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Research Paper

5^{1}Nucleotidase Activity In Albino Rats Treated WITH Camosunate ANTIMALARIAL DRUG

[1]Agbafor K. N., [2]Ossai Emmanuel C., Nwaka A.C.[3] and Ezeilo Uchenna R.[4]

[1]*Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria.*
[2]*Department of Biochemistry, University of Nigeria, Nsukka, Nigeria.*
[3]*Department of Biochemistry, Chukwuemeka Odumegwu Ojukwu University. P. M. B. 02 Uli Anambra State, Nigeria.*
[4]*Department of Chemistry/Biochemistry/Molecular Biology, Federal University Ndufu-Alike Ikwo, Ebonyi State.*

ABSTRACT:- Anti-malaria drugs have been used in the treatment and prevention of malaria, each produces some undesirable effects by various mechanisms. Camosunate, combination therapy containing amodiaquine and artesunate has been used to treat malaria effectively. This research work examined the hepatobiliary effect of the drug in albino rats. Twenty adult male albino rats, distributed into four groups, (A, B, C and D), with five rats in each group, were used in the research. Groups A, B and C were given oral treatment of 5.7, 11.4 and 22.8 mg/kg body weight respectively of drug solution, for seven consecutive days, while group D was kept as the control. Treatment of animals with the drug solution resulted to a decrease in physical activity, body weights and feed and water intake relative to the control. Measurement of the total protein concentration in the serum of the animals did not reveal any significant difference ($P>0.05$) between the test and the control groups. In contrast, the activity of 5^{1} nucleotidase recorded in the treated groups were significantly higher ($P<0.05$) than the control. These effects varied with the doses. These findings suggest that Camosunate may be toxic to the liver or hepatobiliary system.

Keywords:- Camosunate, hepatobiliary system, malaria and antimalarial agent.

I. INTRODUCTION

Malarial infection is an important tropical mosquito - borne infectious disease that kills approximately three million per year. Statistics shows that close to five billion episodes of clinical illness possibly meriting anti malarial therapy occur hiving more than 90% of this burden [1, 2, 3 and 4]. Over the years, different categories of plasmodia specie (plasmodium) falciparum, ovale, vivax, and malariae) evolved with several newly observed mechanisms of resistance. The emergence and spread across sub-saharan Africa of plasmodium falciparum resistance among others to the inexpensive anti malarial [5, 6, 7 and 8]. Chloroquime and sulfadoxine-pyrimethamine have worsened the pandemic and hampered the socioeconomic development of affected countries. Following increased resistance of malaria parasites to conventional drugs in the malarial regions of the world , the WHO has been promoting artemisinin - based combination therapy (ACT) for treating of uncomplicated malaria [9, 10, 11, 12 and 13].

Anti malarial medications, also known as anti malarial, are designed to prevent or cure malaria. Such drugs may be used for some or all of the followings:

- Treatment of malaria in individuals with suspected or confirmed infection. (White, 2004).
- Prevention of infection in individuals visiting a malaria-endemic region who have no immunity (malaria prophylaxis).
- Routine intermittent treatment of certain groups in endemic regions (intermittent preventive therapy).

Some anti-malarial agents, particularly chloroquine and hydroxylchloroquine, are used especially for the treatments of malaria. Current practice in treating cases of malaria is based on the concept combination therapy, since this offers several advantages. Including reduced risk of treatment failure, reduced risk of developing resistance, enhanced convenience, and reduced side-effects, prompt parasitological confirmation by microscopy, or alternatively by rapid diagnostic tests is recommended in all patients suspected of malaria before treatment is

started. Treatment solely on the basis of clinical suspicion should only be considered when a parasitological diagnosis is not accessible [14, 15, 16 and 17].

Artemisinin derivatives are the most recent single drugs approved and to introduced for public anti malaria treatment. Although their recommendation use is for treatment of plasmodium falciparum infection, this drug s also act against other parasites, as well as against tumor cells. The mechanisms of actions attributed to artemisinin include interference with parasite. Transport proteins, distructions of parasites mitochondrial function, modulation of host immune function and inhibition of angiogenesis. Artemisinin combination therapies are currently preferred treatment for malaria [18, 19 and 20].

Camosunate (Artemisimin combination therapy) and its derivative also known as Qinghaosu, are a group of drugs that possess the most rapid action of all current drugs against plasmodium falciparum malaria. Treatments containing an artemisinin derivative (artemisinin- combination therapies, ACTs) are now standard treatment worldwide for P. falciparum malaria [21, 22, 23, 24 and 25].

The starting compound artemisinin is isolated from the plant Artemisia annua, sweet wormwood, a herb employed in Chinese traditional medicine.

Chemically, artemisinin is a sesquiterpene lactone containing an unusual peroxide bridge. This peroxide is believed to be responsible for the drugs mechanism of action. Few other natural compounds with such a peroxide bridge are known. Therapies that combine artemisinin with some other antimalarial drugs are the preferred treatment for malaria and are both effective and well tolerated in patients, The drugs is also increasingly been used in plasmodium Vivax malaria ,as well as being a topic of research in cancer treatment [26, 27, 28, 29 and 30].

Artemisinins are generally well tolerated at the doses used to treat malaria. The side effects from the artemisinins class of medications are similar to the symptoms of malaria: nausea, vomiting, anorexia and dizziness. Mild blood abnormalities have also been noted. A rare but serious adverse effect is allergic reaction [31, 32 and 33].

One case of significant liver inflammation has been reported in association with prolonged use of a relatively high dose of artemisinin for an unclear reason (The patients did not have malaria).The drugs used in combinations therapies can contribute to the adverse effects experienced by these undergoing treatment .Adverse effects in patients with acute plasmodium malaria falciparium treated with artemisinin derivative tends to be higher [31, 32, 33 and 34].

The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions .including: detoxification, protein synthesis and production of biochemicals necessary for digestion. The liver is necessary for survival, there is currently no way to compensate for the absence of liver function long term, although liver dialysis can be used in short term [35, 36, 37 and 38].

5^1 nucleotides (5^1ribonucleotide phosphohydrolase; 5^1NT) an intrinsic membrane glycoprotein is an ecto enzyme in a wide variety of mammalian cells. It hydrolyzes 5^1 nucleotides to their corresponding nucieosides, despite its ubiquitous distribution, serum concentrations of 5^1NT appears to reflect hepatobiliiary diseases with considerable specificity [38, 39, 40, 41 and 42].

Serum 5^1NT is clinically useful for differentia! Diagnosis of hypatobilliary diseases, the enzyme activity being increased only in hypatobilliary disease. Assay of 5^1NT activity may have value as an addition to measurement of non specific total alkaline phosphates (ALP) in patients with suspected hepatobiliiary disease [19].

Aims and objectives

The possible hepatoxic effect of ant-malarial drugs have been widely reported. Hence, this research work investigates the effect of the drug on the hepatobiliary system of albino rats by measuring the serum 5^1 nucleotidase level.

II. MATERIAL AND METHODS

Methods

Collection of samples

Collection of Albino Rats

Twenty male albino rats were purchased from the zoology department of the University of Nigeria Nsukka (U. N. N) and were transported down to Abakaliki.

Collection of drug sample

400mg of camosunate drug (300mg Amodiaquine, and l00mg Artesunate) was bought from Echo drugs pharmacy Abakaliki, Ebonyi State.

Preparation of Drug sample
400mg of comosunate drug (anti malarial) containing 300mg of Amodiaquine base and Artesunate l00mg was dissolved in 200ml of distilled water to obtain a concentration of 10mg/ml.

Animal handling and treatments
Animal groupings
The animals were kept in four cages, five animals per cage and were labeled accordingly.

Measurement of the weight of the Animals
The weight of the animals was taken daily using chemical balance. The results obtained were used to monitor weight changes and determine the volume of the sample to be administered to each of the animals.

Administration of sample
The animals were fed with growers match and water on daily basis for seven consecutive days for acclimatization. The samples were administered to the animals using 1ml syringe. The animals in groups A, B and C were given 5.7, 11.4, and 22.8 mg/kg body weights respectively while the animals in group D (the control) were given distilled water for seven consecutive days.

Collection of samples from the animals
After seven days of treatment with the drugs sample, the animals were starved for 24 hours and their blood samples were collected into a sterile bottle using sterile blade. **Preparation of Working Reagents**

Triethanolamine
Triethanolamine (0.022mg) was dissolved in 20ml of distilled water, the PH was adjusted to 7.9 with NaoH (10mol/L) and diluted to 1L. with distilled water and stored at 40° C.

III. COMBINED REAGENT

(L-Y- Glutamyl Nitroanilide and GlycylglyCine in Water, and stirred constantly in 100ml of buffer at 50° C, at a PH of 8.0 and allowed to precipitate to 3-5 days at room temperature.

Lowry concentrate
The concentrate was prepared by dissolving 20g.of Na2 Co_2 $5H_2o$ in 260ml and of distilled water, 0.4g of $CuSo_4$ $3H_2O$ in 2oml and 0.2 of sodium potassium tartrate was dissolved in 20m! of distilled water. The resultant solutions were mixed to obtain lowry concentrate which consist of 0.3ml of copper reagent o.lml of solduium dodecyl sulphate and 0.1ml of NaoH.

Folin Reagent (0.2N)
Folin reagent was prepared by-mixing 10ml of 0.2nl folin reagent with 90ml of water.

Bovine Serum Albumin Standard Solution
The solution was prepared by dissolving 20mg of the albumin in 10ml of distilled water to give a concentration of 2mg/ml.

Glysine Acid Solution
Serum albumin of glycine acid in 0.1% bovine serum albumin was[1] prepared at PH 5.9. **Measurement of parameters**

Determination of 5[1] nucleotides Level
Determination of 5^1 NT level was done using the methods described by (Reichling and Kaplan) 2001.

Determination of protein concentration
Principle
Under alkaline conditions, divalent copper ions form a complex with peptide bonds in which if is reduced to a monovalent ion. Monovalent copper ions and the radical groups of tyrosine, tryptophan and cysteine react with phenol reagent to produce an unstable product that becomes reduced to molybdenum or tungsten blue.

Procedure: This experiment was carried out on the serum and was performed using the Bovine serum albumin of the standard 0.4ml sample was added to 0.4ml of two times lowry concentration and mixed together. The mixture was incubated at room temperature for 10mins; 0.2ml of 0.2N folin reagent was added, stirred

immediately and incubated for additional 30minutes at room temperature. The absorbance was read at 750nm and was subjected to a standard curve. It is [important to mix the 0.2N folin reagent rapidly to avoid the decomposition of the reagent.

IV. STATISTICAL ANALYSIS

Result was expressed as mean ± standard deviation, the Data were subjected to one way analysis of variance.

V. RESULTS

Physical Observations

On the first day, the animals were very active before the drug was administered. After administration, a remarkable decrease in physical activity was observed. Subsequent days gave rise to further decrease in physical activity especially in groups C, B and A, decrease in food and water intake as the days progressed was also observed, while the animals in the control group increased in physical activity, increase in food and water intake was also noticed among the control group.

Changes in Body Weight

The change in body weight of the rats is represented in table 4.1, the average body weight of animals in groups A, B, C and D after seven (7) days of drug administration is shown below. The animals in Group A, B and C showed an insignificant decrease ($p>0.05$) in weight, while the animals in Group D, which were not treated showed an insignificant increase ($p>0.05$) in weight

Table 1 show changes in the body weight of animals during 7 days of administration.

Day	Group A	Group B	Group C	Group D
1.	120±6.30	78±3.32	82±4.74	91±5.33
2.	117±5.76	78±3.47	77±4.37	100±5.00
3.	116±5.71	68±2.94	71±3.92	111±6.33
4.	113±4.68	66±2.78	61±3.40	111±6.57
5.	112±3.47	62±2.47	61±3.81	113±6.97
6.	109±3.32	62±2.45	60±3.81	115±7.28
7.	96±2.67	58±2.58	60±3.25	116±1.32

Values are the mean weight ± standard deviation; n = 5.

LEGEND
Group A = 5.7mg/kg body weight
Group B = 11.4mg/kg body weight
Group C = 22.8mg/kg body weight
Group D = control

Table 1 above shows the average weight of albino rats treated with camosunate for seven days. The mean body weight of the animals in groups A to C decreased while the control group increased.

Table 2: average protein concentration, 5[1]NT activity and average specific 5[1] NT activity.

Animal Group	Average total protein (mg/ml)	Average 5NT activity (μ/l)	Specific 5NT activity (μ/l/mg/ml).
A.	0.39 ± 0.08^{b}	29.39 ± 2.046^{b}	55.67 ± 1.85^{b}
B.	0.31 ± 0.03^{b}	42.64 ± 2.71^{c}	95.38 ± 12.31^{c}
C	0.23 ± 0.02^{b}	54.49 ± 3.74^{c}	133.68 ± 9.75^{c}
D	0.63 ± 0.04^{a}	11.67 ± 1.09^{d}	14.73 ± 2.03^{d}

Values are mean ± standard deviation, n=5. The values with different superscript differ significantly ($p<0.05$).

LEGEND
Group A = 5.7mg/kg body weight
Group B = 11.4mg/kg body weight
Group C = 22.8mg/kg body weight
Group D = control

Table 2 above shows that the average 5NT activity and average specific 5NT activity in serum of groups A to C were found to be significantly higher ($P<0.05$) than the control and the average total protein concentration of the groups were found to be insignificantly different ($P<0.05$).

VI. DISCUSSION

Serum 5^1 nucleolidase level was investigated with the view of establishing hepatoxicity in albino rats treated with camosunate (Anti-malarials). A decrease in physical activity, food and water intake was observed in the groups that were treated with the drug, while the animals in the control group were physically active. The exact biochemical mechanism responsible for the decrease in the physical activity, food, and water intake is a recommendation for further study. Hence, it may be attributed to the chemical constituents of the drug administered to the rats.

Anti-malarials have been reported to influence various body processes such as muscle contraction, relaxation, and overall body metabolism of the organism. This influence may be as a result of inhibition of cell metabolic enzymes. Vital components of cells such as membranes have also been reported to be affected by anti-malarial compounds [4].

According to [3], treatment of Guinea pig with anti-malarials produced a decrease in physical activity, feed and water intake. The reason behind the decrease in average body weight of the treated rats relative to the control is still not fully understood, this may be partly related to the reported decrease in food and water intake caused by the introduction of the drug solution into the animals.

This finding agrees with the report of [21]. The inability of the drug to produce a significant difference in total protein level in the test and control groups suggest that the chemical constituent of the drug at the doses administered may not influence the rate of protein synthesis and degradation significantly. This report has also been presented by [11] on effects of *Livingia gabonesis* seed extract on albino rats.

The dose dependent nature of the increase in 5^1 NT activity agrees with the general principle of drug effect; the higher the dose, the higher the effect. A similar observation has been made by [10]. In a study isolated 5^1 NT elevation, evaluated in an unselected group of patient at veteran affair hospital, This revealed that most mild elevations of 5^1 NT (about 1.5 times the normal value) resolves with time and not necessarily indicate liver disease [11].

In consideration with the issues that have been discussed in this work, care must be taken and the doses strictly maintained, when this anti-malarials are administered to prevent overdose and the risk of liver disease.

VII. CONCLUSION

The observations made in this work suggest that camosunate may induce metabolic responses, which may include hepatocellular injury. Thus, care must be taken in the use of this anti-malarials for the treatment and the prevention of malarials. Since the drug was able to increase the activity of 5^1NT. However, further research is needed to acertain the actual mechanism behind the reported observation.

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