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Toxicological Assessment of Acetone Thiosemicarbazone Metal Complexes on Body Weight, Biochemical Parameters and Liver Histology of Wistar rats

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Abstract: Toxicological evaluation of 25 and 50 mg/kg body weight of acetone thiosemicarbazone (ACTSC) and its metal complexes were investigated. A total of forty-five (45) male wistar rats used were randomly divided into nine (9) groups (labelled A - I) of five (5) rats each per group i.e. Group A=(Control 5% DMSO); B=(ACTSC 25 mg/kg); C= (ACTSC 50 mg/kg); D=(ACTSC-Cu^{2+} 25 mg/kg); E=(ACTSC-Cu^{2+} 50 mg/kg); F=(ACTSC-Zn^{2+} 25 mg/kg); G=(ACTSC-Zn^{2+} 50 mg/kg); H=(ACTSC-Ni^{2+} 25 mg/kg) and I=(ACTSC-Ni^{2+} 50 mg/kg). After five days of oral test drug administration, the animals were anaesthetized and blood samples collected via cardiac puncture into sample bottles for biochemical analysis (bilirubin total and conjugated, total protein, albumin, globulin. Albumin/globulin ratio), antioxidant status (superoxide dismutase, catalase, and malondialdehyde) and liver enzyme activity (AST, ALP and ALT).Tissues (liver) were also excised for histopathological examination. Analysis of test results obtained indicated both doses of the ACTSC and 50mg/kg body weight of ACTSC–Cu^{2+} caused significant increase ($P< 0.05$) on liver weight and liver/body weight ratio, in the same vein both doses of the ACTSC also caused significant effects ($P< 0.05$) on total and conjugated bilirubin, albumin, globulin and albumin/globulin ratios while only the 50 mg/kg body weight of ACTSC–Zn^{2+} had significant effect ($P< 0.05$) on globulin and albumin/globulin ratio. Antioxidant evaluation of the test samples indicated that both doses of ACTSC and ACTSC-Zn^{2+} complexes caused significant increase ($P< 0.05$) in MDA, however both doses of the ACTSC and 50 mg/kg body weight of ACTSC–Zn^{2+} caused significant decrease ($P< 0.05$) in catalase and superoxide dismutase activity. Investigation of the test samples on liver enzymes showed that both doses of ACTSC and ACTSC-Zn^{2+} caused significant increase in AST and ALP and ALP. Histopathological examination of the liver showed that the groups treated with 25 mg/kg body weight of the complex of ACTSC-Zn^{2+}, 25 and 50 mg/kg body weight of the complexes of ACSTC-Cu^{2+} and ACTSC-Ni^{2+} showed strong similarity with the control group by having well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation.

Keywords: Acetone thiosemicarbazone, Biochemical parameters, Liver enzyme, Antioxidants, Histology.

1 Introduction

Thiosemicarbazones are active pharmacophores, besides having excellent chelatogenic characteristics and their enhanced activity on complexation with metal ion. They can bind to transition metals through the thioketo sulphur and azomethine nitrogen atoms and therefore this type of compounds can coordinate in vivo to metal ions. The importance of thiosemicarbazone metal chelates in medical chemistry is increasing because they have been used as drugs and are reported to possess a wide variety of biological activities against pathogenic organisms. The antitumour activity of such thio compounds was revealed in their ability to inhibit ribonucleotide reductase (RR), a necessary enzyme for deoxyribonucleic acid (DNA) synthesis [1,2,3], other pharmacological activities include: antifungal [4] antitumor, [5] antibacterial, [6,7] antiamoebic, antimalarial and antiviral [8,9,10]. The liver has a central and critical biochemical role in hepatic excretory functions in which organic anions of both endogenous and exogenous origin are extracted from the sinusoidal blood, biotransformed and excreted into the bile or urine. Assessment of this excretory function provides valuable clinical information, frequently used tests involves

the measurement of plasma concentration of endogenously produced compounds such as bilirubin and bile acids and the rate of clearance of exogenous compounds, such as aminopurine, lidocaine and caffeine [11]. Another function is hepatic synthesis where it plays a major role in the regulation of protein, carbohydrate and lipid metabolism. A bidirectional flux of precursors and product such as glucose, amino acids, free fatty acids and other nutrients, occurs across the hepatocyte membrane. Triglycerides, cholesterol and bile acid synthesis also occurs within the liver, a central importance of the liver in metabolic and regulatory pathways is the hepatic metabolic function. The functional expression of the complex, integrated organelle structure includes the metabolism of drugs (activation and detoxification) and disposal of exogenous and endogenous substances such as galactose and ammonia. Liver function tests are routine test performed in the investigation of a liver disease, and these tests are useful in (i) detecting (ii) diagnosing (iii) evaluating severity, (iv) monitoring therapy (v) assessing the prognosis of the liver disease and dysfunction and (vi) they are also useful in directing further diagnostic work up. The array of tests useful for these purposes includes measurement of total bilirubin, protein and albumin concentrations and the activity of enzymes such as aminotransferases e.g. aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma glutamyltransferase (GGT). By using a combination of these tests, it is possible to categorise broad types of liver disease, which can then be more accurately diagnosed through disease specific tests.

2. Material and Methods

2.1 Drugs and Chemicals

Thiosemicarbazide and acetone were ordered from Sigma Aldrich, the metal salts used are $CuSO_4.5H_2O$, $NiSO_4.6H_2O$ and $ZnCl_2$. They were obtained from British Drug House (BDH).The ligand and complexes were synthesized using standard procedures; melting points of complexes were determined using electrically heated Griffin melting point apparatus. The conductivity measurements were taken using Jenway 4510 Conductivity Meter. The CHN Elemental Analysis was done using Thermo Flash 1112 CHNSO Elemental Analyser. Electronic spectra of the ligand and the complexes were recorded in Dimethylsuphoxide (DMSO) solution on Shimadzu 10UV scanning UV-Visible spectrophotometer in the range 200 – 800 nm. The infrared (IR) spectra were recorded on Shimadzu 8400S FTIR spectrophotometer as KBr pellets in the range 4000 – 400 cm^{-1}. The assay kit from Randox Laboratories Limited United Kingdom was used. All other reagents/chemicals obtained from standard suppliers were of analytical grade.

3. Inorganic Synthesis

3.1 Test Compound

The test compound acetone thiosemicarbazone and its transitional metal complexes used for the present study were synthesized and duly characterized by the methods described earlier [12-16].The compounds were also assayed for their pharmacological activity as an antibacterial and antifungal agent [13].

3.2 Acetone Thiosemicarbazone (ACTSC-Ligand)

White crystal. Yield 89%, m.p. 181.9°C. Anal. Calc. For $C_4H_9N_3S$ (131. g/mol): C, 36.62%; H, 6.91%; N, 32.03%; Found: C, 36.51%; H, 6.89%; N, 32.25%; IR (KBr, cm^{-1}): ν(NH_2) 3377, 3230; ν(C=N) 1658; ν(C=S) 866. ^{1}H-NMR (DMSO-d6): δ 1.91, 1.92, (m, 6H, CH_3); 7.51, 7.99 (s, 2H, NH_2); 9.90 (s, 1H, NH).

3.3 Nickel(II) Complex of Acetone Thiosemicarbazone

Brown powder. Yield 69%, m.p. 355 °C (DT). Anal. Calc. For $C_8H_{18}N_6NiO_4S_3$ (417. g/mol): C, 23.04%; H, 4.35%; N, 20.15%; Found: C, 23.34%; H, 4.97%; N, 20.98%; IR (KBr, cm^{-1}): ν(NH_2) 3298, ν(C=N) 1616; ν(C=S) 864.

3.4 Zinc(II) Complex of Acetone thiosemicarbazone

White crystal. Yield 49%, m.p. 290 °C. Anal. Calc. For $C_8H_{18}C_{l2}N_6S_2Zn$ (399. g/mol): C, 23.04%; H, 4.35%; N, 20.15%; Found: C, 23.34%; H, 4.97%; N, 20.98%; IR (KBr, cm^{-1}): ν(NH_2) 3294, 3182; ν(C=N) 1608; ν(C=S) 736.

3.5 Copper(II) Complex of Acetone thiosemicarbazone

Black powder. Yield 65%, m.p. 183.3 °C. Anal.Calc. For $C_{10}H_{24}CuN_6O_5S_3$ (468. g/mol): C, 25.66%; H, 5.17%; N, 17.95%; Found: C, 26.01%; H, 5.86%; N, 17.98%; IR (KBr, cm^{-1}): ν(OH) 3481; ν(NH_2) 3416, 3286; ν(C=N) 1627; ν(C=S) 854. ^{1}H-NMR (DMSO-d6): δ 2.49, 7.45, 7.41, 7.39 (s, 12H, CH_3); 7.76 (s, 2H, NH_2); 9.15 (s, 2H, NH); 10.94 (m, 6H, OH).

4. Experimental Animals

A total of forty-five (45) 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, Delta State University, Abraka. The animals

were kept in standard clean cages and housed in a well-ventilated room at temperature 20-30^{O}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 the experiment. All protocols were performed in accordance with the Institutional Animal Ethnical Committee (IAEC) as per directions of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA).

5. Ethical Clearance

Protocol for the use of wistar rats as animal model for this study was approved by the Research and Bioethics Committee, Faculty of Basic Medical Sciences, Delta State University, Abraka (RBC/FBMS/DELSU/14/10).

6. Biochemical Experimental Design

Forty-five (45) male wistar rats were used, the rats were randomly divided into nine (9) groups of five (5) rats each with two (2) different dose levels used, viz., 25 and 50 mg/kg body weight daily for 5 days: group A=(Control- 5% DMSO); B =(ACTSC 25 mg/kg); C =(ACTSC 50 mg/kg); D =(ACTSC-Cu^{2+} 25 mg/kg), E =(ACTSC-Cu^{2+} 50 mg/kg); F =(ACTSC-Zn^{2+} 25 mg/kg); G =(ACTSC-Zn^{2+} 50 mg/kg); H =(ACTSC-Ni^{2+} 25 mg/kg) and I=(ACTSC-Ni^{2+} 50 mg/kg). The doses were administered orally using oraphageal canulla.

7. Biochemical Analysis

After the experimental period of drug administration, animals in different groups were sacrificed. Blood was collected in tubes without anticoagulant to separate serum for biochemical estimations. The following liver function test were conducted to investigate derangement in the liver of the animals used for the study, aspartate aminotransferase (AST), alanine aminotransferase (ALT) were determined by the colorimetric method of [17] using a commercial assay kits from Randox Laboratories Ltd., Co. Antrim, United Kingdom. Alkaline phosphatase (ALP) was estimated by the colorimetric method of [18] using assay kits from Randox Laboratories Ltd. Serum protein and serum albumin were estimated by Biuret method and Bromo cresol Green (BCG) binding method respectively using a commercial assay kits from Randox Laboratories Ltd. Serum globulin level was calculated as the difference between total protein and albumin, albumin globulin (A/G) ratio was obtained from the division of the values of albumin and globulin, Total and conjugated bilirubin was determined using commercial kits from Randox Laboratory Ltd, using colorimetric method described by [19]. Catalase activity was determined by the method of [20]. Super oxide dismutase (SOD) activity was determined by the methods of [21]. The assay method [22] as modified by [23] was adopted for the assay of Malondialdehyde (MDA) concentration.

8. Histopathological Study

Histopathological Studies of the liver for inflammation, degeneration and dearrangement was done using method described by [24]. Small pieces of liver tissue were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Section of 5-6µm in thickness were cut and stained with hematoxylin and eosin.

9. Statistical Analysis

Data are expressed as the mean of five replicate determinations ± standard deviation, means were analyzed using One Way Analysis of Variance (ANOVA) followed by Posthoc (LSD), $P < 0.05$ were considered as statistically significant.. All statistical analysis was done using Statistical Package for Social Science (SPSS) version 16.

10. Results and Discussion

10.1 Biochemical Parameters in Serum

The 25 and 50 mg/kg body weight of the ligand ACTSC significantly increased ($P < 0.05$) the liver weight and the ratio of the liver weight to body weight in a dose related manner when compared to the control group, in like manner the 50 mg/kg of complexes of ACTSC-$Zn^{2+}$and ACTSC-Cu^{2+} significantly increased the liver weight as a function of the body weight. The increased liver weight and liver to body weight ratio observed in these groups is an indication of liver swelling or fatty liver as a result of liver injury due to the test drugs [25, 26]. Elevated level of bilirubin above the reference value is termed jaundice it is characterized by the appearance of yellow pigmentation in the skin and white of the eye. Jaundice is caused by the excessive breakdown of red cells, impaired liver function or mechanical obstruction of the bile duct [27]. Hence the elevated levels of both bilirubin total and conjugated bilirubin ($P < 0.05$) by both doses of the ligand; ACTSC showed that the rats might have abnormal liver function, or excessively high rate of haemoglobin breakdown [28, 29]. Increased total protein concentration may be due to dehydration, increased immunoglobulin concentration due to infections, and decrease in concentration may occur as a result of over hydration, impaired protein synthesis due to malnutrition, malabsorption, liver disease, hypogammaglobulemia or increased protein loss due to renal, gastrointestinal and skin disorder [30].

Table 1: Effects of ACTSC and its Metal Complexes on Body Weight, Liver Weight and Liver/Body Weight Ratio of Wistar Rats.

GROUPS	BODY WEIGHT BEFORE TREATMENT (g)	BODY WEIGHT AFTER TREATMENT (g)	LIVER WEIGHT (g)	LIVER/BODY WEIGHT RATIO (%)
CONTROL 5% DMSO	101.57 ± 1.06	105.43 ± 1.46	3.33 ± 0.15[a]	3.16 ± 0.11[a]
25 mg/kg ACTSC	102.17 ± 2.61	99.80 ± 3.49	3.52 ± 0.07[b]	3.52 ± 0.05[b]
50 mg/kg ACTSC	105.33 ± 2.08	102.70 ± 2.33	3.67 ± 0.12[c]	3.58 ± 0.04[c]
25 mg/kg ACTSC Zn^{2+}	100.00 ± 2.00	98.57 ± 1.63	3.12 ± 0.13[a]	3.16 ± 0.08[a]
50 mg/kg ACTSC Zn^{2+}	101.33 ± 2.08	99.50 ± 1.78	3.31 ± 0.01[a]	3.32 ± 0.06[d]
25 mg/kg ACTSC Cu^{2+} (ETOH)	112.00 ± 3.61	111.00 ± 3.68	3.53 ± 0.18[a]	3.18 ± 0.06[a]
50 mg/kg ACTSC Cu^{2+} (ETOH)	107.67 ± 2.52	106.20 ± 2.20	3.49 ± 0.02[a]	3.29 ± 0.05[e]
25 mg/kg ACTSC $Ni^{2+}SO_4$	106.57± 2.70	105.67 ± 3.06	3.34 ± 0.12[a]	3.16 ± 0.04[a]
50 mg/kg ACTSC $Ni^{2+}SO_4$	107.00 ± 1.78	105.50 ± 1.49	3.36 ± 0.06[a]	3.19 ± 0.02[a]

Values are mean of five determinations ± 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.

Table 2: Effects of ACTSC and its Metal Complexes on Bilirubin (Bilirubin Total and Conjugated) of Wistar Rats.

GROUPS	BILIRUBIN TOTAL (nmol/L)	CONJUGATED BILIRUBIN (nmol/L)
CONTROL5% DMSO	7.67 ± 1.53[a]	1.13 ± 0.15[a]
25 mg/kg ACTSC	11.00 ± 1.00[b]	1.70 ± 0.10[b]
50 mg/kg ACTSC	14.33 ± 1.53[c]	1.83 ± 0.21[c]
25 mg/kg ACTSC Zn^{2+}	9.33 ± 0.58[a]	1.17 ± 0.21[a]
50 mg/kg ACTSC Zn^{2+}	10.00 ± 1.00[a]	1.23 ± 0.25[a]
25 mg/kg ACTSC Cu^{2+} (ETOH)	8.00 ± 1.00[a]	1.10 ± 0.17[a]
50 mg/kg ACTSC Cu^{2+} (ETOH)	9.67 ± 1.53[a]	1.17 ± 0.12[a]
25 mg/kg ACTSC Ni^{2+} SO_4	8.00 ± 1.73[a]	1.10 ± 0.10[a]
50 mg/kg ACTSC Ni^{2+} SO_4	8.00 ± 2.00[a]	1.17 ± 0.15[a]

Values are mean of five determinations ± 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.

Table 3: Effects of ACTSC and its Metal Complexes on Biochemical Parameters of Total Protein, Albumin, Globulin, Albumin/Globulin Ratio of Wistar Rats.

GROUPS	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)	ALBUMIN/ GLOBULIN RATIO
CONTROL5% DMSO	6.90 ± 0.10[a]	4.33 ± 0.06[a]	2.57 ± 0.11[a]	1.69 ± 0.08[a]
25 mg/kg ACTSC	6.97 ±0.15[a]	3.53 ± 0.06[b]	3.43 ± 0.12[b]	1.03 ± 0.03[b]
50 mg/kg ACTSC	6.93 ± 0.12[a]	3.37 ± 0.21[c]	3.57 ± 0.15[c]	0.95 ± 0.10[c]
25 mg/kg ACTSC Zn^{2+}	6.87 ± 0.15[a]	4.30 ± 0.17[a]	2.57 ± 0.06[a]	1.68 ± 0.09[a]
50 mg/kg ACTSCZn^{2+}	7.03 ± 0.15[a]	4.23 ± 0.25[a]	2.80 ± 0.10[d]	1.37 ± 0.14[d]
25 mg/kg ACTSC Cu^{2+} (ETOH)	6.90 ± 0.17[a]	4.27 ± 0.31[a]	2.63± 0.15[a]	1.63 ± 0.21[a]
50 mg/kg ACTSC Cu^{2+} (ETOH)	6.87 ± 0.15[a]	4.00 ± 0.20[a]	2.93 ± 0.15[e]	1.52 ± 0.15[a]
25 mg/kg ACTSC$Ni^{2+}SO_4$	7.00 ± 0.10[a]	4.43 ± 0.12[a]	2.57 ± 0.15[a]	1.73 ± 0.15[a]
50 mg/kg ACTSC $Ni^{2+}SO_4$	6.93 ± 0.25[a]	4.27 ± 0.38[a]	2.67 ± 0.15[a]	1.61 ± 0.24[a]

Values are mean of five determinations ± 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.

Increased total protein concentration may be due to dehydration, increased immunoglobulin concentration due to infections, and decrease in concentration may occur as a result of over hydration, impaired protein synthesis due to malnutrition malabsorption, liver disease, hypogammaglobulemia or increased protein loss due to renal, gastrointestinal and skin disorder [30]. Total protein of the rats after 5 days treatment regimen showed that total protein of the rats were not significant different ($P > 0.05$) from the control, an indication that the synthetic function of the liver of the rats were not altered [29, 30].

Albumin plays an important physiological role by maintaining osmotic pressure, transport of both endogenous and exogenous substance and serving as protein reserves [30]. The liver's ability to synthesize albumin, globulin is reduced if the normal physiological function of the liver is tempered with [29, 31] hepatitis and liver cirrhosis (liver damage). After 5 days of treatment regimen by the 25 and 50 mg/kg body weight of the ligand; ACTSC serum albumin, globulin, Albumin/globulin ratio were all significantly affected ($P< 0.05$) when compared to the control group, 50 mg/kg ACTSC - Zn^{2+} complex caused significant change ($P < 0.05$) in the levels of globulin and albumin/globulin ratio when compared to the control group, the 50 mg/kg ACTSC - Cu^{2+} complex also brought about significant change ($P < 0.05$) in the value of globulin when compared to the control group. These significant changes in the observed biochemical parameters after five days treatment regimen by the 50 mg/kg of ACTSC and the Zn^{2+} and Cu^{2+}complexes is an indication of altered liver function. These test drugs and may have caused hepatitis or liver cirrhosis (liver damage) [11, 29].

Critical analysis of the result obtained showed that 25 and 50 mg/kg body weight of ACTSC and the complex of ACTSC–Cu^{2+} significantly decreased ($P< 0.05$) SOD and CAT activities at the same time increasing the activity of MDA. Superoxide radicals (anions), a potential destructive radical in the cells is often scavenged by SOD and CAT, radical in the cells is often scavenged by SOD and CAT, hence the observed decrease in the activities of these antioxidant enzymes in these groups portend real danger to the animals in that it makes them vulnerable to oxidative damage that may cause amyotropic lateral sclerosis, a debilitating neurological disorder where motor neurons in the brain and spinal cord degenerates [27].The observed increase in MDA in the groups treated with 25 and 50mg/kg body weight of ACTSC and ACTSC-Zn^{2+} is an indication of increased lipid peroxidation. Lipid peroxidation alters membrane permeability and transport, the result of damage to plasma membrane might result in influx or efflux of ions and other endogenous substances leading to the swelling of the affected cells due to an influx of water [32].

All doses (25 and 50 mg/kg) of ACTSC significantly increased ($P < 0.05$) the levels of AST, ALT and ALP in a dose related manner when compared to the control group, similarly the 50mg/kg body weight of ACTSC-Zn^{2+} complex significantly increased ($P < 0.05$) the values of AST and ALP while only ALT levels were significantly increased ($P < 0.05$) by 50 mg/kg ACTSC - Cu^{2+} complex. Liver disease is the most important cause of elevated transaminase (AST and ALT) and ALP activity in the serum [11]. The elevated levels thus observed are an indication of liver malfunction due toxicity levels of the administered test drugs at the respective dose level.

Table 4: Effects of ACTSC and its Metal Complexes on Liver Antioxidants (Superoxide Dismutase, Catalase and Malondialdehyde) of Wistar Rats.

GROUPS	SUPEROXIDE DISMUTASE (U/mg protein)	CATALASE (U/mg protein)	MALONDIALDEHYDE (nmol/g liver tissue)
CONTROL5% DMSO	7.77 ± 0.15^{a}	92.67 ± 2.52^{a}	382.00 ± 2.65^{a}
25 mg/kg ACTSC	6.77 ± 0.21^{b}	78.00 ± 3.00^{b}	490.00 ± 3.61^{b}
50 mg/kg ACTSC	6.47 ± 0.15^{c}	68.67 ± 2.73^{c}	583.33 ± 4.51^{c}
25 mg/kg ACTSC Zn^{2+}	7.33 ± 0.15^{d}	87.33 ± 2.08^{a}	410.00 ± 3.52^{d}
50 mg/kg ACTSC Zn^{2+}	7.00 ± 0.10^{e}	83.00 ± 2.65^{d}	418.33 ± 3.43^{e}
25 mg/kg ACTSC Cu^{2+} (ETOH)	7.70 ± 0.20^{a}	90.00 ± 2.00^{a}	384.00 ± 2.67^{a}
50 mg/kg ACTSC Cu^{2+} (ETOH)	7.59 ± 0.15^{a}	87.00 ± 2.00^{a}	390.33 ± 2.85^{a}
25 mg/kg ACTSC Ni^{2+} SO_4	7.67 ± 0.15^{a}	91.23 ± 2.00^{a}	385.00 ± 3.51^{a}
50 mg/kg ACTSC Ni^{2+} SO_4	7.62 ± 0.10^{a}	88.00 ± 3.00^{a}	390.00 ± 2.65^{a}

Values are mean of five determinations ± 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.

Table 5: Effects of ACTSC and its Metal Complexes on Liver Enzymes (Aspartate Aminotransferase, Alanine Transferase and Aalkaline Phosphatase) of Wistar Rats.

GROUPS	AST	ALT	ALP
CONTROL5% DMSO	20.67 ± 1.53^{a}	22.33 ± 2.08^{a}	30.67 ± 2.08^{a}
25 mg/kg ACTSC	24.00 ± 1.73^{b}	26.00 ± 1.00^{b}	48.00 ± 3.00^{b}
50 mg/kg ACTSC	25.67 ± 2.52^{c}	27.67 ± 1.53^{c}	54.00 ± 3.05^{c}
25 mg/kg ACTSC Zn^{2+}	21.33 ± 1.53^{a}	23.00 ± 2.00^{a}	32.60 ± 2.08^{a}
50 mg/kg ACTSC Zn^{2+}	24.00 ± 1.00^{d}	24.66 ± 1.53^{a}	36.70 ± 2.52^{d}
25 mg/kg ACTSC Cu^{2+} (ETOH)	21.00 ± 2.00^{a}	22.30 ± 2.05^{a}	32.70 ± 2.50^{a}
50 mg/kg ACTSC Cu^{2+} (ETOH)	21.67 ± 2.08^{a}	23.60 ± 1.52^{e}	33.00 ± 2.00^{a}
25 mg/kg ACTSC Ni^{2+} SO_4	20.33 ± 2.52^{a}	23.70 ± 2.50^{a}	31.00 ± 2.65^{a}
50 mg/kg ACTSC Ni^{2+} SO_4	21.33 ± 1.53^{a}	25.00 ± 2.00^{a}	33.00 ± 3.00^{a}

Values are mean of five determinations ± 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 25 and 50 mg/kg Body Weight of Complexes of ACTSC and its Metal Complexes on Histopathology of the Liver of Wistar Rats.

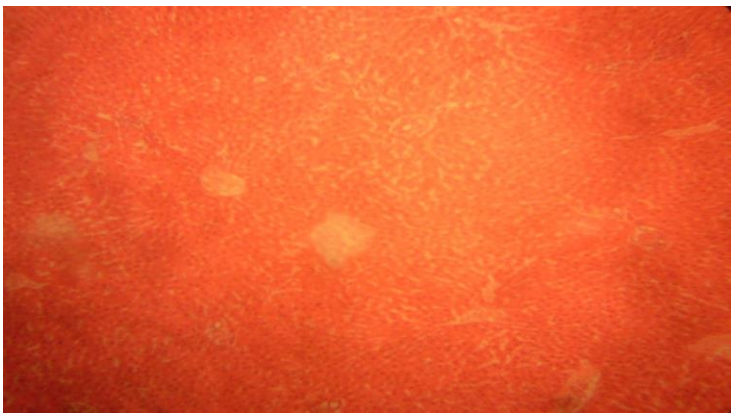

Plate 1: Photomicrograph of rat liver administered with 5% DMSO (CONTROL). Normal rat liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

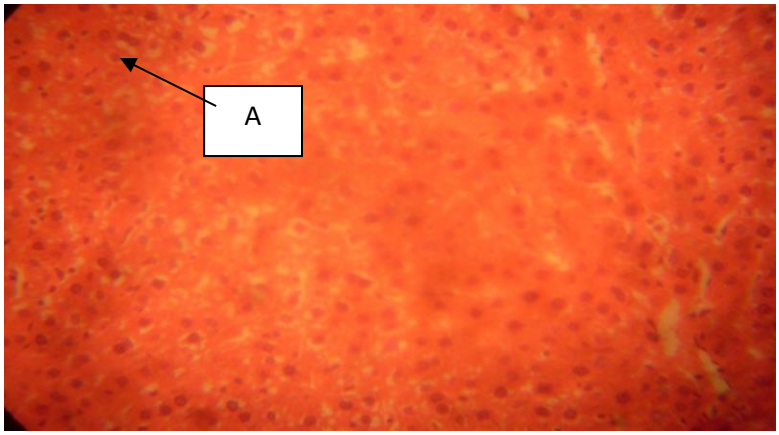

Plate 2: Photomicrograph of rat liver administered with 25 mg/kg body weight of ACTSC, liver showing micro-vesicular steatosis (A), (fatty change). (H & E X 100)

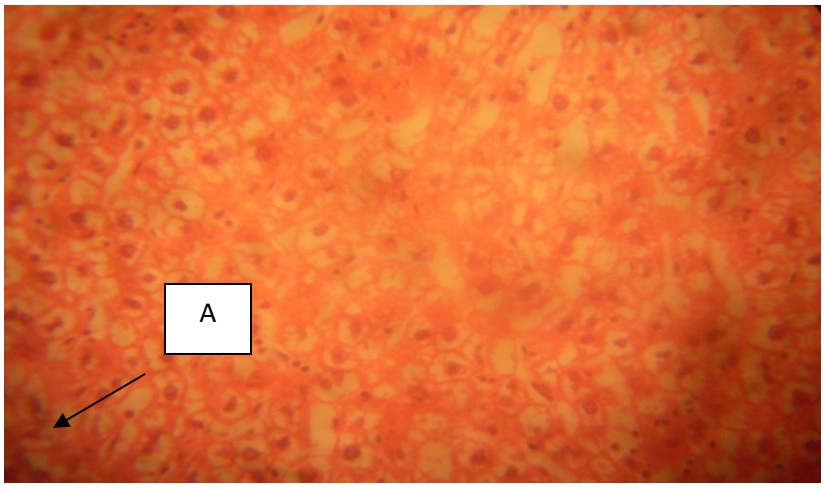

Plate 3: Photomicrograph of rat liver administered with 50 mg/kg body weight of ACTSC, liver showing macro-vesicular steatosis (A), (fatty change). (H & E X 100).

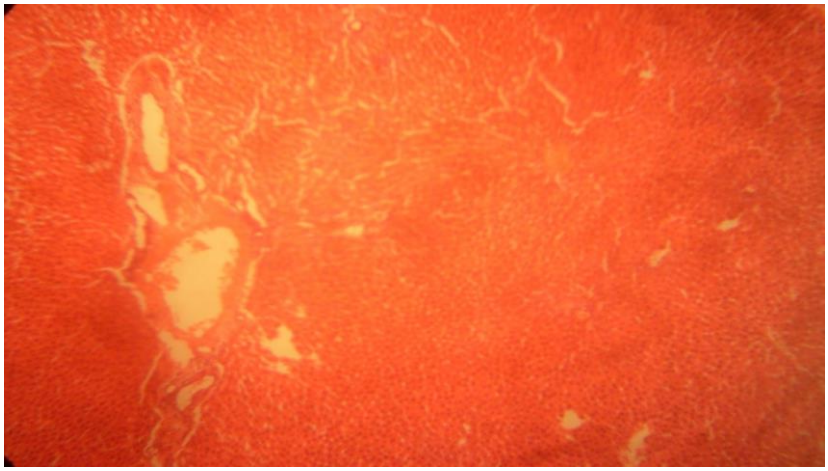

Plate 4: Photomicrograph of rat liver administered with 25 mg/kg body weight of ACTSC–Zn^{2+} complex, liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

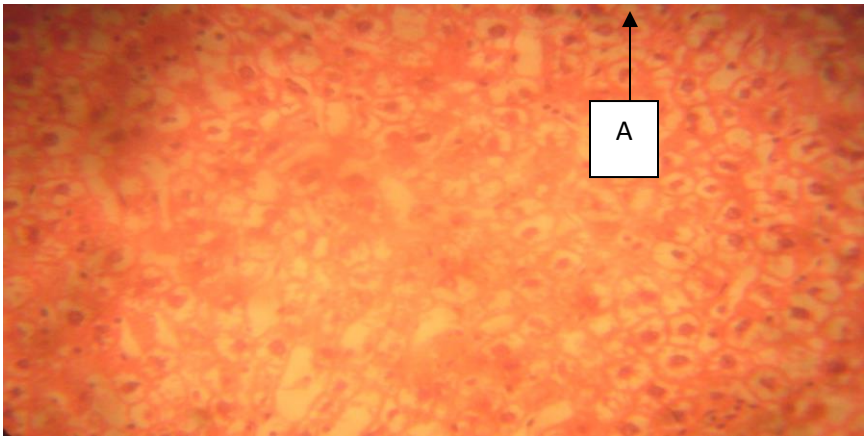

Plate 5: Photomicrograph of rat liver administered with 50 mg/kg body weight of ACTSC-Zn^{2+} complex, liver showing micro-vesicular steatosis (A) (fatty change). (H & E X 100).

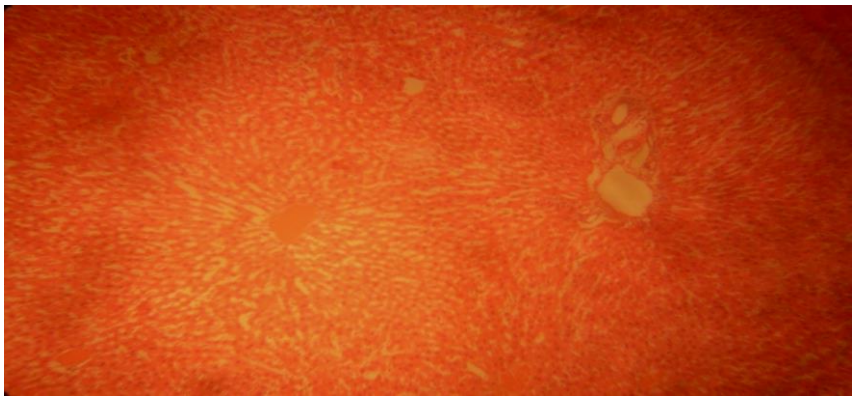

Plate 6: Photomicrograph of rat liver administered with 25 mg/kg body weight of ACTSC–Cu^{2+} complex, liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

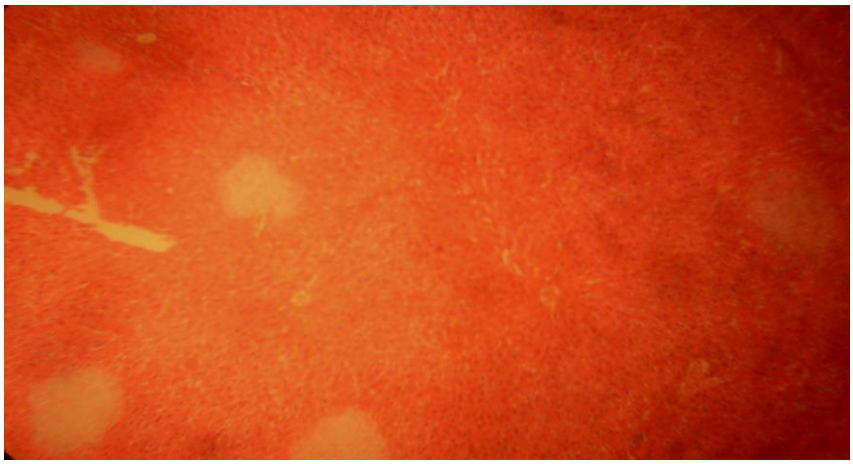

Plate 7: Photomicrograph of rat liver administered with 50 mg/kg body weight of ACTSC-Cu^{2+} complex, liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

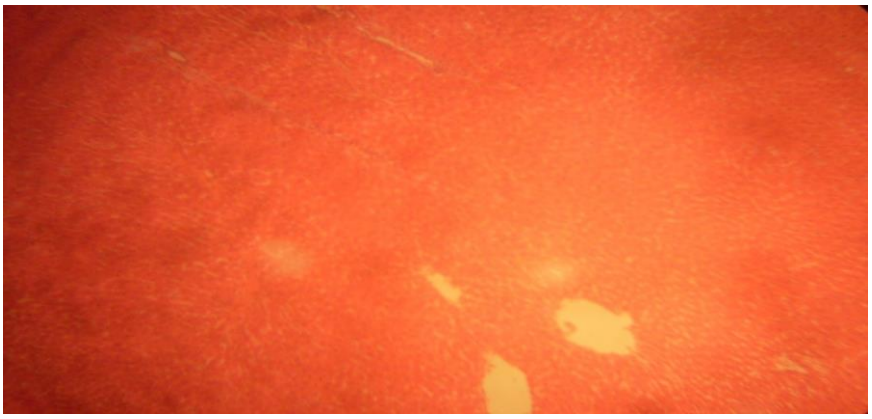

Plate 8: Photomicrograph of rat liver administered with 25 mg/kg body weight of ACTSC-Ni^{2+}complex, liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

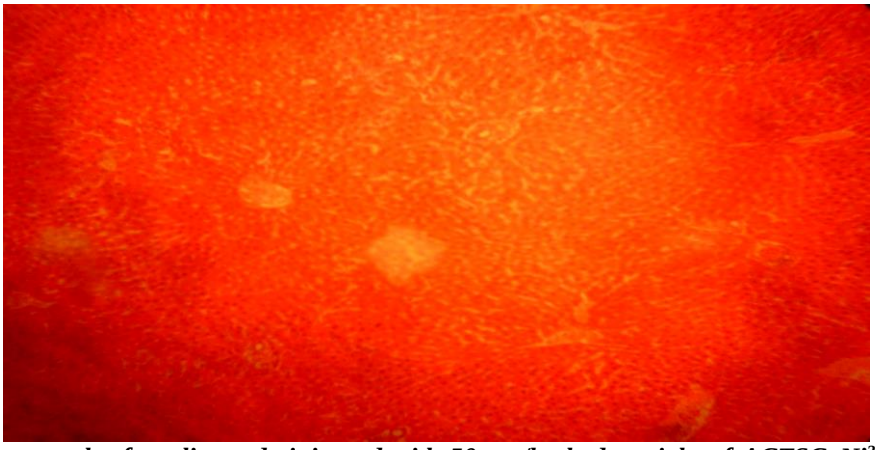

Plate 9: Photomicrograph of rat liver administered with 50 mg/kg body weight of ACTSC–Ni^{2+} complex, liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

Assessments of the liver of those treated with 25 and 50 mg/kg body weight of ACTSC and 5 mg/kg body weight of ACTSC–Zn^{2+} when compared to the control showed moderate to severe alterations, they were characterized with moderate to extensive fatty change/degeneration (micro or macro-vesicular steatosis), area of necrosis and infiltration by inflammatory cells and enlargement of hepatocytes (Plate: 3; 4; 5), an indication of the hepatotoxic nature of these compounds. Examination of the groups treated with 25 mg/kg body weight of ACTSC–Zn^{2+}, 25 and 50 mg/kg body weight of ACSTC–Cu^{2+} and ACTSC–Ni^{2+} showed strong similarity with the control group by having well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (Plate: 2; 6; 7; 8; 9).

11. Animal Rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed. See the experimental part for details

12. Conclusion

Toxicological survey showed that 50 mg/kg of body weight of the ligand exhibits more toxicological effect ($P < 0.05$) on all biochemical parameters evaluated while the histopathological examination of the liver showed that the groups treated with 25 mg/kg body weight of the complex of ACTSC-$Zn^{2+,}$ 25 and 50 mg/kg body weight of the complexes of ACSTC-Cu^{2+} and ACTSC-Ni^{2+} showed strong similarity with the control group by having well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation.

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