

Synthesis, Characterization and Evaluation of Anti-Inflammatory Activity of Acetaminophen Complexes of Copper (II) and Zinc (II) Ions

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Abstract: The aim of this research was to synthesize, characterize as well as to investigate the anti-inflammatory properties of Zn (II) and Cu (II) as the central atoms using acetaminophen as the complexing agent. Cu (II) and Zn (II) Complexes were synthesized and characterized by FTIR spectroscopy, UV- Visible Spectroscopy, X-Ray Diffraction Analysis, Melting Point and Conductivity Measurements. On the basis of this study, it is proven that Acetaminophen acts as a bidentate ligand coordinated to the metal ions through phenol and carbonyl oxygen atom. The acute toxic effect was carried out by the method and the rats were found to be moderately toxic and slightly toxic. The test complexes in general showed maximum inhibition percentage at about 3 hours, after 4 hours it goes on reducing and reaches a minimum at about 5 hours.

Keywords: Acetaminophen, Anti- Inflammatory, Acute Toxicity, Metal Complexes

1. Introduction

The name Acetaminophen is gotten from the actual chemistry of the drug. The chemical name of acetaminophen is para-acetyl aminophenol, and this is where acet-aminophen comes from. Scientists or medical professionals refer to acetaminophen by using Acetyl-para-aminophenol (APAP) [1]. Acetaminophen is a p-aminophenol derivative with analgesic and antipyretic activities.

There are analgesics that are commonly associated with anti-inflammatory drugs but have no anti-inflammatory effects. An example is Acetaminophen. As opposed to Non-Steroidal anti-inflammatory drugs (NSAIDs), which reduce pain and inflammation by inhibiting Cyclooxygenase (COX) enzymes, Acetaminophen been shown to block the reuptake of endocannabinoids [2].

Detailed literature search shows the suitability of Paracetamol and Vanillin in forming low spin, novel heteroleptic, octahedral metal (II) complexes and the potentials of the metal (II) complexes as broad-spectrum antibacterial agents in-vitro. They concluded that Infrared

and electronic spectroscopies, with room temperature magnetic moments indicated a monomeric, 6-coordinate octahedral geometry for all the complexes with the exception of the Cu (II) complex, which was dimeric [3]. The *in-vitro* antibacterial studies on these metal (II) complexes against *B. cereus*, *E. coli*, *P. mirabilis*, *P. aeruginosa*, *K. oxytoca* and *S. aureus* showed that the Cobalt (II) complex exhibited a broad spectrum antibacterial activity against these bacteria [3].

The effects of the vanadium complexes on the selected experimental model of the fibro-vascular tissue were investigated [4]. They concluded that the anti-inflammatory activity of the complexes is moderate, among the complexes reported here, complex-3 [VO (SCA)₂] and 5 [VO (AAMB)₂] have shown high percentage inhibition compared with the standard drug Diclofenac sodium. The anti-inflammatory activities of all oxo-vanadium complexes showed promising results. Among the complexes, complex-3 [VO (SCA)₂] has shown the highest activity and is even more than the standard itself [4].

2. Materials and Methods

All chemicals were of analytical grade. Acetaminophen (product of sigma aldrich) was obtained Bristol chemical, Lagos, Nigeria. Copper Chloride and Zinc Chloride (ZnCl_2) were also obtained.

2.1. Preparation of Cu (II)-AC and Zn (II)-AC

The Copper complex was prepared by the addition of 0.51 g of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in 20ml of distilled water to 0.91 g of acetaminophen in 60 ml of distilled water, the mixture was stirred at room temperature until the dissolution of acetaminophen occurred, then heated for 6 hours. The solution was allowed to cool down overnight after which the precipitate was obtained. The precipitate was filtered off, washed with distilled water and dried for 48 hours. The same procedure was used for the preparation of Zn (II) complex from Zinc chloride [5].

2.2. Physical Measurement

The melting point of Acetaminophen and the dissociation points synthesized complexes were carried out using Automatic melting point SMP40. Molar conductance was carried out using conductance Resistance meter. UV-Visible Spectra was determined using UV-Visible Spectrometer. The infrared spectra were determined using Fourier Transform Infrared Spectrometer [6].

2.3. Acute Toxicity Studies

This method was carried out in two phases. In the first phase, the animals were divided into three groups containing three rats each. All the rats in the groups were administered with Cu (II)-AC at doses of 10, 100 and 1000 mg/kg body weight orally and observed for signs of toxicity and death for 24 hours. In the second phase, three

rats were treated with Cu (II)-AC at doses of 1600, 2900 and 5000 mg/kg [7], and observed for 24 hours for signs of toxicity and death. The same procedure was used for the LD_{50} of Zn (II)-AC.

2.4. Anti-Inflammatory Activity

The rats were divided into five groups ($n=5$), each containing 5 rats. Group 1 served as the control (saline). Group II was given piroxicam orally (20mg/kg) as a standard drug. Groups III, IV and V received 200, 400 and 600 mg/kg of Cu (II)-AC complex by oral administration respectively. One hour later, 0.1ml of freshly prepared carrageenan suspension was injected into the sub planter region of the left hind paw of each rat [8]. The paw diameter was measured with the aid of a digital caliper at 0, 1, 2, 3, 4 and 5 hours, after the injection of carrageenan. The same procedure was used for the Anti-inflammatory activity of Zn (II)-AC. The percentage (%) inhibition of the inflammation was calculated from the formula [8].

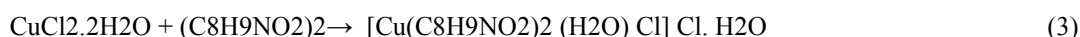
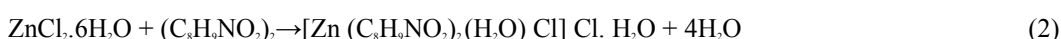
$$\text{Percentage (\%)inhibition} = \frac{[1-Dt]}{[D0]} \times 100 \quad (1)$$

Where D0 was the average inflammation (hind paw edema) of the control group of rats at a given time, Dt was the average inflammation of the sample or piroxicam administered at the same time.

3. Results

3.1. Physical Measurement

The reaction of Acetaminophen with the Zinc (II) chloride gave white solid complex and the reaction of Acetaminophen with copper (II) chloride gave brown solid complex According to the equations below.



The Physical characteristics of the ligand and Zn (II) and Cu (II) complexes are presented in Table 1 below.

3.2. Unit Cell Parameters of the Metal Complexes

The observed unit cell parameters are given in Table 3. The diffraction pattern of complexes is recorded between 2θ ranging from 5° to 65° . The particle size of the samples is

estimated using the Scherrer's formula. According to Scherrer's equation, the particle size is given by $t = 0.9 \lambda / B \cos \theta$, where t is the crystal thickness (in nm), B is half width (in radians), θ is the Bragg angle and λ is the wavelength. The particle size corresponding to each diffraction maxima are determined from the measurement of the half width of the diffraction peak [11].

Table 1. Physical data of Acetaminophen and the solid complexes.

Complexes	color	MP/DT ($^\circ\text{C}$)	Molecular Formula	Percentage yield (%)	Molar Conductance (mS/cm)
AC	white	169.6-170.4	$\text{C}_8\text{H}_9\text{NO}_2$		
Zn (II)-AC	white	165.1-168.1	$\text{ZnC}_{16}\text{H}_{22}\text{N}_2\text{O}_6\text{Cl}_2$	64	0.02
Cu (II)-AC	brown	149.3-150.9	$\text{CuC}_{16}\text{H}_{22}\text{N}_2\text{O}_6\text{Cl}_2$	58	0.05

Table 2. FTIR and UV-Vis Spectral data of Acetaminophen and the solid complexes.

FTIR cm ⁻¹			UV-Vis (nm)
ligand/complexes	V (O-H)	V (N-H)	V (C=O)
AC	3108	3321	1651
Zn (II)-AC	-	3321	1555
Cu (II)-AC	-	3317	1561
			256
			295, 215, 206
			293, 210

Table 3. Unit Cell Parameters of the Complexes.

Complexes	a (Å)	b (Å)	c (Å)	α (°)	β (°)	γ (°)	V (Å) ³	d _{xrd} (nm)
Zn (II)-AC	9.72	7.53	6.81	99.64	105.12	93.38	498	84.03
Cu (II)-AC	12.104	9.421	9.342	97.12	101.24	99.76	1065	58.01

Table 4. Acute lethal effect of CU (II)-AC Administered orally to white Albino Rats.

Experiment	Dose (mg/kg)	No of Dead Rats after 24hrs
Phase 1	10	0/3
	100	0/3
	1,000	0/3
	1,600	0/1
Phase 2	2,900	0/1
	5,000	1/1

Lethal Dose (LD₅₀) = $\sqrt{2900 \times 5000}$
 = $\sqrt{14500000}$
 = 3808 mg/kg

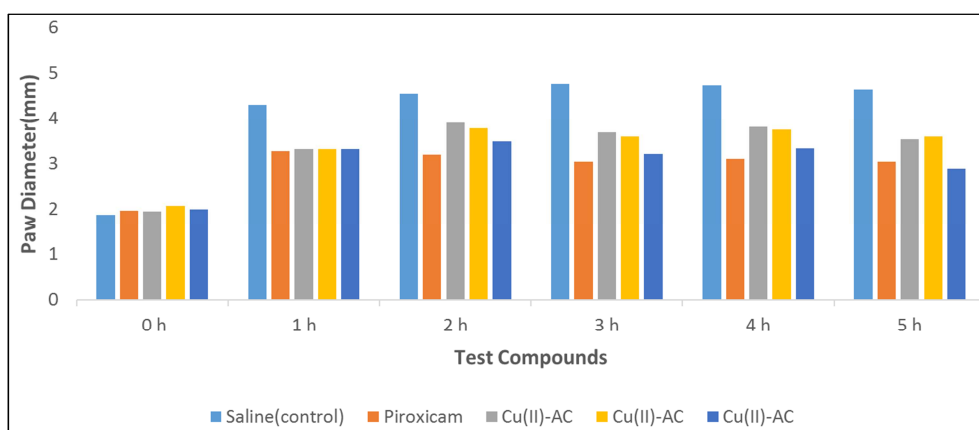
Table 5. Acute lethal effect of Zn (II)-AC Administered orally to white Albino Rats.

Experiment	Dose (mg/kg)	No of Dead Rats after 24hrs
Phase 1	10	0/3
	100	0/3
	1,000	3/3
	140	0/1
Phase 2	225	0/1
	370	1/1
	600	1/1

Lethal Dose (LD₅₀) = $\sqrt{225 \times 370}$ = $\sqrt{83250}$ = 289 mg/kg

Table 6. Effect of Cu (II)-AC on carrageenan- induced rat paw edema.

		edema diameter (mm)/Time interval (hour)					
		[mean of inhibition]					
Group (n=5)	Dose (mg/kg)	0 h	1 h	2 h	3 h	4 h	5 h
Saline (control)	10mL/kg	1.89±0.17	4.31±0.5	4.55±0.4	4.76±0.4	4.74±0.2	4.65±0.20
Piroxicam	20mL/kg	1.98±0.22	3.28±0.4 [23.8]	3.20±0.9 [29.6]	3.04±0.8 [36.1]	3.11±0.9 [34.4]	3.04±0.7 [34.6]
Cu (II)-AC	200mg/MI	1.95±0.19	3.33±0.4 [22.8]	3.91±0.4 [14.0]	3.69±0.9 [22.5]	3.82±0.5 [19.4]	3.54±0.6 [23.8]
Cu (II)-AC	400mg/MI	2.07±0.2	2.87±0.2 [33.3]	3.78±0.7 [16.9]	3.60±0.7 [24.4]	3.76±0.2 [20.7]	3.61±0.3 [22.4]
Cu (II)-AC	600mg/MI	2.008±0.4	2.98±0.3 [30.8]	3.5±0.3 [23.1]	3.2±0.3 [32.4]	3.34±0.4 [29.5]	2.894±0.4 [37.7]

**Figure 1.** Effect of Cu (II)-AC on carrageenan induced rat paw edema.**Table 7.** Effect of Zn (II)-AC on carrageenan- induced rat paw edema.

		edema diameter (mm)/Time interval (hour)					
		[mean of inhibition]					
		0h	1h	2h	3h	4h	5h
Saline (control)	10mL/kg	2.178±0.2	4.25±0.3	4.962±0.3	5.526±0.7	4.674±0.4	5.138±0.4
piroxicam	20mL/kg	2.172±0.1	2.764±0.4 [35]	4.962±0.2 [41.5]	3.168±0.5 [42.7]	3.096±0.5 [33.8]	2.83±0.2 [44.9]
Zn (II)-AC	50mg/MI	2.178±0.2	3.754±0.2 [11.7]	4.072±0.4 [17.9]	4.126±0.6 [25.3]	3.83±0.5 [18.1]	3.634±0.4 [29.3]
Zn (II)-AC	25mg/MI	2.028±0.1	3.708±0.2 [12.8]	4.962±0.7 [8.5]	4.042±0.5 [26.9]	3.76±0.6 [19.6]	3.414±0.4 [33.6]
Zn (II)-AC	12.5mg/MI	2.166±0.14	4.246±0.3 [0.09]	4.962±0.7 [0.7]	4.09±0.5 [26.0]	3.978±0.2 [14.9]	3.742±0.4 [27.1]

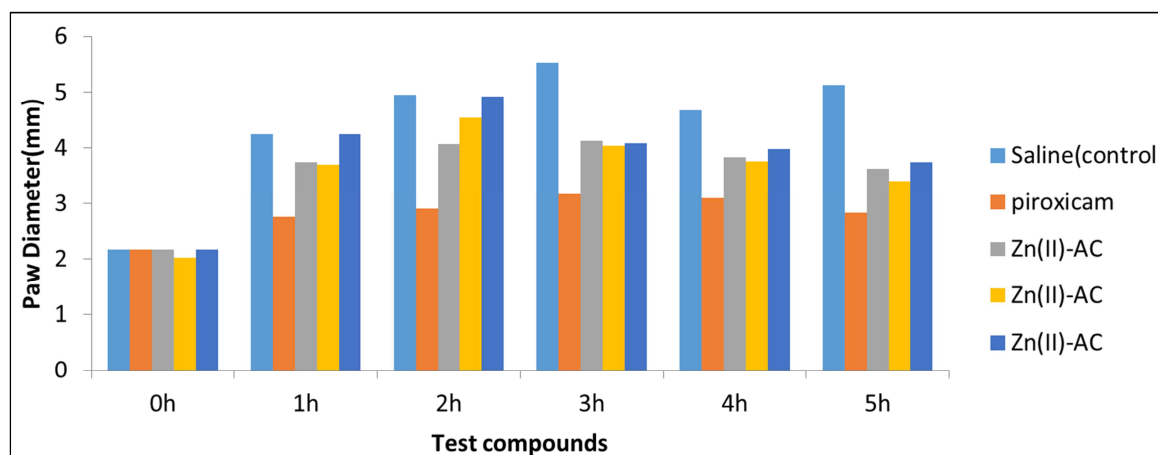


Figure 2. Effect of Zn (II)-AC on carrageenan induced rat paw edema.

4. Discussion

The solubility of the complexes was carried out by dissolving complexes in some solvents such as ethanol, water, methanol and Dimethyl sulfoxide. The complexes were soluble in ethanol, methanol and Dimethyl sulfoxide but were found to be sparingly soluble in water. The melting point of Acetaminophen was in the range 169.6-170.4°C, whereas the metal complexes decomposed in the range 149.3-168.9°C, confirming coordination.

The molar conductivities of the complexes were determined in Methanol. The molar conductance values in Table 1 were found to be in the range of 0.02mS/cm to 0.05 mS/cm showed that the molar conductance are of very low values, indicating the non-electrolytic or covalent nature of these complexes [3]. Analytical data of the compounds, together with their physical properties are consistent with proposed molecular formula.

The electronic absorption bands showed the absorption spectra of Acetaminophen and the complexes were recorded in methanol solvent in the range of (200-400 nm) and they are shown in Table 2. The electronic spectra of Acetaminophen show bands at (256nm). The bands were shifted in the metal complexes due to coordination [3]. As shown in Table 2, where Cu (II)-AC and Zn (II)-AC showed electronic transition at (210, 293 nm) and (206, 209 293 nm).

The Fourier Transform Infrared Spectrum of acetaminophen (Table 2) showed some characteristic stretching bands at 3321, 3108 and 1650 cm^{-1} . In the analysis of acetaminophen, -NH group showed absorption band at 3321 cm^{-1} , the absorption band at 3108 cm^{-1} can be assigned to -OH stretching vibration. The strong absorption band at 1651 cm^{-1} in acetaminophen spectrum is indicative of a carbonyl group [9].

4.1. Infrared Spectra of Zn (II)-AC and Cu (II)-AC

Infrared spectrum of Zn (II)-AC was also studied. The

shift in stretching frequency of -OH bands is observed after the formation of Zn(II)-AC complex due to the coordination (Table 2). The insignificant shift in the -NH group is an indication that -NH is not involved in the formation of Zn(II)-AC [10].

By the analysis of Cu(II)-AC, several bands have been modified. The shift in the characteristic -OH bands at 3108 cm^{-1} is an evidence of coordination through this group. The shifting of the carbonyl group from 1651 to 1561 cm^{-1} is also an indication of coordination through the carbonyl group. Comparing the absorption bands of AC and Cu (II)-AC, since there is no significant shift in the -NH group, then the -NH group is not involved in complex formation [10].

4.2. XRD Patterns and Unit Cell Parameters of the Metal Complexes

The Powder XRD patterns of Cu(II)-AC and Zn(II)-AC complexes are shown in Table 3. The observed unit cell parameters are given. The Powder XRD patterns of acetaminophen and its complexes were recorded. Unit cell parameters were found by using trial and error methods (Kavitha *et al.*, 2013). All compounds are monoclinic with different unit cell parameters. The XRD patterns of all complexes are similar and suggest that all the complexes possess similar structure (Kavitha *et al.*, 2013).

4.3. Proposed Structure of the Acetaminophen Complexes

The proposed structures of Acetaminophen complexes with Zn (II) and Cu (II) ions have been confirmed from the Melting point, IR, molar conductance, UV-Vis and XRD analysis data. Thus, from the IR spectra, it is concluded that acetaminophen behaves as a neutral bidentate ligand coordinated to the metal ions oxygen atoms of hydroxyl and carbonyl groups. From the molar conductance data, it is found that the complexes seem to be non-electrolytes. On the basis of the above observations, the investigated complex structures can be given below.

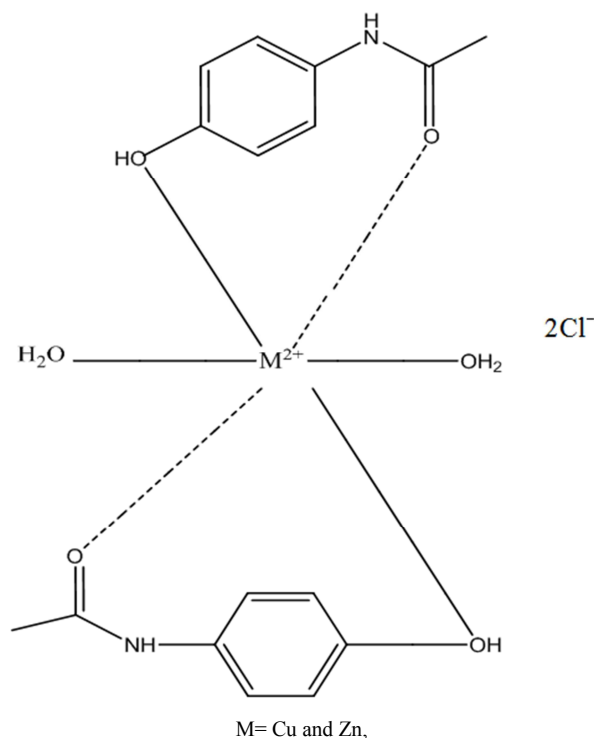


Figure 3. The proposed structure of metal (II) complexes.

4.4. Acute Toxicity

In lethal dose determination (LD_{50}), the result for Acute toxicity study of Cu(II)-AC on rats shows that no mortality was recorded in any of the test groups within 24 hours in the first phase for Cu(II)-AC. In the second phase, mortality was recorded at 5000 mg/kg.

The result for Acute toxicity study of Zn(II)-AC on rats shows that all the rats in the third group died within 24 hours in the first phase for Zn(II)-AC. In the second phase, mortality was recorded at 370 and 600 mg/kg.

Based on the results, further specific doses were administered to calculate the LD_{50} .

According to Hodge and Sterner toxicity (2005) in lethal dose determination, substances with lethal dose (LD_{50}) ≥ 5000 mg/kg are considered to be practically non-toxic, 500 - 5000 mg/kg are considered to be slightly toxic, 50 - 500 mg/kg are considered to be moderately toxic, 1 - 50 mg/kg are considered to be highly toxic, Less than 1 mg/kg are considered to be extremely toxic.

From Table 4 and 5, the LD_{50} of Zn(II)-AC and Cu(II)-AC were 289 mg/kg and 3808 mg/kg respectively. So the two complexes were considered to be moderately toxic and slightly toxic [12].

4.5. Anti-Inflammatory Activity

The test complexes in general show maximum inhibition percentage at about 3 h. After 4 h it goes on reducing and reaches a minimum at about 5 h. The percentage Anti-inflammatory effect of Cu(II)-AC (Table 6) at the peak of carrageenan-induced edema at the third hour were 22.5%, 24.4%, 32.4% at respective doses of 200, 400 and 600mg/kg

compared to 36.1% for piroxicam at 20mg/kg. At the fourth and fifth hour, there was an increase in the percentage Anti-inflammatory effects and a decrease in the volume of edema.

From Table 7, the percentage Anti-inflammatory effect of Zn(II)-AC at doses of 50, 25 and 12.5 mg/ml at the 3rd hour was 25.3%, 26.9% and 26%. The percentage Anti-inflammatory effect of Piroxicam at the third hour was 42.7% at 20mg/kg. There was an increase in percentage Anti-inflammatory effect at the 4th and 5th.

5. Conclusion

Cu (II)-AC and Zn (II)-AC have been synthesized and characterized by FTIR spectroscopy, electronic spectroscopy, Conductivity measurement and XRD. It was proven that the formation of complexes occurs by both C=O and -OH groups from Acetaminophen.

Based on the observations made, the complexes were considered according to Hodge and sterner (2005) to be moderately toxic and slightly toxic.

There was also a general decrease in the diameter of the edema and increase in the percentage Anti-inflammatory effect of at the fourth and fifth hour for the complexes and the percentage Anti-inflammatory effect at its peak was at the third hour. The percentage Anti-inflammatory effect of piroxicam at the 3rd hour was 46.5%. since the percentage Anti-inflammatory effects of the complexes are higher than that of the normal saline, we can conclude that the complexes have anti-inflammatory activity.

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