ANTIMICROBIAL, ACUTE TOXICITY AND ANTI-INFLAMMATORY INVESTIGATIONS OF MG(II) COMPLEX OF ACETAMINOPHEN

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Abstract: Magnesium complex of Acetaminophen was synthesized and characterized by infrared spectroscopy, UV-Visible spectroscopy, melting point, X-Ray diffraction analysis and conductivity measurements. On the basis of this study, it is proven that acetaminophen acts as a bidentate ligand coordinated to the metal ion through phenol and carbonyl oxygen atom. Antimicrobial and antifungal activities of magnesium complex and acetaminophen were determined against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeroginesa* and *Candida albicans*. The results indicate that acetaminophen showed sensitivity against *Pseudomonas aeroginesa.* Magnesium complex of acetaminophen showed activity against *Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeroginesa*. The lethal dose determination (LD_{50}) of magnesium complex was 1265 mg/kg. The complex was considered to be slightly toxic. Anti-Inflammatory activity of the synthesized complex has been carried out by paw edema method in rats. The Maximum Percentage inhibition of Magnesium complex was 18.9%.

Keywords: Acetaminophen, magnesium, antimicrobial, anti-inflammatory, toxicity, metal, complex

Introduction

Acetaminophen (AC) consists of a benzene ring core, substituted by one hydroxyl group and the nitrogen atom of an amide group in the para (1,4) pattern (Bertolini *et al.,* 2006). The amide group is acetamide (ethanamide). It is an extensively conjugated system, as the lone pair on the hydroxyl oxygen, the benzene pi cloud, the nitrogen lone pair, the p orbital on the carbonyl carbon, and the lone pair on the carbonyl oxygen are all conjugated. The presence of two activating groups also make the benzene ring highly reactive toward electrophilic aromatic substitution. As the substituents are ortho, para-directing and para with respect to each other, all positions on the ring are more or less equally activated (Bertolini *et al.,* 2006). The conjugation also greatly reduces the basicity of the oxygen and the nitrogen, while making the hydroxyl acidic through delocalization of charge developed on the phenoxide anion.

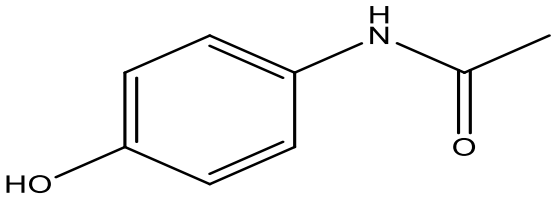

Fig. 1: Acetaminophen

Acetaminophen is not considered a Non-steroidal anti-inflammatory drug (NSAID) because it does not exhibit significant anti-inflammatory activity (Viswanathan *et al.,* 2008). This is despite the evidence that paracetamol and NSAIDs have some similar pharmacological activity (Byrant *et al*., 2013)

An Antimicrobial is an agent that kills microorganisms or stops their growth (Kellermeyer *et al*., 2013). Some sources distinguish between antibacterial and antibiotic; antibacterial are used in soaps and disinfectants, while antibiotics are used as medicine (Rollin *et al.,* 2016). The choice of antibiotic given will also be based on its cost. Identification is critically important as it can reduce the cost and toxicity of the antibiotic therapy and also reduce the possibility of the emergence of antimicrobial resistance (Rollin *et al.,* 2016). Anti-inflammatory or anti-inflammatory refers to the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system to block pain signaling to the brain (Ottani *et al.,* 2006).

Detailed literature search shows that mixed drug complexes of sulfamethoxazole and vanilin have been reported (Lawal and Obaleye, 2007; Refat *et al.,* 2013). However, less information is available on the anti-inflammatory activity of magnesium complex of acetaminophen. Thus, we present the antimicrobial and anti-inflammatory activities of magnesium complex of acetaminophen (Mg(II)-AC), with the aim of investigating the potentials of its magnesium (II) complex as broad spectrum antimicrobial and anti-inflammatory agent.

Materials and Methods

All chemicals were of analytical grade. Acetaminophen (product of sigma aldrich) was obtained Bristol chemical, Lagos, Nigeria. Magnesium Chloride ($MgCl_2$) was also obtained.

Preparation of Mg(II)-AC

Magnesium complex of acetaminophen was prepared by the addition of (0.61 g, 3 mmol) of $MgCl_2.6H_2O$ in 20ml of distilled water to (0.91 g, 6 mmol) of acetaminophen in 60 ml of distilled water, the mixture was stirred using Magnetic stirrer at room temperature until the dissolution of acetaminophen occurred, then heated using water bath for 6 hours. The solution was allowed to cool down overnight after which the precipitate was obtained. The precipitate was filtered off using Whatman filter paper, washed with distilled water and dried in a desiccator for 48 hours (Oluwatoosin *et al.,* 20014).

Physical measurement

The melting point and decomposition temperature of the synthesized complex were carried out using Automatic melting point SMP40. Molar conductance was carried out using conductance Resistance meter. The solubility of the complex was carried out by dissolving the complexes in ethanol, water, methanol and Dimethyl sulfoxide. UV-Visible Spectra was determined using Cary 300 UV-Visible Spectrometer. The room temperature magnetic susceptibility at 303K was measured. The infrared spectra were determined using Cary 630 FTIR (Refat *et al*., 2013). X-ray diffraction pattern were recorded using X-Ray diffractometer.

Antimicrobial activities of Mg(II)-AC

The antimicrobial activity of Magnesium complex (Mg(II)-AC) was determined using some pathogenic microbes, the minimum inhibitory concentration of Mg(II)-AC was determined using broth dilution method, and minimum

bactericidal or minimum fungicidal concentrations were carried out (Aamer *et al*., 2015).

Acute toxicity studies of Mg(II)-AC (LD_{50})

The LD_{50} 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 Mg(II)-AC at doses of 10, 100 and 1000 mg/kg body weight orally and observed for signs of toxicity and death for 24 h. In the second phase, three rats were treated with Mg(II)-AC at doses of 1000, 1600 and 2900 mg/kg and observed for 24 h for signs of toxicity and death (Lorke, 1983).

Anti-inflammatory activity of Mg(II)-AC

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 (20 mg/kg) as a standard drug. Groups III, IV and V received 200, 150 and 100 mg/kg of Mg(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. 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 percentage (%) inhibition of the inflammation was calculated from the formula (Winter *et al*., 1962).

$$percentage(\%)\ inhibition = \frac{[1 - Dt]}{[D0]}\ x\,100 \quad (1)$$

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 administered or piroxicam at the same time.

Results and Discussion

Physical measurement

The reaction of Acetaminophen with magnesium (ii) chloride gave white solid complex according to equation 2 below. The purity of Magnesium complex was checked by taking the melting points.

$MgCl_2.6H_2O + 2(C_8H_9NO_2) \rightarrow [Mg(C_8H_9NO_2)_2\ (H_2O)\ Cl]\ Cl.\ H_2O + 4H_2O$ (2)

The formation of the metal complex was confirmed by molar conductance, solubility, XRD, infrared and electronic spectroscopies. Acetaminophen melt at 169.6 – 170.4^{o}C, whereas the metal complex decomposed in the range 165.6 – 167.8^{0}C, confirming coordination. The complex was supportive of octahedral geometry with a moment 0.44 B.M. (Raman *et al*., 2004).

The Physical characteristics of the ligand and Mg(II) complex prepared are presented in Table 1. The complex was soluble in ethanol, methanol and Dimethyl sulfoxide but was found to be slightly soluble in water (Table 1). Molar conductivity of the complex was determined in Dimethyl sulfoxide. The molar conductance value of the complex in Table 1 (0.03 mS/cm) shows that the molar conductance is of very low values, indicating the non-electrolytic or covalent nature of magnesium complex (Refat *et al.,* 2013).

Table 1; Physical data of acetaminophen and the solid complex

Complexes	color	MP/DT (^{o}c)	Molecular Formula	Percentage yield (%)	Molar Conductance (mS/cm)
AC	white	169.6-170.4	$C_8H_9NO_2$		
Mg(II)-AC	white	165.6-167.8	$MgC_{16}H_{22}N_2O_6Cl_2$	60	0.03

MP= melting point, DT= Decomposition temperature

Table 2: UV-Vis and FTIR Spectral data of Acetaminophen and Magnesium complex

FTIR cm^{-1}				UV-Vis (nm)
ligand/complexes	V(O-H)	V(N-H)	V(C=O)	
AC	3108, 3160	3321	1651	256
Mg(II)-AC		3324	1547	293, 209, 206

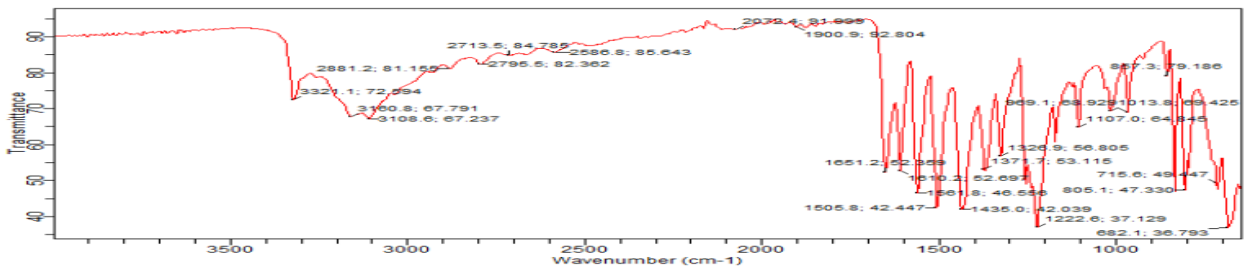

Fig. 2: FTIR spectra of acetaminophen

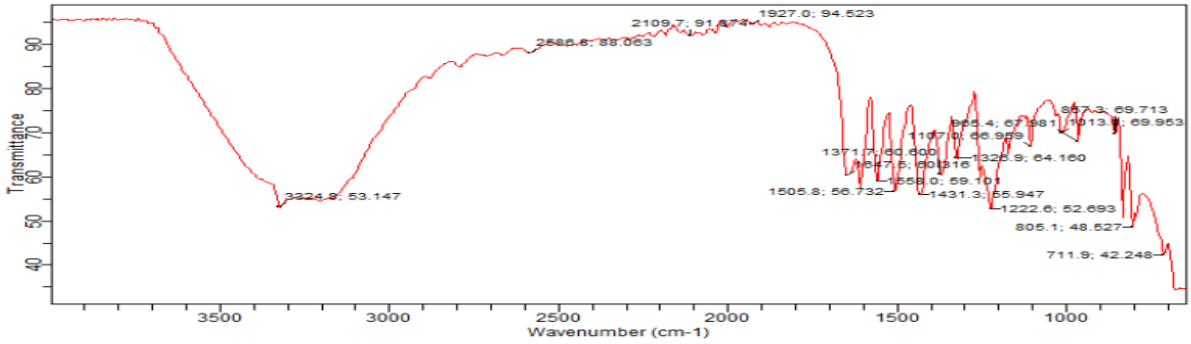

Fig. 3: FTIR spectra of Mg(II)-AC Complex

Table 3: Unit cell parameters of the complex

Complexes	a (Å)	b (Å)	c (Å)	α(^{0})	β (^{0})	ϒ(^{0})	V (Å)^3	d_{xrd} (nm)
Mg(II)-AC	7.864	16.625	5.651	90	90	90	739	73.36

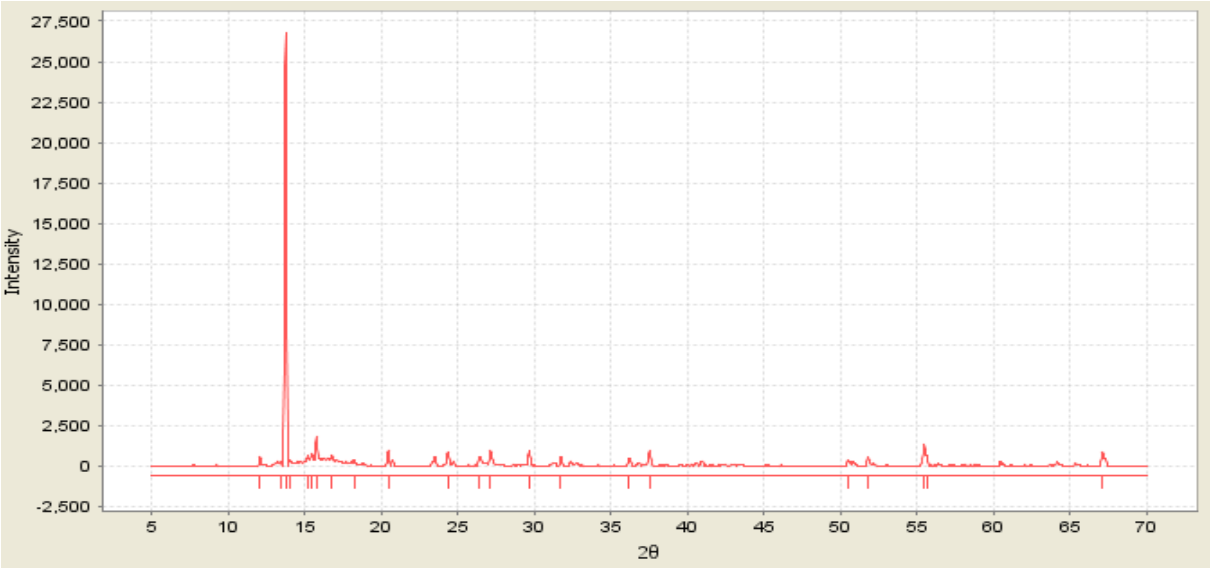

Fig. 4: Mg(II)-AC XRD pattern
Wavelength:Wavelength Cu Ka1 1.54056Å

Electronic spectra acetaminophen and magnesium complex

The Electronic spectra of Acetaminophen show bands at (256 nm). The bands were shifted in the metal complexes due to coordination (Aamer *et al*., 2015). As shown in Table 2, where Mg(II)-AC showed electronic transition at 206, 209 293 nm.

Infrared spectra of acetaminophen and Mg(II)-AC

The Fourier Transform Infrared Spectrum of acetaminophen (Table 2 and Fig. 2) shows some characteristic stretching bands at 3321, 3108 and 1650 cm^{-1}. In the analysis of acetaminophen, -NH group shows 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 (Coates, 2000).

In the analysis of Mg(II)-AC (Fig. 3), some bands have been modified. The disappearance of the characteristic –OH band at 3108 cm^{-1} is an evidence of coordination through this group. The shifting of the carbonyl group from 1651 to 1547 cm^{-1} is also an indication of coordination through the carbonyl group. Comparing the absorption bands of AC and Mg(II)-AC, since there is no significant shift in the –NH group, then the –NH group is not involved in complex formation (Gulam and Manohar, 2014).

XRD patterns and unit parameter of magnesium complex

The powdered XRD patterns of Mg(II)-AC is shown in 'Table 3'. The observed unit cell parameters are also given in Table 3. The diffraction pattern of complexes is recorded between 2θ ranging from 5° to 65°. The particle size of the sample is estimated using the Scherrer's formula. According to Scherrer's equation, the particle size is given by t = 0.9 λ/Bcosθ, 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 measurement of the half width of the diffraction peak (Kavitha *et al*., 2013).

Proposed structure of magnesium complex of acetaminophen

The proposed structure of magnesium complex of acetaminophen was derived from decomposition temperature, Infrared, molar conductance, UV–Visible and X-Ray Diffraction analysis data. Thus, from the Infrared spectra, the acetaminophen is seen as a bidentate ligand, coordinated to the metal ion- oxygen atoms of hydroxyl and carbonyl groups. From the molar conductance data, the complex is found to be

non-electrolytes. On the basis of the above observations, octahedral geometry structure is suggested for the investigated complex Fig. 5.

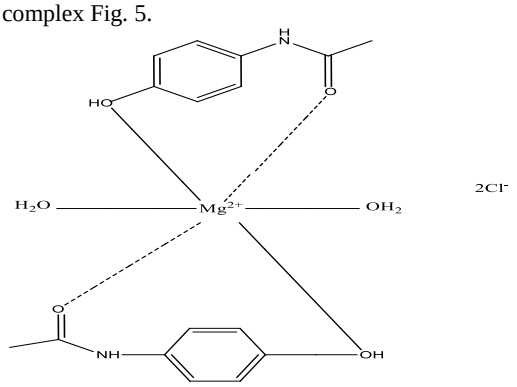

Fig. 5: The proposed structure of $[Mg(C_8H_9NO_2)_2 (H_2O) Cl] Cl.H_2O$

Table 4: The susceptibity test of acetaminophen (AC) at varying concentrations

Test organisms	Diameter of zone of inhibition (mm) at varying concentrations (mg/mL)			
	50	25	12.5	6.25
S. aureus	0	0	0	0
B. subtilis	0	0	0	0
E. coli	0	0	0	0
S. typhi	0	0	0	0
P. aeruginosa	18	15	0	0
C. albicans	0	0	0	0
A. niger	0	0	0	0

Table 5: The susceptibility test of Mg(II)-AC at varying concentrations

Test organisms	Diameter of Zone of inhibition (mm) at varying concentrations (mg/mL)			
	50	25	12.5	6.25
S. aureus	19	15	13	0
B. subtilis	0	0	0	0
E. coli	0	0	0	0
S. typhi	15	12	0	0
P. aeruginosa	27	18	13	0
C. albicans	0	0	0	0
A. niger	0	0	0	0

Table 6: The sensitivity test of ciprofloxacin/fluconazole (controls) at varying concentrations

Test organisms	Diameter of zone of inhibition (mm) at varying concentrations (mg/mL)			
	50	25	12.5	6.25
S. aureus	38	30	25	20
B. subtilis	39	32	28	25
E. coli	30	25	22	19
S. typhi	37	32	28	25
P. aeruginosa	25	21	18	16
C. albicans	36	32	28	25
A. niger	25	20	15	13

Note: ciprofloxacin is for *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*, while Fluconazole is for *Candida albicans and Aspergillus niger*

Antimicrobial activity of acetaminophen and magnesium complex

The sensitivity of the test organisms at various concentrations (100, 50, 12.5 and 6.25 mg/ml) of the metal complex and acetaminophen were carried out. Table 4 shows the activity of Acetaminophen against *Pseudomonas aeruginosa* (18 and 15 mm) at 50 and 25 mg/ml, while it showed no significant effect on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans*.

The susceptibility of Mg(II)-AC is presented in Table 5, Mg(II)-AC showed sensitivity against three of the six microorganisms, *Staphylococcus aureus* (Gram-positive), *Salmonella typhi* (Gram-negative), *Pseudomonas aeruginosa* with the inhibitory zones of 19.0, 15.0 and 13.0 mm respectively at the concentrations of 50, 25 and 12.5 mg/ml respectively for *Staphylococcus aureus*, 15 and 12 mm at 50 and 25 mg/ml for *Salmonella typhi* and 27, 18 and 13 mm for *Pseudomonas aeruginosa*. Mg(II)-AC was inactive against *Bacillus subtilis* (Gram-positive), *Escherichia coli* (Gram-negative), *Candida albicans* (Fungus) and *Aspergillus niger* (fungus). The test of Mg(II)-AC on some bacteria isolates revealed that Mg(II)-AC possess antimicrobial activities compared to acetaminophen which showed activity against only *Pseudomonas aeruginosa.* This is due to chelation, which reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with donor groups of the ligand, which increases lipophilic character, favouring its permeation through lipid layers of the bacterial membrane (Osowale *et al*., 2014).

Ciprofloxacin (Table 6) showed inhibitory activity against all the bacteria *Bacillus subtilis* (39, 32, 28 and 25 mm), *Staphylococcus aureus* (38, 30, 25 and 20 mm), *Escherichia coli* (30, 25, 22 and 19 mm), *Salmonella typhi* (37, 32, 28 and 25 mm) and *Pseudomonas aeruginosa* (25, 21, 18 and 16 mm) at 50, 25, 12.5 and 6.25 mg/ml. while Fluconazole showed activity against *Candida albicans* (36, 32, 28 and 25 mm) and *Aspergillus niger* (25, 20, 15 and 13 mm).

Table 7: Acute lethal effect of Mg(II)-AC administered orally to white albino rats

Experiment	Dose (mg/kg)	No of dead rats after 24 h
Phase 1	10	0/3
	100	0/3
	1,000	1/3
Phase 2	600	0/1
	1,000	0/1
	1,600	1/1
	2,900	1/1

Lethal Dose (LD_{50})=$\sqrt{1000\times1600} = \sqrt{1600000} = 1265$ mg/kg

Acute toxicity (LD_{50})

The results for Acute toxicity study of Mg(II)-AC on rats showed that one rat died within 24 h in the first phase for Mg(II)-AC. In the second phase, mortality was recorded at 1600 and 2900 mg/kg. The result was used to calculate the LD_{50}. In lethal dose determination, substances with lethal dose (LD_{50}) ≥5000mg/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 (Hodge and Sterner, 2005). From Table 7, the LD_{50} of Mg(II)-AC was 1265 mg/kg. So the complex is considered to be slightly toxic.

Table 8: Effect of Mg(II)-AC on carrageenan- induced rat paw edema

Group(n=5)	Dose(mg/kg)	edema diameter(mm)/Time interval (hour) [mean of inhibition]					
		0h	1h	2h	3h	4h	5h
Saline(control)	10mL/kg	2.148±0.0627	4.51±0.1339[a]	4.606±0.0501	4.738±0.0883[a]	4.654±0.0509	4.626±0.081[a]
piroxicam	20mL/kg	2.09±0.0453	2.47±0.0278[b,c] [45.1]	2.476±0.0427[b,c] [47.1]	2.53±0.0673[b,c] [46.5]	2.414±0.0682 [49.5]	2.322±0.0445[b,c] [49.6]
Mg(II)-AC	200mg/Ml	2.12±0.0432	3.63±0.0249[b,d] [18.8]	3.816±0.0238[b,d] [18.1]	.844±0.0277[b,d] [17.9]	3.836±0.0273 [17.9]	3.8±0.0141[b,d] [19.7]
Mg(II)-AC	150mg/Ml	2.14±0.077	3.66±0.1964[b,d] [19.5]	3.774±0.1839[bd] [17.2]	3.888±0.0622[b,d] [18.9]	3.82±0.1074 [17.6]	3.714±0.1023[b,d] [17.9]
Mg(II)-AC	100mg/Ml	2.004±0.1753	3.992±0.1254[b,d] [11.5]	4.276±0.2085[d] [7.8]	4.206±0.2015[b,d] [7]	4.424±0.2424 [4.9]	3.936±0.2647[b,d] [10.6]

ANOVA: indicates that comparison at the 5% level is statistically significant. Means having different superscripts ([a,b,c,d]) are significantly different (P<0.05), [ ] = % Inhibition

Anti-inflammatory activity

Anti-inflammatory activity was determined by the paw edema method in rats. The rats were divided into five groups, the injection of 0.1ml of freshly prepared carrageenan suspension into the sub planter region of the left hind paw of Group 1 (normal saline) produced an edema reaching its maximum at the third hour (4.764±0.4). The test complex showed maximum inhibition percentage at about 3 h. After 4 h it goes on reducing and reaches a minimum at about 5 h.

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 Mg(II)-AC (Table 8 and Fig. 7) and the percentage Anti-inflammatory effect at its peak was at the third hour (for the three doses :200, 150 and 100 mg/kg) were 17.9%, 18.9% and 7%. The percentage Anti-inflammatory effect of piroxicam at the 3rd hour was 46.5%. Mg(II)-AC produced a significant ($p<0.05$) decrease in the size of the paw oedema as shown in Table 8.

Conclusion

Mg(II)-AC complex has been synthesized and characterized by FTIR spectroscopy, electronic spectroscopy, Conductivity measurement and XRD. It was proven that the formation of the complex occurred via both C=O and –OH groups from Acetaminophen. The antibacterial studies showed all the magnesium complex had better antibacterial activity than Acetaminophen. Mg(II)-AC (200, 150 and 100 mg/kg) was more effective than normal saline, but not as effective as piroxicam (10 mg/kg)

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