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RESEARCH ARTICLE

TOXICITY PROFILING OF THE CO-ADMINISTRATION OF THE ESSENTIAL OIL OF *PIPER GUINEENSE* AND QUININE IN RATS

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Abstract

Background: The co-administration of medicinal plant products with orthodox drugs is not uncommon with a view to enhancing efficacy and improving treatment outcomes. However, reports indicated that such combinations may also enhance associated toxic effects. We recently reported that though the co-administration of essential oil of *P. guineense* (EOPG) and Quinine has the potential to improve treatment outcomes in experimental cerebral malaria, the observed deaths associated with higher co-administered doses necessitated the need for further toxicological evaluation.
Method: Rats were randomly divided into 12 groups (n=10), consisting of control, quinine, EOPG (12.5, 25, 50, 100, and 150 mg/kg), and their respective Quinine combinations. Control received vehicle (5% Tween 80 in distilled water), Quinine was given at a dose of 20 mg/kg stat, then 10 mg/kg twice daily for the next two days, while other groups were treated once daily for 3 days. All doses were administered intraperitoneally and rats were assessed for weights and novelty-induced behaviors (NIB). On day 4, rats were randomly sub-grouped into Treated and non-dosing Recovery (n=5) and sacrificed on day 4 and 18 respectively. Blood and organ samples were processed for hematological, biochemical, and histopathological evaluation.
Results: In this study, analysis of our results showed that co-administration of EOPG and Quinine revealed significant alterations in body and organ weights, rearing, grooming, and locomotion, as well as biochemical and hematological, and liver histoarchitecture, with potential for persistent toxicity.
Conclusion: We propose that the earlier reported death associated with the co-administration of EOPG with Quinine in experimental cerebral malaria may be associated with increased toxicity on the liver and risk of heart-related diseases. This study concludes that despite the beneficial effects of EOPG/Quinine co-administration at lower doses, caution is advised.
Keywords: Biochemical, essential oil, hematology, histology, *Piper guineense*, toxicity.

INTRODUCTION

Plants contribute a crucial part to the world of medicine[1-3]. Its use in medicine is as old as the existence of mankind[2,3]. The knowledge of plant use in medicine emanated from man's interaction with his environment, and this early knowledge came from instinct and experience mostly without any scientific basis[3]. About 80% of the population in developing countries relies on plants as sources of primary health care because of cultural beliefs and the absence of modern health facilities. Also, about 11% of the 252 drugs considered "basic and essential" were mainly of plant origin[2].

Crude drugs of natural or biological origin are used to describe plant parts or whole plants with medicinal properties. Both plant-based drugs and plant-derived drugs are of enormous importance in treating different diseases and infections[1,2]. Recent research shows that medicinal plants play a great role, not just in the

treatment of diseases, but also in prevention[2,4]. Plants owe their relevance in medicine to the presence of certain phytochemicals or bioactive ingredients in them, which can be isolated and/or synthesized for use in orthodox medicine. Common examples are quinine, morphine, codeine, vincristine, aspirin, digitalis, etc[5,6]. Some of these plant phytochemicals may need to be structurally optimized into new drugs for better efficacy for use in modern medicine. Thus, plants remain the most abundant natural primary sources of active drugs and are of immeasurable benefit to medicine.

Despite breakthroughs in modern medicine, there are still many diseases and infections for which suitable drugs are yet to be found. Emerging resistance cases with synthetic drugs have fueled great exploration into the use of medicinal plants and the development of more potent drugs[5-7]. A major approach to these emerging problems is the complementary usage of both orthodox medicine and herbal medicine in the management of diseases and infections[8,9]. Herbal medicines are normally mixtures of several complex and diverse phytochemicals, and available reports have indicated that they may sometimes be associated with very toxic phytochemicals present alongside the active principles responsible for their pharmacological effects[10,11]. The multi-component nature of herbal medicines increases the tendency to interact with orthodox drugs[12]. Such interactions can be very toxic at the pharmacokinetics or pharmacodynamics level; hence the combination of herbal medicine and orthodox medicine need to be carefully assessed for possible toxicity[12].

Piper guineense Schum & Thonn (Piperaceae), is among the commonly used plants in many households within the West African region, where the fruits and leaves have been used as condiments to flavor food in both domestic and commercial cuisines[13,14]. The nutritive values of the plant are associated with its spicy taste, high fiber, and mineral content, with traces of protein, carbohydrates, and essential vitamins[15,16]. Several scientifically validated pharmacological uses have been attributed to various parts of the plant, including antinociceptive, anti-inflammatory, anti-oxidant, antihyperglycemic, anti-plasmodial, analgesic, anticonvulsant, muscle relaxant, sedative, and antipsychotic activities[13,14,17-20]. Recently, we reported the antimalarial and neuroprotective effects of the essential oil of *P. guineense* in experimental cerebral malaria[21]. While our report showed that the combination of the essential oil of the dried fruit of *P. guineense* and Quinine has the potential to improve treatment outcomes in mouse models of cerebral malaria, the administration of the combination at doses greater than 6.25 mg/kg caused deaths in tested animals within 24 hours of commencement of treatment, necessitating the need for further evaluation of the toxicity profile of the combination. Moreover, the potential for abusive use of the seeds of *P. guineense* has been reported[16]. Therefore, in this study, we investigated the toxicity potential of the co-administration of Quinine and EOPG, especially at higher doses, with a view to guiding proper usage, prevent toxic damage to vital organs, and take advantage of its beneficial effects for the good of mankind.

MATERIALS AND METHODS

Materials for the study

The following materials were used: plastic animal cages, feed and water containers, dissecting set and board, syringes and needles, disposable gloves, EDTAK3 and universal sample bottles, animal observation cage, cotton wool, tissue papers, microscope, and glass wares. Also used are Tween 80, Quinine, formalin, and distilled water. All the chemicals and reagents were of analytical grade.

Processing of collected plant materials

The sourcing, preparation, and processing of the dried fruits of *P. guineense* by hydro-distillation to obtain essential oil were as earlier reported[21]. The voucher specimen (FPI 2312) was earlier deposited in the Pharmacy herbarium, Obafemi Awolowo University (OAU), Ile-Ife. The oil was prepared in 5% Tween 80 shortly before administration.

Humane care of experimental rats

Healthy adult rats of both sexes and weights ranging from 130-150g were obtained from the Department of Pharmacology Animal House Facility, Faculty of Pharmacy, OAU, Ile-Ife, Nigeria. The rats were kept in standard plastic cages with free access to feeds and water *ad libitum*. Proper hygienic conditions were maintained through constant cleaning and changing of cage beddings. The rats were maintained and cared for according to the international guidelines[22,23], and in line with approved protocols by the Animal Health Research and Ethic Committee of the Institute of Public Health, OAU, Ile-Ife, Nigeria, with ethical clearance number IPH/OAU/12/1782.

Pilot study

The determination of the median lethal dose (LD_{50}) was carried out using a modification of Lorke's method[24] as earlier described[21]. A preliminary assessment was carried out using the proposed highest dose of 150 mg/kg, and its combination with Quinine, to determine the humane endpoint criteria[22,25,26].

Experimental setup and dosage administration

Administration was carried out via the intraperitoneal route for 3 days. Rats were randomly allocated into twelve groups consisting of 10 rats each (two controls and ten test groups). Group 1 is the negative control in which 1 ml/kg 5% Tween 80 was administered. Group 2 is the positive control and was given Quinine standard regimen (20 mg/kg stat, followed by 10 mg/kg twice daily for the next two days). Groups 3-7 were administered 12.5, 25, 50, 100, and 150 mg/kg doses of the essential oil respectively. Groups 8 – 12 were co-administered with the above-graded doses of the essential oil and Quinine standard regimen. After the last administration, surviving animals were randomly divided into two sub-groups – Treated and Recovery. The Treated sub-group was sacrificed 24 hours after the last administration, while the Recovery sub-group was sacrificed after 14 days of a non-dosing recovery period.

Novelty induced behavior observation

Novelty-induced behavior of each animal which includes grooming, rearing, and locomotion; was assessed daily before administration using standard procedures[20,27]. For the recovery set, observational assessments were continued every other day of the 14 days of the non-dosing recovery period.

Weights and weight ratio determination

The body weight of each animal was assessed daily and before sacrifice for both the Treated and Recovery sub-groups. Relative body weights were calculated as the percentage of daily weights relative to the weight on day 0. Also, relative organ weights were calculated as the percentage of organ weight relative to the body weight on the day of sacrifice. The organ-brain weight ratio is the ratio of organ weights relative to the weight of the brain in each animal.

Sample collection

Animals were sacrificed via cervical dislocation. Blood was collected by cardiac puncture into K3 EDTA sample tubes for hematological and biochemical analysis. The brain, liver, and kidney were harvested and weighed. Randomly selected livers were prepared for homogenization and biochemical assessment. Other livers and kidneys were preserved in 10% formalin for histopathological assessment.

Assessment of hematological indices

The hematological indices, including red blood cell (RBC), white blood cell (WBC), hemoglobin concentration (HBC), packed cell volume (PCV) or hematocrit, as well as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), were auto-determined using auto-analyzer as earlier reported[28,29].

Evaluation of plasma and liver homogenate biochemical indices

The K3 EDTA blood samples were subjected to centrifugation at 3000 rpm for 5 minutes to obtain the plasma. The liver homogenate was prepared as earlier described[30]. Quantitative assessment of biochemical parameters including aspartate aminotransferase (AST) and alanine aminotransferase (ALT)[31], cholesterol (CHOL), triglycerides (TRIG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL)were conducted using standard laboratory kits (Randox Laboratories Limited, Crumlin, County Antrim, BT294QY, United Kingdom) in line with manufacturer's protocols.

Histopathological examination

The tissue specimen was prepared on slides mounted in DPX (Distrene Plasticizer and Xylene) with a cover slip using standard histology protocols as earlier reported[28,29,32]. The staining was carefully reviewed under the microscope, and photomicrographs were taken with a LEICA DM750 microscope connected to a digital camera (LEICA ICC 50) and a desktop computer at x400 magnification.

Statistical analysis

Quantitative data were expressed as Mean ± Standard error of the mean (Mean±SEM) and analyzed using one-way analysis of variance (ANOVA) and/or pairwise comparison with Student's T-Test. Data were considered significant at $p< 0.05$.

RESULTS

Effect on the body weight and organ–weight ratio

Results for relative weight change are given in Figure 1A. Decreases in weights as compared to the starting weights were observed in all the Treated and Recovery sub-groups, except with Q/25 and Q/100. The changes in relative weights were significant with EOPG-12.5, EOPG-150, Q/25, and Q/150 for the Treated, and Q/25, Q/100, and Q/150 for the Recovery when compared to Control and/or Quinine (Figure 1A).

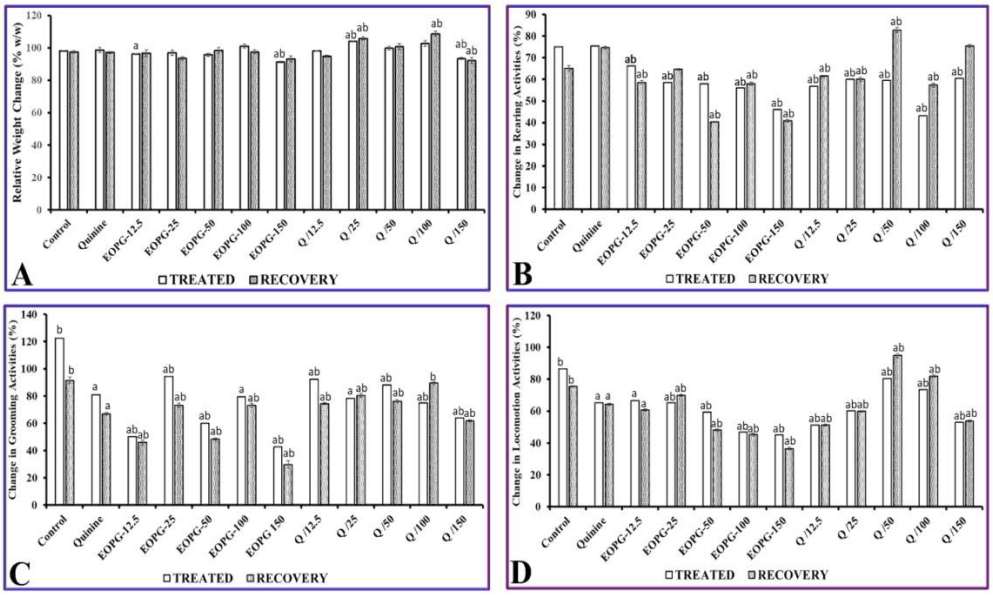

Figure 1: Relative change in weight, rearing, grooming, and locomotion in rats following co-administration of Quinine with EOPG.

Table 1: Organ weight ratios following co-administration of EOPG and Quinine.

	Brain/body	Liver/body	Kidney/body	Liver / brain	Kidney/brain
			Treated		
Control	0.0119±0.0001	0.0324±0.0011	0.0066±0.0004	2.7580±0.0689	0.5570±0.0238
Quinine	0.0113±0.0003	0.0335±0.0013	0.0072±0.0003	2.9800±0.0445	0.6418±0.0138
EOPG-12.5	0.0123±0.0005	0.0395±0.0023	0.0084±0.0001^{ab}	3.2132±0.1039^{a}	0.6840±0.0251^{a}
EOPG-25	0.0112±0.0002	0.0426±0.0022^{a}	0.0083±0.0002^{a}	3.9041±0.0845^{ab}	0.7503±0.0062^{ab}
EOPG-50	0.0119±0.0007	0.0460±0.0018^{ab}	0.0080±0.0002^{a}	3.9267±0.0972^{ab}	0.6771±0.0329
EOPG-100	0.0117±0.0004	0.0542±0.0013^{ab}	0.0094±0.0001^{ab}	4.7082±0.1071^{ab}	0.8121±0.0322^{ab}
EOPG 150	0.0126±0.0004	0.0525±0.0027^{ab}	0.0096±0.0002^{ab}	4.3291±0.0651^{ab}	0.7735±0.0263^{ab}
Q /12.5	0.0123±0.0004	0.0355±0.0004	0.0070±0.0005	3.8863±0.1238^{ab}	0.5718±0.0128^{b}
Q /25	0.0116±0.0006	0.0478±0.0027^{ab}	0.0072±0.0003	4.1355±0.1154^{ab}	0.6191±0.0246
Q /50	0.0125±0.0002	0.0467±0.0003^{ab}	0.0087±0.0001^{ab}	3.7499±0.0512^{ab}	0.7026±0.0114^{a}
Q /100	0.0116±0.0005	0.0490±0.0010^{ab}	0.0092±0.0002^{ab}	4.2570±0.0891^{ab}	0.8036±0.0113^{ab}
Q /150	0.0108±0.0002^{a}	0.0507±0.0037^{ab}	0.0099±0.0004^{ab}	4.6820±0.0811^{ab}	0.9165±0.0261^{ab}
			Recovery		
Control	0.0122±0.0001	0.0368±0.0004^{b}*	0.0075±0.0004	3.0249±0.0069^{b}*	0.6158±0.0032^{b}*
Quinine	0.0127±0.0004*	0.0345±0.0001^{a}	0.0084±0.0004	2.7341±0.0164^{a}*	0.6657±0.0018^{a}
EOPG-12.5	0.0111±0.0001^{ab}	0.0352±0.0006	0.0077±0.0004	3.2266±0.0307^{ab}	0.6994±0.0047^{ab}
EOPG-25	0.0114±0.0007	0.0365±0.0011*	0.0076±0.0001*	3.2166±0.0251^{ab}*	0.6693±0.0042^{ab}*
EOPG-50	0.0102±0.0008	0.0376±0.0012*	0.0072±0.0004	3.7173±0.0191^{ab}	0.7223±0.0052^{ab}*
EOPG-100	0.0123±0.0001	0.0350±0.0008*	0.0073±0.0005*	2.8728±0.0166^{ab}*	0.5926±0.0021^{ab}*
EOPG 150	0.0112±0.0005	0.0383±0.0007^{b}*	0.0078±0.0003*	3.4237±0.0193^{ab}*	0.6915±0.0023^{ab}*
Q /12.5	0.0134±0.0003^{a}*	0.0353±0.0013	0.0075±0.0001	2.6283±0.0118^{ab}*	0.5587±0.0050^{ab}
Q /25	0.0117±0.0004	0.0367±0.0012*	0.0065±0.0003^{b}	3.1377±0.0092^{ab}*	0.5573±0.0022^{ab}*
Q /50	0.0129±0.0004	0.0378±0.0002^{b}*	0.0070±0.0005*	3.9435±0.0428^{ab}*	0.5402±0.0027^{ab}*
Q /100	0.0113±0.0001^{a}	0.0333±0.0006^{a}*	0.0079±0.0004*	2.9430±0.0207^{ab}*	0.6935±0.0029^{ab}*
Q /150	0.0148±0.0002^{ab}*	0.0377±0.0003^{b}*	0.0085±0.0004	2.5728±0.0148^{ab}*	0.5733±0.0015^{ab}*

*, ^{a} and ^{b} indicate significant different when compared to respective Treated sub-group, Control, and Quinine (p<0.05) respectively.

While there were slight improvements in weights following the non-dosing recovery period, such were not significantly different from the Treated, suggesting a persistent nature of toxicity. The results of organ–body weight and organ–brain weight ratios are shown in Table 1. In Treated sub-groups, the brain-body weight ratio did not show any significant changes except the observed significant reduction at Q/150 when compared with Control. However, following the recovery period, more significant changes were observed with increases with Q/12.5, and Q/150, and reduction with EOPG-12.5 and Q/100. These increases were also significantly higher than their respective Treated sub-groups, suggesting potential for persistent toxicity. On the other hand, the liver/body weight ratio revealed a more pronounced toxic effect, with significant increases in all EOPG and Q/EOPG combination doses when compared with Control and Quinine, except with EOPG-12.5, and Q/12.5, suggesting that the toxic effects are dose-dependent and lie more with the higher dose. This is consistent with our earlier published work where the combination of lower doses of EOPG with Quinine showed improved antiplasmodial and neuroprotective effects[21]. Following recovery, the new liver/body weight ratio showed that the effects appear completely reversed when compared to respective Treated sub-groups, though, there were still significant changes at EOPG-150 and higher doses of the combination when compared with recovery Control and/or Quinine.

The observed effects on kidney/body, liver/brain, and kidney/brain ratios were similar to the effects on liver/body weight ratio (Table 1). However, contrary to the liver/body ratio, both the Treated and Recovery sub-groups for kidney/body, liver/brain, and kidney/brain ratios maintained similar significant changes. But, while changes in Treated appear to be dose-dependent increases in ratios, the recovery was not dose-dependent with some showing significant reduction. In general, the Recovery sub-groups showed a significant reduction in these weight ratios when compared with their corresponding Treated group, suggesting a potential for reversal of toxic effects following withdrawal or cessation of administration.

Analysis of novelty-induced behavioral observations

The results for rearing, grooming, and locomotion are presented in Figure 1 (B–D). In Treated, all sub-groups showed a significant decrease in rearing activities when compared to Control and Quinine. Following the recovery period, significant decreases were maintained in all except EOPG-25 and Q/150, which were not significant, and Q/50, which showed significantly higher rearing activities. For grooming and locomotion activities, the animals showed significant decreases in activities when compared to Control and Quinine. However, similar to rearing, Q/50 showed a significantly higher locomotion activity following recovery. The majorly significant reduction in rearing, grooming, and locomotion activities for treatment and recovery period, suggests a form of persistent toxic effects.

Effect on haematological indices

The analysis of blood indices (Table 2) showed varying degrees of significant changes when compared with Control and/or Quinine. While red blood cells (RBC), white blood cells (WBC), hematocrits or packed cell volume (PCV), and hemoglobin concentration (HBC) showed mostly dose-dependent decreases in values,

mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were mostly significantly increased when compared to Control and Quinine. The observed dose-dependent reduction in RBC, PCV, and HBC, as well as the corresponding significant increases in MCV, MCH, and MCHC, suggest potential toxic effects on the blood cells, which may be unconnected with any extraneous infections as indicated by reduced WBC. The situation is similar following the recovery period, and though there were signs of reversal with RBC, WBC, PCV, and HBC, the dose-dependent significant increases in MCV, MCH, and MCHC, which were also significantly higher than the respective Treated sub-groups, may be an indication of persistent toxicity.

Biochemical Result Analysis

The results of biochemical assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol (CHOL), triglycerides (TRIG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and protein levels using liver homogenates and plasma are presented in Table 3 and Table 4. The results from the analysis of the liver biochemical indices (Table 3) showed a dose-dependent significant reduction in liver proteins with EOPG alone, and varying degrees of significant reduction with Quinine/EOPG combination when compared with Control and Quinine. The recovery showed a similar pattern, except for a significant reduction in values when compared with the treated groups, indicating a potential for reversal of toxic effects. The significant dose-dependent increases in CHOL/HDL, TRIG/HDL, and LDL/HDL ratios, as well as non-HDL (the bad lipids), which were especially higher with higher doses of EOPG alone and in all combinations, is an indication of increased potential risk of heart problems. However, while these lipid ratios showed a good sign of recovery, recovery from AST/ALT alteration may take a longer time, suggesting a potential for persistent toxic effects.

Furthermore, the results from the plasma biochemical evaluation (Table 4) were similar to that of the liver homogenate, with some variations. Using EOPG alone, dose-dependent increases in values were observed with non-HDL, as well as AST/ALT, CHOL/HDL, and LDL/HDL ratios, as well as dose-dependent decreases in TRIG/HDL ratio. On the other hand, with the combination of Quinine with EOPG, dose-dependent increases were seen with AST/ALT, CHOL/HDL, TRIG/HDL, and non-HDL, while LDL/HDL showed a dose-dependent decrease. Unlike in the liver homogenate, LDL/HDL was not associated with increased values in all tested groups, but instead, TRIG/HDL, which was low in liver samples. In any case, significantly higher values of AST/ALT, CHOL/HDL, TRIG/HDL, and non-HDL are clear indications of toxicity, especially an increased risk of heart disease. However, similar to liver samples, there were signs of potential recovery, as seen with a significant reduction in values when compared with the Treated group. However, the rate of reduction appears quite slow, confirming a high potential for persistent toxicity.

Table 2: Effects of co-administration of EOPG and Quinine on hematological indices.

	RBC x 10^6 (cell/µL)	WBC x 10^3 (cells/µL)	PCV (%)	HBC (g/dl)	MCV (fl/cell)	MCH (pg/cell)	MCHC (g/dl)
Treated							
Control	11.80±0.18	6.57±0.38	41.00±0.21	14.26±0.66	34.92±0.82	12.22±0.19	34.95±0.24
Quinine	10.47±0.53	5.86±0.15	38.00±1.15	13.56±1.08	36.48±0.21	12.92±0.50	35.68±0.70
EOPG-12.5	8.55±0.58^{a}	5.81±0.16	41.33±1.20	13.28±1.04	48.70±1.02^{ab}	15.52±0.49^{ab}	32.16±0.58^{ab}
EOPG-25	12.05±1.00	5.85±0.29	32.00±1.53^{a}	14.30±0.38	26.76±1.48^{ab}	12.02±0.25	44.82±1.45^{ab}
EOPG-50	9.00±1.38	5.30±0.37	27.33±1.20^{ab}	13.01±1.17	31.51±0.77^{b}	14.92±0.19^{ab}	47.40±1.29^{ab}
EOPG-100	9.20±0.90	5.42±0.11	36.00±0.65^{a}	13.21±0.86	39.35±0.18^{ab}	14.79±0.25^{a}	37.36±0.77
EOPG 150	6.72±0.31^{ab}	4.89±0.12^{ab}	29.33±1.33^{ab}	10.83±0.47^{a}	43.93±1.34^{ab}	16.19±0.14^{ab}	37.17±0.11^{a}
Q/12.5	5.67±0.68^{ab}	5.40±0.14	40.00±1.15	13.56±0.56	43.58±1.05^{ab}	23.78±0.19^{ab}	34.05±0.32
Q/25	7.47±0.53^{ab}	5.01±0.67	31.33±0.71^{ab}	12.86±0.62	41.93±1.37^{ab}	17.28±0.26^{ab}	41.05±1.06^{ab}
Q/50	7.10±0.37^{a}	5.41±0.54	38.67±0.67	12.16±0.97	42.61±0.64^{ab}	13.49±0.36	31.48±0.71^{ab}
Q/100	6.68±0.35^{ab}	4.81±0.44	26.50±0.50^{ab}	10.43±0.47^{a}	39.53±0.12^{ab}	15.55±0.40^{ab}	39.31±0.36^{ab}
Q/150	5.25±0.82	4.25±0.18^{ab}	39.33±0.33^{a}	12.04±0.39	42.60±1.06^{ab}	13.18±0.46	30.89±0.79^{ab}
Recovery							
Control	11.28±0.53	6.18±0.17	46.67±0.08^{b}*	13.87±0.18	41.47±0.28^{b}*	12.36±0.14^{b}	29.78±0.67*
Quinine	12.23±0.49	6.31±0.19	42.33±0.09a*	13.16±0.28	34.78±0.16^{a}	10.79±0.13a*	31.12±1.09*
EOPG-12.5	11.50±0.24*	6.15±0.16	43.67±0.08^{ab}	12.81±0.29	38.99±0.80b*	11.36±0.12a*	29.36±0.92
EOPG-25	9.69±0.19b	5.95±0.18	47.00±0.10^{b}*	15.24±0.41^{b}	47.01±0.24^{ab}*	16.00±0.35^{ab}*	34.25±0.31a*
EOPG-50	11.25±0.39	5.55±0.13	43.50±0.50a*	12.22±0.46	38.78±0.81^{b}*	10.98±0.16a*	28.27±1.08*
EOPG-100	8.93±0.41b	5.40±0.12^{ab}	40.00±0.20^{ab}*	13.37±0.28	45.39±0.38^{ab}*	15.30±0.21^{ab}	33.56±1.02
EOPG 150	8.54±0.01^{ab}*	6.39±0.24*	43.50±0.50a*	15.58±0.31^{ab}*	50.94±1.02^{ab}*	18.24±0.32^{ab}*	35.91±0.39^{ab}
Q/12.5	8.97±0.03^{ab}*	6.31±0.28*	43.67±0.96	13.37±0.17	50.20±1.40^{ab}*	15.29±0.23^{ab}*	30.58±0.65*
Q/25	8.20±0.06^{ab}	6.37±0.09	37.67±0.33^{ab}*	13.30±0.42	45.95±0.08^{ab}*	16.22±0.17^{ab}*	35.30±0.17^{ab}*
Q/50	9.51±0.24^{b}	5.28±0.18^{ab}	39.00±0.30^{ab}	15.03±0.32^{b}*	42.39±1.18^{b}	16.41±0.11^{ab}	38.63±0.13^{ab}*
Q/100	9.31±0.44^{b}*	6.68±0.22*	45.67±0.28^{b}*	15.73±0.28^{ab}*	49.06±0.57^{ab}*	17.03±0.13^{ab}*	34.72±0.22^{a}*
Q/150	8.17±0.22^{ab}	5.12±0.17^{ab}*	41.00±0.10^{ab}	15.45±0.15^{ab}*	49.58±1.08^{ab}*	19.22±1.06^{ab}*	39.03±0.17^{ab}*

*, ^{a} and ^{b} indicate significant different when compared to respective treated sub-group, Control, and Quinine (p<0.05) respectively.

Table 3: Effects of co-administration of EOPG and Quinine on liver biochemical indices.

	Protein	AST/ALT	CHOL/HDL	LDL/HDL	TRIG/HDL	non-HDL
Treated						
Control	1.37±0.01^{b}	0.76±0.06	1.95±0.02^{b}	0.45±0.02^{b}	1.03±0.05^{b}	2.09±0.06^{b}
Quinine	1.68±0.04^{a}	0.85±0.03	2.44±0.08^{a}	1.14±0.06^{a}	0.65±0.07^{a}	3.07±0.10^{a}
EOPG-12.5	1.21±0.02^{ab}	4.24±0.06^{ab}	2.00±0.04^{b}	0.46±0.00^{b}	0.47±0.02^{a}	3.28±0.05^{a}
EOPG-25	1.11±0.03^{ab}	7.32±0.03^{ab}	1.70±0.01^{ab}	0.55±0.00^{ab}	0.47±0.01^{a}	5.25±0.09^{ab}
EOPG-50	1.03±0.02^{ab}	10.52±0.06^{ab}	1.68±0.05^{ab}	0.84±0.04^{ab}	0.23±0.01^{ab}	4.50±0.32^{ab}
EOPG-100	0.87±0.01^{ab}	8.38±0.07^{ab}	2.48±0.07^{a}	1.08±0.01^{a}	0.26±0.01^{ab}	9.48±0.50^{ab}
EOPG 150	0.75±0.02^{ab}	3.79±0.01^{ab}	2.70±0.04^{ab}	1.08±0.00^{a}	0.16±0.01^{ab}	7.84±0.23^{ab}
Q /12.5	0.91±0.02^{ab}	5.16±0.02^{ab}	2.18±0.04^{a}	0.58±0.00^{ab}	1.80±0.03^{ab}	2.99±0.17^{a}
Q /25	1.28±0.05^{b}	4.94±0.16^{ab}	2.19±0.07	0.77±0.03^{ab}	1.32±0.01^{ab}	5.41±0.35^{ab}
Q /50	1.08±0.02^{ab}	3.31±0.03^{ab}	4.56±0.10^{ab}	3.04±0.10^{ab}	1.15±0.05^{b}	7.72±0.07^{ab}
Q /100	1.23±0.04^{b}	3.12±0.03^{ab}	4.64±0.03^{ab}	3.42±0.02^{b}	0.49±0.02^{a}	8.01±0.08^{ab}
Q /150	1.03±0.05^{ab}	2.13±0.04^{ab}	5.53±0.07^{a}	4.39±0.04^{a}	0.45±0.01^{a}	8.11±0.15^{ab}
Recovery						
Control	1.55±0.03^{a}	0.98±0.04	1.62±0.11^{b}	0.43±0.04^{b}	0.74±0.03^{b}	1.86±0.29
Quinine	1.01±0.09^{b}	0.99±0.02	2.17±0.06^{a}	0.80±0.04^{a}	1.29±0.08^{a}	2.51±0.13
EOPG-12.5	1.02±0.03^{a}	3.64±0.06^{ab}*	3.06±0.09^{ab}*	1.49±0.01^{ab}*	0.97±0.02^{ab}*	6.73±0.30^{ab}*
EOPG-25	0.79±0.02^{a}	4.04±0.16^{ab}*	1.61±0.05^{b}	0.21±0.01^{ab}*	0.34±0.00^{ab}*	4.65±0.39^{ab}*
EOPG-50	0.80±0.03^{a}	7.10±0.30^{ab}*	1.42±0.04^{b}	0.26±0.01^{ab}*	0.26±0.01^{ab}	2.45±0.21*
EOPG-100	0.86±0.03^{a}	5.42±0.17^{ab}*	3.29±0.08^{ab}*	1.95±0.03^{ab}*	0.27±0.00^{ab}	5.73±0.27^{ab}*
EOPG 150	0.91±0.05^{a}	2.66±0.12^{ab}*	1.20±0.02^{ab}*	0.29±0.01^{b}*	0.08±0.00^{ab}*	2.05±0.25*
Q/12.5	1.17±0.03^{a}	3.36±0.06^{ab}*	2.62±0.06^{ab}*	1.18±0.02^{ab}*	1.29±0.04^{a}*	5.88±0.31^{ab}*
Q/25	1.05±0.03^{a}	2.82±0.09^{ab}*	2.25±0.12^{a}	0.86±0.09^{a}	0.89±0.07^{b}*	5.72±0.16^{ab}
Q/50	1.09±0.10^{a}	2.68±0.05^{ab}*	2.21±0.03^{a}*	0.68±0.03^{a}*	1.00±0.01^{ab}*	4.21±0.06^{ab}*
Q/100	1.13±0.04^{a}	2.20±0.05^{ab}*	1.79±0.08^{b}	0.52±0.02^{b}*	0.49±0.02^{ab}	2.50±0.12
Q/150	1.18±0.03^{a}	2.22±0.06^{ab}	2.04±0.01^{a}*	0.88±0.01^{a}*	0.40±0.01^{ab}	3.46±0.06^{ab}*

*, ^{a} and ^{b} indicate significant different when compared to respective treated sub-group, Control, and Quinine (p<0.05) respectively.

Histology Result

The histological assessment of the kidney in the Treated sub-groups (Figure 2) revealed that all the sub-groups show normal histoarchitecture with well-delineated glomeruli, proximal convoluted tubules, and distal convoluted tubules, except Q/150 which shows some signs of necrosis, and EOPG-12.5, EOPG-50 and Quinine which show signs of distorted proximal and distal convoluted tubules when compared with the other groups. During the following period of non-dosing recovery, all the Recovery sub-groups showed and/or maintained near-to-normal histoarchitecture with well-delineated glomeruli, proximal convoluted tubules, and the distal convoluted tubules, except Q/25 where the distal convoluted tubules are not well represented. This suggests potential for reversal of kidney toxicity (Figure 3).

In addition, the assessment of liver histology in Treated sub-groups (Figure 4) revealed that EOPG-25 and EOPG-150 showed some aberrations in the histoarchitecture of the hepatocytes when compared with the other groups. Treated sub-groups Q/12.5, Q/25, and Q/100 showed signs of necrosis and distortion of the hepatocytes with dilated sinusoids when compared with the others (Figure 4). Thus, the result showed that EOPG alone or in combination with Quinine has the potential to induce liver toxicity. Following the period of recovery, all the Recovery sub-groups showed normal hepatocytes (Figure 5). However, Quinine showed deposition of plaques, while Q/100 and Q/150 showed dilated sinusoid and deposition of brownish plaques respectively (Figure 5), suggesting a form of persistent liver toxicity at higher doses of EOPG combination with Quinine.

DISCUSSION

Toxicology involves an observational and data-gathering phase and the use of those data to predict likely outcomes upon exposure to human populations[34,35]. This is very vital, especially in the use of herbal medicines, which are as old as human civilization[3,10]. Plants are useful not only in the treatment and management of many disease conditions but also in the discovery of potential bioactive molecules[5-7]. However, alongside the beneficial components present in plants are also some toxic secondary metabolites produced as natural defense mechanisms[11]. With over 80% of the world's population still depending on the use of medicinal plants for their primary healthcare needs, there the growing concerns about the potential toxicity associated with the use of these medicinal plants, and thus, the need to establish their safety[36]. Also, the conscious or unconscious co-administration of orthodox medicine with medicinal plant products is not uncommon, and though some beneficial effects of such combination have been reported[8,12,37,38], concerns about their safety remain. We have earlier reported that the combination of the essential oil of the dried fruit of *P. guineense* and Quinine has the potential to improve treatment outcomes in mouse models of cerebral malaria.

Table 4: Effects of co-administration of EOPG and Quinine on plasma biochemical indices.

	Protein	AST/ALT	CHOL/HDL	LDL/HDL	TRIG/HDL	non-HDL
Treated						
Control	0.76±0.02^{b}	1.53±0.07^{b}	1.91±0.01^{b}	0.88±0.06^{b}	0.40±0.01^{b}	1.61±0.13^{b}
Quinine	0.94±0.04^{a}	4.84±0.05^{a}	3.12±0.02^{a}	1.83±0.05^{a}	0.53±0.02^{a}	4.90±0.08^{a}
EOPG-12.5	1.88±0.02^{ab}	3.32±0.02^{ab}	2.06±0.05^{b}	0.15±0.01^{ab}	2.09±0.03^{ab}	2.80±0.09^{ab}
EOPG-25	1.19±0.03^{ab}	2.99±0.08^{ab}	2.21±0.06^{ab}	0.65±0.02^{ab}	1.24±0.02^{ab}	4.76±0.02^{a}
EOPG-50	1.35±0.07^{ab}	3.08±0.05^{ab}	2.33±0.05^{ab}	1.14±0.02^{ab}	1.16±0.02^{ab}	5.56±0.13^{ab}
EOPG-100	1.92±0.06^{ab}	2.97±0.03^{ab}	2.88±0.01^{ab}	1.78±0.06^{a}	0.32±0.01^{ab}	7.60±0.14^{ab}
EOPG 150	1.75±0.02^{ab}	3.03±0.04^{ab}	3.16±0.03^{a}	2.01±0.02^{a}	0.29±0.01^{ab}	8.74±0.09^{ab}
Q/12.5	0.59±0.01^{b}	4.11±0.02^{ab}	2.95±0.01^{ab}	1.85±0.05^{a}	0.75±0.03^{ab}	2.78±0.01^{ab}
Q/25	0.67±0.03^{b}	4.65±0.04^{a}	2.89±0.08^{a}	1.50±0.05^{ab}	1.67±0.04^{ab}	2.98±0.03^{ab}
Q/50	0.68±0.01^{b}	4.90±0.01^{a}	3.13±0.02^{a}	1.53±0.03^{ab}	3.88±0.03^{ab}	3.24±0.01^{ab}
Q/100	0.78±0.01^{b}	4.64±0.02^{ab}	3.94±0.08^{ab}	1.37±0.00^{ab}	4.74±0.04^{ab}	3.91±0.03^{ab}
Q/150	0.88±0.03	5.23±0.01^{ab}	4.16±0.05^{a}	1.19±0.03^{ab}	5.69±0.01^{ab}	4.23±0.06^{ab}
Recovery						
Control	0.90±0.01	2.63±0.01^{b}	4.08±0.02^{b}	2.40±0.01^{b}	1.35±0.04^{b}	2.41±0.04^{b}
Quinine	0.85±0.02	4.80±0.04^{a}	1.09±0.01^{a}	0.20±0.01^{a}	0.27±0.01^{a}	0.39±0.03^{a}
EOPG-12.5	1.48±0.02^{ab}	2.60±0.02^{b}	1.54±0.06^{ab}	0.12±0.01^{ab}	1.68±0.07^{ab}	1.57±0.15^{ab}
EOPG-25	1.53±0.03^{ab}	3.13±0.02^{ab}	1.97±0.02^{ab}	0.58±0.01^{ab}	0.95±0.01^{ab}	5.37±0.15^{ab}
EOPG-50	1.69±0.03^{ab}	3.66±0.03^{ab}	2.10±0.05^{ab}	0.91±0.02^{ab}	0.79±0.00^{ab}	6.14±0.04^{ab}
EOPG-100	1.70±0.00^{ab}	3.44±0.03^{ab}	2.16±0.03^{ab}	1.60±0.03^{ab}	0.58±0.00^{ab}	7.17±0.17^{ab}
EOPG 150	1.85±0.02^{ab}	3.55±0.04^{ab}	2.72±0.07^{ab}	1.71±0.02^{ab}	0.35±0.01^{ab}	8.74±0.09^{ab}
Q/12.5	0.80±0.02^{a}	3.73±0.03^{ab}	1.81±0.03^{ab}	1.52±0.03^{ab}	0.92±0.04^{ab}	2.71±0.07^{ab}
Q/25	0.86±0.03	3.74±0.06^{ab}	2.60±0.03^{ab}	0.50±0.05^{ab}	0.97±0.04^{ab}	3.23±0.10^{ab}
Q/50	0.89±0.06	3.49±0.02^{ab}	2.67±0.02^{ab}	0.94±0.03^{ab}	2.44±0.01^{ab}	3.35±0.19^{ab}
Q/100	0.95±0.04	3.42±0.03^{ab}	2.79±0.03^{ab}	0.94±0.06^{ab}	2.61±0.03^{ab}	3.85±0.05^{ab}
Q/150	1.03±0.03^{ab}	3.22±0.04^{ab}	2.86±0.05^{ab}	0.71±0.02^{ab}	3.54±0.06^{ab}	4.83±0.21^{ab}

*, ^{a}and^{b}indicate significant different when compared to respective Treated sub-group, Control, and Quinine ($p< 0.05$) respectively.

However, the observed deaths associated with the co-administration of the EOPG and Quinine at doses greater than 6.25 mg/kg EOPG within 24 hours of the commencement of treatment[21], necessitated the need for further toxicological evaluation.

Body and organ weights are useful parameters in assessing the toxicity of substances which can be revealed through an increase or decrease in weight[39,40]. Weight reduction has been associated with consequent depression of cellular metabolism and growth or cell death, and a dose capable of causing a 10% decrease in body weight, is considered a toxic dose[39,40]. While the observed mostly reduction in weights in the Tested and Recovery sub-groups (Figure 1A) may not be significant when compared with the starting weights of the animals, the significant changes in relative weights at EOPG-12.5, EOPG-150, Q/25, and Q/150 for Treated set, and Q/25, Q/100 and Q/150 for Recovery, and lack of significant different between Treated and Recovery sub-groups (Figure 1) demonstrated that the toxic effects of EOPG and its combination with Quinine on weights may be persistent.

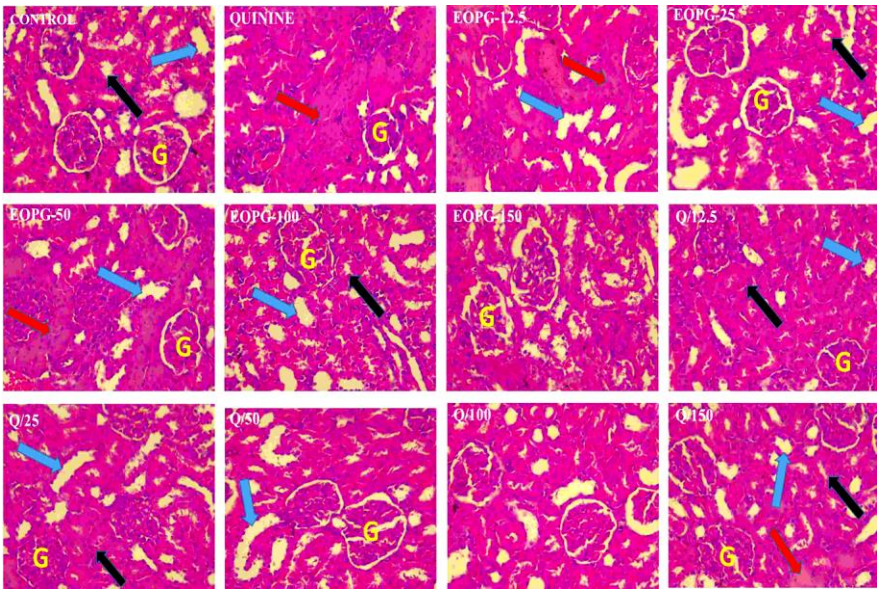

Figure 2: Photomicrographs of rat's kidney following co-administration of EOPG and Quinine.

Glomeruli (G), proximal convoluted tubules (blue arrows), and the distal convoluted tubules (black arrows). Red arrows indicate signs of necrosis and/or distorted proximal and distal convoluted tubules.

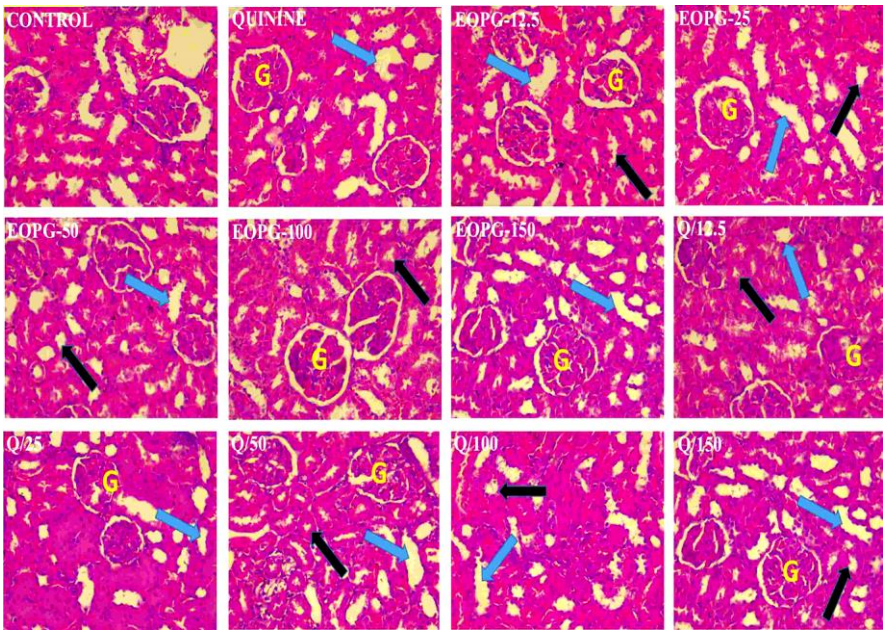

Figure 3: Photomicrographs of rat's kidney following non-dosing recovery period.
Glomeruli (G), proximal convoluted tubules (blue arrows), and the distal convoluted tubules (black arrows).

Organ weight analysis is a useful endpoint in determining organ toxicity[39,41-43]. An increase in liver weight, which may be in the form of hepatocellular hypertrophy (increase in size) or hyperplasia of organelles (increase in number), is majorly indicative of toxicity[29,44]. Obtained results showed a more persistent toxic effect on the liver compared to the kidney, and though alteration in organ/body and organ/brain ratios remains significant when compared with Control and Quinine, the kidney stands a better chance of faster recovery (Table 1). This may not be unexpected since the liver has greater exposure to tested substances than any other body organ. The histology results (Figure 2 – Figure 5) also confirmed these observations and clearly showed that liver toxicity is more persistent. Furthermore, it confirmed that toxicity to the kidneys may be more associated with EOPG or Quinine alone rather than with their respective combination, suggesting that the Q/EOPG co-administration may have potential kidney protective roles at EOPG doses less than 150 mg/kg (Figure 2 – Figure 5). Histological examination is a golden standard for evaluating treatment-related pathological changes in tissues and organs. This complements chemical pathological data with morphological pathological findings and gives a piece of more holistic information on toxicity. Our current histological findings pointed to the fact that the use of EOPG alone or with quinine may precipitate organ damage. Hence, there is a need to exercise caution in the long-term use of the oil.

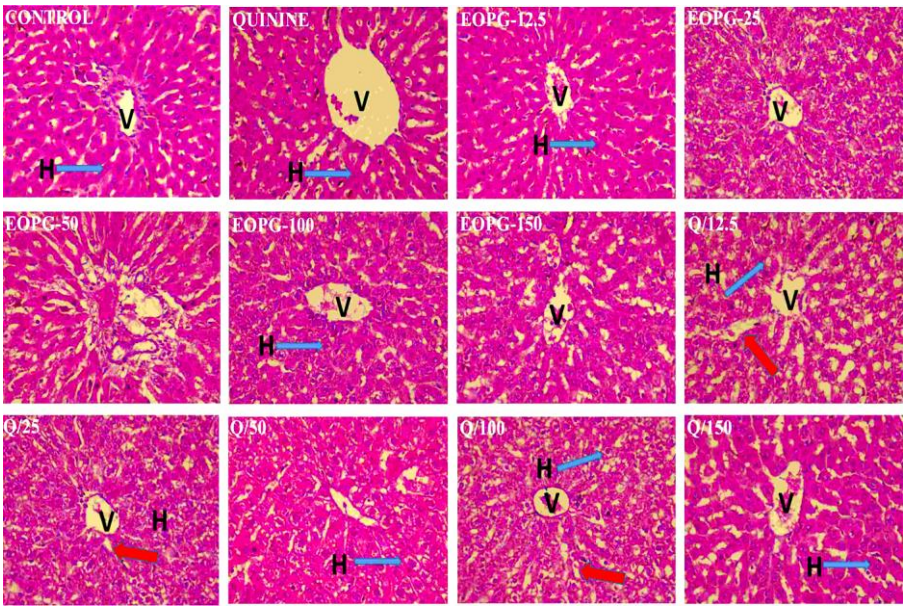

Figure 4: Photomicrographs of rat's liver following co-administration of EOPG and Quinine.
H: Hepatocytes; V: Central vein; dilated sinusoid (red arrows)

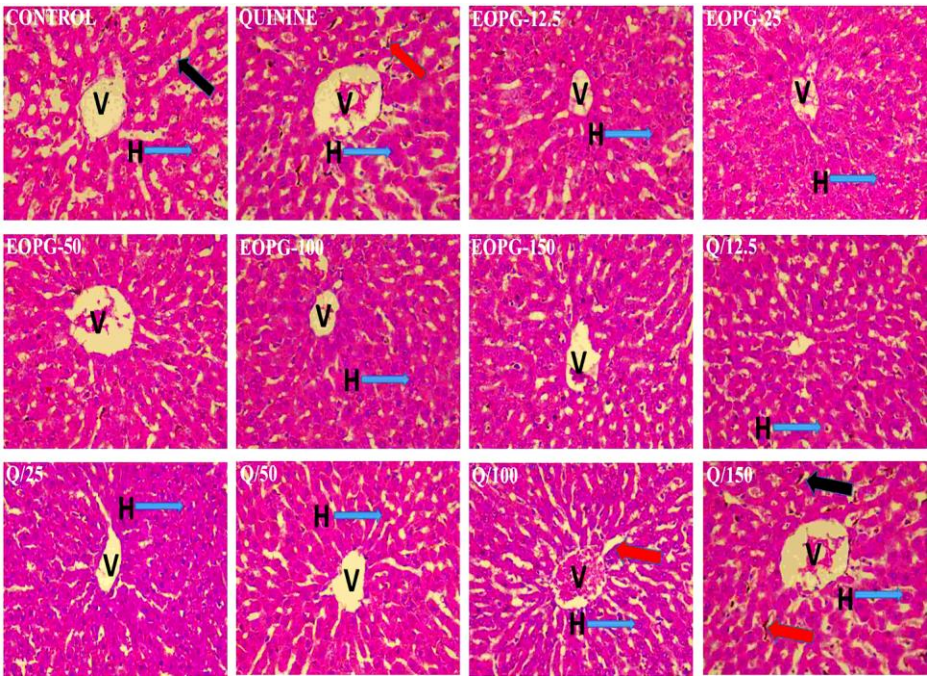

Figure 5: Photomicrographs of rat'sliver following non-dosing recovery period.
H: Hepatocytes; V: Central vein

In addition, the biochemical analysis of liver homogenate and plasma further confirmed that the liver is adversely affected by the test substances (Table 3 and Table 4). The biochemical assessment is a fundamental tool in evaluating not just the extent and severity of organ damage but also the type of organ damage[29,45,46]. Liver biomarkers in the biochemical assessment include aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline phosphatase (ALP), etc. ALP is located in the cytoplasm and is released into circulation after cellular damage. ALT is found in the cytoplasm, liver, kidney, heart, and muscle but its highest concentration is in the liver[47]. ALT is a more specific indicator of liver damage as AST elevations may be caused by damage to other organs[45,47]. Elevation of the level of these enzymes indicates cellular damage, leakages, and loss of functional integrity of the hepatic cell membrane[45,47,48]. Elevation in the cholesterol and triglyceride levels of the liver, due to their accumulation within the hepatocytes can result in steatosis and primary lipotoxicity[49]. With significant increases in proteins, AST, ALT, cholesterol (CHOL), triglyceride (TRIG), low-density lipoproteins (LDL), non-HDL (high-density lipoprotein) and the correspondingly higher ratios of AST/ALT, CHOL/HDL, TRIG/HDL, and LDL/HDL, in both Treated and Recovery sub-groups, our results confirmed the superior and persistent assaults on the liver. However, in general, even though AST/ALT ratios are significantly higher with EOPG alone, the significantly lower values of CHOL/HDL, TRIG/HDL, and LDL/HDL ratios when compared with corresponding Q/EOPG combination, suggest that the risk of heart problems is less likely with EOPG alone and more likely with the combination. This may explain the reason for the 100% deaths following EOPG/Quinine co-administration in parasitized mice as reported in our previous study[21]. Such toxic effects may also be exacerbated by the load of the malaria parasite. Furthermore, novelty-induced behavior (NIB) change is a sensitive endpoint in assessing how animals react to changes in their internal or external environment. Reduction in NIB may be a result of neuro-suppressant activity or physiological changes causing weakness. In this study, the observed majorly significant, and yet persistent reduction in rearing, grooming, and locomotion activities confirmed the neuro-behavioural activities of EOPG as earlier reported[20,21].

Meanwhile, the assessment of hematological parameters can be used to determine the extent of the toxic effects of xenobiotics, including plant extracts, on the blood. Blood components are diagnostic and can be used for predicting human toxicity[29,50].White blood cells are the first line of cellular defense that fight off infectious agents and respond to any inflammation or tissue injury, and their elevation may indicate induction of immune response by the test substance[51]. Red blood indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are useful parameters in diagnosing anemia[52,53]. With the dose-dependent reduction in RBC, PCV, and HBC, as well as the corresponding significant increases in MCV, MCH, and MCHC in Treated and Recovery sub-groups, our results showed higher chances of blood-related toxic effects, especially with higher doses of EOPG alone and in combination with Quinine.

Limitations of the study

This is an animal-based research output and may only be used to guide further evaluation in humans or provide guidance for the safe use of the plant in humans.

CONCLUSIONS

In present study safety profile of the co-administration of a standard dosage regimen of Quinine with EOPG using neuro-behavioral, biochemical, hematological, and histopathological assessments was explored. Obtained results revealed varying degrees of alteration in weights, organ/weight ratios, biochemical and hematological indices, and histopathological features. It was proposed that the earlier reported death associated with the co-administration of EOPG with Quinine in experimental cerebral malaria may be associated with increased toxicity on the liver and risk of heart-related diseases, coupled with increased parasite load. We conclude that while the use of EOPG/Quinine co-administration at a lower dose was beneficial in experimental cerebral malaria, there is a need for caution in its use.

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AUTHOR'S CONTRIBUTION

DANIYAN MO: conceptualization and project administration. **OGUNYEMI TH:** initial draft of the manuscript. **OLAWUNI IJ:** quantitative and qualitative data analysis and interpretations. **ASIYANBOLA ID:** performed the experiments. **ADELODUN ST:** data analysis and interpretations. **OPOGGEN TS:** performed the experiments. **OYEMITAN IA:** data analysis and interpretations. All authors reviewed and edited the manuscript, and approved the final submission.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The Authors declare no conflict of interest.

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