
# The Effects of Co-Administration of Magniferin and Arthemeter-Lumefantrine on Serum Lipids, Some Cardiac Enzymes, And Histology of The Heart of Plasmodium *Berghei Berghei* – Infected Swiss Albino Mice.

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ABSTRACT

Malaria is a vector-borne infectious disease caused by Plasmodium parasites transmitted by infected female Anopheles mosquitoes. In tropical and subtropical countries, herbal medicine has gained popularity due to its therapeutic benefits, low cost, and bioavailability. This study evaluated the effect of co-administration of mangiferin and arthemeter-lumefantrine on serum lipids, cardiac enzymes, and heart histology in plasmodium berghei-infected Swiss albino mice. Forty-two (42) albino swiss mice were divided into seven groups, with four groups infected with the blood of highly parasitized P. berghei-infected mice, while three were control groups. Arthemeter Lumefantrine (AL) was administered as the standard drug, and Mangiferin was used for treating the parasite. Group 1 was the normal control and received normal feed and water ad libitum. Group 2 was infected with P. berghei; Groups 3 and 4 were treated with 8 mg/kg body weight of arthemeter-lumefatrine and 410.79 mg/kg of mangiferin, respectively. Groups 5 and 6 were infected with P. berghei and treated with 8 mg/kg body weight of Arthemeter-Lumefantrine and 410.79 mg/kg of Mangiferin, respectively. Group 7 was infected with P. berghei and treated with Arthemeter-Lumefantrine and Mangiferin at doses of 8 mg/kg and 410.79 mg/kg body weight, respectively. The study discovered that giving mangiferin and AL together had a big effect on the lipid profile and heart function of mice that had been paralyzed, which suggests that the heart might be protected. The study recommends investigating possible drug interactions between mangiferin and other commonly used drugs in Africa to ensure that combining treatments doesn't negatively impact or decrease their effectiveness

**Keywords:** Malaria, Mangiferin, Arthemeter Lumefantrine (ACT), Serum Lipids, and Cardiac Enzymes

## Introduction

In non-industrialized cultures, herbs are often used for medicinal purposes, such as treating diseases like otitis media, boils, hypertension control, and malaria therapy. Mangifera indica, a plant belonging to the Anacarchacae family, has been found to have therapeutic properties against malaria. Herbalists in southeast Nigeria use the leaves of M. indica to create a concoction for patients experiencing malaria symptoms.

Malaria affects around 280 to 290 million people annually, with Quinine and artemisinin being natural antimalarial drugs. However, most current antimalarial drugs are no longer effective against Plasmodium falciparum. An investigation into the potential synergistic effects of mangiferin when combined with existing antimalarial drugs and its impact on cardiac health could lead to improved treatment strategies less prone to resistance development. Malaria is a significant global health concern, especially in areas with limited access to modern medical treatment. Herbal remedies are widely used in conjunction with mainstream treatments, particularly in areas with little availability of modern medical treatment. In 2020, the WHO African Region was estimated to be responsible for 95% of all malaria cases and 96% of malaria-related deaths worldwide. In 2019, Nigeria contributed to around 27% of worldwide malaria infections and 23% of global malaria deaths.

The lack of thorough testing on the co-administration of standard antimalarial drugs and alternative therapies raises concerns about their safety and effectiveness. Standardization and regulation are crucial for effective malaria management. The cost and availability of Artemisinin-Based Combination Therapy (ACT) in remote regions of Nigeria present a significant barrier to effective malaria treatment. Rural regions also face insufficient healthcare infrastructure, restricted medical services, and high poverty rates.

This study aims to evaluate the effect of co-administration of magniferin and arthemeter-lumefantrine on serum lipids, cardiac enzymes, and heart histology in plasmodium berghei berghei-infected Swiss albino mice.

## Objectives of the Study:

The Objectives of this study are to investigate the Synergistic Effects of Co-Administration of mangiferin and arthemeter-lumefantrine on :

i. Serum lipids concentration (TC, TG, HDL, LDL,VLDL) of Plasmodium *berghei berghei* infected Swiss albino mice.
ii. Cardiac function parameters; serum creatine kinase, lactate dehydrogenase, troponin I of *Plasmodium berghei berghei* infected Swiss Albino Mice.

## Literature Review:

Malaria, caused by P. falciparum, can lead to cardiac abnormalities and decreased heart function in individuals. A study by Günther et al. (2022) found that a small percentage of malaria patients had higher levels of troponin-T but no increases in CK-MB. Myoglobin levels were high in 6.2% of the patients, all of whom were old and had elevated blood concentrations of cystatin C. Cardiac abnormalities were observed in 23 individuals based on ECG results.

The seclusion of contaminated red blood cells (RBCs) may contribute to cardiovascular symptoms, as the pathogen alters the appearance and properties of RBCs to enhance adhesion. This can compromise microcirculation and contribute to the development of lactic acidosis. Cardiac factors can also contribute to some pulmonary problems and potentially lead to sudden cardiac fatalities.

Phosphokinase (CPK) is an enzyme produced by different organs and cell types that facilitates the transformation of creatine using adenosine triphosphate (ATP) to produce phosphocreatine (PCr) and adenosine diphosphate (ADP). It is measured in blood tests to assess damage to tissues rich in CK, such as in cases of myocardial infarction, rhabdomyolysis, muscular dystrophy, autoimmune myositides, and acute kidney injury.

Cardiac troponin I (cTnI) is a protein group present in both cardiac and skeletal muscles and plays a significant role in the laboratory diagnosis of myocardial infarction. Lactate dehydrogenase (LDH) is an enzyme found in almost all living cells that facilitates the transformation of lactate into pyruvate and vice versa, while simultaneously converting NAD+ into NADH and vice versa. Elevated levels of LDH can be useful for determining whether a

patient has had a myocardial infarction if they come to doctors several days after an episode of chest pain.

## Materials and Methods:

### Collection and Preparation of leaves of *Mangifera indica*

Mangifera indica leaves that had just reached maturity were collected from Nkit Itam in Itam, the Local Government Area of Akwa Ibom State in Nigeria. The University of Uyo, Uyo's Botany Department handled the authentication procedure.For the Mangifera indica sample, specimen voucher number UUH4361 (Itam) was assigned. The leaves of the Mangifera indica plant were gathered, carefully cleaned, and then let to air dry for five days at room temperature.

### Purification of Mangiferin

Method**: (**Jutiviboonsuk and Sardsaengjun, 2010).

The leaves of Mangifera indica L. var Alphonso were collected, air-dried, and turned into a powder. The defatted powder was separated using aluminum foil and cotton wool. The defatted leaves were then extracted with 70% methanol for 72 hours, resulting in a semi-solid mass. This mass was divided into five parts using dichloromethane and dissolved in 50% ethanol. The resulting ethanolic phase was hydrolyzed, and the ethyl acetate fraction was divided four times using 100 milliliters of ethanol. The resulting ethyl acetate fraction was dissolved in ethanol and refrigerated overnight. The precipitate was separated using column chromatography. The dried extract was then isolated using three separate columns, containing hexane, dichloromethane, ethyl acetate, and ethanol. The isolated compound was then separated and dried into pale yellow, needle-shaped crystals. The isolated compound was further studied using various techniques, including UV/VIS spectroscopy, Thin Layer Chromatography, melting point studies, gas chromatography, and High Performance Liquid Chromatography. The results were compared with a mangiferin reference standard. The extract was collected and stored in a sterile tube at room temperature.

### Procurement of Artemeter-Lumefantrine and Malaria Parasite

The reference antimalarial (Malenter DS) was obtained from Siban Pharmacy, 27 Ikpa Road, Akwa Ibom State, Nigeria, and it was a product of Norvartis Pharmaceuticals UK Ltd.

*Plasmodium Berghei* was acquired from the Department of Microbiology and Parasitology, National Institute for Medical Research, Yaba, Lagos. It was sub-passaged and kept in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria.

### Experimental Animals

42 Swiss male mice, weighing between 20 and 30 grams, were procured from the animal house of the Pharmacology and Toxicology department, Faculty of Pharmacy, University of Uyo. The animals were made to acclimatize for ten (10) days. Their bedding was changed every two days in well-ventilated cages with a wooden bottom and a wire mesh top, kept at a constant temperature of 29±2°C. The animals were fed on commercial mice pellets manufactured by Phizer Livestock Ltd, Aba but purchased from Uyo main Market, Uyo and also allowed free access to water.

### Inoculum Preparation

Anowi *et al*. (2015) reported that 0.2 milliliters of parasitized red blood cells were dissolved in normal saline (2 in 10 milliliters) and given intraperitoneally to mice that had been taken from the malaria-infected group in order to induce malaria in them. The animals that were induced were given therapy after three days, during which time blood was extracted from veins near their tails to determine the extent of parasitemia.

### Determination of Parasitemia and Calculation

Anowi et al. and Banti et al. conducted a study on parasitemia determination using modified techniques. Infected mice were given blood samples from their tails and placed on a sterile glass slide. The slide was fixed with methanol, stained with Giemsa stain and buffer, and examined with an oil-coated microscope lens. Parasitemia was assessed by counting infected red blood cells among 200 randomly selected red blood cells. The parasitemia percentage was calculated using the formula: % Parasitemia = (Number of Parasite-Infected RBCs / 200) × 100.

### Experimental Design

Exactly 42 male Swiss Mice weighing (20-30 g) were grouped into 7 groups, each containing six mice as seen in Table 3.1.

**Table 3.1**
**Experimental Design showing Mice grouping and treatment**

| Groups | No. ofAnimals | Treatment |
|---|---|---|
| 1. | 6 | Normal feed and water only |
| 2. | 6 | Parasitized (Normal Feed and water only) |
| 3. | 6 | Normal + 8 mg/kg A-L only twice daily for 3 days |
| 4. | 6 | Normal + 400 mg/kg MI mangeferin only twice daily for 3 days |
| 5. | 6 | Parasitized + 8mg/kg A-L twice daily for 3days |
| 6. | 6 | Parasitized + 400 mg/kg Mi mangiferin extract twice daily for 3days |
| 7. | 6 | Parasitized + 8 mg/kg A-L + 400 mg/kg MI twice daily for 3days |

NC: Normal Control, PC: Parasite Control, A-L: Arthemeter-Lumefantrine, M: Mangiferin, P+A-L: Parasite and Arthemeter-Lumfantrine, P+M: Parasite and Mangiferin, P+A-L+M: Parasite, Artemeter-Lumfantrine and Mangiferin
**Source**: Compiled by the researcher (2023).

**Extract and Drug Administration**

The mice were given both the extract and the drug in a single and combined dosage by carefully inserting an oral canular using the oral method of drug delivery. After three days of doing this twice a day, blood samples were taken for examination.

**Termination of Experiment and Samples Collection for Analysis**

Animals were anesthetized with chloroform and blood samples were collected from their hearts. The blood samples were then placed in an EDTA-filled container and centrifuged to separate the serum. The supernatant contained serum and sediments, which were stored at 20°C for biochemical analysis. After removal, the heart was cleaned with normal saline and preserved in 10% formalin for histological analysis. The process involved a series of steps to ensure accurate results.

**Determination of Cardiac Function Indices**

Lactate dehydrogenase was determined using Spectrum Diagnostic Assay Kit

Troponin I was assayed using Enzyme-Linked Immunosorbent Assay (ELISA) Kit.

**Determination of Creatine Kinase using Colorimetric method.**

The enzyme creatine kinase (CK) reversibly transfers a phosphate group from phosphocreatine to ADP. Hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6P-DH) are two enzymes that mediate related processes.

$$\text{Phosphocreatine} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}.$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{Glucose-6-phosphate}$$

$$\text{G6P} + \text{NADP} \xrightarrow{\text{G6P-DH}} \text{6Phosphogluconate} + \text{NADPH} + \text{H}$$

The amount of CK in the sample is directly related to the rate of NADPH production at 340 nm, which can be measured using a photometer (Abbot et al., 1984).

**Lipid Profile Estimation**

Triglycerides, total cholesterol, HDL, and LDL were measured by spectrometer methods using diagnosis kits, following the good laboratory practices. VLDL was measured by enzyme-linked immunosorbent assay

**Histopathological Procedures**

This was done using Hematoxylin and Eosin Method (Brancroft *et al*., 1996)**.**

**Statistical Analysis**

The data was analyzed with the Microsoft Excel spreadsheet tool and the Statistical Package for Social Sciences version 17 (SPSS INC, Chicago, Illinois). The data was presented using the mean value together with its corresponding standard deviation. The utilization of analysis of variance (ANOVA) was utilized to evaluate the existence of variances among distinct groups. A two-tailed P value that exceeds 0.05 was considered to have statistical significance.

**Results**

**Parasite Count before and after Treatment**

Table 4.0 shows as compared to the host, Plasmodium berghei significantly ($P<0.05$) raised the plasmodium count in the parasite control group. The group of parasitized mice treated with ACT showed a substantial ($P<0.05$) drop in parasite count compared to the parasite control group. When compared to normal mice treated with arthemeter lumefantrine, parasitized mice treated with Mangiferin showed a substantial ($P<0.05$) increase in the number of parasites; however, when compared to both parasite control and parasitized mice treated with ACT, there were significant ($P<0.05$) reductions in the number of parasites. Parasitized mice treated with ACT and Mangiferin showed significant ($P<0.05$) decreased when placed side by side to Parasite control and Parasitized mice treated with Mangiferin.

**Effect of Mangiferin and Arthemeter Lumefantrine on Lactate Dehydrogenase, roponin I and Creatinine Kinase Levels of Plasmodium Infected Swiss Albino Mice**

The study found that creatine kinase levels decreased significantly in Groups 4, 5, and 6 compared to Group 1, while Group 6 showed increased creatine kinase levels. The mangiferin-treated group had lower troponin I levels compared to the parasite control and the group treated with arthmeter lumefantrin (A-L). The Parasite + ACT + Mangiferin group showed no significant difference in troponin I levels compared to the normal control group. The combined treatment of M and A-L showed significantly decreased LDH concentration values compared to normal, healthy mice treated with A-L. The healthy group treated with M showed significant decremental and incremental values for troponin I concentration compared to both the parasite control and Normal + A-L. The combined treatment of M and A-L also showed significantly decreased LDH concentration values compared to normal + A-L.

**Serum Lipids Concentration (mg/dL) of Plasmodium Infected Swiss Albino Mice**

The study found that the total cholesterol (TC) of the Parasite + A-L + Mangiferin group increased significantly compared to the normal control group and the Parasitized + AL group. A significant drop was observed between the parasitized + AL group of triacylglycerol (TG) and the parasitized control group of mangiferin. The Parasite + Mangiferin (M) group of TG c showed a substantial decrease compared to the Normal Control group, the Parasitized Control group, the AL group, and M. The values of high-density lipoprotein cholesterol (HDL-c) were substantially higher than in the groups that were parasitized and normal. There was a significant rise in HDL-c values of the parasitized + AL group compared to the normal control group, the parasitized control group, and the AL group.

A significant decrease was observed in the Parasite + ACT group of low-density lipoprotein (LDL-c) when compared to the Parasite Control group and the AL group. A significant decrease was observed in the Parasite + M group of LDL-c when compared to the Parasite Control group and the AL group.

A significant decrease was also observed in the Parasitized + AL group of VLDL-c when compared to the Parasitized Control group and the AL group.

**Table 4.0: Parasite Count before and after Treatment**

| Post infection | Day 1 | Day 2 |
|---|---|---|
| Host | 3.00 ± 0.00 | 3.00 ±0.00 |
| Normal mice treated with arthemeter-lumefantrine | 0.00 ±0.00 | 0.00 ± 0.00 |
| Parasite control | 8.17 ± 1.90 | 32.40 ± .17* |
| Parasite + Arthemeter | 10.17 ±3.64 | 0.00 ± 0.00[b] |
| Lumefantrine | | |
| Parasite + Mangiferin | 14.33 ± 4.92[a] | 10.50 ±1.44[a,b,c] |
| Lumefantrine + Mangiferin | | |
| Parasite + Arthemeter | | |
| Lumefantrine + Mangiferin | 8.90 ± 3.25 | 0.00 ± 0.00[b,d] |

Values are expressed as mean ±SEM.
* = p<0.05 vs host
a = p<0.05 vs normal mice treated with arthemeter-lumefantrine
b = p<0.05 vs parasite control
c = p<0.05 vs parasite + ACT
d = p<0.05 vs parasite + Mangiferin

**Figure 4.0: Bar Chart showing Lactate Dehydrogenase Levels of Treated and Untreated Plasmodium *berghei berghei* Infected Swiss Albino Mice**

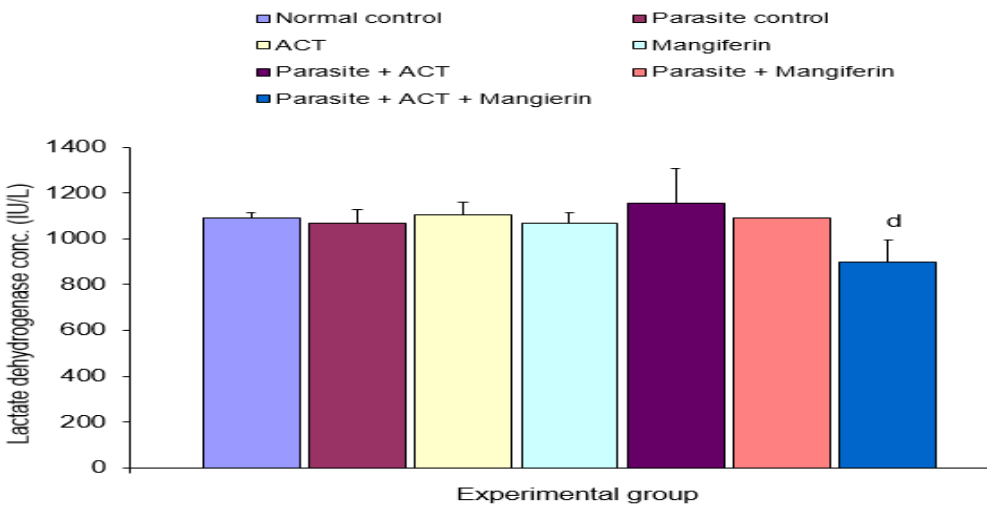



Figure 12: Lactate dehydrogenase concentration in the different experimental groups.

Values are expressed as mean +SEM, n = 3.
d = p<0.05 vs parasite + ACT

**Figure 4.1 Bar Chart showing Creatine Kinase Levels of Treated and Untreated Plasmodium *berghei berghei* Infected Swiss Albino Mic**

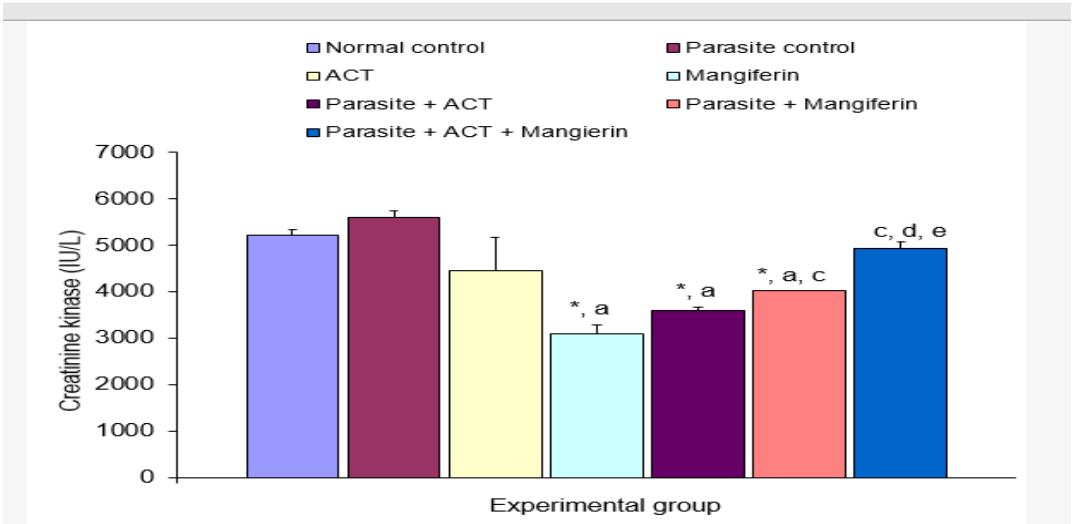



Figure 14: Creatinine kinase concentration in the different experimental groups.

Values are expressed as mean +SEM, n = 3.
* = p<0.05 normal control
a = p<0.05 vs parasite control
b = p<0.05 vs ACT
c = p<0.05 vs mangiferin
d = p<0.05 vs parasite + ACT
e = p<0.05 vs parasite + ACT + mangiferin

**Figure 4.2 Bar Chart showing Troponin Levels of Treated and Untreated Plasmodium *berghei berghei* Infected Swiss Albino Mice**

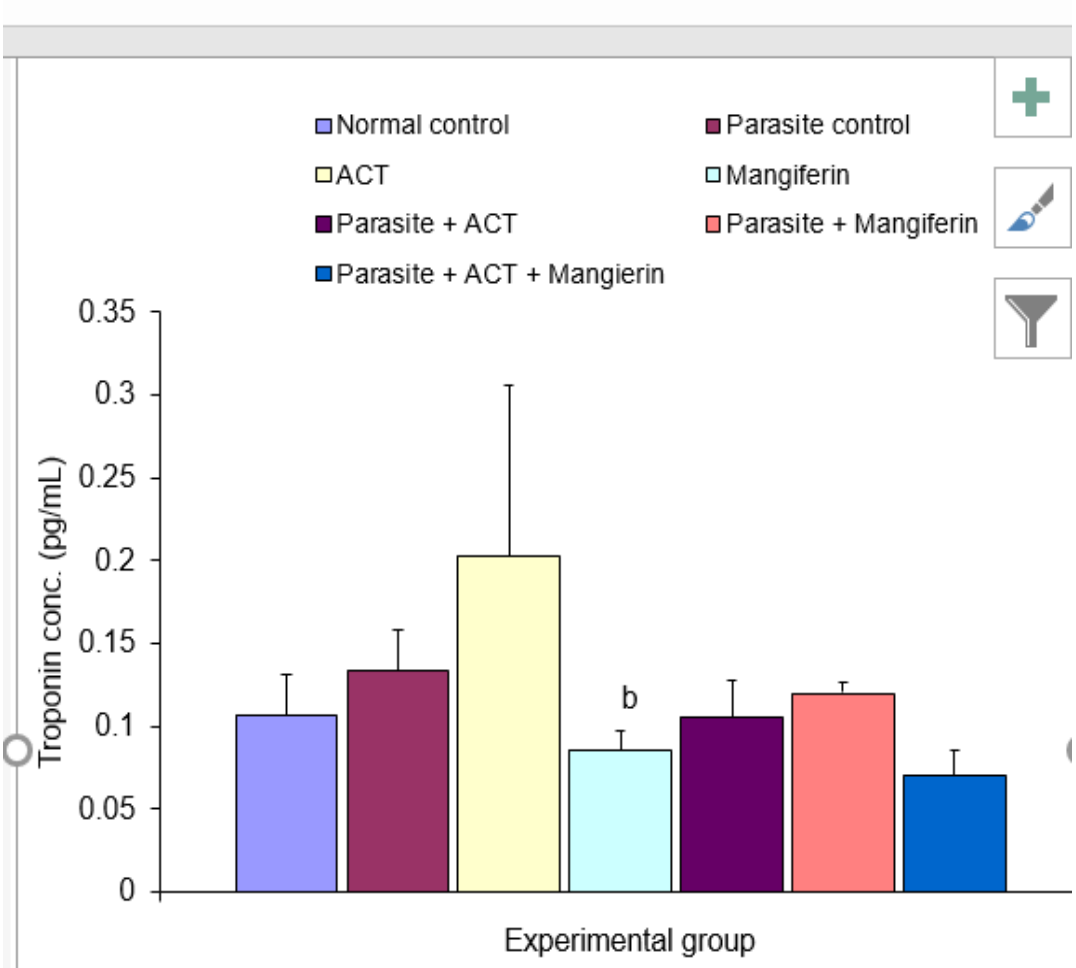



Figure 13: Tropnin concentration in the different experimental groups.

Values are expressed as mean +SEM, n = 3.
b = p<0.05 vs ACT

**Table 4.1: Effect of Mangiferin and Arthemeter Lumefantrine on Serum Lipids Concentration (mg/dL) of Plasmodium Infected Swiss Albino Mice**

| Groups | TC | TG | HDL-c | VLDL-c |
|---|---|---|---|---|
| **NC** | 104.08±5.40 | 88.17±3.25 | 60.46±4.84 | 17.63±0.65 |
| **PC** | 130.61±7.36 | 102.00±11.42 | 82.35±8.99 | 20.40±2.28 |
| **A-L** | 136.05±4.91 | 96.16±2.93 | 80.92±13.89 | 19.23±0.32 |
| **M** | 118.20±5.37 | 84.94±1.61 | 55.13±5.04 | 16.99±0.32 |
| **P+ AL** | 105.44±7.85 | 75.27±8.25$^{b.c}$ | 29.58±3.43$^{a,b}$ | 15.05±1.65$^{a,b}$ |
| **P + M** | 121.45±0.60 | 60.12±0.12$^{*,a,b,c}$ | 41.01±0.27$^{a,b}$ | 12.02±0.02$^{*,a,b,c}$ |
| **P + AL+M** | 147.76±28.52$^{*,d}$ | 49.55±3.92$^{*,a,b,c,e}$ | 78.71±22.52$^{d,e}$ | 19.35±2.07$^{d,e}$ |

Values are expressed as mean ± SEM, n = 3.
**Keys** * = significantly different from normal control at p<0.05

a = differs significantly from parasite control at p<0.05
b = differs significantly from ACT at p<0.05
c = differs significantly from Mangiferin at p<0.05
d = differs significantly from parasite + ACT at p<0.05
e = differs significantly from parasite + Mangiferin at p<0.05
M = Mangiferin
AL = Arthemeter-lumefantrine
TG = Triacylglycerol
TC = Total cholesterol
HDL-c = High-density lipoprotein cholesterol
LDL-c = Low-density lipoprotein cholesterol
VLDL-c = Very low-density lipoprotein cholesterol

**RESULTS OF HISTOPATHOLOGY OF THE HEART**
**Heart Photomicrographs**

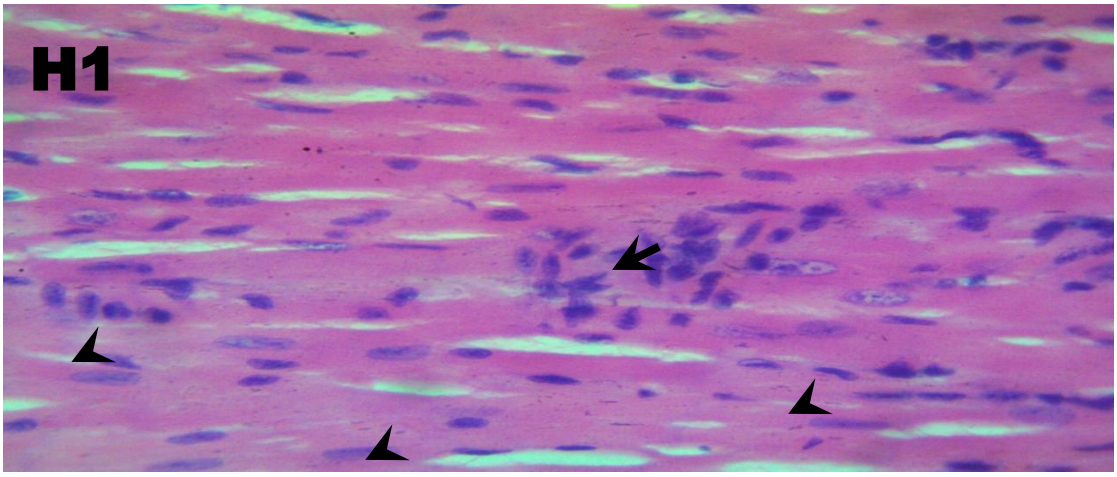



**Figure 4.1.1:** Photomicrograph of cross section of the Heart/Cardiac Tissue (Group 1) stained in with H and E method at mag. X 400.

**H1 :** Photomicrographs of the cross sections of the Heart of group 1 animals given normal feed and water and showing, (Black arrow head) centrally placed nuclei, (

**Inferences:** (H1) – Appears normal (O/++)

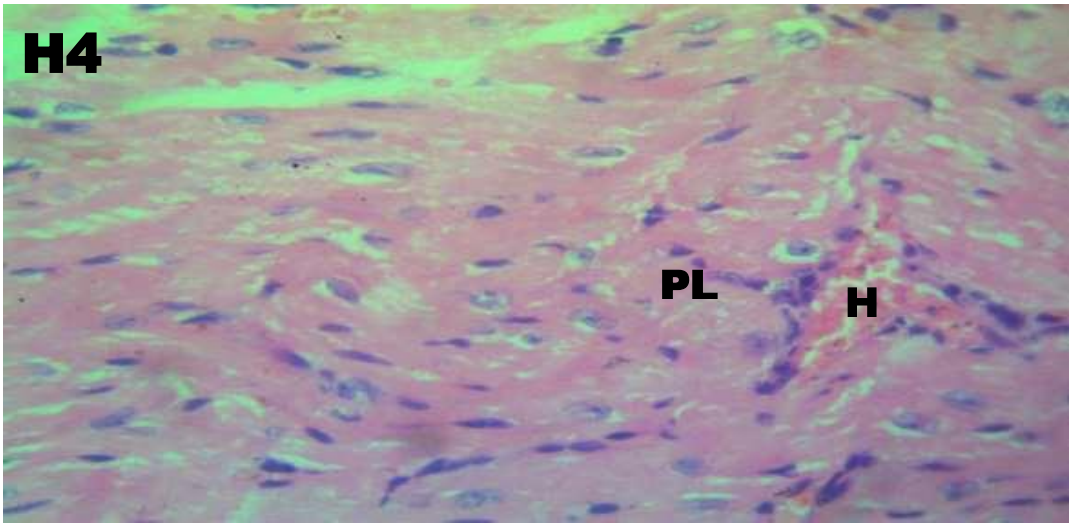
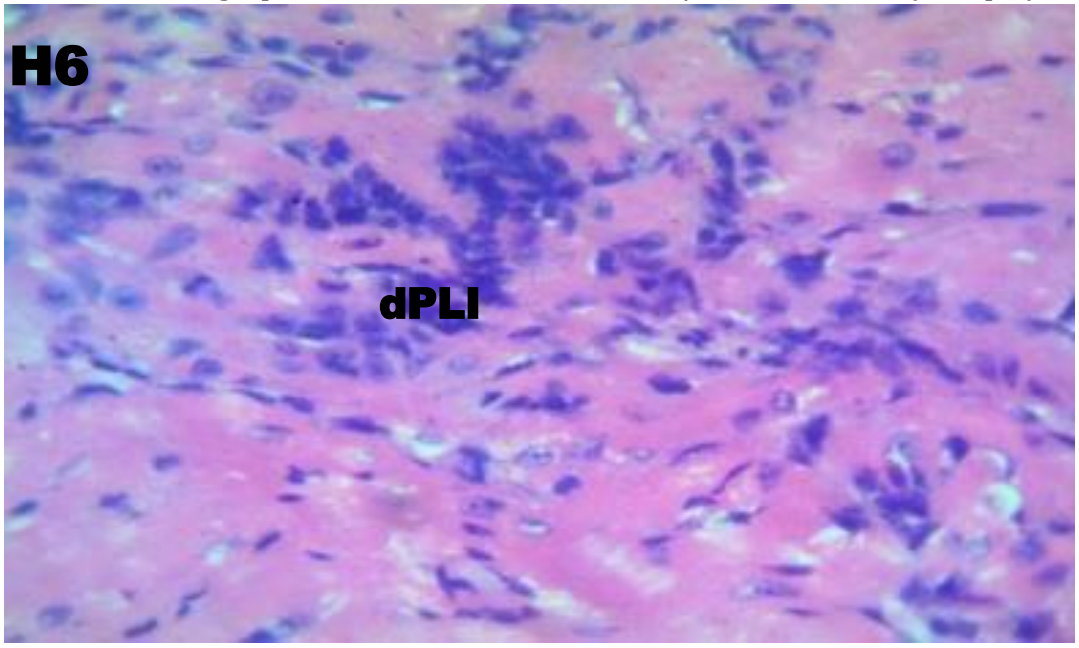



**Figure 4.1.2:** Photomicrograph of cross section of the Heart/Cardiac Tissue (Group 2) stained in with H and E method at mag. X 400.

**H4:** Photomicrographs of the cross sections of the Heart of group 2 animals, parasitized and given normal feed and water showing cardiac tissues with Polymorphonuclear leukocytic infiltration (PLI), Haemorrhage (H)

**Inferences:** (H4) - Moderately affected (+)

**Figure 4.1.3:** Photomicrograph of cross section of the Heart/Cardiac Tissue (Group 3) stained in with




H and E method at mag. X 400.

**H6:** Photomicrographs of the cross sections of the Heart of group 3 animals, given 8mg/kg , dense polymorphonuclear leukocytic infiltration (dPLI)

**Inferences:** (H6) - Severely affected (+++)

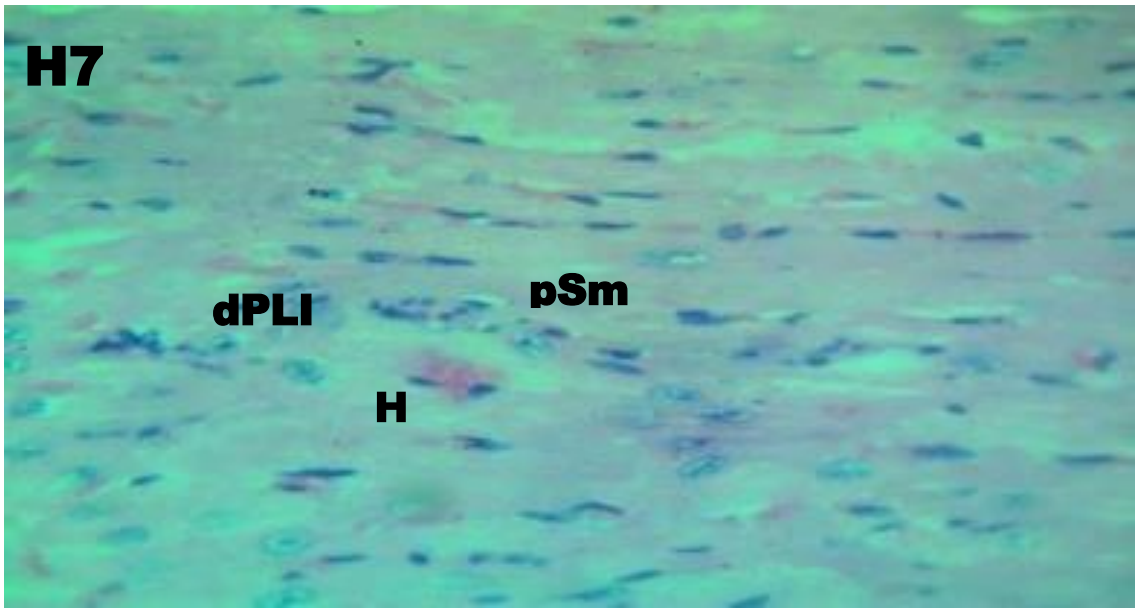



**Figure 4.1.4:** Photomicrograph of cross section of the Heart/Cardiac Tissue (Group 4) stained in with H and E method at mag. X 400.

**H7 :** Photomicrographs of the cross sections of the Heart of group 4 animals, given 400mg/kg body weight of Mangiferin twice daily for three days, showing cardiac tissue with Pale staining myocytes , dense polymorphonuclear leukocytic infiltration (dPLI), Haemorhage (H)

**Inferences:** (H7) - Moderately affected (++).

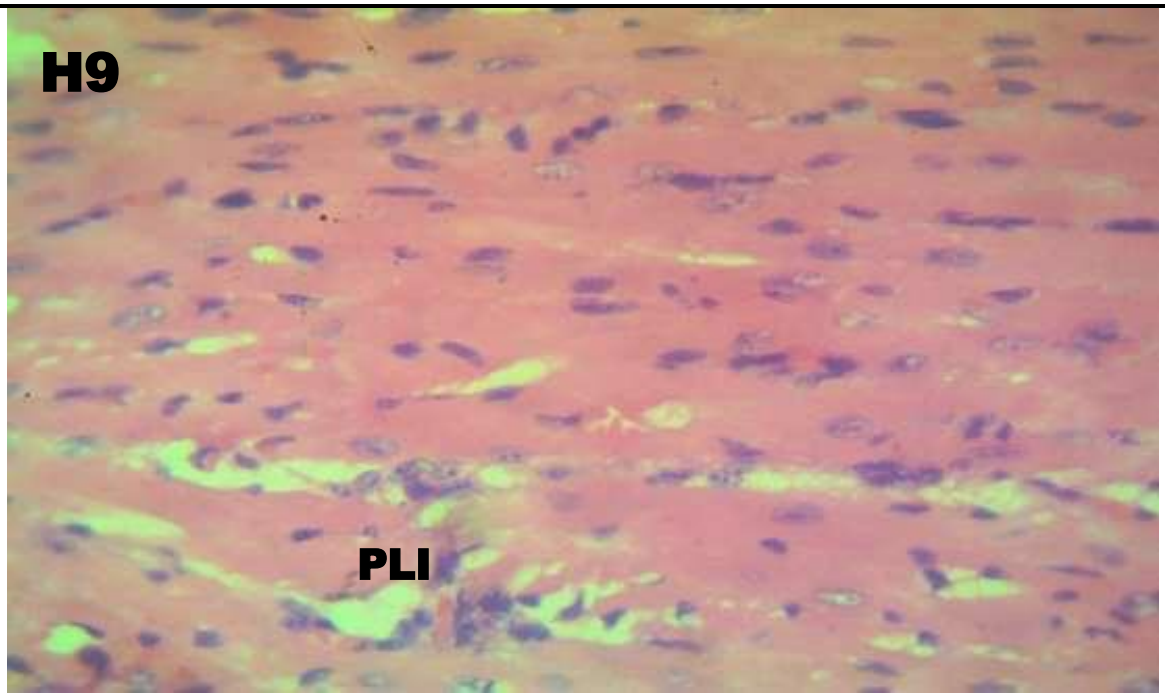



**Figure 4.1.5:** Photomicrograph of cross section of the Heart/Cardiac Tissue (Group 5) stained in with H and E method at mag. X 400.

**H9 :** Photomicrograph of the cross sections of the Heart of group 5 animals, parasitized and given 8mg/kg body weight of Arthemeter-Lumefantrine twice twice daily for three days,s showing mild to moderately affected (++) cardiac tissue, and (PLI) Polymorphonuclear leukocytic infiltration.

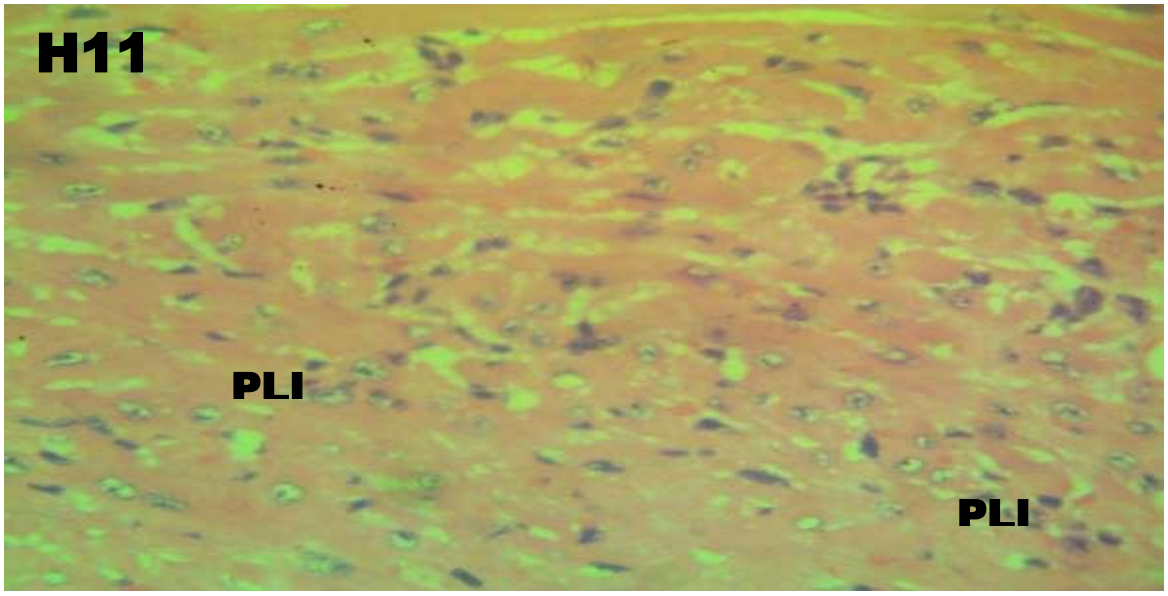



**Figure 4.1.6**: Photomicrograph of cross section of the Heart/Cardiac Tissue (Group 6) stained in with H and E method at mag. X 400.

**H11:** Photomicrographs of the cross sections of the Heart of group 6 animals, parasitized and given 400mg/kg body weight of Mangiferin twice daily for three days, showing mild to moderately affected (++) cardiac tissue, and Polymorphonuclear leukocytic infiltration (PLI)

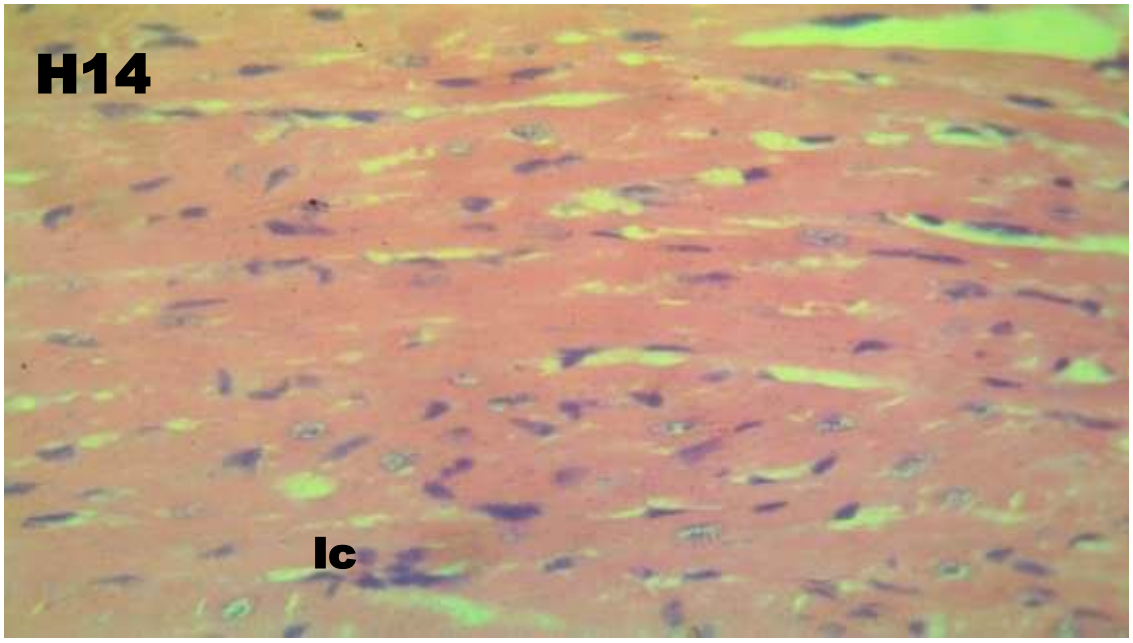



**Figure 4.1.7:** Photomicrograph of cross section of the Heart/Cardiac Tissue (Group 7) stained in with H and E method at mag. X 400.

**H14:** Photomicrographs of the cross sections of the Heart of group 7 animals, parasitized and given 400mg/kg body weight of Mangiferin and 8mg/kg body weight of Arthemeter-Lumefantrine twice daily for three days, showing: cardiac tissue with (Ic),Inflammatory cells

**Inferences:** (H14) - Mildly affected (+)

**Histological Section of the Heart Revealed that:**

**Group 1** - was not affected
**Group 2** - was moderately affected
**Group 3** - was severely affected
**Group 4** - was moderately affected
**Group 5** - was mild to moderately affected mild
**Group 6** - was mild to moderately affected
**Group 7** - was mildly affected

**Conclusion**

Co-administration of mangiferin and AL has significant effect on the lipid profile and cardiac function of Plasmodium berghi berghei paratisized mice suggesting potential cardiac protection.

**Recommendation**

Drug Interactions: It is crucial to look into possible interactions between mangiferin and other drugs that are often used in Africa to make sure that combining treatments won't have a negative impact or decrease their effectiveness. Impact of co-administration on other vital organs: It is important to consider the impact of co-administration on other vital organs like the liver and kidney, as well as hematological indices.

**REFERENCES**

1. Anowi, C. F., Ike, C., Ezeokafor, E., & Anikpe, N. A. (2015). Investigation into the anti-malarial activity of the aqueous leaf extract of Nauclea latifolia (Rubiaceae) using curative method.
2. Balogun, E. O., Nok, A. J. and Kita, K. (2016). Global Warming and the Possible Globalization of Vector-borne Diseases: A Call for Increased Awareness and Action. *Tropical Medicine and Health,* 7:173-186.
3. Banti, C. N., Kyros, L., Geromichalos, G. D., Kourkoumelis, N., Kubicki, M., & Hadjikakou, S. K. (2014). A novel silver iodide metalo-drug: Experimental and

computational modelling assessment of its interaction with intracellular DNA, lipoxygenase and glutathione. *European journal of medicinal chemistry*, 77, 388-399.

4. Gardner, M. J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R. W., ... & Barrell, B. (2002). Genome sequence of the human malaria parasite Plasmodium falciparum. *Nature*, 419(6906), 498-511.
5. Ginsburg, H., & Deharo, E. (2011). A call for using natural compounds in the development of new antimalarial treatments–an introduction. *Malaria journal*, 10, 1-7.
6. Omosun, G., Okoro, I. A., Ekundayo, E., Ojimelukwe, P. C., & Ibe, O. (2013). Ethnobotanical study of medicinal plants useful for malaria therapy in eight local government areas of Abia State, Southeast Nigeria. *Advancement in Medicinal Plant Research*, 1(2), 39-44.
7. Sardsaengjun, C., & Jutiviboonsuk, A. (2010). Effect of temperature and duration time on polyphenols extract of Areca catechu Linn. seeds. *Thai Pharmaceutical and Health Science Journal,* 5(1), 14-17.
8. Van, W, B. E., & Wink, M. (2018). *Medicinal plants of the world*. Cabi, 6.