Activities of aqueous extracts of Mangifera Indica on Parasitaemia level and blood profile of Plasmodium Berghei - infected Albino Mice

Olayode Ahmed A[1]*, Saka Olusola S[1], Ajayi Olusoji C[2], Agbaje Mojeed A[1]
[1] Department of Anatomy and Cell Biology, Faculty of Basic Medical Science
[2] Department of Pharmacognosy, Faculty of Pharmacy
Obafemi Awolowo University, Ile-Ife, Nigeria
* olayodeahmed01@gmail.com

ABSTRACT

This study was carried out to assess the effect of Mangifera indica on the percentage parasitaemia and some blood parameters of Plasmodium - infected Albino mice. Forty-two male albino mice, weighing between 20 and 25 g, were used for this research. The mice were randomly assigned into six groups A, B, C, D, E and F of seven mice each. Group A was the negative control, group B was positive control. Mice in groups C, D and E were orally administered with Mangifera indica extract (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) respectively for 5 days starting from the 4th day of inoculation while mice in group F were orally administered with Artesunate for 5 days (3 mg/kg b.w on the first day and 1.5 mg/kg b.w for the next 4 days) starting from the 4th day of inoculation. Blood smears were taken every 2 days to check the parasitaemia level for 14-day post-treatment withdrawal period. The extract has a dose dependent reducing effect on the level of parasitaemia. It also showed that the increase in the rate of reticulocytosis and consequent increase in the number of red blood cells in a dose dependent manner.

Keywords: Mangifera indica, Percentage Parasitaemia, Reticulocytosis, Erythropoiesis, Plasmodium parasites.

INTRODUCTION

Malaria, the world's most devastating human parasitic infection [1], is a vector-borne infectious disease which is caused by various single celled protozoans of the genius *Plasmodium* and it is transmitted by female Anopheles mosquitoes [2].

Most serious illnesses and deaths are attributed to *Plasmodium falciparum* which cause fevers that sometimes occur at 48-hour intervals. Symptoms like high fever, head ache, drowsiness, confusion occur as the parasites spread to several other organs to cause some other symptoms like diarrhea, kidney failure and jaundice. The malarial parasites have developed resistance to a number of anti-malarial medicines in many parts of the world [2]. Prompt and effective treatment with Artemisinin-based combination therapies, usage of mosquito nets or insecticidal nets by people at risk and indoor residual spraying of insecticide to control the vector mosquitoes are the persistent control of malaria [3].

Severe malaria can be fatal or lead to anaemia, heart and kidney failure, and/or coma; untreated infections can cause recurrent illness for years. Unfortunately, there is not yet a successful vaccine against malaria.

Malarial transmission to the human host is established by sporozoites infection to the liver. The malarial sporozoite once injected into the blood by the bite of female anopheles mosquitoes is attached to the hepatocytes through the receptor for thrombospondin and properdin. Here these sporozoites become mature to form tissue 'schizonts' or become dormant 'hypnozoites'. Tissue 'schizonts' amplify the infection by producing large number of 'merozoites' [4]. Merozoite infects and ruptures the liver cells in an attempt to escape back into the circulation and continues the infection.

In the case of malarial anaemia which may be caused by hemolysis, erythrophagocytosis, dyserythro-poiesis or ineffective erythropoiesis, there may be low reticulocytosis indicating reduced red blood cell (RBC) output [5]. It was demonstrated [6] that low reticulocytosis (i.e. reduced red blood cell output) which leads to malarial anaemia is usually as a result

of *Plasmodium* products, especially hemozoin and suppression of erythropoietin (Epo) - induced erythropoiesis, but the mechanism involved is not clear. It was reported that severe malaria causes sequestration of parasitized red blood cells which leads to deposition of hemozoin (Hz) (malarial pigment) that reduce the reticulocytosis [7]. Studies suggested that pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-12 and macrophage migration inhibitory factor (MIF), as well as *Plasmodium* products such as Hz, contribute to the pathogenesis of malarial anemia [8]. It was stated that blunted Epo-induced growth and maturation of erythroid precursors contribute to suppressed erythropoiesis in *P. chabaudi* AS-infected mice [9], and was concluded that parasite factors like Hz contribute to erythropoietic suppression, low reticulocytosis and malarial anemia by inhibiting Epo-induced proliferation of RBC precursors [6].

In contrast to the outcome of the studies that malarial anemia leads to low reticulocytosis by inhibiting erythropoiesis, it was stated that there was increase in the rate of reticulocytosis when hypoxic anemia is noticed [10]. The hypoxia- inducible factor (HIF) is discovered as a key mediator of cellular adaptation to low oxygen. In response to reduced delivery of oxygen to the cellular and tissue level, the chemoreceptors would have identified the malarial anemia as hypoxic anemia. This might have triggered the secretion of HIF which could antagonize the suggested suppression of erythropoiesis by increasing reticulocytosis [10]. It has been suggested that HIF promotes erythropoiesis through coordinated cell type-specific hypoxia responses, which include increased erythropoiesis in production in the kidney and liver, enhance iron uptake and utilization that facilitate erythroid progenitor maturation and proliferation [10].

As the parasitaemia level increases, there seems to be reduction in packed cell volume in the infected mice, the regulatory factors in the coordination of the parasitaemia level in relation with the blood profile needed keen examination, hence this study.

MATERIALS AND METHODS

Animal Care and Management

Forty-two male Albino mice, weighing between 20 and 25 g, obtained from the Animal Holding of Faculty of Basic Medical Sciences, Obafemi Awolowo University Ile-Ife, were used for this research. The mice were housed in plastic cages in the Animal Holding of the Department of Anatomy and Cell Biology, Obafemi Awolowo University Ile-Ife. They were maintained on standard laboratory animal pellets before the start of the experiment and water was provided ad libitum. The mice received humane care according to the guidelines of Health Research and Ethics Committee of the Institute of Public Health, Obafemi Awolowo University, Ile Ife. The mice were randomly assigned into six groups A, B, C, D, E and F of seven mice each.

Plant Material and Preparation of Extract

Fresh leaves of mango tree were obtained from plantations in Ile-Ife and authenticated by a plant taxonomist in the Department of Botany, Obafemi Awolowo University, Ile-Ife. A voucher specimen was deposited at the department's Herbarium for future reference with voucher number IFE-17465. The leaves were cleaned and air-dried at room temperature. The dried leaves were pulverized using an electric blender. The pulverized leaves were soaked in distilled water for 24 hours with continuous shaking in an electric shaker. The dissolved extract in water was concentrated in a vacuum rotary evaporator at 40^0C and later freeze-dried.

Inoculation of Plasmodium Parasites

This was done in the Department of Medical Microbiology and Parasitology, College of Health Sciences, Obafemi Awolowo University, Ile-Ife. Parasites of P. berghei were used to infect the mice in Groups B, C, D, E and F. Infection of mice with *Plasmodium* parasites was done intraperitonially with 0.2 ml of mixture of normal saline and infected blood of the donor mouse whose parasitaemia level was approximately 36 %.

Experimental Design

Group A was the normal control group served with equivalent volume of normal saline;

Group B was infected with Plasmodium parasite (PP) + equivalent volume of normal saline;

Group C was infected with Plasmodium parasite (PP) and treated with MI (50 mg/kg body weight) after 72 hours post - infection for 5 days;

Group D was infected with PP and treated with MI (100 mg/kg body weight) after 72 hours post - infection for 5 days;
Group E was infected with PP and treated with MI (150 mg/kg body weight) after 72 hours post - infection for 5 days; and
Group F was infected with PP and treated with Artesunate after 72 hours post - induction for 5 days (3 mg/kg body weight on first day and 1.5 mg/kg body weight for the next 4 days).
The animals were left for another 14 days after treatment, during which blood smears were taken every 2 days to check the parasitaemia level.

Determination of Parasitaemia Level

Blood samples were collected from the tail vein of the mice and thin blood films on microscope slides were prepared, air-dried and fixed with methanol. The fixed thin smears of blood were stained with 10% Giemsa stain diluted with buffered water (pH = 7.2) for 20 - 30 minutes. The percentage parasitaemia was determined by counting the number of parasitized red blood cells out of 1 000 blood cells in ten randomly selected microscopic fields using oil immersion lens (×100)[11].

Stereological Parameters

Kupffer Cells

Photomicrographs were analyzed using Motic Image Plus (MIPlus) 2.0 ML version by importing the photomicrograph unto the MIPlus, then a 1 mm^2 square box was drawn and the number of Kupffer cells found within the area of the square were counted and recorded.

Percentage Reticulocyte

Photomicrographs of blood smears were taken at × 400 magnifications whereby the polychromatic staining was used to identify the reticulocytes. The photomicrographs were imported unto the ImageJ 1.48 version (National Institutes of Health, USA) where the Grid plug in was activated before the manual cell counter was used to count thirty (30) randomly selected grids out of eighty - four (84) grids. Two cell types (Cell 1 - reticulocytes and Cell 2 - erythrocytes) were selected for counting. Each tick displayed a particular colour for the type of cell selected and the manual counting was displayed at the end of each count, this was recorded. The percentage reticulocyte was calculated for each using:

$$Percentage\ Reticulocyte\ (\%) = \frac{Total\ Reticulocytes\ counted}{(Reticulocyte + Erythrocyte) counted} \times 100\%$$

Red Blood Cell Count

Photomicrographs from the blood smear were imported unto the Motic Image Plus (MIPlus) 2.0 ML version. A squared box measuring an area of 0.01 mm^2 was used to count the red blood cells (both reticulocytes and erythrocytes) at ten different areas on each photomicrograph. The area was later converted to 1 mm^2 by multiplying the data derived from the counting by 100.

Statistical Analysis

One-way ANOVA was used to analyze data, followed by Student Newman-keuls (SNK) test for multiple comparisons. GraphPad Prism 5 (Version 5.03, Graphpad Inc.) was the statistical package used for data analysis. Data obtained were analyzed using descriptive and inferential statistics. Significant difference was set at $p<0.05$.

RESULT

Level of Parasitaemia in Control and Experimental Groups

Table 1 and Fig. 1 showed the level of parasitaemia across the control and treated groups. The result presented a significant decrement in the levels of parasitaemia in groups C (16.22±0.498), D (14.69±0.643), E (14.02±0.275) and F (11.74±0.293) when compared with group B (33.80±0.941). There was no significant change in the parasitaemia level between the treated groups.

Table 1:The level of parasitaemia in the control and treated groups

Day	A (-ve control)	B (+ve control)	C (50 mg/kg MI)	D (100 mg/kg MI)	E (150 mg/kg MI)	F (Artesunate)
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3	0.00±0.00	16.78±0.882	18.75±1.86	17.82±1.43	19.34±0.513	17.55±1.09
8	0.00±0.00	21.39±0.963	20.34±0.681	18.00±0.413	17.59±0.586	16.48±0.342
10	0.00±0.00	23.76±0.999	21.23±0.560	20.33±0.546	19.56±0.382	18.75±0.213

12	0.00±0.00	24.43±0.743	20.76±0.951	19.45±0.583	18.49±0.321	17.56±0.258
14	0.00±0.00	25.23±1.25	19.88±0.991*	18.01±0.639*	17.76±0.266*	16.66±0.221*
16	0.00±0.00	28.02±0.718	18.76±0.690*	16.85±0.661*	16.45±0.387*	15.60±0.234*
18	0.00±0.00	29.59±0.668	17.48±0.410*	15.92±0.673*	15.63±0.354*	14.89±0.259*
20	0.00±0.00	31.68±0.935	16.87±0.519*	15.13±0.652*	14.76±0.409*	12.86±0.272*
22	0.00±0.00	33.80±0.941	16.22±0.498*	14.69±0.643*	14.02±0.275*	11.74±0.293*

Values expressed as Mean±SEM

*: Significant decreased when compared with B (Positive control)

Fig. 1: Showing the effect of *Plasmodium berghei* parasites, *Mangifera indica* and Artesunate on the Parasitaemia level in Control and the Treated groups.

Inoculation of *Plasmodium* parasites

Administration started

Administration ended & Beginning of 2-week withdrawal period

2-week withdrawal period ended

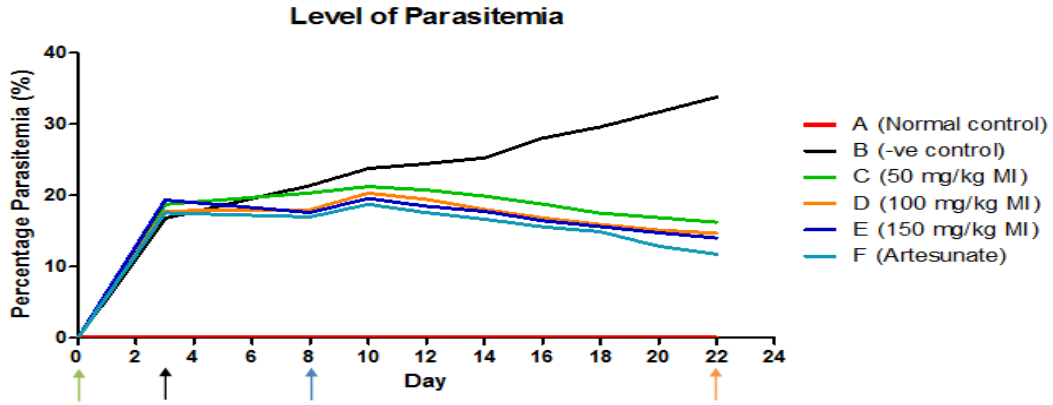

Fig. 1: Showing the effect of *Plasmodium berghei* parasites, *Mangifera indica* and Artesunate on the Parasitaemia level in Control and the Treated groups.

Inoculation of *Plasmodium* parasites

Administration started

Administration ended & Beginning of 2-week withdrawal period

2-week withdrawal period ended

Evaluation of Suppressive Activity of the Extract

The suppressive test is a standard test commonly used for antimalarial screening and the determination of percent suppression of parasitaemia is the most reliable parameter (Gitua *et al*., 2012). Table 2 showed that administration of *Mangifera indica* extract at dosages of 50 mg/kg b.w/day, 100 mg/kg b.w/day and 150 mg/kg b.w/day were able to suppress parasite growth by 52.01%, 56.54% and 58.52% respectively. Therefore, the total ED_{50} value for the extract of Mangifera indica administered on oral route was 170.33 mg/kg b.w/day.

Table 2:The Suppressive Test for Antimalarial Extract

	Percentage Parasitaemia (%)	Percentage of Suppression(%)
A (Negative Control)	0.00±0.000	-
B (Positive Control)	33.80±0.941	0
C (50 mg/kg MI)	16.22±0.498	52.01
D (100 mg/kg MI)	14.69±0.643	56.54
E (150 mg/kg MI)	14.02±0.275	58.52
F (Artesunate)	11.74±0.293	65.27

Percentage Reticulocyte in Blood Smear

Table 3 and Fig. 2 showed the percentage reticulocyte in the blood smears of the control and treated group. There was significant increase across groups B (23.0±0.564), C (26.5±0.562), D (18.4±0.417), E (11.2±0.393) and F (21.6±0.429) when compared with group A (4.78±0.294). There was significant increase in group C (26.5±0.562) when compared with group B (23.0±0.564), but there was significant decrease across groups D (18.4±0.417), E (11.2±0.393) and F (21.6±0.429) when compared with group B (23.0±0.564).There was significant increase in group C (26.5±0.562) when compared with group F (21.6±0.429), but there was significant decrease across groups D (18.4±0.417) and E (11.2±0.393) when compared with group F (21.6±0.429). There were significant differences across groups C (26.5±0.562), D (18.4±0.417) and E (11.2±0.393) when compared with one another.

Table 3:Percentage Reticulocyte and Red Blood Cell Count in Blood Smears of Control and Treated Groups

	Percentage Reticulocyte (%)	Red Blood Cell Count ($\times 10^3$ cells/mm^2)
A (Negative Control)	4.78±0.294	7.00±0.107
B (Positive Control)	23.0±0.564*	3.34±0.0782*
C (50 mg/kg MI)	26.5±0.562*#^	3.84±0.0649*#^
D (100 mg/kg MI)	18.4±0.417*#^	4.64±0.896*#^
E (150 mg/kg MI)	11.2±0.393*#^	6.06±0.0571*#^
F (Artesunate)	21.6±0.429*#	5.40±0.0655*#

Values expressed as Mean±SEM

*: Significantly different when compared with A (Negative control);

#: Significantly different when compared with B (Positive control);

^: Significantly different when compared with F (Artesunate)

Red Blood Cell Count in Blood Smear

Table 3 and Fig. 3 showed the red blood cell count in the blood smears of the control and treated group. There were significant reductions in red blood cell count across groups B (3.34±0.0782), C (3.84±0.0649), D (4.64±0.0896), E (6.06±0.0571) and F (5.40±0.0655) when compared with group A (7.00±0.107). There was significant increase in red blood cell across groups C (3.84±0.0649), D (4.64±0.0896), E (6.06±0.0571) and F (5.40±0.0655) when compared with group B (3.34±0.0782). There was significant decrease across groups C (3.84±0.0649) and D (4.64±0.0896) when compared with group F (5.40±0.0655) but a significant increase was seen in group E (6.06±0.0571) when compared with group F (5.40±0.0655). A significant decrease was noticed across groups C (3.84±0.0649) and D (4.64±0.0896) when compared with group E (6.06±0.0571).

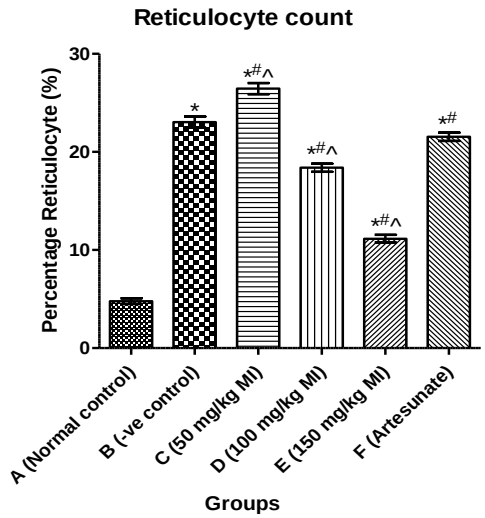

Fig. 2: Showing the Percentage Reticulocyte in Control and the Treated Groups

Values expressed as Mean±SEM

*: Significantly different when compared with A (Negative control);

#: Significantly different when compared with B (Positive control);

^: Significantly different when compared with F (Artesunate)

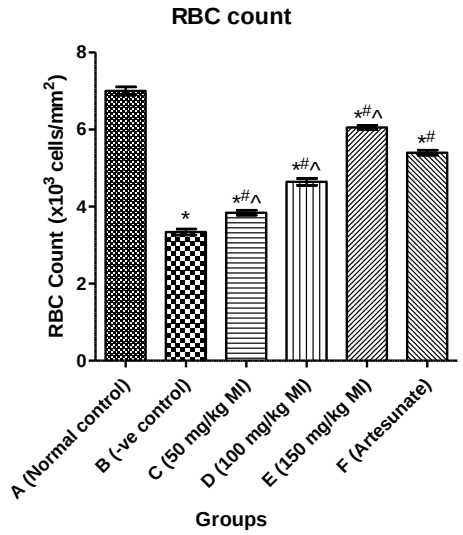

Fig. 3: Showing the Red Blood Cell Count in Control and the Treated Groups

Values expressed as Mean±SEM

*: Significantly different when compared with A (Negative control);

#: Significantly different when compared with B (Positive control);

^: Significantly different when compared with F (Artesunate)

DISCUSSION

The increase in the rupture of the liver cells leads to increase in merozoites in circulation which in turn leads to the increase in number of parasitized red blood cells (PRBCs) in circulation. Our result showed that the positive control group presented a gradual significant increase in the percentage parasitaemia while the levels of parasitaemia in the treated groups reduced significantly when compared with the positive control group. This significant reduction of percentage levels of parasitaemia by MI was dose dependent. The presence of triterpenoid in MI might have inhibited the stages of infection thereby reducing the parasitaemia. Triterpenoid possesses dual antimalarial activity as inhibitors of erythrocytic and liver stages of Plasmodium infections [12]. Artesunate reduced the number of PRBCs in circulation and this was in line with earlier result [13].

Results from the RBC density showed that many erythrocytes were lysed due to parasite infection, which is one of the indications of anemia as the number of red blood cells in the positive control and the treated groups have reduced significantly when compared with that of the negative control group. MI extract possesses haemopoietic property and may enhance erythropoietic processes and it may also increase resistance to oxidative damage to red blood cells membranes [14]. Increase in serum iron binding capacity, one of the properties of mangiferin, may be responsible for the haemopoietic property of MI as reported [15]. In the case of malarial anaemia which may be caused by hemolysis, erythrophagocytosis, dyserythropoiesis or ineffective erythropoiesis, there may be low reticulocytosis indicating reduced red blood cell (RBC) output [5]. It was demonstrated [6] that low reticulocytosis (i.e. reduced red blood cell output) which leads to malarial anaemia is usually as a result of Plasmodium products, especially hemozoin and suppression of erythropoietin (Epo) - induced erythropoiesis, but the mechanism involved is not clear. It was reported that severe malaria causes sequestration of parasitized red blood cells which leads to deposition of hemozoin (Hz) (malarial pigment) that reduce the reticulocytosis [7]. Studies suggested that pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-12 and macrophage migration inhibitory factor (MIF), as well as Plasmodium products such as Hz, contribute to the pathogenesis of malarial anemia [8].

Also evident in our study was polychromasia in the extract treated group which is an indication that there were prompt reticulocytosis for the replacement of the disrupted RBC. This property of MI may be responsible for the release of immature RBCs into blood circulation. The increase in the reticulocyte count in the mice that were administered with different doses of the extract may be an indication of increased erythropoietic processes, this may be sensed as a result of low delivery of oxygen to the cellular level [10]. The hypoxia- inducible factor (HIF) is discovered as a key mediator of cellular adaptation to low oxygen. In response to reduced delivery of oxygen to the cellular and tissue level, the chemoreceptors would have identified the malarial anemia as hypoxic anemia. This might have triggered the secretion of HIF which could antagonize the suggested suppression of erythropoiesis by increasing reticulocytosis [10]. It has been suggested that HIF

promotes erythropoiesis through coordinated cell type-specific hypoxia responses, which include increased erythropoiesis in production in the kidney and liver, enhance iron uptake and utilization that facilitate erythroid progenitor maturation and proliferation [10]. This observation is in agreement with our result as the percentage reticulocyte in positive control was seen to increase which was contrary to suppressed reticulocytosis earlier stated.

CONCLUSION

In conclusion, Mangifera indica has shown its possession of haemopoietic activities and has the ability to cause increase in reticulocytosis and in turn increases erythropoiesis.

Acknowledgement: The effort of Mr Adelodun Stephen on the help provided during the infection procedures in the mice is hereby acknowledged.

REFERENCES

1. Nicholas JW, Breman JG. "Malaria and Babeiosis; Diseases Caused by Red Blood Cell Parasites in Harrisons Principle of Medicine". McGraw Hill, New York, 2005; 1218.
2. WHO. Malarial Chemotherapy. Tech. Reg. Ser. WHO, Geneva, 1998.
3. Palmer J. "WHO gives indoor use of DDT a clean bill of health for controlling malaria". WHO, 2006.
4. Kausar MW, Moeed K, Asif A, Rizwi F, Raza S; Correlation of Bilirubin with Liver Enzymes in Patients of Falciparum Malaria; International Journal of Pathology; 2010; 8(2); 63-67.
5. Lamikanra AA, Brown D, Potocnik A, Casals-Pascual C, Langhorne J, Roberts DJ; Malarial anemia of mice and men. Blood; 2007; 110; 18 - 28.
6. Thawani N, Tam M, Bellemare MJ, Bohle S, Olivier M, Souza JB, Stevenson MM. Plasmodium products contributor to severe malarial anemia by inhibiting erythropoietin - induced proliferation of erythroid precursors. Journal of Infectious Diseases 2013; 409.
7. Srivastava HC, Sharma SK, Bhatt RM, Sharma VP. Studies on Plasmodium vivax relapse pattern in Kheda Hospital, Gujarat. India Journal of Malariology 1996; 33; 175-179.
8. Thawani N, Tam M, Stevenson MM. STAT6-mediated suppression of erythropoiesis in an experimental model of malarial anemia. Haematologica 2009; 94; 195-204.
9. Chang KH, Stevenson MM. Malarial anemia: mechanism and implications of insufficient erythropoiesis during blood-stage malaria. International Journal of Parasitology 2004; 24; 1501 - 16.
10. Haase VH; Hypoxia regulation of erythropoiesis and iron metabolism; American Journal of Physiology (Renal Physiology); 2010; 299(1); 1-13.
11. Brecker G, Schneiderman M; A time saving device for the counting of reticulocytes; American Journal of Clinical Pathology; 1950; 20; 1079-1083.
12. Ramalhete C, da Cruz FP, Mulhovo S, Sousa IJ, Fernandes MX, Prudencio M, Ferreira MJU; Dual-stage triterpenoids from an African medicinal plant targeting the malaria parasite; Bioorganic & Medicinal Chemistry 2014; 22 (15); 3887-3890.
13. Georgewill UO, Ebong OO; A Comparative Study on the Efficacy of Some Artemisinin Combination Therapies on Plasmodium berghei in Swiss Albino Mice; Pharmacology & Pharmacy 2012; 3; 109 - 112.
14. Ogbe RJ, Adenkola AY, Anefu E; Aqueous Ethanolic Extract of Mangifera indica Stem Bark Effect on the Biochemical and Haematological Parameters of Albino Rats; Arch. Appl. Sci. Res; 2012; 4 (4); 1618 - 1622.
15. Pardo-Andreu GL, Barrios MF, Curti C, Hernandez I, Merino N, Lemus Y, Martinez I, Riano A, Delgado R; Protective Effects of Magnifera indica L extract (Vimang) and its major component mangiferin, on iron-induced oxidative damage to rat serum and liver; Pharmacology Research; 2008; 57(1); 79 - 86.