/kg**	**Iron Complex 20 mg/kg**
Liver	ALT (U/L)	35.4 ± 4.3 (a)	42.6 ± 5.0 (ab)	51.2 ± 5.6 (b)	57.8 ± 6.1 (b)
	AST (U/L)	78.5 ± 6.1 (a)	84.3 ± 6.7 (ab)	96.1 ± 7.2 (b)	103.7 ± 8.0 (b)

	ALP (U/L)	110.2 ± 10.4 (a)	122.7 ± 11.1 (ab)	135.5 ± 12.3 (b)	140.8 ± 13.0 (b)
	Total Bilirubin (mg/dL)	0.62 ± 0.07 (a)	0.70 ± 0.08 (ab)	0.79 ± 0.09 (b)	0.85 ± 0.10 (b)
	Total Protein (g/dl)	6.91 ± 0.33 (a)	6.55 ± 0.30 (ab)	6.21 ± 0.27 (b)	6.10 ± 0.28 (b)
	Albumin (g/dl)	3.95 ± 0.21 (a)	3.72 ± 0.19 (ab)	3.48 ± 0.20 (b)	3.42 ± 0.18 (b)
Renal function	BUN (mg/dl)	19.3 ± 1.5 (a)	22.6 ± 1.6 (ab)	26.2 ± 1.9 (b)	28.5 ± 2.0 (b)
	Creatinine (mg/dl)	0.52 ± 0.04 (a)	0.61 ± 0.05 (ab)	0.71 ± 0.06 (b)	0.76 ± 0.07 (b)
	Uric Acid (mg/dl)	2.8 ± 0.3 (a)	3.2 ± 0.3 (ab)	3.6 ± 0.4 (b)	3.9 ± 0.5 (b)

* ($p < 0.05$) variation statistics between groups indicated by letters.

All treatment groups had markedly higher levels of liver function biomarkers, especially the alanine aminotransferase (A.L.T), and aspartate aminotransferase (AST), with the iron(III) complex group showing the highest values. These increases are indicative of hepatocellular injury, likely resulting from membrane destabilization and leakage of intracellular enzymes into the circulation [23]. The hydrazide moiety is known to undergo hepatic biotransformation, producing reactive intermediates such as hydrazine radicals and nitrogen-centered species capable of initiating oxidative stress and lipid peroxidation [24]. This oxidative burden disrupts mitochondrial function and compromises hepatocyte integrity [25].

Hepatic impairment is further supported by the notable decrease in serum total protein and albumin levels seen at higher dosages. Only the liver can synthesize albumin., and its depletion reflects impaired synthetic capacity or increased degradation under oxidative conditions [26]. These findings suggest that both hepatocellular damage and metabolic stress contributed to the observed biochemical disturbances.

Renal function markers—contain creatinine, blood -urea nitrogen (BUN), and uric acid were also markedly elevated, especially in the hydrazide 50 mg/kg and iron(III) complex groups. These elevations may be linked to glomerular filtration impairment or tubular injury, often associated with mitochondrial toxicity and oxidative damage within renal epithelial cells [27]. Hydrazide derivatives can interfere with mitochondrial respiratory complexes and promote reactive oxygen species (ROS) accumulation, leading to renal cellular apoptosis [28,29]. Elevated uric acid levels may also result from enhanced purine catabolism and impaired excretion, both hallmarks of nephrotoxicity [30].

The iron(III) complex appeared more toxic than the hydrazide compound alone, which may be explained by its increased lipophilicity and cell membrane permeability, leading to broader tissue distribution. Moreover, the iron center may participate in Fenton chemistry, catalyzing the formation of hydroxyl radicals from hydrogen peroxide, thereby intensifying oxidative stress [31,32]. These mechanisms likely account for the enhanced hepatic and renal toxicity observed in this group. Collectively, the data support the need for further long-term safety assessments, including histopathological and antioxidant profiling.

2-Interpretation of Hematological Parameters

Hematological analysis showed dose-dependent alterations, particularly in (red and white) blood cell indices. Red blood cell (R.B.C) counts declined from 6.6 ± 0.5 $\times 10^6/\mu L$ in the control group to 5.5 ± 0.5 $\times 10^6/\mu L$ and 5.2 ± 0.4 $\times 10^6/\mu L$ in the hydrazide 50 mg/kg and iron complex groups, respectively. Hemoglobin levels followed a similar trend, dropping from 13.8 ± 0.6 g/dL to 11.7 ± 0.8 g /dl and 11.3 ± 0.9 g/dL, alongside reductions in hematocrit (control: 41.2 ± 2.5%, iron complex: 35.2 ± 3.0%). These findings indicate mild normocytic anemia. White blood cell (WBC) counts increased from 7.2 ± 0.6 $\times 10^3/\mu L$ to 9.5 ± 1.0 $\times 10^3/\mu L$ and 10.1 ± 1.2 $\times 10^3/\mu L$, suggesting a leukocytic response. Neutrophil percentages increased from 26.3 ± 3.2% to 37.4 ± 4.1%, while lymphocyte percentages decreased from 64.7 ± 4.1% to 53.2 ± 5.4%, reflecting a shift toward neutrophilia, often associated with inflammatory or stress responses. Other indices such as MCV and MCH showed modest, non-significant elevations but remained within physiological limits.

Table 7. Hematological Parameters in Wistar Rats Following Subacute Treatment

Parameter	Control Group	Hydrazide 25 mg/kg	Hydrazide 50 mg/kg	Iron Complex 20 mg/kg
RBC (×10⁶/μL)	6.6 ± 0.5 (a)	6.1 ± 0.4 (ab)	5.5 ± 0.5 (b)	5.2 ± 0.4 (b)
Hemoglobin (g/dL)	13.8 ± 0.6 (a)	12.7 ± 0.7 (ab)	11.7 ± 0.8 (b)	11.3 ± 0.9 (b)
Hematocrit (%)	41.2 ± 2.5 (a)	39.1 ± 2.6 (ab)	36.7 ± 2.9 (b)	35.2 ± 3.0 (b)
MCV (fL)	82.3 ± 3.1	84.7 ± 3.5	87.9 ± 3.8	90.2 ± 4.1 (b)
MCH (pg)	20.9 ± 1.0	20.8 ± 1.1	21.3 ± 1.2	21.8 ± 1.3
WBC (×10³/μL)	7.2 ± 0.6 (a)	8.2 ± 0.8 (ab)	9.5 ± 1.0 (b)	10.1 ± 1.2 (b)
Neutrophils (%)	26.3 ± 3.2 (a)	30.1 ± 3.4 (ab)	34.1 ± 3.7 (b)	37.4 ± 4.1 (b)
Lymphocytes (%)	64.7 ± 4.1 (a)	60.2 ± 4.5 (ab)	56.0 ± 5.0 (b)	53.2 ± 5.4 (b)
Monocytes (%)	5.2 ± 0.8	5.5 ± 0.9	5.7 ± 1.0	6.0 ± 1.1
Eosinophils (%)	2.1 ± 0.4	2.4 ± 0.5	2.6 ± 0.6	2.8 ± 0.6

*($p < 0.05$) variation statistics between groups indicated by letters .

The hematological alterations observed in treated Wistar rats indicate that both the hydrazide derivative and its iron(III) complex exert subacute hematotoxic effects, particularly at higher doses. Animals given 50 mg/kg of the hydrazide and 20 mg/kg of the iron(III) complex showed a notably lower red blood cell (RBC) count, the hemoglobin concentration and hematocrit parts. These findings suggest the onset of anemia, likely of macrocytic type, as evidenced by concurrent elevations that explain corpuscular high (MCV) and mean corpuscular hemoglobin (MCH). Such macrocytic changes may result from impaired DNA synthesis or disrupted erythropoiesis, potentially due to oxidative stress induced by the hydrazide moiety and metal-mediated redox cycling, which can generate reactive oxygen species (ROS)[33]. ROS accumulation interferes with erythroid precursor cell survival in the bone marrow and may lead to shortened erythrocyte lifespan in circulation [34].

Moreover, the decline in hemoglobin may be attributed not only to reduced erythropoiesis but also to heme degradation or interference in iron incorporation into hemoglobin, particularly in the presence of the iron(III) complex. Despite iron being essential for erythropoiesis, excess or poorly coordinated iron can catalyze oxidative reactions, damaging hematopoietic cells [35].

Significant leukocytosis, especially neutrophilia, was observed in the iron complex-treated group. This indicates a systemic inflammatory response, possibly resulting from mild tissue injury or immune system stimulation due to the xenobiotic nature of the metal–ligand complex. Elevated neutrophils suggest acute-phase activation, while the concurrent reduction in lymphocyte percentage may reflect a shift in leukocyte distribution typical of stress leukograms [36]. Transition metals such as iron in complexed forms have been shown to activate toll-like receptors (TLRs) and stimulate pro-inflammatory cytokine production, thereby modulating immune cell profiles [37,38]. Although monocyte and eosinophil counts remained within normal limits and did not show statistically significant variation, the upward trends observed could reflect subtle immune modulation. Eosinophilia may be triggered by oxidative stress or allergic-type responses to foreign chemical entities [39]. Collectively, these hematological shifts highlight that repeated exposure to hydrazide derivatives and their iron(III) complexes can impair hematopoiesis and induce systemic inflammation, effects that must be carefully considered in future pharmacological development and safety evaluation.

4. Conclusion

The reaction between (2-Hydroxy acetophenone with 4-Hydroxybenzohydrazide) with drops of G.A.A effectively synthesizes ligand. Data from elemental analysis, mass spectroscopy, ^{1}HNMR, ^{13}CNMR, and FTIR showed that the ligand and its complex were synthesized. The 28-day subacute toxicity evaluation of the synthesized hydrazide Schiff base and its iron(III) complex in male Wistar rats revealed dose-dependent alterations in

liver and kidney function markers, as well as hematological parameters. While higher doses caused significant biochemical and hematological changes, the low-dose groups (such as 25 mg/kg of hydrazide and 20 mg/kg of the iron complex) exhibited only mild effects, indicating a relatively safer profile at these levels. These findings suggest that the compound may be considered for use at lower doses, although further long-term studies and histopathological assessments are necessary to fully ensure its safety. Although no severe toxicity was observed, additional evaluations are essential before considering its pharmacological application

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