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Research Article

In-vivo Anti-plasmodial Activity of Mama Decoction® with or without Paracetamol in Mice

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ABSTRACT

Mama Decoction® (MD) is an aqueous antimalarial herbal preparation obtained from combination of *Mangifera indica*, *Alstonia boonei*, *Morinda lucida* and *Azadirachta indica* leaves with each component plant reported to have anti-plasmodial activity. Management of plasmodial infections is usually accompanied by co-administration of antipyretic and analgesic agent with paracetamol being the most common. Therefore, the study investigated the biological implications of co-administration of MD with paracetamol (PA) in *in-vivo* anti-plasmodial model in mice. The anti-plasmodial activity of MD (1mL/kg bw), chloroquine phosphate (Cq) (10mg/kg bw) alone and co-administration with PA (15mg/kg bw) (PMD and PCq) were evaluated by oral administration using established malaria infection test (Rane's test). Chloroquine phosphate and distilled water were used as positive and negative controls respectively. Haematological analysis and histopathological evaluation of the liver and kidney were done using standard procedures. Reduction in parasitaemia at day 4 of treatment with MD, PMD, Cq, PCq were 34.80%, 42.36%, 86.84% and 77.25% respectively. PA significantly reduce anti-plasmodial effect of Cq ($p=0.0239$), while the enhanced activity on MD was not significant ($p=0.3090$). Significant reduction ($p=0.0027$) in PCV and Hb in MD, PA and untreated parasitized group were observed. Hepatotoxicity were observed in parasitized treated and non-treated groups with more effect by co-administration with PA. Different levels of renal tubular epithelial necrosis were observed in the parasitized untreated and Cq groups. The results confirmed anti-plasmodial activity of MD, which though non-significantly reduced by its co-administration with paracetamol has hepatotoxic potential

Keywords: *Mama Decoction, Paracetamol, Parasitaemia, Co-administration*

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INTRODUCTION

The use of herbal medicinal products is on the increase worldwide and the practice of co-administration of herbal medicinal products with western medicines is raising a public health concern globally and most importantly in the developing countries (Bamidele *et al.*, 2018). This growing increase in the practice of co-administration of herbal medicinal products with western medicines is enhanced by the availability of these products as food/dietary supplements without prescription with little or no regulation. However, the fact that natural products are not evaluated for possible interactions with conventional drugs has raised a growing concern about the possible interactions of these conventional drugs with herbal medicinal products (Gardiner, *et al.*, 2008).

Over the years, several clinically relevant herb-drug interactions have been reported, resulting in altered bioavailability, pharmacological activity, toxicity/adverse reactions that are sometimes life threatening (Elvin-Lewis, 2001; Mohamed and Frye, 2012; Alzubaidi, and Hussain, 2015; Obiageri, 2018). For instance, ginseng was reported to induce mania when concurrently administered with phenelzine (Mustapha *et al.*, 1996), while an enhanced hypoglycemic effect was reported when chlorpropamide was taken with meal containing garlic and *Mormodica charantia* in the treatment of type 2 diabetes (Aslam and Stockley, 1979). For gingko biloba, haemorhage associated with concomitant use with aspirin, warfarin and acetaminophen/ergotamine caffeine preparation have been documented (Rowin and Lewis, 1996; Gianni and Dreitlein,

1998). Also, the leaves of *Carica papaya* (Pawpaw) with antimalarial activity was reported to antagonize antimalarial properties of artesunic acid in *Plasmodium berghei*-infected mice (Onaku, 2011). However, synergistic herb-drug interactions were reported for *Vernonia amygdalina* with chloroquine (Nandakumar *et al.*,2006) and curcumin (isolated from *Curcuma longa*) with artemisinin (Iwalokun, 2008) when co-administered respectively. Similarly, Adepiti *et al.*, (2016) reported a total clearance of parasitaemia when Mama Decoction® (MD) was co-administered with amodiaquine in CQ-resistant *Plasmodium berghei* infected mice at their therapeutic doses.

Mama Decoction® (MD) is an aqueous antimalarial herbal preparation obtained from *Mangifera indica*, *Alstonia boonei*, *Morinda lucida* and *Azadirachta indica* leaves with each component plant reported to have anti-plasmodial activity (Awe *et al.,* 1998; Isah *et al.,* 2003). Also, antimalarial constituents of *A. indica* and *M. lucida* leaves have been isolated (Khalid *et al.,* 1989; Cimanga *et al.*, 2006). A significant suppressive and about 50% curative activities of MD was reported by Odediran *et al.*, (2014), while previous study on sub-chronic toxicity study of MD reported normal kidney and liver tissues morphology in non-parasitized animals (Akanmu *et al.,* 2013).

The symptom of malaria is usually accompanied by pyrexia and analgesia, thus, management of plasmodial infections is most times accompanied by co-administration with antipyretic and analgesic agents with paracetamol being the most common analgesic agent. In view of the reported safety issues accompanying co-administration of herbal products and orthodox drug compounds, there is the need to regularly evaluate such co-administration, hence, this study reported the safety profile and possible pharmacological implications of co-administering MD with paracetamol using *in-vivo* anti-plasmodial model.

MATERIALS AND METHODS

Reagents: Giemsa stain, glacial acetic acid, methanol, formalin, chloroform (Aldrich Chemical Company Ltd, England) and paracetamol powder. All other chemicals were of analytical grade.

Procurement of Mama Decoction® and paracetamol powder: Mama Decoction® (MD) with batch number FP15001 and Paracetamol powder (Tianju Bofa Pharmaceutical Corporation Ltd, India) were obtained from Drug Research and Production Unit (DRPU), Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Identification and assay of paracetamol powder: Ultraviolet spectroscopic analysis, melting point and chemical content determination for paracetamol powder were carried out using official method (BP 2013).

Experimental animals: Thirty-five (35) healthy Swiss albino mice of both sexes weighing 20-26g were obtained from the animal house of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The animals were housed under a 12-h light/dark cycle with free access to water and commercial food pellets (Vital Feed Nig. Ltd, Jos, Nigeria). They were acclimatized for about two weeks before use and were used in accordance with the National Institute of Health (NIH) "Guide for the Care and Use of Laboratory Animals" 1985.

Drug and herbal product administration: Chloroquine phosphate (reference antimalarial drug) was administered at 10mg/kg bw of chloroquine base once daily for two (2) days and 5mg/kg bw on day 3. Mama Decoction® was administered at 1mL/kg bw 12 hourly for four days (based on the dosage instruction) and paracetamol (15mg/kg bw) was administered 8 hourly for three days. The dosages were based on the standard dosages for the three preparations. All the drugs were administered by oral gavage.

Parasite strain: Chloroquine-sensitive *Plasmodium berghei* NK65 strain was obtained from the Department of Chemistry, Faculty of Science, OAU, Ile-Ife, Nigeria. The parasites were maintained in continuous blood passage in mice. The parasitized blood donor mice with high parasitaemia was used for inoculation by first anaesthetizing the mouse with chloroform, and through cardiac puncture the blood was collected using sterile heparinized bottles. The percentage parasitaemia was determined. Appropriate volume of blood was then obtained from the donor mouse and diluted with sterile normal saline so that the final inoculum (0.2mL) for each mouse contains 10_7 parasitized red blood cells.

Rane's curative antiplasmodial test: The curative effect of MD and its interaction with paracetamol was assessed by the method described by Ryley and Peters (1970). Thirty mice were inoculated intraperitoneally, each with 0.2mL standard inoculum of infected blood containing 10_7 CQ-sensitive *Plasmodium berghei* NK 65 parasitized red blood cells on the first day. After the parasitaemia was established in the blood (72 hours later), the mice were randomly divided into six (6) groups (n=5, both sexes) per group. The infected untreated group (UM) was the negative control and the seventh group was uninfected healthy (HM) untreated group. Both groups were treated with 0.2mL distilled water. On the other hand, infected groups were treated with chloroquine (Cq), paracetamol (PA), MD, PA co-administered with MD (PMD), and PA co-administered with Cq (PCq) respectively. The administration was done by oral gavage. Giemsa stained thin blood smears prepared from tail snips were used to monitor the level of parasitaemia on days 1, 2, 3, 4, 7 and 9 by microscopic examination. The parasitaemia was determined by obtaining the percentage of the ratio of parasitized RBC to the total number of RBC. Percentage reduction (or parasite clearance) was calculated as 100× [(A–B)/A], where A is the average parasitemia of day 0 prior to treatments and B is the average parasitemia on days 1, 2, 3, 4, 7 and 9.

Haematological and biochemical analyses: At the end of the experiment, the animals were anaesthetized with chloroform and blood was obtained using cardiac puncture technique. Blood (5ml) each was collected into the pre-labelled ethylenediaminetetraacetic acid (EDTA) sample bottles, which were gently rolled to allow the blood to mix thoroughly

with the anticoagulant. The blood was evaluated for packed cell volume (PCV), haemoglobin level (Hb), WBC, platelets count, neutrophils and lymphocytes counts using standard methods (Baker, *et al.*, 1998).

Histopathology: The liver and the kidney were removed, stored in sample bottles containing 10% buffered formalin solution and preserved for histopathological evaluation using standard procedures (Soujanya, *et al.*, 2013).

Statistical analysis

Analysis of variance (one-way and two-way ANOVA) and Chi-square test were used for the various experimental test conditions at $p<0.05$ compared with their controls. The post hoc test of Dunnett`s Multiple Comparison Test (DMC) was used at $p=0.05$ significance level.

RESULTS

Identification and assay of paracetamol powder: Ultraviolet spectroscopic analysis for paracetamol powder showed maximum absorption at 248.34nm while its melting point was 168o-170oC. The assay of paracetamol powder in triplicate gave a chemical content of 99.9%w/w which correspond to 99-101% of the official method (BP 2013).

Antiplasmodial activity: The parasitaemia level was reduced by the administration of MD, PMD, Cq and PCq while increase was observed with UM and PA groups (Figure 1). The reduction in parasitaemia at day 4 of treatment were 34.80%, 42.36%, 86.84% and 77.25% for MD, PMD, Cq and PCq respectively (Figure 2). The increase in percentage reduction of parasitaemia with co-administration of PA with MD (PMD) was not significant ($p=0.3090$). However, a significance decrease ($p=0.0239$) in percentage reduction in parasitaemia was observed when PA was co-administered with Cq (PCq) (Figure 2). Antiplasmodial activity of MD and PMD groups were significantly lower than Cq and PCq (Figure 2).

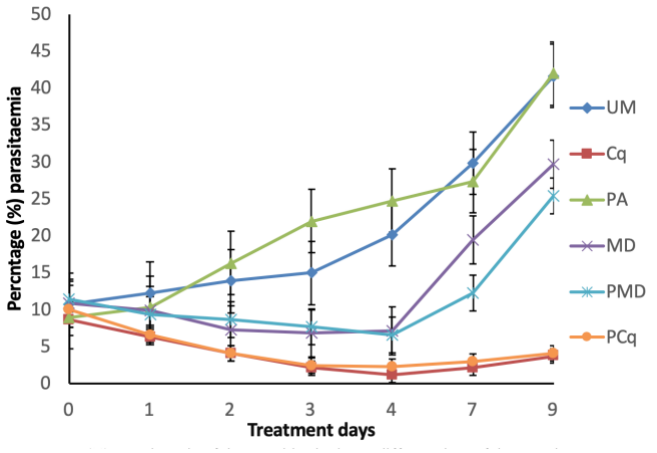

Figure 1
Percentage (%) parasitaemia of the parasitized mice at different days of drugs and Mama Decoction® (MD) treatments (day 0, 1, 2, 3, 4, 7 and 9 of drugs and Mama Decoction administration). Data are expressed as Mean ± S.E.M, ($p<0.05$).

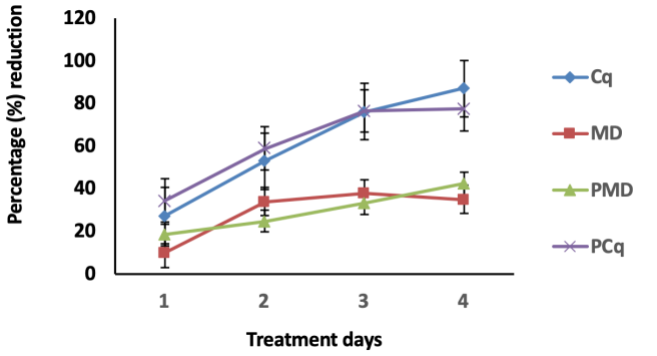

Figure 2
Percentage (%) reduction in parasitaemia level of the parasitized mice at different days of drugs and Mama Decoction® (MD) treatments (day 1, 2, 3 and 4 of drugs and Mama Decoction® (MD) administration). Data are expressed as Mean ± S.E.M, ($p<0.05$).

Table 1
Effects of MD, PA, Cq and their combinations on haematological parameters of parasitized and non-parasitized mice day 6 post treatments

Code	PCV (%)	HB (g/dL)	WBC (×10⁹/L)	Platelet (×10⁹/L)	NEU (%)	LYM (%)
HM	28.0 ± 2.26	9.32 ± 0.75	7.54 ± 1.20	183 ± 6.98	24.40 ±2.66	75.60 ±2.66
UM	6.67 ± 1.67a	2.23 ± 0.53 a	12.53 ± 3.47	148 ± 2.00	22.33 ±2.96	77.67 ±2.96
PA	8.00 ± 2.16 a	2.68 ± 0.71 a	13.50 ± 2.78	157 ± 8.48	20.75 ±5.02	79.25 ±5.02
MD	10.00 ±2.08 a	3.33 ± 0.69 a	11.50 ± 0.71	175 ± 18.58	41.67 ±9.17	58.33 ±9.17
PMD	17.50 ±2.50	5.85 ± 2.15	13.80 ± 1.80	182 ± 21.00	36.00 ±6.00	63.50 ±6.50
Cq	22.33 ±2.60	7.43 ± 0.87	9.03 ± 2.41	85 ± 36.20 a, b	17.33 ±4.63	82.33 ±4.91b
PCq	18.0 ± 1.47	6.00 ± 0.48	9.28 ± 0.75	131 ± 9.51	21.00 ±4.92	79.00 ±4.92

Data are expressed as mean ± S.E.M, ($p<0.05$).
a= statistically significant when compared with the control
b= statistically significant when Cq was compared with MD.

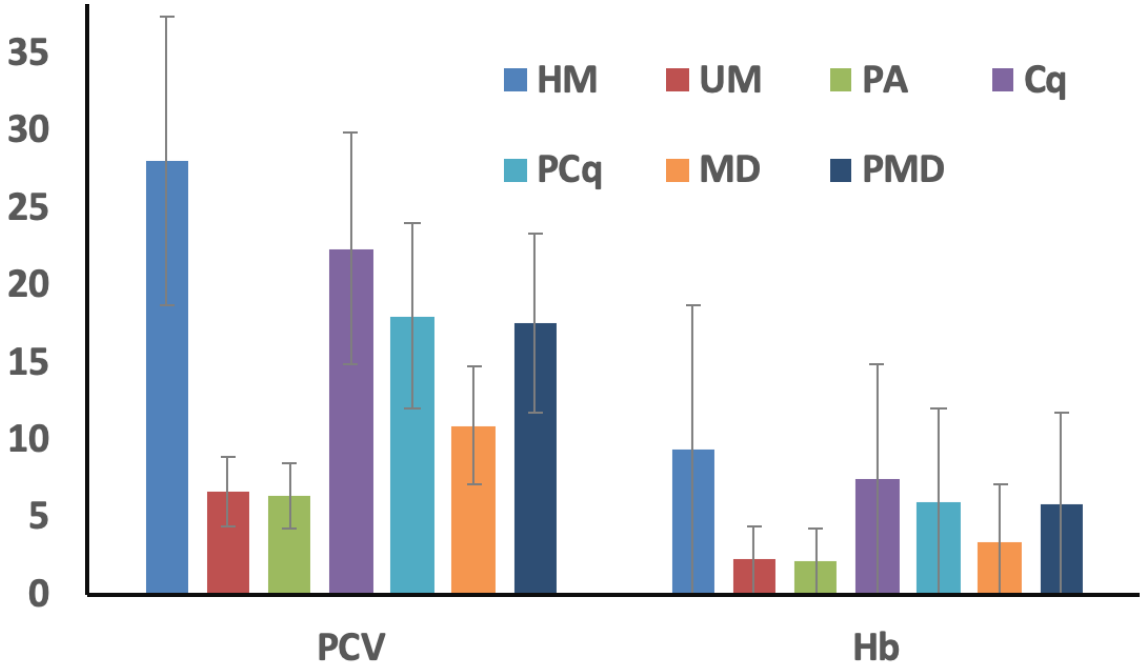

Figure 3
PCV (%) and Hb (g/dL) of the parasitized and non-parasitized mice day 9 of treatments with PA, Cq, PCq, MD and PMD. Data are expressed as mean ± S.E.M, ($p<0.05$)

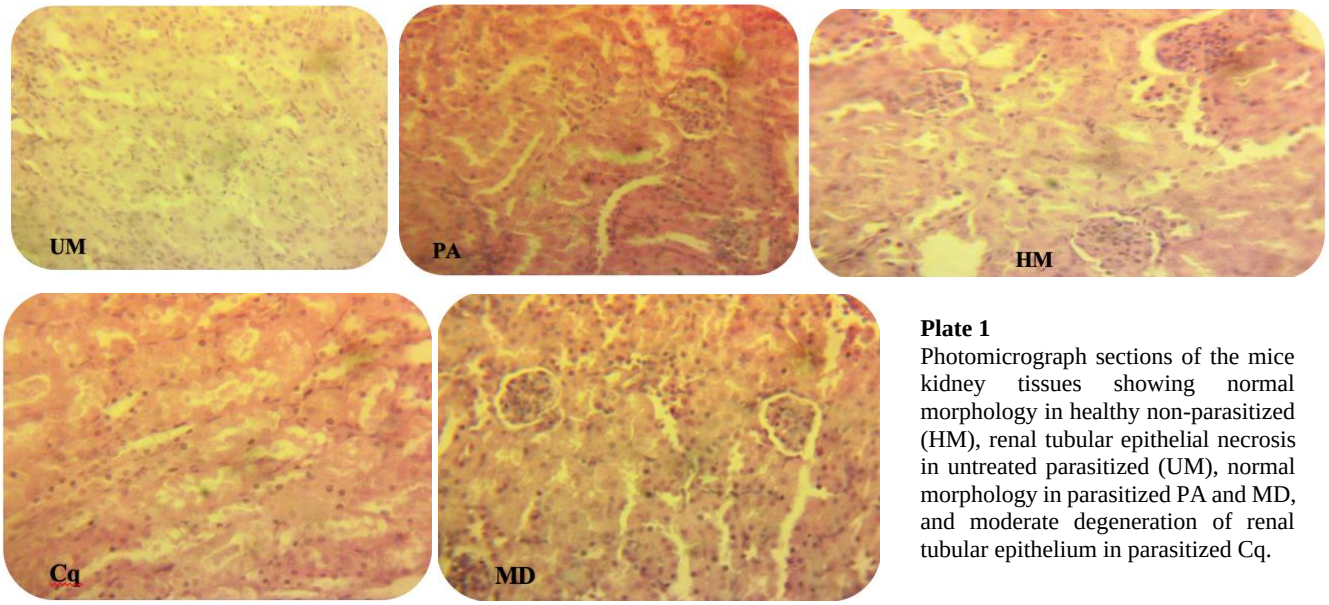

Plate 1
Photomicrograph sections of the mice kidney tissues showing normal morphology in healthy non-parasitized (HM), renal tubular epithelial necrosis in untreated parasitized (UM), normal morphology in parasitized PA and MD, and moderate degeneration of renal tubular epithelium in parasitized Cq.

Histopathological evaluation: Histopathological analyses showed that kidney tissues showed renal degeneration in UM and Cq groups (Plate 2) and the liver tissues with varying degrees of hepatocytes damage in all the parasitized treated and untreated groups, non-parasitized group maintained normal morphology.

DISCUSSION

The symptoms of malaria are usually accompanied by pyrexia and analgesia; hence its treatment usually involves the use of antipyretic and analgesic agents alongside antimalarial drugs. A previous study reported the use of PA with herbal products including MD in 50% of the respondents (Bamidele *et al.*, 2018). This is due to the ease of availability and effectiveness of PA in the management of pyrexia and pain associated with malaria. This study on the implication of co-administration of MD and PA focused on pharmacodynamics, haematological and histopathological assessment.

A significant reduction in parasitaemia was observed with Cq and MD ($p< 0.0001$) and their co-administration with PA, however, the parasitaemia reduction for Cq was significantly higher than MD ($p< 0.0001$, Figure 2). The increase in parasitaemia observed in PA corroborates the fact that paracetamol possess no anti-plasmodial activity.

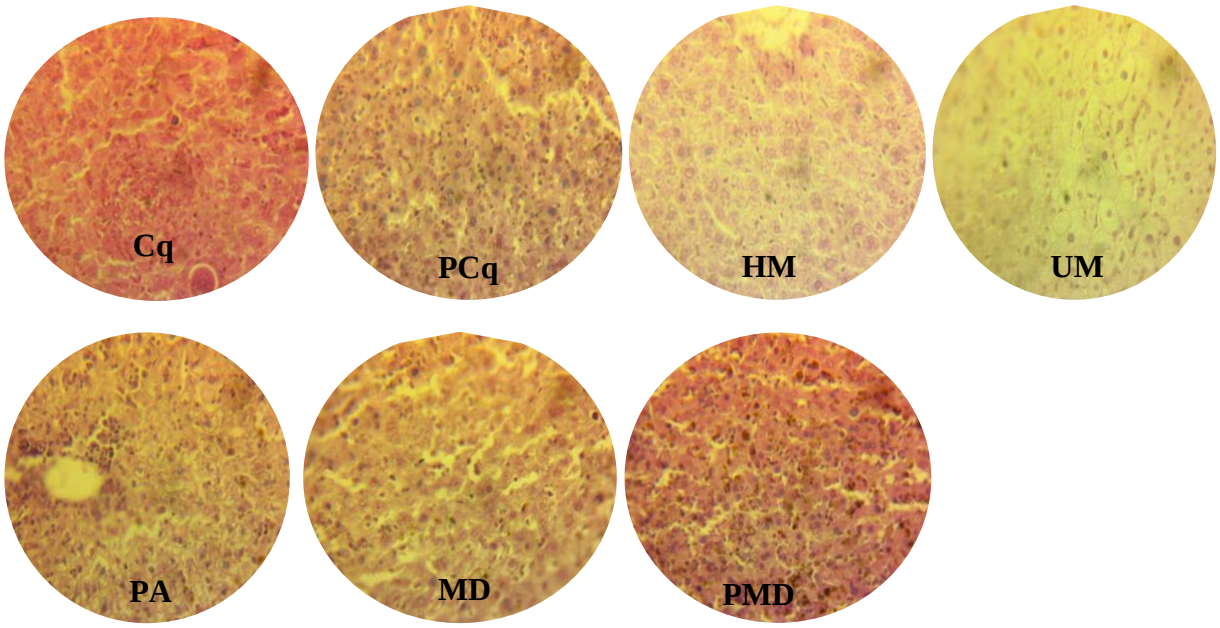

Plate 2
Photomicrograph sections of the mice liver tissues showing normal morphology in healthy non-parasitized (HM), diffuse hepatocellular degeneration (swelling) and formation of megalocytes in untreated parasitized (UM), perivasculitis, centrilobular hepatocellular degeneration and necrosis with kupffer hyperplasia in PA, focal necrotizing hepatitis with diffuse kupffer hyperplasia and parasitic section in Cq, hepatocellular degeneration and necrosis in PCq, centrilobular degeneration and necrosis of hepatocytes with a few inflammatory cells in MD, and kupffer hyperplasia and hepatocellular necrosis in PMD

The anti-plasmodial activity of MD obtained in this study though lower than Cq justifies its use in the treatment of malaria, which corroborates the report by Adepiti *et al.*, (2014) and Odediran *et al.*, (2014). Increased parasitaemia reduction when PA was co-administered with MD (PMD) was not statistically significant (p=0.3090), however, PA significantly decrease (p=0.0239) parasitaemia reduction of Cq (PCq) (Figure 2). This indicates that while paracetamol may not affect the efficacy of MD, it could compromise the effectiveness of Cq.

Meanwhile, there was no total clearance in parasitaemia as at the 4th day of administration for the completion of treatments, while a gradual increase in parasitaemia was observed at days 7 and 9 (days 3 and 5 after completion of drug and herbal treatments) (Figure 1). This indicates recrudescence which was higher for MD than Cq ($p<0.05$), with paracetamol not having any effect on the rate of recrudescence of PMD.

The anti-plasmodial activity of MD could be due to the presence of saponins and anthraquinones which have been associated with anti-plasmodial activity of plants (Philipson and Wright,1991; Christensen and Kharazmi, 2001). Behavioral assessment observed that the groups administered with paracetamol were more active indicating the pharmacodynamics activity of PA in militating against pain associated with plasmodial infection.

Haematological parameters are important indices of the physiological and pathological status for both animals and humans (Obianime and Aprioku, 2011). The findings in this study observed a significant decrease (p= 0.0027) in PCV and Hb in the treated parasitized groups (MD and PA) and the untreated parasitized group (UM) as compared to the healthy control group (HM) (Table 1). This decrease in PCV and Hb of the parasitized mice may be due to the haemolytic effect of *Plasmodium berghei* (Chinchilla *et al.*, 1998) and not the drug/herb products. This could be justified by a previous report of insignificant reduction in PCV, RBC and Hb in healthy albino rats treated with MD (Bamidele *et al.*, 2018). However, treatment with Cq, PCq and PMD had no significant effect ($p>0.05$) on PCV and Hb (Table 1).

Similary, the difference observed in PCV and Hb of Cq and MD, Cq and PCq, PCq and PMD, MD and PMD were equally not significant ($p>0.05$). This could be related to their extent of reducing parasitaemia corresponding to higher percentage reduction in Cq (86.84%), PCq (77.25%) and PMD (42.36%) compared to MD with 34.80% (Figure 2). Hence, it can be deduced that reduction in PCV and Hb was as a result of haemolysis of red blood cell (RBC) caused either by parasite multiplication or by spleen reticuloendotelial cell action (Chinchilla *et al.*, 1998). This decrease may also be due to multiple causes, of which repeated haemolysis of infected red cells takes preference.

On the other hand, other haematological parameters showed no significant difference ($p>0.05$) when compared with HM except for Cq with significant reduction (p= 0.0053) and increase (p= 0.0023) in platelet and lymphocytes counts respectively. However, the difference in platelet counts between Cq and PCq was not significant. Platelet helps in blood clotting to prevent wound infections, and a low platelet count could expose the rats to various infections. Low platelet counts cause clot retraction to be deficient leading to poor constriction of ruptured vessels, as well as inability of blood

to coagulate effectively. Thus, the decrease in platelet counts in Cq group is of clinical significance. However, low platelet counts have been reported in plasmodium infected people (Kotepui *et al.,* 2014; Tarig, *et al.*, 2016).

The hepatocytes in healthy group were relatively intact, however, varied degrees of hepatoxicities in the parasitized treated groups could be attributed to the destructive effect of parasitaemia on the liver tissues (Viriyavejakul, *et al.,* 2014; Onyesom and Onyemakonor, 2011). Also, the kidney tissues showed different levels of renal degeneration in UM and Cq (Plate 1) and varying degrees of liver damage (hepatotoxicity) in the parasitized groups. A previous study reported normal morphology for liver and kidney tissues in non-parasitized animal treated with MD in sub-chronic toxicity study (Akanmu *et al.,* 2013), thus, the observed hepatocytes damage is most likely not as a result of MD treatment. However, it can be deduced from this study that the co-administration of MD with PA (PMD) at normal dose is accompanied by higher level of hepatotoxicity when compared with MD alone compared to what was obtained with PA alone.

In conclusion, the results confirmed the anti-plasmodial activity of Mama Decoction® (MD) which was significantly lower than chloroquine. However, co-administration of paracetamol with Mama Decoction® resulted in a slight enhancement of anti-plasmodial effect which may not be clinically significant. Furthermore, co-administration of Mama Decoction® with paracetamol should be with caution in view of the observed hepatotoxicity.

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