

**Original Research Article****Spectroscopic Characterization, Antimicrobial and Toxicological Properties of Derivatised Thiosemicarbazone Transition Metal Complexes**Kpomah B<sup>1\*</sup>, Egboh, S.H.O<sup>2</sup>, Agbaire, P.O<sup>2</sup>, Kpomah, E.D<sup>3</sup><sup>1</sup>Chemistry Department, Delta State College Physical Education, Mosogar Nigeria<sup>2</sup>Chemistry Department, Delta State University, Abraka Nigeria<sup>3</sup>Biochemistry Department, Federal University Otuoke, Bayelsa State, Nigeria**\*Corresponding Author:**

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**Abstract:** Complexes of Schiff base were synthesized by direct condensation of acetaldehyde with thiosemicarbazide in the presence of FeCl<sub>3</sub>.6H<sub>2</sub>O and CuSO<sub>4</sub>.5H<sub>2</sub>O. The products were characterized by elemental analysis, molar conductance, infrared as well as electronic spectra study. The complexes are of 1:2 (metal:ligand) stoichiometry and are non-electrolytes in solution. On the basis of these spectra data, the general formulas: Cu(ADTSC)<sub>2</sub>SO<sub>4</sub>H<sub>2</sub>O and Fe(ADTSC)<sub>2</sub>Cl<sub>2</sub>.3H<sub>2</sub>O and an octahedral geometry was proposed for the complexes. The ligand and its complexes were screened for their antibacterial activities against *Salmonella typhi*, *Shigella sp*, *Escherichia coli*, *Klebsiella sp*, *Staphylococcus aureus* *Pseudomonas aeruginosa* and *Vibrio cholera*. The results of these studies showed that the metal complexes displayed more antibacterial activities against most of the species as compared to the uncomplexed ligand. The minimum inhibitory concentrations (MICs) of the compounds were also determined by two fold serial dilution method, the results showed an MIC value in the range of 500-250 µg/ml for the free ligand and 250-62.50 µg/ml range for the complexes. Results obtained from the toxicity effects of administration of the ligand and metal complexes at the doses of 25 and 50mg/kg body weight daily for 5 days showed that both doses of acetaldehyde thiosemicarbazone significantly increased ( $P < 0.05$ ) the levels of AST, ALT and ALP when compared to the control group, 50mg/kg body weight of acetaldehyde thiosemicarbazone copper(II) complex equally increased significantly ( $P < 0.05$ ) the level of ALT based on comparison with the control group. The toxicities of other compounds were considerably non-significant ( $P \geq 0.05$ ).

**Keywords:** Synthesis, Cu(II), Fe(III), acetaldehyde, thiosemicarbazone, spectral, antibacterial activities, toxicity

**INTRODUCTION**

Thiosemicarbazones derived from the reaction of a thiosemicarbazide and an aldehyde or ketones are very versatile Schiff base ligands. They show a variety of coordination modes in metal complexes [1-3]. Thiosemicarbazones can act as a monodentate ligand that binds to the metal ion through the thioketo sulphur atom or as a bidentate ligand that coordinates to the metal ion through the sulphur atom and one of the nitrogen atoms of the hydrazine moiety to form four or five membered chelate rings [1-4]. Thiosemicarbazone complexes have an imine group (-N=CH-) which imparts the biological activity and chelating properties towards the central metal atom.

Thiosemicarbazones and their complexes have received considerable attention because of their pharmacological activities which include: antifungal as reported by [5, 6], antitumor [7], antibacterial [8-11], antiamoebic, antimalarial and antiviral [12,13,14]. Some thiosemicarbazones are relatively specific inhibitors of ribonucleotide reductase, which is an

important metabolic target for the development of chemotherapeutic agents against cancer [15]. The biological activities of thiosemicarbazones are considered to be due to their ability to form chelates with metals, activities of metal complexes differ from those of either ligands or the metal ions, an increase and/or decreased biological activities are reported for several transition metal complexes [8]. Thiosemicarbazone derivatives and their metal complexes are also reported to have diverse analytical applications [16]. Pavon *et al* reported the 4-phenyl-3-thiosemicarbazone of biacetylmonoxime as an analytical reagent[17]. Thiosemicarbazones are also reported to have corrosion inhibition properties [18]. Metal complexes of thiosemicarbazone have shown variable bonding properties and structural diversity, two structural isomers (E-, Z -form) are possible and they can co-ordinate to the metal either as a neutral ligand or as a deprotonated ligand through the NS atoms [19, 20, 8].

## EXPERIMENTAL SECTION

### Materials/ Reagents

Thiosemicarbazide was ordered from Sigma Aldrich. The metal salts used for the synthesis,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  were obtained from British Drug House (BDH). Other reagents and solvents like methanol, ethanol, acetaldehyde chloroform, dichloromethane, acetic acid, dimethylsulphoxide (DMSO), and concentrated hydrochloric acid were also obtained from BDH. All reagents used were of analytical grade, with no further purification. The assay kit for the determination of AST, ALT and ALP were products of Randox Laboratory Ltd., Co-Atrim, UK. All the synthesized compounds were screened for their antibacterial activity using sensitivity disc method; the minimum inhibitory concentrations (MICs) of the compounds were also determined by two fold serial dilution method.

### Experimental Microorganism/Animals

All the bacterial strains were freshly isolated from patients with infections from different clinical sources from the Central Hospital Eku, Delta State, Nigeria. They were identified by conventional methods and cultivated on solid media and incubated at  $37^\circ\text{C}$  for 24 hrs. A total of thirty five (35) healthy male rat

(Wister strain), 2.5 -3 months old, weighing between 190-200 g were obtained from the animal house unit of the Department of Biochemistry University of Port Harcourt, River State. The animals were kept in a clean cage and housed in a well-ventilated room at temperature  $20\text{-}30^\circ\text{C}$  under natural light and dark cycle with free access to grower's mash and water for a period of one week to acclimatized prior to the commencement of experiment. All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) as per directions of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA).

### Synthesis of acetaldehydethiosemicarbazone (ADTSC)

10mmol, (1.92 g) thiosemicarbazide was dissolved in methanol (60 mL) by refluxing at  $50^\circ\text{C}$ . In the refluxing solution, acetaldehyde 10 mmol, (1.56mL) solution in methanol (30 mL) was added; this was then followed by the addition of few drops of concentrated HCl. The reaction mixture was continuously stirred and refluxed for 4 hrs at  $60^\circ\text{C}$ . The volume of reaction mixture was reduced and kept in the refrigerator overnight. White crystals of ADTSC precipitated out, the crystals was washed with methanol and dried in the desiccator over silica gel [8, 21].

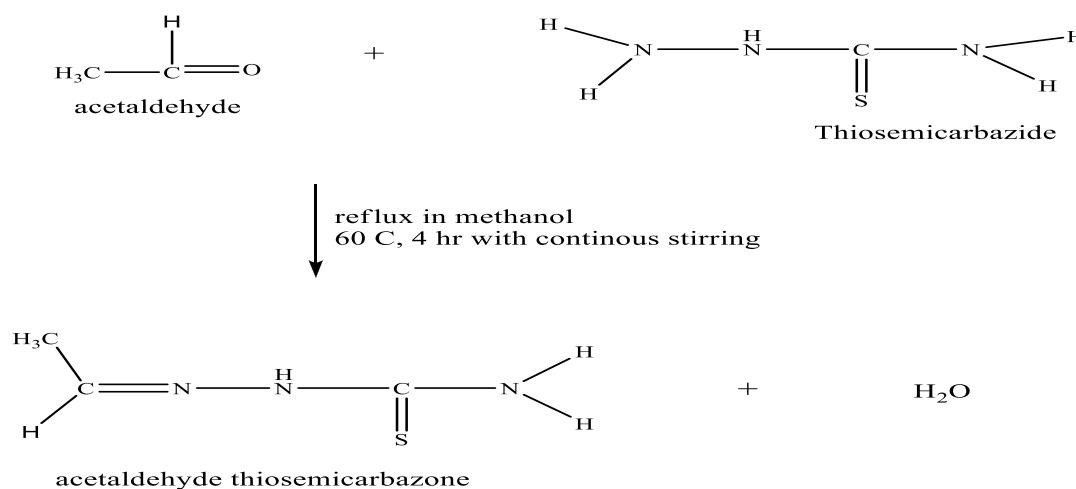


Fig-1: Synthesis of acetaldehyde thiosemicarbazone

### Synthesis of metal complexes of acetaldehyde thiosemicarbazone

The copper and iron metal complexes of ADTSC were prepared by a slow addition of 1 mmol of the metal salt solution in 15 mL methanol to hot stirring methanolic solution (30 mL) of ADTSC (2 mmol, 0.234 g) in the molar ratio of 2:1. The reacting mixture was continuously stirred and refluxed for 2-3 hrs. The product formed was collected by filtration, washed with cold methanol, and dried in the desiccator over silica gel [8, 21]

## RESULTS AND DISCUSSION

### Physical characteristics and microanalytical data of ADTSC and its metal complexes

The colour exhibited by the metal complexes in Table 1 could be due to d-d electron transition or as a result of electron transfer (lone pair) from the ligand to the central metal [22, 23]. The higher melting point displayed by the complexes as compared with the ligand may be as a result of increase in molecular mass. The molar conductance measurements of the complexes in DMSO indicate that they are both non-electrolytes.

The results of partial elemental analysis are in good agreement with assigned formulations.

**Table 1: Physical characteristics and microanalytical data of ADTSC and its metal complexes**

Formulation & empirical formula	Molecular Weight (g/mol.)	Colour	Yield (%)	M.p (°C)	Elemental Analysis Found/ (Calcd) (%)			EC 10 <sup>-3</sup> Min DMSO (ohm <sup>-1</sup> cm <sup>2</sup> mol <sup>-1</sup> )
					C	H	N	
ADTSC C <sub>3</sub> H <sub>7</sub> N <sub>3</sub> S	117	White crystals	84	189.9	29.87 (30.75)	5.98 (6.02)	35.22 (35.03)	
[Fe(ADTSC) <sub>2</sub> Cl <sub>2</sub> .3H <sub>2</sub> O] C <sub>6</sub> H <sub>20</sub> Cl <sub>2</sub> FeN <sub>6</sub> O <sub>3</sub> S <sub>2</sub>	415	Brown crystals	54	306	17.19 (17.36)	4.91 (4.86)	19.85 (20.24)	15.12
[Cu(ADTSC) <sub>2</sub> SO <sub>4</sub> H <sub>2</sub> O] C <sub>6</sub> H <sub>16</sub> CuN <sub>6</sub> O <sub>5</sub> S <sub>3</sub>	412	Black crystals	59	283	16.74 (17.49)	3.75 (3.91)	20.53 (20.40)	18.23

EC = Electrical Conductance, 10<sup>-3</sup> M solution in DMSO, Ohm<sup>-1</sup> cm<sup>2</sup> mol<sup>-1</sup>

**Table 2: Electronic spectra nm, (cm<sup>-1</sup>) of ADTSC and its metal complexes**

Compound	d <sup>n</sup> Configuration	n→π*	π→π*	Charge Transfer	d-d Transition
ADTSC	-	198 (50505) 223 (44843)	304 (32894)	-	-
[Cu(ADTSC) <sub>2</sub> SO <sub>4</sub> H <sub>2</sub> O]	d <sup>9</sup>	204 (49019)	270 (37037) 289 (34602)	323(30959) 355(28169)	436 (22935) <sup>2</sup> B <sub>1g</sub> → <sup>2</sup> E <sub>g</sub>
[Fe(ADTSC) <sub>2</sub> Cl <sub>2</sub> .3H <sub>2</sub> O]	d <sup>6</sup>	208 (48076)	276(36231) 301 (33222)	311 (32154)	-

#### Electronic spectra of ADTSC

Electronic spectra data of ADTSC metal complexes are listed in Table 3. It showed five absorption bands in the region 198 nm (50505 cm<sup>-1</sup>), 223 nm (44843 cm<sup>-1</sup>) and 304 nm (32894 cm<sup>-1</sup>) corresponding to n→π\* and π→π\* transitions. Upon complexation a blue shift was observed due to the polarization of the C=N bond caused by the metal ligand electron interaction during chelation. This also clearly indicates the coordination of azomethine nitrogen to the metal atom [24-26].

#### Electronic spectra of [Cu(ADTSC)<sub>2</sub>SO<sub>4</sub>H<sub>2</sub>O]

The electronic absorption spectra of [Cu(ADTSC)<sub>2</sub>SO<sub>4</sub>H<sub>2</sub>O] showed three detected absorption bands between 204 -289 nm and two bands at 323 and 355 nm assigned to n→π\*/π→π\* and intraligand transitions respectively. These bands were formed from the small hyperchromic shift of the bands found in the uncomplexed ligand upon complexation,

which is in accordance with the studies of previous electronic spectra of copper(II) thiosemicarbazone complexes [27, 28]. The d-d transition of the complex was also recorded in visible region by concentrating the solution giving 436 nm (22935 cm<sup>-1</sup>) corresponding to <sup>2</sup>B<sub>1g</sub> → <sup>2</sup>E<sub>g</sub> [24-26].

#### Electronic Spectra of [Fe(ADTSC)<sub>2</sub>Cl<sub>2</sub>.3H<sub>2</sub>O]

The electronic spectra of [Fe(ADTSC)<sub>2</sub>Cl<sub>2</sub>.3H<sub>2</sub>O] exhibited band at 208 nm (48076 cm<sup>-1</sup>) attributed to n→π\* and only two bands at 276 nm (36231 cm<sup>-1</sup>) and 301 nm (33222 cm<sup>-1</sup>) corresponding to π→π\*. No band was observed at the visible region. Iron has a d<sup>6</sup> configuration like Mn(II) and as such series of weak transitions are expected. These bands in most cases are usually obscure by charge transfer, ligand-metal charge transfer is usually common and occurs between 222-384 nm (45000-26000 cm<sup>-1</sup>). This is in accordance with those having octahedral structure [27, 28].

**Table 3: The main IR in (cm<sup>-1</sup>) of (ADTSC) and its metal complexes**

IR Band Assignment (KBr, cm <sup>-1</sup> )	ADTSC	Cu(ADTSC) <sub>2</sub> SO <sub>4</sub> H <sub>2</sub> O	Fe(ADTSC) <sub>2</sub> Cl <sub>2</sub> .3H <sub>2</sub> O
V(OH), H <sub>2</sub> O		3375m	3441 s
V(N-H)	3373 br 3263 br	3288 m	3286 s 3180 s
V(C=N)	1645 s	1633 s	1599 s
n(C-S)+n(C-N)	1286 m	1284 s	1269 s
V(N-N)	1095 m	1147 m	1145 s
V(C=S)	999 s	817 m	738 s
M-N		461 w	540 s
M-S		430 w	441 s

S = strong, w = weak, m = medium, br. = broad

### IR of (ADTSC) and its metal complexes

In the IR spectra of ADTSC free ligands, the bands observed at 1286 and 999  $\text{cm}^{-1}$  regions are assigned to  $\nu(\text{C-S}) + \nu(\text{C-N})$  and  $\nu(\text{C=S})$  stretchings, respectively, [29, 30]. Coordination of sulfur with the metal ion would result in the displacement of electrons towards the latter, thus resulting in the weakening of the  $\nu(\text{C=S})$  bond. Hence, on complexation  $\nu(\text{C=S})$  stretching vibrations should decrease as observed in the spectra of the metal complexes at 1284  $\text{cm}^{-1}$  for the copper complex and 1269  $\text{cm}^{-1}$  for iron complex assigned to  $\nu(\text{C-S}) + \nu(\text{C-N})$ . Similarly, the  $\nu(\text{C=S})$  stretchings experienced a shift to lower frequency as the bands for copper and iron complexes appeared at 817 and 738  $\text{cm}^{-1}$  respectively, meanwhile those of  $\nu(\text{N-N})$  increased upon complexation [30], these increase is due to the increase in the bond strength. This kind of shift on hydrazinic nitrogen describes the presence of

electron withdrawing groups thereby confirming the coordination via the azomethine nitrogen. This agrees well with previous studies of metal complexes of thiosemicarbazones [31,32, 21]. A strong intense band appears at 1645  $\text{cm}^{-1}$  in the spectrum of the ligand due to  $\nu(\text{C=N})$  vibrations, has undergone a frequency shift of about 13-46  $\text{cm}^{-1}$  in the metal complexes. This shift indicates the coordination of nitrogen to metal ion. All these peculiar changes and the appearance of some non-ligand bands in the far infrared region are evidence of the metal-sulfur and metal-nitrogen bonds [33, 34]. This is in agreement with previous observations [35, 36, 2]. The possibility of thione-thioltautomerism ( $\text{H-N-C=S}$ ) ( $\text{C=N-SH}$ ) in the ligand has been ruled out as no band was displayed in the infrared absorption around 2600-2500  $\text{cm}^{-1}$  which is the characteristics of the thiol group [26, 37].

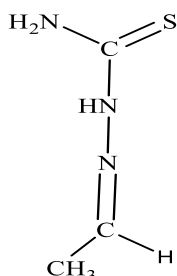


Fig-2: Acetaldehyde thiosemicarbazone

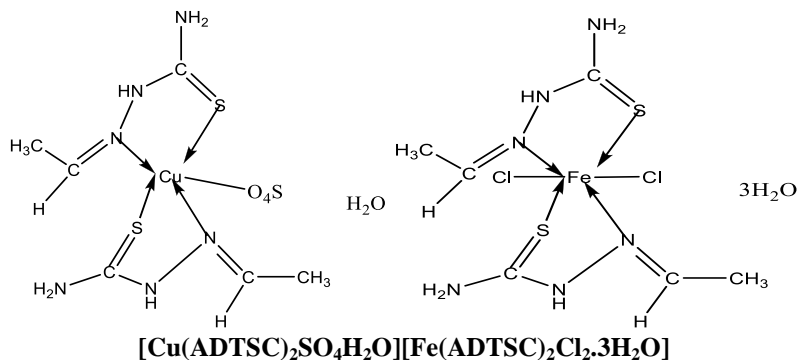


Fig-3: Metal Complexes of Acetaldehyde thiosemicarbazone

Table 4: Antibacterial activity data of ADTSC and its metal complexes after 24 hours using sensitivity disc (30  $\mu\text{g/mL}$ ) zone of inhibition in (mm)

Test Samples	<i>Salmonella typhi</i>	<i>Shigella species</i>	<i>Escherichia coli</i>	<i>Klebsiella sp</i>	<i>Staph. aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Vibrio Cholerae</i>
Control (DMSO)	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
ADTSC	10.33±.58**	8.67 ± 2.08**	8.33 ± 1.53**	9.76±2.52**	7.67±0.58**	10.76± 1.15**	11.00±2.00**
Cu(ADTSC) <sub>2</sub> SO <sub>4</sub> H <sub>2</sub> O	20.30±.58**	21.30±2.04**	25.52±2.50**	22.00±2.00**	19.33±1.53**	28.67± 3.51**	23.67±1.53**
Fe(ADTSC) <sub>2</sub> Cl <sub>2</sub> .3H <sub>2</sub> O	22.00±0.00**	19.60±0.60**	21.13±2.52**	20.33±1.53**	18.66±1.15**	23.00±3.00**	20.00± 2.00**

All values are mean of triplicate determinations  $\pm$  standard deviation, values in the same column with different superscript letters (\*) are significantly different from the control (\*) ( $P < 0.05$ ), one way analysis of variance (ANOVA) followed by post hoc LSD

### Antibacterial activities of the ligand and the complexes

The Schiff base ADTSC and its copper and iron metal complexes were screened for their antibacterial activities against *Salmonella typhi*, *Shigella species*, *Escherichia coli*, *Klebsiella sp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio Cholerae*, to investigate their biopotency. The results obtained from antimicrobial susceptibility testing are depicted in Table 4. The quantitative antimicrobial activity test results proved that both the ligand and the complexes combinations have specific anti-microbial activity, depending on the pathogenic microbial species tested. The antimicrobial screening shows that the metal chelates exhibit more inhibitory effect than the parent ligand. Chelation induced

significant changes in the biological activity of the ligand. A possible explanation for the observed increased activity upon chelation is that the positive charge of the metal in chelated complex is partially shared with the ligand's donor atoms so that there is  $\pi$ -electron delocalization over the whole chelate ring [38]. Subsequently, this reduces the polarity of the metal ion and which in turn will increase the lipophilic character of the metal chelate and favors its permeation through the lipid layers of the bacterial membranes [39]. Lipophilicity is a property that has a major effect on absorption, distribution, metabolism, excretion and toxicity properties as well as on pharmacological activity because drugs cross biological membranes through passive transport, and the ability to do this is strongly dependent on their lipophilicity [40].

**Table 5: Minimum inhibition concentration in (bactericidal) ( $\mu\text{g/mL}$ ) of ADTSC and its complexes**

Test Samples	<i>Salmonella typhi</i>	<i>Shigella species</i>	<i>Escherichia coli</i>	<i>Klebsiella sp</i>	<i>Staph. aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Vibrio Cholerae</i>
Control (DMSO)	00	00	00	00	00	00	00
ADTSC	250	250	500	500	500	500	500
[Cu(ADTSC) <sub>2</sub> SO <sub>4</sub> H <sub>2</sub> O]	125	125	250	62.50	62.50	62.50	62.50
[Fe(ADTSC) <sub>2</sub> Cl <sub>2</sub> .3H <sub>2</sub> O]	62.50	125	62.50	62.50	125	62.50	125

### Minimum inhibition concentration in (bactericidal) of ADTSC and its complexes.

The metal complexes exhibit good antibacterial activities against tested microorganism with high values of MIC when compared to the values reported in related work [41, 42]. The MIC value is in 500-250  $\mu\text{g/mL}$  range for the free ligands and in the 250-62.50  $\mu\text{g/mL}$  range for the complexes. The results suggest that coordination to the transition metal complexes could be an interesting strategy for dose

reduction [41, 42]. Comparing the MIC values of the free ADTSC with the values obtained for the complexes, it can be seen that, the activity of the complexes are much higher and can be presented as approximately 2 to 3-folds higher activities than the respective free ligand. Taking into consideration that the molecular weight of the complexes is about 4 times than of the ligand, it may be assumed that the antibacterial activity are significantly enhanced upon coordination to transition metals.

**Table 6: Effects of Complexes of AHDT on liver Enzymes of wistar rats**

Groups	Aspartate Aminotransferase (AST)	Alanine Transferase (ALT)	Alkaline Phosphatase (ALP)
Control 5% DMSO	20.67 $\pm$ 1.53 <sup>a</sup>	22.33 $\pm$ 2.08 <sup>a</sup>	30.50 $\pm$ 2.08 <sup>a</sup>
25 mg/kg ADTSC (L)	23.33 $\pm$ 2.08 <sup>b</sup>	26.00 $\pm$ 1.00 <sup>b</sup>	49.67 $\pm$ 1.53 <sup>b</sup>
50 mg/kg ADTSC	26.33 $\pm$ 1.53 <sup>c</sup>	28.33 $\pm$ 1.53 <sup>c</sup>	57.33 $\pm$ 2.08 <sup>c</sup>
25 mg/kg [Cu(L) <sub>2</sub> SO <sub>2</sub> ]	21.33 $\pm$ 1.53 <sup>a</sup>	23.33 $\pm$ 2.08 <sup>a</sup>	32.00 $\pm$ 2.52 <sup>a</sup>
50 mg/kg [Cu(L) <sub>2</sub> SO <sub>2</sub> ]	22.70 $\pm$ 1.15 <sup>a</sup>	26.33 $\pm$ 2.52 <sup>d</sup>	33.20 $\pm$ 2.08 <sup>a</sup>
25 mg/kg [Fe(L) <sub>2</sub> Cl <sub>2</sub> ]	21.00 $\pm$ 1.00 <sup>a</sup>	23.00 $\pm$ 2.00 <sup>a</sup>	32.30 $\pm$ 2.50 <sup>a</sup>
50 mg/kg [Fe(L) <sub>2</sub> Cl <sub>2</sub> ]	21.67 $\pm$ 1.53 <sup>a</sup>	26.00 $\pm$ 1.00 <sup>c</sup>	35.30 $\pm$ 2.08 <sup>d</sup>

Values are mean of five determinations  $\pm$  standard deviation, values in the same column with different superscript letters are significantly different from the control ( $P < 0.05$ ), one way analysis of variance (ANOVA) followed by post hoc LSD

### Effects of Complexes of AHDT on liver Enzymes of wistar rats.

Appraisal of the results obtained in Table 6.0 showed that both doses of acetaldehyde thiosemicarbazone significantly increased ( $P < 0.05$ ) the levels of AST, ALT and ALP when compared to the control group, 50 mg/kg body weight of acetaldehyde thiosemicarbazone copper(II) complex equally increased significantly ( $P < 0.05$ ) the level of ALT based on comparison with the control group..

Values of AST, ALT and ALP has often been used as an index of monitoring pathological conditions of liver, elevated levels above the reference point of ALP is a positive detector of intrahepatic and extrahepatic bile obstruction, infiltrative diseases of the liver and all bone diseases associated with osteoblastic activity [43,44], hence values that differs significantly from the control is suggestive that the affected ligands and complexes may have adverse drug reaction on the liver and bones of the test rats.

### CONCLUSION

The complexes have NS donor bidentate nature. The biological behavior revealed that the ligand shows a weak activity against the test bacterial strains. The resulting metal complexes enhanced the antimicrobial activity of the free ligand as they were effective against gram positive bacteria (*Klebsiella pneumoniae* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Chelation induced significant changes in the biological activity of the ligand.

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