Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(8):4066-4072

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Ni(II) complex of mefloquine-pyrimethamine: Synthesis, toxicological and antimalarial activities against *Plasmodium Berghei*

[1*]Adediji Johnson F, [2]Obaleye Joshua A and [1]Akinremi Caroline A

[1]Department of Chemistry. Federal University of Agriculture Abeokuta. P.M.B 2240. Abeokuta. Ogun State. Nigeria.
[2] Department of Chemistry, University of Ilorin, P.M.B 1515, Ilorin. Kwara state. Nigeria.

ABSTRACT

Malaria is a tropical disease that has constitutes a major health problem and big challenges for drug discovery because of the frequent chemoresistance to most available drugs in the market. In the present study, Nickel(II) Chloride Hexahydrate were used to complex the mixed Mefloquine and Pyrimethamine (an antimalarial drugs). The complex of the type ML_1L_2 were formed [Where M=Ni, L_1=Mefloquine and L_2=Pyrimethamine]. The compound was characterized by elemental analysis, conductivity, IR, UV-VIS and magnetic measurements. The chelate do not show toxicity against alkaline phosphatase (ALP) activities of enzymes from the homogenates of liver, kidney and serum of the experimental rats. Antimalarial activities of the complex were investigated using mice infected with Plasmodium berghei. The results show that the mixed metal complex exhibited significant higher antimalarial activity with the highest percentage inhibition of 70.3% at 25mg/kg dosage. Overall, the metal chelate possesses a better physical properties and enhanced biological activities due to complexation with metal as compared to their parent compounds.

Key words: Chemoresistance, Characterized, Toxicity, Antimicrobial

---

INTRODUCTION

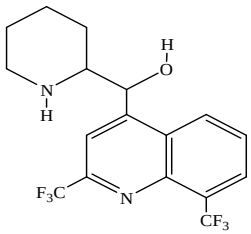
Quinoline methanol and amino pyrimidine drugs are used in malarial treatment due to their esquizonticide effect [1]. They constitute an important class of synthetic antimalarial agents which have been subjects of intensive study. Mefloquine which was presented in 1971, were screened for antimalarial activity during the Vietnam War at the Walter Reed Army Institute (WRAI) in U.S.A[2,3]. It is active against Chloroquine resistance-falciparum.

Pyrimethamine is a 2,4-diamino pyrimidine derivative, almost white crystalline powder with antimalarial properties. The antimalarial activity of Pyrimethamine is due to its ability to inhibit plasmodial dihydrofolate reductase enzyme, for which the drug has a pronounced affinity [4]. Mefloquine plus sulphadoxine and Pyrimethamine combination have been registered under the trade name Fansimef by Roche [5]. To treat multi resistance malarial infection. The compound used in this study has shown improved pharmacokinetic properties and a broad spectrum of activities against malaria chemotherapy.

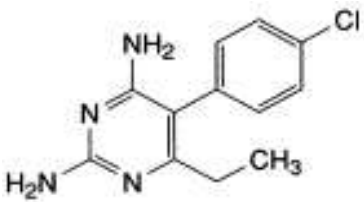
Metal coordination to biologically active molecule can be employed as a strategy to enhance their activity and overcome resistance, for instance, metal complexes of thiosemicarbazones can be more active than the free ligand, or can be employed as a vehicle for activation of the ligand as the cytotoxic agent.[6]

It has also been reported that metal complexes as a chemotherapeutic drugs has become a vibrant and growing area of research in recent time and some of the metal based drugs are already in the market for treating various diseases and ailments.[7]

In this context, the present work describes the synthesis of Ni(II) complex having as a ligands mixed Mefloquine and Pyrimethamine. We report the synthesis and biological activities (antimalarial and toxicological studies), various spectroscopic and analytical techniques were also employed.



Structure of Mefloquine



Structure of pyrimethamine

EXPERIMENTAL SECTION

All chemicals used were of the purest laboratory grade(Merck) and were used as received. Mefloquine hydrochloride and Pyrimethamine were obtained from SWISS Pharmaceutical Limited .Lagos Nigeria. Carbon Hydrogen and Nitrogen contents were determined using a Perkin-Elmer CHN 2400. The metal estimation was done using an Alpha 4 Atomic Absorption Spectrophotometer with PM 8251 simple pen recorder. Infrared spectra were recorded on KBr disc in the range 40000-600cm^{-1} on PUC scientific model 500 FTIR spectrometer. The UV/Visible spectra were on aquamate spectrophotometer model V4.60. Molar conductivities were carried out using Jenway 4010 conductivity meter. The molar magnetic susceptibilities of the powdered samples were measured using Faraday Balance model 7650 using Hg[Co(SCN)$_4$] calibrant. Thin layer chromatography was carried out using TLC plate coated with silica gel.

ALP assay kits were obtained from Randox Laboratories Limited, Antrim, United Kingdom. Albino rats (*Wistar Strain*) were obtained through the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Treatment of animals

Male albino rats (Wistar strain), weighing between 160 - 180 g were obtained commercially from Ilorin, Kwara state, Nigeria and housed in the animal house. They were kept in wire meshed cages and fed with commercial rat chow (Bendel Feeds Nigeria Ltd) and supply water *ad libitum*. Eighteen rats were divided into three groups of 6 rats per group. The first group was used as control and received distilled water. The second group of rats was treated with free mixed ligand (Mefloquine-Pyrimethamine) while the third group treated with metal complex [Ni(L_1L_2)]. The distilled water, mixed ligand and solution of metal complex were administered orally to the rats of various groups three times daily for seven days at the dose of 1.0 mg/Kg body weight. The animals were sacrificed 24 hrs after the last treatment.[8]

Preparation of serum and tissue homogenates

The method described by Yakubu *et al*. [9] was used to prepare the serum. The rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac punctures into clean, dry centrifuge tube after which they were left for 10 min at room temperature. The tubes were then centrifuged for 10 min at 3000 x g in an MSC (Essex, UK) bench centrifuge. The clear supernatant (serum) was aspirated using a Pasteur pipette into clean, dry sample bottles and then frozen overnight before use.

The liver and kidney excised from rat, blotted of blood stains was rinsed in 1.15% KCl and homogenized in 4 volumes of ice-cold 0.01 mol dm^{-3} potassium phosphate buffer (pH 7.4). The homogenates were centrifuged at 12,500 x g for 15 min at 4°C and the supernatants, termed the post-mitochondrial fractions (PMF) were aliquoted and used for enzyme assays.

Determination of serum and tissue ALP activities

Serum and tissue's ALP activities were determined using Randox diagnostic kits.. ALP activity determination was based on the method of Wright *et al* [10]. The yellow colour p-nitrophenol formed was monitored at 405 nm. Protein determination of serum and all fractions was estimated by the method of Lowry *et al.* [11] as modified by Yakubu *et al.* [9] using bovine serum albumin as standard.

Albino Swiss mice (NK-65) were obtained from the animal house of Nigeria Institute of Medical Research, Lagos. *Plasmodium berghei* used in this study were obtained through the same source.

Statistical analysis
The data were analysed using one way ANOVA followed by Duncan multivariable post-hoc test for comparison between control and treated rats in all groups. Values of p less than 0.05 were considered statistically significant.

SYNTHESIS
The complexes were prepared following the reported procedure with slight modifications.[12] Ethanolic solutions of the metal chlorides were Prepared in round bottom flasks (0.01 mole of $NiCl_2.6H_2O$). 0.01mol (4.148 g) of mefloquine was mixed with 0.01 mol (2.487 g) of Pyrimethamine in a beaker. The mixed ligands were dissolved in 20 ml of ethanol and added to the solution of the corresponding metal salt dissolved previously in 10 ml of ethanol in a round bottom flask fitted with a condenser. 10% methanolic ammonia solution was used to maintain the pH. The solution was refluxed for 2 h. The metal chelates crystallized, after leaving the reacting solution for about 30 min in a refrigerator. The metal complex thus separated were filtered and washed with ethanol and then with distilled water to remove unreacted ligand and metal; finally the solid complex was dried in a desiccator.

Colour: Lemon green. **Nature**: powder. **Melting Pt**: 185^oC. **% yield**: 67.5%. **Soluble in**: Hot distilled water, ethanol and methanol. **IR (KBr)**: 3466.3(N-H)str.,3137.1(O-H)overlapped, 1733.6(C=N), 1137.2(C-N), 718.3(M-L). **UV/Visible data (nm)**: 201.0, 206.5. **Anal. Calcd(%)** $Ni(C_{29}H_{29}F_6N_6OCl)$ M.Wt: 667.0g/mol. C(52.09%), H(4.35%), F(17.09%), N(12.46%) O(2.40%), Cl(2.55%); Found (%) C(52.08), H(4.32), N(12.42). Metal(8.85%) calculated, Found(8.80%). μ_{eff} (BM)=3.39. **Conductivity** ($\Omega^{-1}cm^{-1}dm^{-1}$) =1.15 x$10^{-5}$.

ANTIMALARIA ACTIVITIES
TREATMENT OF ANIMALS
8-week-old non-infected male Swiss albino mice weighing 18-20g were used. The animals were housed in standard cages, with standard feed and water given ad libitum, and they were acclimatized for 10 days prior to the experiments. All animal experiments were performed according to the guidelines for experimentation, Nigeria Institute of Medical Research (NIMR).

All the experiments were carried out in a suitable laboratory setting that has ambient illuminations and under controlled temperature of 20^oC close to the temperature of where the animals were obtained. [13]

ACUTE TOXICITY STUDY
The antimalarial mixed metal complex was evaluated for their toxicity in *plasmodium berghei* non-infected mice. Ten mice were randomly divided into two groups of five rats per cage. Before oral administration of a single dose of the metal-drug, the mice were fasted for two hours [14, 15]. Then the mice in group 1 were given orally 0.2ml of metal-drug at a single dose of 25mg/kg body weight. The mice in group 2 (Control group) received 0.5ml of 3% Tween 80. The mice were observed continuously for 24 hours intermittently at interval of 4 hours. They were observed for gross behavioural changes such as feeding, hair erection, lacrimation, mortality and other signs of toxicity manifestations [16].

PARASITE INOCULATION
Test were performed in a 4-day suppressive standard test using the methods of David and Lorke at al. [16,17]. The plasmodium species, that is most widely employed in rodent malaria parasite-plasmodium berghei NK-65 (Chloroquine sensitive strain), obtained from NIMR was used to infect mice for a four day suppressive test. The *p.berghei* was subsequently maintained in the laboratory by serial blood passage from mouse to mouse. To infect the mice, blood sample was collected from auxiliary vessels of a donor mouse with a rising parasitaemia of about 30-37%. Then, the blood was diluted in normal saline so that each 0.2ml contained approximately 1x10^6 infected red blood cells. Each animal received inoculums of about 1x10^6 parasite via intraperitoneal route(IP). The inoculated mice were then randomized into five mice per cage and maintained in the animal house on a commercial diet and water ad libitum.

EVALUATION OF SCHIZONTOCIDAL ACTIVITY ON EARLY INFECTION (4-DAY TEST).
The Schizontocidal activity of Ni(Mef-Pyr) on early *p.berghei* was evaluated in a four day test. The Peters' and Knight et al. 4-day suppressive test against *p. berghei* infection in mice was employed [18].

Briefly, adult Swiss male albino mice weighing 18-20g were inoculated by intra-peritoneal (IP) injection with 1x10^6 infected erythrocytes. The mice were randomly divided into groups of five per cage and treated during 4 consecutive day with daily doses of the metal drug by oral route (5,15 and 25mg/kg). Two control groups were used in each experiment, one treated with Chloroquine at total dose of 25mg/kg while the other group was kept untreated given normal saline as placebo. All experiments were done in triplicate.

On the fifth day blood sample was collected from tail snip of each mouse. Thin smears were prepared and stained with 10% Geimsa solution. Then, each stained slide was examined microscopically (1000xMagnification) power to evaluate the percent suppression of the metal drug with respect to the control groups.

The percent suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice. Chloroquine was used as positive control while normal saline was used as a negative control. For each group of mice treated with metal drug or Chloroquine, the mean percentage chemosuppression was then calculated as 100 [(A-B)/A], where A was the mean percentage parasitaemia of the mice treated only with saline containing 0.5% Tween-80(the negative control) and B was the mean parasitaemia in the test group.

STATISTICAL ANALYSIS

Data were compared using student's t tests, with a p value of <0.05 being considered statistically significant.

RESULT AND DISCUSSION

The metal chloride salt reacted with the mixed ligands according to the following proposed general equation:

$$\mathbf{M + L_1 + L_2 = ML_1L_2}$$

Where; L_1 = mefloquine, L_2 = Pyrimethamine, M = Ni^{2+}

The synthesised complex was found to be non-hygroscopic solids with lemon green colour. The complex is well soluble in DMF and DMSO, and also in ethanol, methanol and water. The complex have sharp melting point, with no decomposition observed at the reported melting point. The average percentage yield is 67.5%. R_f, the retention factor values were calculated from the developed single spot for the complex indicating the purity of the compound.[19]. The retention factor of the metal complex was found to be higher than that for the ligand. Comparing the conductivity at room temperature of the ligand with that of its metal complex, it is possible to infer their non-electrolytic nature. The analytical data of the mixed ligand antimalaria metal complex showed that the metal chelate has a 1:1:1 stoichiometry.

The UV/Visible spectra of the ligands and its complex have been interpreted in terms of charge transfer transitions of the metal to the antibonding orbital of the ligand and in terms of the $\pi \rightarrow \pi^*$ transitions of the ligands[20].

Following our recent study of free mefloquine, two absorption bands at 272 and 207 nm were assigned to the $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions, respectively. These bands undergo a hypochromic shift in the mixed ligand metal complex due to complexation. Three absorption bands were observed in the spectra of the mixed complex and it has been interpreted charge transfer transitions.

From the UV/Visible data it is possible to infer that there is no $d \rightarrow d$ transitions, and the two ligands used were active in the coordination.

The infrared data showed the results of the most informative and indicative region. The assignments have been interpreted based on literature values obtained for similar structural compounds [21].

The shifts observed in the absorption bands between Mefloquine, Pyrimethamine and its metal complex showed that there is coordination. Metal-Ligand bands were observed in the ranges of 610 - 950 cm^{-1} in the metal complex. The Ni(II) complex shows a μ_{eff} value of 3.39 BM, which corresponds to high spin (octahedral) stereochemistry [22,24].
Figs 1. Show the results of ALP activities on the serum, kidney and liver. There was no significant reduction ($p<0.05$) in serum ALP activities of mixed ligand and its metal complex compared with control, this suggests that the integrity of the plasma membrane of the cells in the various tissues might have not been adversely affected. This is because ALP is a membrane-bond enzyme often used to assess the integrity of the plasma membrane and endoplasmic reticulum [8]. The observed significant increase in the ALP activities in the liver and kidney of the rat administered with metal complex suggests an enhancement of the activities of the existing enzymes by the drugs and their metabolites. The increase may be as a result of stress imposed on the tissue by the drug, which may lead to loss of the enzyme molecule through leakage into extra-cellular fluid, which has been significantly noticed in the serum. In a bid to offset this stress, the tissue may increase the de novo synthesis of the enzyme, thus accounting for the increase in activities in these tissues [23].

Overall, the integrity of the cell membranes of the various tissues (especially kidney and liver) was not adversely affected by the mixed ligand and metal complex.

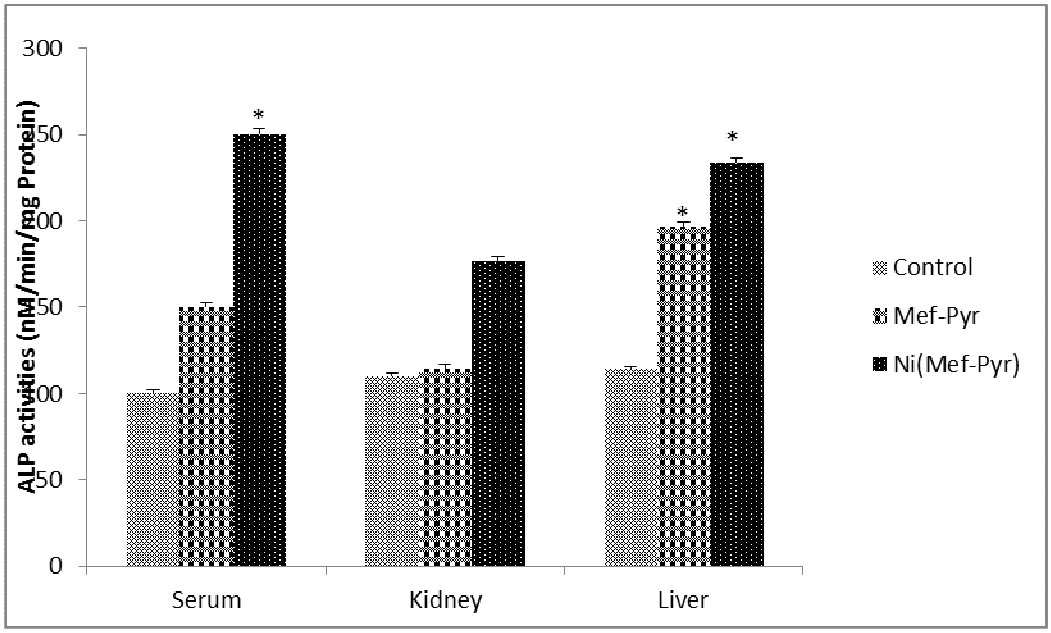


Fig. 1: Effect of administration of mixed ligand and the metal complex on the activities of alkaline phosphatase of rat serum, kidney and liver.

** Significantly different from the control ($p<0.05$)*

In the acute toxicity tests, all the mice administered antimalaria metal complex at 5-25mg/kg exhibited insignificant signs of toxicity, ranging from writhing and gasping (LD_{50} of >25mg/kg) to decreased respiratory rate, decreased limb tone, and death. The LD_{50} was calculated to be >25mg/kg. The present results indicate that metal-drug possesses useful blood Schizontocidal when used at doses that cause no marked toxicity in mice.

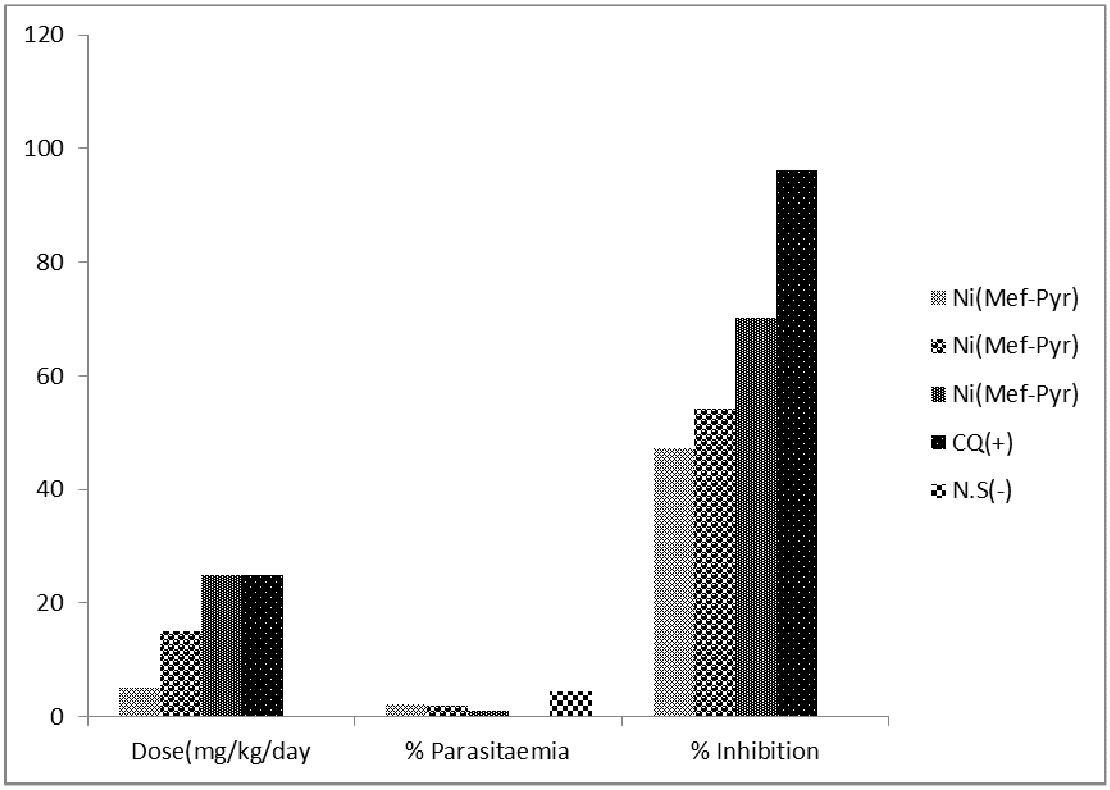


Figure 2: Antimalarial Activity of Ni(Mef-Pyr) complex and Chloroquine in mice infected with *plasmodium berghei*.

The metal drug complex used in this study was observed to show some antimalarial activity judging by its percentage chemosuppression in comparison with Chloroquine in the 4-day suppressive test (figure 2). Treatment of mice infected with *p.berghei* with metal drug showed a dose-dependent chemosuppression in comparison with Chloroquine treated controls with the 25mg/kg treated group of mice showing the highest percent chemosuppression. The activity might be attributed to presence of antimalarial properties of the ligands (Mefloquine and Pyrimethamine) enhanced by coordination of cobalt ion in the complex.

Structural Interpretation

The structural information from this complex is in agreement with the data reported in this paper based on the elemental, IR and UV/visible spectra, molar conductance, and magnetic moment measurements.

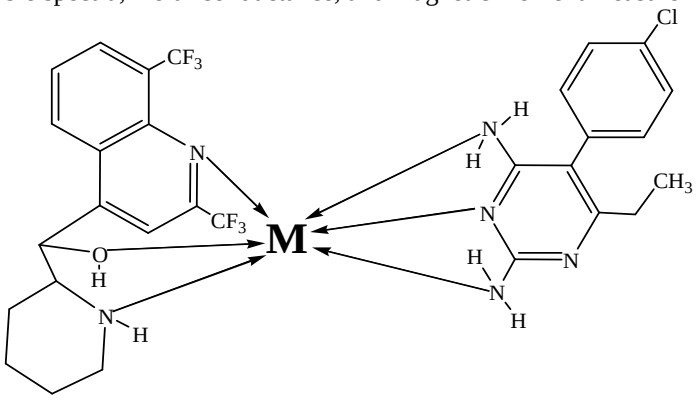


Fig 3: The proposed coordination mode of the metal complex.

CONCLUSION

The Mefloqine (Mef) and Pyrimethamine (Pyr) coordinates to the Ni(II) ion using the N:N:O donor atoms in the compound. The assignment of octahedral geometry proposed has been based on the information obtained by magnetic and infrared measurements. In the absence of no suitable crystal for single crystal X-ray structure, the proposed coordination modes of the complexes are presented in Fig. 3.The metal complex possesses greater physical and biological properties compare to their parent compound. The metal complex possesses antimalaria properties with best clearance of 70.3% at 25mg/kg body weight.

REFERENCES

[1] JA Obaleye, AC Tella and RO Arise *Adv. In Nat. Appl.Sci*.,**2009**, 3 (1): 43-48.
[2] W.H.O Drug information. **1987**, Vol 1. N0 3, 127
[3] CJ Ohnmacht, AR Patel and RE Lutz. *J.Med. Chem*. **1971**,14, 926
[4] R Ferone, JJ Burchall and GH Hitching. *Molec. Pharmacy*. **1969**,5, 49-59
[5] WH Wernsdorfer, and RL Kousnetov,: Bull. W.H.O. **1980**,28, 341
[6] MM Ligia; VA Mauro; SL Maria Cristina, AFM Flavio, PS Bazerra Ana. *Eur.J of Med. Chem*. **2009**, 44:4107-4111
[7] AC Tella and JA Obaleye. *Bull. Chem. Soc. Ethiop*. 2011,25(3), 371-380.
[8] JF Adediji**,** SA Amolegbe and A Lawal. *Jour. of Chem. and Pharm. Research*, 2012, 4(3):1511-1518.
[9] MT Yakubu; MA Akanji. and AT Oladiji, *Asian J. Androl.*, **2005**, 7: 399 – 404.
[10] PJ Wright; DT Plummer and PT Leathwood, *Enzymologia*, **1972**, 42: 317 – 327.
[11] OH Lowry; NJ Rosebrough; AL Farr and RJ Randall, *J. Biol. Chem*., **1951**, 193: 265 – 275.
[12] JF Adediji; ET Olayinka; MA Adebayo and O Babatunde, *Int. J. Phys. Sci*., **2009**, 4: 529 – 534.
[13] IT Peter, and VK Anatoli. ASM press. W.D.C.; **1998**, Pp.11-22
[14] The Organization of Economic Co-operation and development(OECD). **2001**.The OECD guide line for testing of chemical. 420 Acute oral toxicity. France;
[15] W Peters. *Exp. parasitol* **1965**, 17:80-89.
[16] D Lorke,. *Arch Toxicol*, **1983**, 54: 275-287.
[17] AF David, JR Philip, LC Simon, B Reto, and N Solomon. *Nature Reviews*. **2004**, 3:509-520.
[18] DJ Knight and W Peters. *Ann. Trop. Med.parasitol*. **1980**, 74:393-404.
[19] GG Mohamed; and ZH Abdel-Wahab, S*pectrochimica Acta. Part A: Mol. Biomol. Spec.*, **2005**, 9: 2231 – 2238.
[20] HD Williams,; I Flemming. Methods in Organic Chemistry, **1980,** 4th ed. McGraw-Hill Book Ltd, London.
[21] JA Obaleye EA Balogun. and OG Adeyemi, *BioChemistry*, **1999**, 9: 23 – 27.
[22] SK Kamruddin and A Roy. *Indian J. Chem*. 2001,40a(2):P.211-212.
[23] MA Akanji, OA Olagoke, OB Oloyede. *Toxicology* **1993**, *81*, 173–179.

[24] SA Aly, *J.Chem. Pharm. Res*; **2011**, 3(6): 1028-1036.