July 2024, Volume 18, Number 3

Iranian Journal of Toxicology
ARAK UNIVERSITY OF MEDICAL SCIENCES

Research Paper

Reduced Toxicity of Methylphenyl Ketone Compounds by Combining them with Metal Ions

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How to cite this paper:
Kpomah B, Kpomah ED, Ugbune U, Agbaire PO, Apiamu A. Reduced Toxicity of Methylphenyl Ketone Compounds by Combining them with Metal Ions. Iranian Journal of Toxicology. 2024; 18(3):120-129. **doi:** 10.32592/IJT.18.3.120

doi: 10.32592/IJT.18.3.120

Article info
Received: 19 Jan 2024
Accepted: 26 Jul 2024
Published: 20 Jul 2024

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ABSTRACT

Background: The human race is currently burdened with cases of multi-drug-resistant pathogenic microorganisms, causing a menace to the efficacy of the existing antimicrobials. This has created a critical need for innovative drugs with minimal toxicity. The present study aimed to assess the sub-chronic toxicity of some transition metal complexes containing Schiff base ligand with 2,2-bipyridine.

Methods: A total of 36 male rats weighing 178.12±5.48 g were assigned to six groups of six per group. *Group A:* administered 5% DMSO; *Group B:* treated with 200 mg kg-1 BW of 2, 2-Bipyridine; *Group C:* treated with 200 mg kg-1 BW MPK-TSC; *Group D:* treated with 200 mg kg-1 body weight [Cu (MPK-TSC) (Bipy)Cl_2]; *Group E:* treated with 200 mg kg-1 BW [Ni (MPK-TSC) (Bipy)Cl_2]; and *Group F:* treated with 200 mg kg-1 BW [Zn (MPK-TSC) (Bipy)Cl_2]. Blood was collected via cardiac puncture for hematological analysis. Liver and kidney homogenates were used for plasma enzymes, liver and renal functions, and the antioxidant indices. The histological sections of rats' liver and kidney tissue samples were also examined.

Results: Animals from the control and different treated groups exhibited normal body weight gain throughout the dosing period of 28 days, except those treated with Bipy, with a marked decrease in body weight. The results of the study indicated that Bipy and MPK-TSC had significant adverse effects (P<0.05) on various biochemical indices.

Conclusion: As evidenced by the obtained results, combining MPK-TSC with metal ions resulted in a significant reduction in the toxicity of these compounds.

Keywords: 2,2-Bipyridine, Metal ion complexation, Methylphenyl ketone, Sub-chronic toxicity, Thiosemicarbazone

Introduction

The world is currently facing numerous cases of multi-drug resistant pathogenic microbes due to their mutation over the years, posing serious threats to the therapeutic efficacy of existing antimicrobial agents. Mohammad and Asad described this problem as multidimensional since it affects health care systems, the economy, and social and human issues. The effects of drug resistance include prolonged treatment, costly therapy, morbidity, high mortality, and economic loss. As reported by the World Bank [1, 2], by 2050, the annual global gross domestic product will drop by 1.1% and 3.8% in minimally and heavily impacted antimicrobial resistance areas, respectively. This has, therefore, created a critical need for innovative medicines to mitigate these threats. Metal complexes of thiosemicarbazone (TSC) ligands and their derivatives have been reported to exhibit satisfactory medicinal properties and offer advantages in terms of producing minimal toxicity but with great efficacy [3].

Over the years, derivatives and metal complexes of TSCs have been used owing to their pharmacological properties [4, 5]. Pharmacological applications of TSC derivatives and their metal complexes range from the treatment of heavy metal toxicity [6], anticancer [7], antimicrobial [8], anti-inflammatory [9], anti-diabetes [10], and neurological disorders [11]. The relative stability of transition metals in diverse oxidation states earns them a significant function in biological redox interactions. Moreover, there are extensive therapeutic applications for metal complexes [12]. Schiff bases are the condensation products of ketones or aldehydes with primary amines. They act as flexi-dentate ligands and coordinate commonly via the *O* atom of the

deprotonated phenolic group and the *N* atom of the azomethine group [13].

As a pharmacological compound, TSC is a major class of Schiff bases. The intense chelating capability of TSC can be attributable to the hydrazine nitrogen atom and thioketone sulfur, thereby making them excellent chromogenic agents for metal ions. The heightened biological activity of TSC is reportedly due to excellent chelation potentials with trace metal ions present in biological systems [14]. Over the years, TSCs and their metal derivatives have attracted much attention due to their wide range of pharmacological properties. Having been spurred from our previous research [5], the current study aimed to assess the sub-chronic toxicity profile of a 28-day repeated dose of some metal complexes of methyl phenyl ketone (MPK) with 2,2-Bipyridine (Bipy) in male Wistar rats.

Materials and Methods

Synthesis of primary ligand: The ligand methylphenyl ketone thiosemicarbazone (MPK-TSC) is produced by the reaction of an equimolar methanolic solution of MPK with TSC. After the addition of limited drops of concentrated HCl to the mixture, it was refluxed with continuous stirring at 60°C for 4 h. The mixture was reduced in volume and refrigerated overnight, and white crystals precipitated [5].

Synthesis of mixed ligand complexes: Two mmol (0.387 g) MPK-TSC in 30 mL methanolic solution was added slowly to 15 mL hot methanolic solution of the metal salt. For the copper complex, 1 mmol (0.170 g) of $CuCl_2$. $2H_2O$ mixture was used. The reaction mixture was constantly stirred and refluxed for 30 min until precipitated. Thereafter, 2 mmol (0.312 g) of Bipy in 30 mL methanol solution was added slowly. Upon the addition of 2, 2'-bipyridine, the reaction mixture became apparent and was continuously stirred and refluxed for another 4 h at 60^{O}C. The mixture was reduced in volume and refrigerated overnight, resulting in the formation of a black powder [5].

Test compounds: The complexes used in the current study had previously been prepared [5] and characterized using spectroscopic and CHN elemental analysis. The results of the previous research are introduced below:

Complex 1: [Cu(MPK-TSC)(Bipy)Cl_2] $C_{19}H_{19}Cl_2CuN_5S$ (482.00 g/mol); mp. 250.18 ^{O}C; 57 % yield as a black powder. IR(KBr) cm^{-1} 3736 (br) 3308 (s), 2074 (s), 1641 (s), 1076 (s). 1037 (s) 723 (s), 473 (w), 468 (w), 418 (w). Anal Calcd.: C: 47.16, H: 3.96, N: 14.47; Found: C: 47.57, H: 4.08, N: 14.86.

Complex 2: [Ni(MPK-TSC)(Bipy)Cl_2] $C_{19}H_{19}Cl_2N_5NiS$ (477.01 g/mol); mp. 310. 40 DT; 54 % yield as a dark green powder. IR(KBr) cm^{-1} 3119 (s), 3061 (s), 2063 (s), 1615 (s), 1257 (m), 1035 (s). 1064 (s) 767 (s), 512 (w), 431 (w), 416 (w). Anal Calcd: C: 47.64, H: 4.00, N: 14.62; Found: C: 47.70, H: 4.11, N: 14.79

Complex 3: [Zn(MPK-TSC)(Bipy)Cl_2] $C_{19}H_{19}Cl_2N_5SZn$ (483.00 g/mol); mp. 210.10 ^{O}C; 75 % yield as a white powder. IR (KBr) cm^{-1} 3728 (br), 3495 (br), 3296 (s), 3173 (s), 2070 (s),1627 (s), 1068 (s) 725 (s), 563 (w), 466 (w), 437 (w). Anal Calcd.: C: 46.98, H: 3.94, N: 14.42; Found: C: 48.12, H: 4.06, N: 14.51

Experimental animals: A total of 36 male healthy adult Wistar rats weighing 178.12±5.48 g were purchased from the animal house section of the Department of Biochemistry, Delta State University, Abraka, Nigeria. They were kept in standard clean metabolic cages and housed in a well-ventilated room at 25C°°C,-30°C, under natural light and dark cycle. The rats had free access to grower mash and tap water during seven days of acclimatization plus the experimental period of 28 days. All procedures were performed in accordance with the Institutional Animal Ethical Committee (approval code: REL/FOS/22/04).

Experimental design: The rats were assigned to six main groups of six each as follows: *Group A* was administered dimethyl sulfoxide (5% DMSO); *Group B* was treated with 200 mg/kg 2, 2-Bipy; *Group C* was given 200 mg/kg MPK-TSC*; Group D* was treated with 200 mg/kg [Cu (MPK-TSC) (Bipy)Cl_2]; *Group E* was given 200 mg/kg [Ni (MPK-TSC) (Bipy)Cl_2]; and *Group F* was administered 200 mg/kg [Zn (MPK-TSC) (Bipy)Cl_2]. All complex doses were administered orally.

Changes in body weights: Rats in all groups were weighed on the first day and upon the completion of their treatment periods, and the percent of body weight changes were documented consistently in all groups [15].

$$\%\ change\ in\ body\ weight = \frac{final\ body\ weight - initial\ body\ weight}{initial\ body\ weight} \times 100\%$$

Kidneys and liver as a ratio of body weights: The kidneys and liver were removed from each aminal under anesthesia and weighed immediately. The ratio of the organ versus body weight was determined as a percentage in each animal group [15].

$$Organ\ ratio\ (\%) = \frac{weight\ of\ organ\ (g)}{body\ weight\ (g)} \times 100\%$$

Animal handling and tissue samples: After 28 days, animals were euthanized under anesthesia using a diethyl ether chamber. Blood specimens were collected from the rats by cardiac puncture and added to EDTA-containing tubes for the analyses of blood parameters. The liver and kidneys from each rat were dissected immediately and separated into two portions. One portion was used for histological studies, while the second one was homogenized and kept for biochemical analyses.

Biochemical assay kits: The assay kits for liver function indices and plasma proteins were obtained from Teco Diagnostics Ltd., USA (Anaheim, CA). The renal function indices and antioxidants assay kits were obtained from Randox Laboratories Ltd. (Crumlin, UK). All other reagents and chemicals were purchased from local suppliers at analytical grade.

Hematological analyses: A full blood count was carried out on each blood sample of the respective groups using an Erba Mannheim Automatic Hematology Analyzer (Elite 580) by adopting the Coulter method [16].

Liver and kidney homogenates: The liver and kidney tissue samples were homogenized (10% w/v) in ice-cold 0.1 M Tris-HCl buffer (pH 7.4). The homogenates were centrifuged at 3000 rpm for 15 min at 4ºC, and the supernatant was used for biochemical analyses.

Liver function biochemical analyses: Liver function parameters were determined by established methods [17-21]. These parameters included alkaline Phosphatase (ALP), Lactate dehydrogenase (LDH), total protein (TP), and albumin. The serum globulin (Glo) level was calculated as the difference between total protein and albumin, while the albumin globulin (A/G) ratio was determined by dividing the values of albumin over globulin [21].

Renal function biochemical analyses: Blood urea nitrogen (BUN) was evaluated by the modified Berthelot method, according to Tobacco et al. [22]. Creatinine (CRT) was assayed by the method of Bartels et al. [23] and uric acid (UA) was evaluated using the method proposed by Duncan et al. [24].

Antioxidant enzymes analyses: The catalase (CAT) activity was estimated using the method of Cohen et al. [25]. Superoxide dismutase (SOD) activity was determined using the methods of Misra and Fridovich [26]. The determination of glutathione peroxidase (Gpx) activity was achieved by the method of Chance and Maehly [27]. The assay method of Gutteridge and Wilkins [28] was adopted for the assay of Malondialdehyde (MDA) concentration.

Organs histopathology: For histopathological examinations, the liver and kidney slices were fixed in 10% formal saline and embedded in paraffin wax blocks. Thereafter, 5μm -thick sections were made, stained with hematoxylin and eosin (H&E), and examined under a light microscope for the determination of pathological alterations [29].

Statistical analyses: Experimental values were expressed as mean±standard deviation ($n = 6$). To determine differences between the groups, one-way analysis of variance (ANOVA) and Duncan post-hoc tests were used to compare the group means, with the statistical significance set at P<0.05. We used SPSS software (version 23.0) for the statistical analyses, and the charts were plotted using GraphPad Prism (version 8).

Results

Effects on body and organ weights: The effects of MPK-TSC and its metal complexes on body, organ weights, and the relative body-to-organ weight ratio of rats are presented in Table 1. Compared to controls, the results demonstrated significant decreases (P<0.05) in the body weights of the rats treated with 2, 2-Bipyridine, MPK-TSC, [Cu(MPK-TSC) (Bipy)Cl_2] and [Ni(MPK-TSC) (Bipy)Cl_2]. In addition, the rats treated with 2, 2-Bipyridine exhibited significant differences (P<0.05) in their liver and kidney weights as compared to controls. Nonetheless, the corresponding data for the animals treated with [Zn(MPK-TSC) (Bipy)Cl_2] were insignificant compared to those of controls.

Effects on hematological indices: The effects of MPK-TSC and its metal complexes on the hematological indices of rats are presented in Table 2. The result of experimental animals indicated significant decreases in the RBC, Hb, and MCHC values across all groups compared to the control group (P<0.05). In a similar vein, all groups experienced a significant increase in white blood cell count compared to the control group except for the Zn-(MPK-TSC) (Bipy) Cl_2 treated group (P<0.05). The PCV, MCV, MCH, and platelet levels decreased significantly (P<0.05) except for the group treated with Zn-(MPK-TSC)(Bipy)Cl_2 as compared to controls.

Table 1. Effects of MPK-TSC and its metal complexes on body weight, liver & kidney weights, and organ to body weight ratio in rats.

Weight (g) /Animal groupings	Control (5% DMSO)	2, 2-Bipyridine	MPK-TSC	[Cu(MPK-TSC) (Bipy)Cl_2]	[Ni(MPK-TSC) (Bipy)Cl_2]	[Zn(MPK-TSC) (Bipy)Cl_2]
Initial body weight	178.00 ± 4.28	181.12 ± 4.54	182.47 ± 4.63	180.42 ± 4.55	182.31 ± 4.61	175.83 ± 4.25
Final body weight	182.56 ± 4.71	174.60 ± 4.23	186.88 ± 4.65	184.75 ± 4.43	186.22 ± 4.67	180.31 ± 3.89
Changes in body weight	**2.56^a**	**-3.60^b**	**2.42^c**	**2.40^d**	**2.14^e**	**2.55^a**
Liver weight	4.98 ± 0.61	6.64 ± 0.73	5.20 ± 0.59	5.12 ± 0.51	5.21 ± 0.58	4.87 ± 0.59
Relative Liver weight (%)	**2.73^a**	**3.80^b**	**2.78^a**	**2.77^a**	**2.80^a**	**2.70^a**
Kidney weight	1.21 ± 0.05	1.83 ± 0.09	1.29 ± 0.07	1.25 ± 0.05	1.31 ± 0.06	1.18 ± 0.04
Relative kidney weight (%)	**0.66^a**	**1.05^b**	**0.69^a**	**0.68^a**	**0.71^b**	**0.65^a**

Data are mean ± standard deviation per group (n=6). The mean values on the same row with different superscript letters are significantly different (P<0.05), one-way analysis of variance followed by posthoc LSD.

Table 2. Effects of MPK-TSC and its Metal Complexes on Haematological Indices of wistar Rats.

Haematological parameters	Control (5% DMSO)	2,2-Bipyridine	MPK-TSC	Cu(MPK-TSC) (Bipy)Cl_2	Ni(MPK-TSC) (Bipy)Cl_2	Zn(MPK-TSC) (Bipy)Cl_2
PCV (%)	42.31±2.45^a	34.22±2.42^b	38.55±2.49^c	39.32±2.53^d	38.87±2.18^e	40.68±1.98^a
RBC $(\times 10^6 \mu L^{-1})$	7.60±0.98^a	5.13±0.71^b	6.90±0.92^c	7.01±0.95^d	6.96±0.93^e	6.94±0.90^f
Hb (gdL^{-1})	13.83±2.45^a	9.21±2.00^b	10.99±2.11^c	12.09±2.21^d	11.54±2.23^e	12.78±2.32^f
WBC $(\times 10^3 \mu L^{-1})$	9.86±1.97^a	13.83±2.47^b	11.93±2.13^c	10.78±2.07^d	11.06±2.11^e	10.21±2.19^a
MCV (fL)	67.23±1.54^a	50.44±1.43^b	58.14±1.55^c	61.51±1.52^d	60.11±1.32^e	64.19±1.61^a
MCH (pg)	33.16±1.35^a	25.23±1.27^b	27.17±1.21^c	30.33±1.23^d	28.88±1.29^e	31.59±1.32^a
MCHC (gdL^{-1})	43.44±1.99^a	31.31±1.86^b	35.38±1.94^c	39.47±1.92^d	37.89±1.76^e	40.07±1.81^f
Platelet $(10^9 L^{-1})$	9102±19.67^a	6783±17.34^b	7765±18.69^c	8543±19.65^d	8321±19.49^e	8903±19.77^a

Data are mean ± standard deviation (n=6). Mean values on same row with different superscript letters are significantly different ($p < 0.05$), one-way analysis of variance followed by posthoc LSD.

Effects on liver function: The effects of MPK-TSC and its Cu, Ni, and Zn complexes on liver function indices of rats are presented in Figure 1. As observed in this Figure, 2, 2BiPy illustrated significantly high toxicity in all groups against the four liver enzymes, alanine transaminase (ALT) and aspartate transaminase (AST), ALP, and LDH, compared to that of controls ($P<0.05$). On the contrary, the complexes of MPK-TSC with Cu, Ni, or Zn particles significantly lowered the toxicity levels, with the Zn complex showing no toxicity, similar to that of controls.

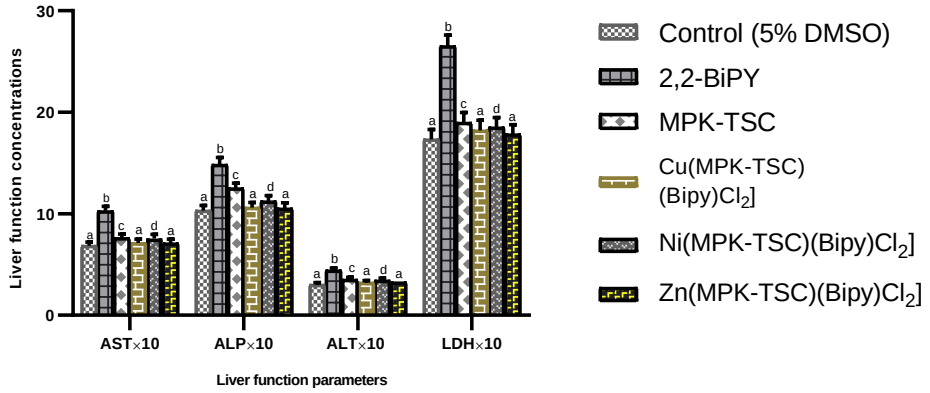


Figure 1. The effect of MPK-TSC and its metal complexes on the liver enzymes.
Data are means ± Standard deviations (n=6). Same parameters with different superscript letter are significantly different ($P<0.05$), one-way ANOVA.

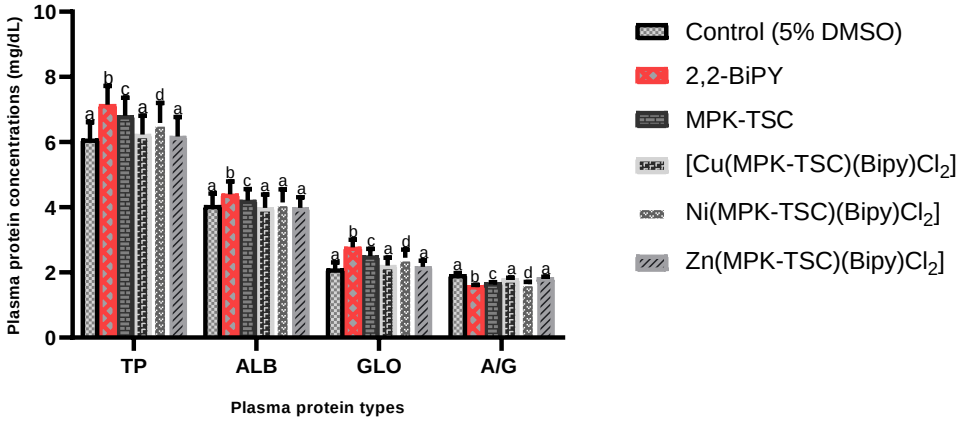


Figure 2. The effect of MPK-TSC and its metal complexes on plasma protein levels.
Data are means ± Standard deviations (n=6). Same parameter with different superscript letter are significantly different ($P<0.05$), one-way ANOVA. *Key:* TP: total protein; ALB: albumin; GLO: globulin; A/G: albumin globulin ratio.

Effects on plasma proteins: The effect of MPK-TSC and its metal complexes on plasma proteins are depicted in Figure 2, demonstrating TP, albumin (ALB), globulin (GLO), and albumin globulin ratio (A/G). As illustrated in Figure 2, the plasma levels of TP, ALB, and GLO are significantly higher than that of controls but below the corresponding levels induced by Bipy. On the contrary, the A/G ratios for the MPK-TSC metal complexes were consistently but insignificantly above that level found for Bipy, as compared to the control.

Effects on renal function: The effects of MPK-TSC and its metal complexes on the renal function of rats are presented in Figure 3, based on the blood levels of CRT, BUN, and UA. All three renal parameters were significantly lower than that induced by Bipy but insignificantly higher or similar to those found in the blood collected from control rats. Notably, the zinc complex of MPK-TSC caused the lowest levels of CRT, BUN, and UA compared to those induced by the other two metal complexes of MPK-TSC compounds (Figure 3).

Effects on antioxidant indices: The effects of MPK-TSC and its metal complexes on antioxidant parameters of rats are displayed in Figure 4, based on the blood levels of CAT, superoxide dismutase (SOD), glutathione peroxidase (Gpx), and malondialdehyde (MDA). The blood levels of CAT, SOD, and Gpx were significantly higher in all of the six rat groups than in those treated with Bipy. Nevertheless, MDA levels were significantly lower in experimental groups and controls than in the rats treated with Bipy.

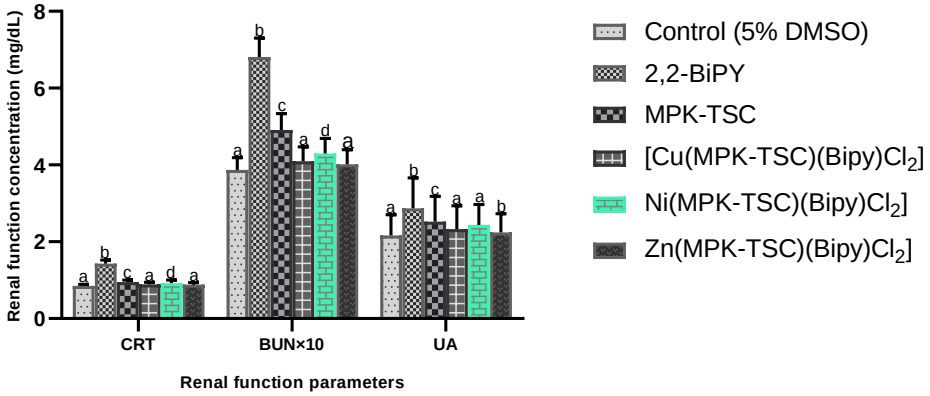


Figure 3. The effects of MPK-TSC and its metal complexes on renal function of male wistar rats. Data are the mean ± standard deviation (n=6). Same parameter with different superscript letter are significantly different (P<0.05), one-way ANOVA. *Key:* CRT: creatinine; BUN; blood urea nitrogen; UA: uric acid.

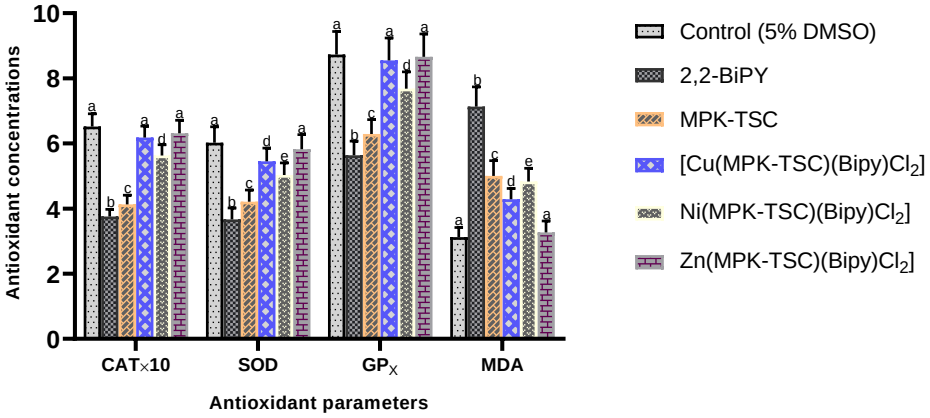


Figure 4. The effects of MPK-TSC and its metal complexes on the antioxidant status of male wistar rats. Data are the means ± Standard deviations (n=6). Same parameter with different superscript letter are significantly different (P<0.05), one-way ANOVA. *Key:* CAT: catalase, SOD: superoxide dismutase, GPx: glutathione peroxidase, and MDA: malondiadehyde.

Liver and kidney histopathological examinations

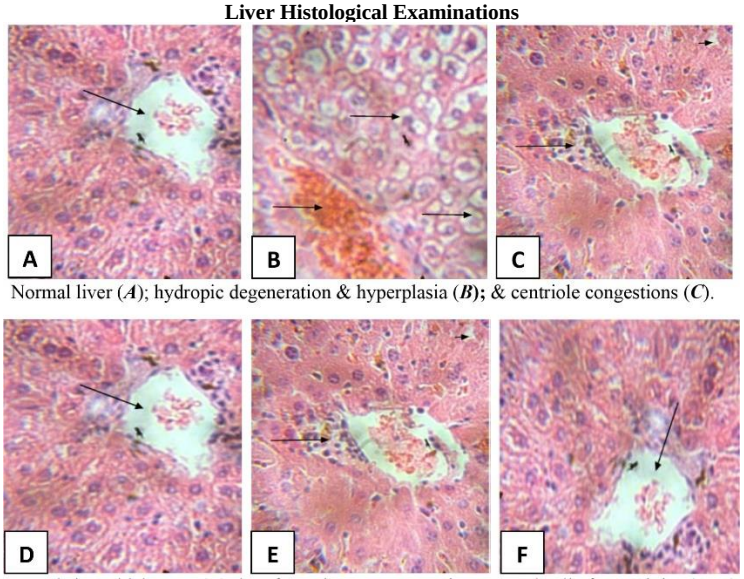
Liver histopathology: The normal and pathological changes in liver tissue samples are displayed in Figure 5. They represent the six distinct experimental conditions, the microscopic results of which are observed in Figure 5, panels A to F. In the control group, micrographs illustrated normal liver histology, showing visible centrioles and fenestrated sinusoidal areas. The hepatocytes appeared distinct with well-differentiated nuclei. In the rat group treated with Bipy, the microscopic images exhibited hydropic degeneration, hyperplasia, and some multifocal areas of congestion (arrows). In the rat group treated with MPK-TSC**,** the liver tissue illustrated pathological changes, such as fatty changes and hydropic degeneration. Furthermore, there were multifocal coagulations of hepatocytes and some portal biliary hyperplasia.

In the three rat groups treated with MPK-TSC complexed with the three metal particles, healthy centrioles were found with well-fenestrated sinusoidal spaces. The hepatocytes appear distinct with well-differentiated nuclei, and the liver histology appeared essentially normal. In the group that received the nickel compound, less fatty changes and hydropic

degeneration of the hepatocytes were observed. In addition, there were fewer multifocal congestions of the centriole; nonetheless, occasional neutrophilic infiltrates were noted close to the centrioles. In the rat group treated with MPK-TSC combined with zinc, distinct centrioles were observed with well-fenestrated sinusoidal spaces. Moreover, the hepatocytes appeared distinct with well-differentiated nuclei, and the liver histology appeared essentially normal.

Kidney histopathology: As displayed in Figure 6, the microscopic examinations of rats' kidney tissues revealed some histopathological alterations under the six experimental conditions as discussed for the liver of rats. The control kidney sections showed normal histological and microanatomical features. These sections indicated detailed cortical parenchyma and the renal corpuscles that appeared dense and round structures. Tissue samples from the rats treated with MPK-TSC and Bipy, respectively, showed mild to moderate distortions, disruptions, and edema in the microanatomy of the renal cortex and parenchyma. Nonetheless, the rats exposed to MPK-TSC complexed with metal nanoparticles demonstrated renal histological integrity essentially the same as those observed in controls.

Liver Histological Examinations

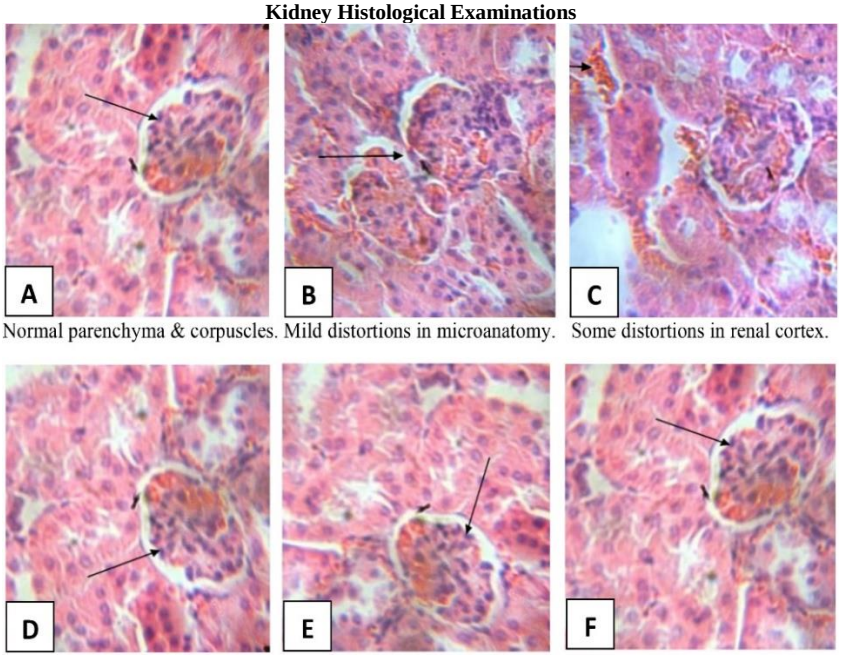


Normal liver (***A***); hydropic degeneration & hyperplasia (***B***); & centriole congestions (***C***).

Normal sinusoidal space (***D***); low fatty changes, congestion; normal cells & centrioles (***E, F***).

Figure 5. Normal and pathological alterations of liver tissue upon histological examinations exposed to six experimental treatments (x40 magnification).
Keys: **A**: Control; **B**: Bipy; **C**: MPK-TSC; **D**: MPK-TSC + Cu; **E**: MPK-TSC + Ni; **F**: MPK-TSC + Zn.

Kidney Histological Examinations



Normal parenchyma & corpuscles. Mild distortions in microanatomy. Some distortions in renal cortex.

Kidney histology in D, E and F sections showed normal cortical paryncheма, corpuscles and microanatomical features.

Figure 6. Normal and pathological alterations of the kidney tissue samples noted upon histological examinations after exposure to six experimental treatments (x40 magnification).
Keys: **A**: Control; **B**: Bipy; **C**: MPK-TSC; **D**: MPK-TSC + Cu; **E**: MPK-TSC + Ni; **F**: MPK-TSC + Zn.

Discussion

Alteration in body weight is a salient indicator of the overall health status of animals [30]. It is also a major pointer to the adverse effects of xenobiotics [31]. An estimated loss of body weight between 15%-29% in an interval of five to seven days is critical [32]. Weight reduction arising from the xenobiotics challenge could be attributed to a distressed cytochrome P_{450} during hepatic metabolism [33]. In descriptive statistics, we expressed the body weight of rats in percentage, as presented in Table 1. After 28 days of treatment, all the rat groups experienced progressive increases in body weight except for the rats administered with the Bipy. They showed a significant weight loss ($P<0.05$), indicating that the physical status of this group was compromised, probably due to acute loss of fluid, proteolysis, and lipolysis induced by the toxic agent.

The findings of this study suggested that not all of the test drugs had adverse effects on body weight except for Bipy. Organ weight is also regarded as a delicate measure of drug toxicity. This is commonly demonstrated as significant differences in organ weights between treated and control animals, occurring in the absence of morphological changes [34]. Therefore, The assessment of alterations in organ weight in the presence of body weight differences can result in the use of an organ-to-body weight ratio to assess the given treatment effects in toxicity studies [35]. This approach also helps evaluate the safety of the administered drugs. It is also a common practice to compare organ weight relative to the body weight of animals, aiding researchers in eliminating the bias originating from differences in animal body weights. This approach, which supports the use of relative organ weights, was employed in the current study. Our findings indicated a marked increase in liver and kidneys, as well as the relative weights of liver and kidneys ($P<0.05$) of the group administered with Bipy. All other test drugs, however, led to no significant differences ($P<0.05$). The changes detected in the groups treated with Bipy may be due to enzyme induction in the tested organs. A study has suggested that increases in organ weight, e.g., liver and kidney, without microscopic lacerations, are likely to be linked to such processes as enzyme induction [34].

Haemoglobin, as the most commonly used biomarker of anemia, plays a significant role in the perfusion of bodily tissue. On the other hand, the red blood cell (RBC) count reflects the number of circulating RBCs and is particularly useful in identifying erythrocytosis. A decline in RBC count, hemoglobin, and/or the blood levels of MCH, MCV, and MCHC is an indication of anemia [36]. Platelets maintain the integrity of the vascular tree and produce platelet plug in the first phase of clotting and platelet factor 3, an essential component of the coagulation cascade [37]. Platelets are rapidly deployed to tissue injuries or infection sites and potentially modulate inflammatory processes by interacting with leukocytes, secreting cytokines, chemokines, and other inflammatory mediators. Platelets are of great importance in the initiation of thrombosis; therefore, their morphological and functional changes are closely correlated with the occurrence and development of coronary artery thrombosis [38].

Platelet count and blood level can also be employed to determine the thrombopoietic activity of the bone marrow. Furthermore, an increase or decrease in platelet numbers can point to disorders of hemostasis [39] or liver disease [40]. White blood cells (WBCs) are cellular elements involved in humoral and cell-mediated immunity. Elevated WBC count is considered a risk factor for cardiovascular disease and mortality [41]. Subsets of WBC, such as neutrophils, lymphocytes, monocytes, or ratio of neutrophil to lymphocyte counts (N/L), have been identified as easy, simple, inexpensive, and reliable prognostic indices to evaluate the host immunity [42]. Blood is acknowledged as one of the most critical body fluids that controls our various vital functions, including metabolite transport and defense against xenobiotic attacks. Moreover, blood is a major indicator of our pathological exposure to toxicants and adverse agents [43]. The assessment of hematological parameters can be used to explain the function of a chemical compound in an organism and further provides information regarding the status of bone marrow activity and hemolysis. It can also be used to define the degree of deleterious effects of foreign compounds on blood constituents in an animal.

The current study assessed the effects of MPK-TSC and its metal complexes on some hematological indices. The results indicated a significant decrease ($P<0.05$) in the levels of RBC, Hb, and MCHC across all the groups as compared to the control group. In a similar vein, all groups experienced a marked increase ($P<0.05$) in WBC as compared to the control except the Zn (MPK-TSC) $(Bipy)Cl_2$ treated group. The blood levels of PCV, MCV, MCH, and platelets were decreased significantly ($P<0.05$) except in the rat group that received Zn(MPK-TSC) $(Bipy)Cl_2$, as compared to controls.

The liver is the largest and one of the most active and complex organs in the human body. It plays an important function in the intermediary metabolism of itself and other organs in maintaining the internal body milieu. It is essentially involved in the regulation, synthesis, storage, and secretion of essential nutrients and chemicals. It also plays a major role in the biotransformation of xenobiotics [20], making the liver susceptible to damage caused by various noxious compounds that enter the body. Serum AST, ALT, ALP, and LDH are cytoplasmic enzymes released into the circulation following cellular damage. They are the

most sensitive biomarkers used in the diagnosis of hepatic lesions associated with some infections [44]. An increase in the values of these enzymes is directly linked to the degree of tissue damage, which is also an indication of loss of tissue functional integrity and cell membranes and, consequently, leakage into the bloodstream.

Aminotransferases are ubiquitous pyridoxal-5'-phosphate-dependent enzymes that catalyze the reversible transfer of amino groups from amino acids to α-keto acids. They play a key role in the metabolism of amino acids in all species. Aspartate aminotransferase (AST) exists at high concentrations in the hepatic, renal, cardiac, and skeletal muscle tissues, as well as erythrocytes. Damage to any of these tissues increases the AST levels in the blood. Alkaline phosphatase (ALP) is a ubiquitous membrane-bound glycoprotein that catalyzes the hydrolysis of phosphate monoesters at basic pH values. This enzyme is a vital serum analyte, and its elevation in the serum correlates with ongoing bone and liver diseases [45]. Decreased levels of ALP are less common than its rise, probably occurring due to such conditions as hypophosphatasia, postmenopausal women receiving estrogen therapy, men with new heart surgery, malnutrition, magnesium deficiency, hypothyroidism, severe anemia, and children with achondroplasia [45].

Alanine aminotransferase (ALT) is found ubiquitously in the kidney, myocardium, skeletal muscle, brain, pancreas, spleen, and lungs. More precisely, the highest tissue level of ALT activity can be detected in the cytosol of hepatocytes. This enzyme catalyzes the transfer of amino groups from the L-alanine to alpha-ketoglutarate, and the conversion products are L-glutamate and pyruvate. The process is critical in the liver in the tricarboxylic acid cycle. Pyruvate can be used in the citric acid cycle to produce cellular energy. The release of ALT from dying or damaged hepatocytes results in increased serum ALT levels, and therefore, it is more specific for hepatic damage [21]. The cytoplasmic enzyme LDH is present in most body tissues but at high levels in muscles, liver, and kidneys. It is a central enzyme in the anaerobic metabolic pathway. It belongs to the class of oxidoreductases with an enzyme commission number. The function of this enzyme is to catalyze the reversible conversion of lactate to pyruvate with the reduction of NAD^+ to NADH and vice versa. Factors that can cause increased LDH in the blood may include liver diseases, anemia, heart attack, bone fractures, muscle trauma, cancers, and such infections as meningitis, encephalitis, and HIV [46].

The lactate dehydrogenase enzyme functions as a major indicator of acute and chronic diseases. After 28 days of treatment with the test drugs, 2, 2-Bipyridine, MPK-TSC, and [Ni (MPK-TSC) (Bipy)Cl_2] had a significant effect (P<0.05) on the concentrations of AST, ALT, ALP, and LDH levels. It can be suggested that these test drugs, after 28 days, had marked toxicological effects on the liver of the treated rats. Chen et al. [47] corroborated these findings. These compounds are thought to exert their toxicity primarily through redox cycling to produce free radicals, such as superoxide anions, and diminishing NADPH [48]. The core plasma protein fractions are albumin, globulin, and fibrinogen. Plasma proteins are present at well-adjusted concentrations, with a normal albumin-to-globulin ratio (A/G) being the standard. This ratio could be altered in liver diseases [20]. Amplified total protein concentration may be due to dehydration or increased immunoglobulin concentration due to infections. Reduction in total protein concentrations may occur as a result of overhydration, impaired protein synthesis due to malnutrition, malabsorption, liver disease, and hypo-gammaglobulinemia. Alternatively, it can be ascribed to increased protein loss due to renal, gastrointestinal, and skin disorders [49]. Albumin synthesis is a vital function of the liver, performing a significant physiological role in preserving osmotic pressure, transporting both endogenous and exogenous substances, and serving as a protein reservoir. Albumin concentrations depend on infections, nutritional deficiency, catabolism, hormonal factors, and urinary and gastrointestinal losses. All experimental test drugs caused significant changes (P<0.05) in the values of plasma proteins of total protein, albumin, globulin, and ALB/GLO ratio except for the Zn-(MPK-TSC) (Bipy)Cl_2 and Cu(MPK-TSC) (Bipy)Cl_2 treated group as compared to controls. It is believed that the integrity and synthetic ability of the rats' liver were not compromised by these two test drugs.

Renal function is essential for homeostasis. The kidneys play important pleiotropic functions, encompassing the removal of metabolic wastes and the maintenance of water-electrolyte balance and blood pressure [15]. The assessment of renal function is the key to ensuring drug administration safety and recognizing acute kidney damage at early onset. The non-significant changes(P<0.05) in renal function parameters (creatinine, urea, and uric acid) of the rats administered with [Cu (MPK-TSC) (Bipy)Cl_2], [Ni (MPK-TSC) (Bipy)Cl_2], [Zn (MPK-TSC) (Bipy)Cl_2] as compared to the control group is an indication of the non-adverse effect of these test drugs on the kidney. Nonetheless, the 2, 2-Bipyridine and MPK-TSC caused significant changes (P<0.05) in the renal function indices as compared to the controls. The significantly decreased activities of SOD, CAT, and GPx antioxidant enzymes coupled with increased activities of MDA in the groups treated with Bipy and MPK-TSC compared to the controls support the fact that these antioxidants are easily deactivated by the test drugs through the release of ROS and lipid peroxides. In contrast, the other test compounds did not display a significantly marked effect on these antioxidant indices (P<0.05).

Histomorphological check of the liver and kidneys in the control and test groups administered with [Cu (MPK-TSC)(Bipy)Cl_2], [Ni (MPK-TSC) (Bipy)Cl_2]and[Zn (MPK-TSC) (Bipy)Cl_2] (Figures 5 and 6) demonstrated liver cells with visible centrioles with well fenestrated sinusoidal areas. The hepatocytes appeared distinct, with well-differentiated nuclei at both low and high magnification, while the kidney section showed normal histological features. The section indicated detailed cortical parenchyma, and the renal corpuscles appeared dense and rounded. The liver in the rat groups treated with Bipy and MPK-TSC exhibited visible fatty changes and hydropic degeneration of the hepatocytes besides multifocal hepatocytic coagulation and portal biliary hyperplasia. The histology of kidney sections revealed varying degrees of distortion and disruption in microanatomy of the renal cortex, including queried edema, as compared to controls.

Conclusions

As evidenced by the results of this study, Bipy and MPK-TSC had significant adverse effects on various biochemical indices that were evaluated. However, complexing the compounds with metal ions demonstrated a significant reduction in the margin of toxicity, as indicated by the levels of biochemical parameters evaluated and the histology findings from the organs.

Conflict of Interests

The authors declare no conflict of interest with any internal or external entity. They also declare no financial or commercial interests in conducting this project.

Funding

Funding for this study was provided by the first author and the Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria.

Acknowledgement

The authors are thankful to the professors, management, and staff of the Departments of Chemistry, Biochemistry, and Clinical Science, Delta State University, Abraka, Nigeria.

Compliance with Ethical Guidelines

The protocol for the use of Wistar rats as an animal model for this study was approved by the Research and Ethics Committee, Faculty of Science, Delta State University, Abraka (REL/FOS/22/04)

Authors' Contributions

The initial concept and research protocol were developed by the first and second authors. They implemented all aspects of the study and analyzed the data in collaboration with effective inputs from other authors. All authors contributed fairly equally in drafting the initial and final drafts of the manuscript. All authors have read and approved the final version of this paper prior to submission for publication.

References

1. Ahmad M, Khan AU. Global economic impact of antibiotic resistance: A review. J Glob Antimicrob Resist. 2019;**19**:313-6. [**doi:** 10.1016/j.jgar.2019.05.024] [**pmid:** 31176071]
2. Bank W. By 2050, drug-resistant infections could cause global economic damage on par with 2008 financial crisis. The World Bank. 2016. [Link]
3. Kpomah B, Kpomah ED. Toxicological Assessment of Acetone Thiosemicarbazone Metal Complexes on Body Weight, Biochemical Parameters and Liver Histology of Wistar rats. J Pharm Appl Chem .2017;**3**(3):215-24. [**doi:** 10.18576/jpac/030306]
4. Kpomah B, Egboh SHO, Agbaire PO, Kpomah ED. Spectroscopic Characterization, Antimicrobial and Toxicological Properties of DerivatisedThiosemicarbazone Transition Metal Complexes. Saudi J Med Pharm Sci. 2016;**2**:(12):318-25. [**doi:** 10.36348/sjmps.2016.v02i12.001]
5. Kpomah B, Ugbune U, and Idu TE. 3D Series Metal Complexes Containing Schiff Base Ligand with 2,2'-Bipyridine: Synthesis, Characterization and Assessment of Antifungal Activity. International Journal of Chemical Sciences. 2021;**30**(10):34-45. [**doi:** 10.9734/CSJI/2021/v30i1030258]
6. Abeer AE, Sohair RF, Mohamed AE, Osama E, Ayman SM, Ahmed AE. Protective roles of novel thiosemicarbazone derivatives on hepatotoxicity and nephrotoxicity induced by cadmium chloride in rats. Acta Poloniae Pharmaceutica. 2021;**78**(2):193–204. [**doi:** 10.32383/appdr/135247]
7. Sibuh BZ, Gupta PK, Taneja P, Khanna S, Sarkar P, Pachisia S, et al. Synthesis, In Silico Study, and Anticancer Activity of Thiosemicarbazone Derivatives. Biomedicines. 2021; **19**(10):1375. [**doi:** 10.3390/biomedicines9101375] [**pmid:** 34680491]
8. Gupta S, Singh N, Khan T, Joshi S. Thiosemicarbazone derivatives of transition metals as multi-target drugs: A review. Results in Chemistry. 2022; 4 100459 [**doi:** 10.1016/j.rechem.2022.100459]
9. Saini RP, Kumar V, Gupta AK Gupta GK. Synthesis, characterization, and antibacterial activity of a novel heterocyclic Schiff base and its metal complexes of first transition series. Medicinal Chemistry Research. 2013;**23**(2):690–8. [**doi:** 10.1007/s00044-013-0657-6]
10. Shehzad MT, Imran A, Njateng GSS, Hameed A, Islam M, al-Rashida M, et al. Benzoxazinone-thiosemicarbazones as antidiabetic leads via aldose reductase inhibition: synthesis, biological screening and molecular docking study. Bioorg Chem. 2019;**87**:857-66. [**doi:** 10.1016/j.bioorg.2018.12.006] [**pmid:** 30551808]
11. Bharti SK, Singh SK. Metal based drugs: current use and future potential. Der Pharmacia Lettre. 2009;**1**(2):39-51. [Link]
12. Bagchi A, Mukherjee P, Raha A. A review on transition metal complex-modern weapon in medicine. International Journal of Recent Advances Pharmaceutical Research. 2015;**5**(3):171–80. [Link]
13. Song J, Pan R, Li G. Synthesis and anticancer activities of thiosemicarbazonesderivative of thiochromanones and related scaffolds. Med Chem Res. 2020;**29**:630-42. [**doi:** 10.1007/S00044-020-02503-w]
14. Pandeya SN, Dimmock JR. Recent evaluations of thiosemicarbazones and semicarbazones and related compounds for antineoplastic and anticonvulsant activities. Pharmazie. 1993;**48**(9):659-66 [**pmid:** 8234398]
15. Kpomah ED, Arhoghro EM. Investigation into the intake of a popular polyherbal drug (Jalin Herbal Mannex Liquid) on selected biochemical indices of male wistar rats. African Journal of Biochemistry Research. 2022;**16**(4):55-62. [**doi:** 10.5897/AJBR2022.1150]
16. Coulter WH. Means for counting particles suspended in a fluid. United States patent US. 1953. [Link]
17. Reitman S, Frankel AS. A colometric method of determination of serum glutamic pyruvic transaminase. Am J Clin Pathol. 1957;**28**(1):56-63. [**doi:** 10.1093/ajcp/28.1.56] [**pmid:** 13458125]
18. Rec GSCC. Optimized standard colorimetric methods. Journal of Clinical Chemistry. 1972;**10**:182-4. [Link]
19. Emery AE, Moores GE, Hodson V. The determination of lactate dehydrogenase (LDH) isoenzymes with urea. Clinica Chimica Acta. 1968;**19**(1):159-61. [**doi:** 10.1016/0009-8981(68)90202-7]
20. Ochei J, Kolhatkar A. Medical Laboratory Science, Theory and Practices. Tata McGraw-Hill, New York. 2008:163-75. [Link]
21. Kpomah ED, Kpomah B. Microstructural Tissue Assessment, Sex

Hormones and Biochemical Investigations Following Acute Administration of Piper guineenseSchumach&Thonn. On female Rattusnovergicus. IOSR Journal of Applied Chemistry. 2018;**11**(5):9-17. [**doi:** 10.9790/5736-1105010917]
22. Tobacco A, Meiattini F, Moda ETarli P. Simplified enzymic/colourimetric serum urea nitrogen determination. Clin Chem. 1979;**25**(2):336-7. [**pmid:** 759035]
23. Bartels H, Bohmer M, Heuerli C. Micro-determination of creatinine. Clin Chim Acta. 1971;**32**(1):81-5. [**doi:** 10.1016/0009-8981(71)90467-0] [**pmid:** 5096431]
24. Duncan P, Bayse D, Burnett R, Carey N, Carter R, Fellows WD, et al. A candidate reference method for uric acid in serum. II. Inter-laboratory testing. Interlaboratory testing. Clin Chem. 1982;**28**(2):291-3. [**pmid:** 7055950]
25. Cohen GD, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. Anal Biochem. 1970;**34**:30-8. [**doi:** 10.1016/0003-2697(70)90083-7] [**pmid:** 5440916]
26. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;**247**(10):3170-5. [**pmid:** 4623845]
27. Maehly AC, Chance B. The assay of catalases and peroxidases. Methods Biochem Anal. 1954;**1**:357-424. [**doi:** 10.1002/9780470110171.ch14] [**pmid:** 13193536]
28. Gutteridge JM, Wilkins S. Copper dependant hydroxyl radical damage to ascorbic acid; formation of thiobarbituric acid reactive products. FEBS Lett. 1982;**137**(2):327-30. [**doi:** 10.1016/0014-5793(82)80377-3][**pmid:** 6277694]
29. Krause WJ. The art of examining and interpreting histologic preparations: A student handbook. CRC Press. 2001. [Link]
30. Arhoghro EM, Kpomah ED. Alanine Aminotransferase and AspertateAminotransaminase Activities in Wistar Rats Fed with Musa paradisiaca (Plantain) Stem Pulp in Aluminium Chloride Induced Hepatic Oxidative Stress. Journal of Applied Sciences and Environmental Management. 2022;**26**(6):1057-62. [**doi:** 10.4314/jasem.v26i6.9]
31. Alemán CL, Noa M, Más R, Rodeiro I, Mesa R, Menéndez R, et al. Reference data for the principal physiological indicators in three species of laboratory animals. Lab Anim. 2000;**34**(4):379-85. [**doi:** 10.1258/002367700780387741] [**pmid:** 11072858]
32. Olfert ED, Cross BM, McWilliam AA. (eds). Guide to the care and use of Experimental animals. 1993. [Link]
33. Adnan N, Bukhari U, Raza I. Evaluation of Weight Changes On Wistar Rats Induced By Erythromycin And Aqueous Neem Leaves Extract. Journal of Bahria University Medical and Dental College. 2019;**9**(1):3-7. [Link]
34. Ramakrisna N, Vinod KS, Santanu J, Santosh KP, Anil G. What suits best for organ weight analysis: Review of relationship between organ weight and body/brain weight for rodent toxicity studies. International Journal of Pharmaceutical sciences and research. 2014;**5**(4):1525-32. [**doi:** 10.13040/IJPSR.0975-8232.5(4).1525-32]
35. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, et al. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicol Pathol. 2007;**35**(5):742-50. [**doi:** 10.1080/01926230701595292] [**pmid:** 17849357]
36. Trent RJA. Diagnosis of the Haemoglobinopathies. Clin Biochem Rev. 2006;**27**(1):27-38. [**pmid:** 16886045]
37. Erhabor O, Muhammad HA, Muhammad K, Onwuchekwa C, Egenti NB. Interpretation of Full Blood Count Parameters in Health and Disease. International Journal of Haematology. 2021;**5**(1) [**doi:** 10.23880/hij-16000180]
38. Ding L, Sun L, Wang F, Zhu L, Zhang T. Clinical Significance of Platelet Volume and Other Platelet Parameters in Acute Myocardial Infarction and Stable Coronary Artery Disease. Arq Bras Cardiol. 2019;**112**(6):715-9. [**doi:** 10.5935/abc.20190058] [**pmid:** 30942294]
39. Radaelli F, Colombi M, Calori R, Zilioli VR, Bramanti S. Analysis of risk factors predicting thrombotic and/or haemorrhagic complications in 306 patients with essential thrombocythemia. Hematol Oncol. 2007;**25**(3):115-20. [**doi:** 10.1002/hon.816] [**pmid:** 17464935]
40. Adams LA. Biomarkers of liver fibrosis. J Gastroenterol Hepatol. 2011;**26**(5):802-9. [**doi:** 10.1111/j.1440-1746.2010.06612.x] [**pmid:** 21198831]
41. Madjid M, Fatemi O. Components of the complete blood count as risk predictors for coronary heart disease: in-depth review and update. Tex Heart Inst J. 2013;**40**(1):17-29. [**pmid:** 23467296]
42. Jee SH, Park JY, Kim HS, Lee TYSamet JM. White blood cell count and risk for all-cause, cardiovascular, and cancer mortality in a cohort of Koreans. Am J Epidemiol. 2005;**162**(11):1062-9. [**doi:** 10.1093/aje/kwi326] [**pmid:** 16221804]
43. Olafadehan OA. Changes in haematological and Biochemical Diagnostic Parameters of Red Sokoto Goats Fed Tannin-Rich Pterocarpuserinaceus Forage Diets. VeterinarskiArhiv. 2011;**81**:471-83. [Link]
44. Ige SF, Akhigbe RE, Edeogho O, Ajao FO, Owolabi OQ, Ohekunle, OS. Hepatoprotective activities of Allium cepa in cadmium treated rats. Int J Pharm Pharm Sci. 2011;**3**(5):60-3. [Link]
45. Sharma U, Pal D, Prasad R. Alkaline Phosphatase: An Overview. Indian J Clin Biochem. 2014;**29**(3):269-78. [**doi:** 10.1007/s12291-013-0408-y] [**pmid:** 24966474]
46. Khan AA, Allemailem KS, Alhumaydhi FA, Gowder SJT, Rahmani AH. The Biochemical and Clinical Perspectives of Lactate Dehydrogenase: An Enzyme of Active Metabolism. Endocr Metab Immune Disord Drug Targets. 2020;**20**(6):855-68. [**doi:**10.2174/1871530320666191230141110] [**pmid:** 31886754]
47. Chen J, Li J, Jiang H, Yu J, Wang H, Wang N, et al. Developmental co-exposure of TBBPA and titanium dioxide nanoparticle induced behavioral deficits in larval zebrafish. Ecotoxicol Environ Saf. 2021;**215**:112176. [**doi:** 10.1016/j.ecoenv.2021.112176] [**pmid:** 33780780]
48. Constable EC, Housecroft C. The Early Years of 2,2' Bipyridine-A Ligand in Its Own Lifetime. Molecules. 2019;**24**(21):3951. [**doi:** 10.3390/molecules24213951] [**pmid:** 31683694]
49. Kpomah ED, Kpomah B, Arhoghro EM. Histomorphological and Biochemical Changes Induced in Male Wistar Rats by Chronic Oral Doses of Piper guineenseSchumach. &Thonn. Nigerian Journal of Pharmaceutical and Applied Science Research. 2018;**7**(1):44-51. [Link].