Synergistic effect of aqueous extract of Telfaria occidentalis on the biological activities of artesunate in Plasmodium berghei infected mice

Adegbolagun Olayemi M[1*], Emikpe Benjamin O[2], Woranola Ibukun Oluwa O[1] and Ogunremi Yetunde[3]

1. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan.
2. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.
3. Department of Clinical Pharmacy and Pharmacy Management, Faculty of Pharmacy, University of Ibadan.

Abstract

Background: Resistance to most antimalarial drugs has encouraged the use of herbal preparations along with prescribed orthodox drugs.

Objective: this study investigated effect of co-administration of aqueous extract of *T. occidentalis* leaves, commonly used as antimalarial and haematinic agent in Nigeria, and artesunate using *P. berghei* animal model.

Methods: In vivo curative antiplasmodial effect of *T. occidentalis* (200mg/kg) alone and in combination with artesunate (2mg/kg) were evaluated using albino mice infected with 10^6 parasitized erythrocytes of *P. berghei* intraperitoneally. The haematological parameters: haemoglobin level, red blood cells and white blood cells and packed cell volume were monitored using standard methods.

Results: Aqueous extract of *T. occidentalis,* artesunate and the combination gave 72.17±4.07%, 70.43± 4.27% and 85.43± 3.65% reduction in parasitaemia after 48hours respectively. A significant enhancement of the PCV was obtained with the co-administration of artesunate and aqueous extract ($p < 0.01$). Similar trends were also observed with heamatological parameters at 72 hours of administration.

Conclusion: This study revealed a synergistic effect of the co-administration on parasite clearance rate of *P. berghei* infection in mice, with a significant enhancement of haematological parameters within 48 hours of administration. This indicates a rapid rate of recovery from plasmodial infections with the co-administration.

African Health Sciences 2014;14(1): 111-118 http://dx.doi.org/10.4314/ahs.v14i1.17

Introduction

Malaria is a life threatening parasitic infection caused by Plasmodium species and the most virulent to man is *Plasmodium falciprum*. Malaria is a common disease in most tropical countries with over 120 million cases reported annually resulting in the death of about 3 million people annually [1]. The disease which isendemic in Sub-Saharan Africa with children under the age of five and pregnant women at risk [2] is among the top three deadly communicable diseases and it is the most deadly tropical disease despite various efforts towards its control [3].

***Correspondence author:**
Adegbolagun Olayemi M.
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, 200005, Oyo State, Nigeria .
Mobile phone: 2348023451393,
E-mail: duplag03@yahoo.com, om.adegbolagun@mail.ui.edu

The provision of effective chemoprophylaxis and treatment of malaria is still a major problem in tropical countries [4].

Chemotherapeutic management of malaria has been bedeviled by various problems as a result of development of resistance to most of the earlier drugs like chloroquine, amodiaquine and other drugs [5]. This has led to the development of newer drugs of which artemisinine derivatives have been widely accepted. Artemisinin antimalarial drugs derived from the extract of a Chinese herb '*Quinhaosu*' used for the treatment of fevers have in the past three decades been reported to be efficacious in clinical management of chloroquine-resistant malaria [6, 7].

Artesunate, a pro-drug of dihydroartemisinine is about the most widely used of all the artemisinin derivatives. It is the most rapidly acting of the artemisinin derivatives, exhibiting rapid absorption after oral and intramuscular administration and rapid elimination [8]. Artesunate have been reported to be effective in uncomplicated, severe and multidrug resistant malaria. It is a key member of the artemisinin – combination therapy (ACT) currently

approved for the management of malaria by WHO.

The current regimen for the management of malaria comprising the use of artemisinin derivatives with other antimalarial agents is currently having problems with implementation in developing countries due to affordability and accessibility constraints. The rising cost of prescription drugs in the maintenance of personal health has resulted in increased interest in the use of medicinal plants as a re-emerging health aid [9]. This has led to re-awakened interest in the exploration of medicinal plants for the management of malaria. Herbal medicine is becoming increasingly popular in both developed and developing countries [10, 11].

The level of confidence placed on herbal preparations has encouraged their use along with prescribed orthodox drugs and over-the-counter (OTC) drugs for various diseased conditions [12,13,14,15,16].

Telfarias occidentalis, a member of the Cucurbitaceae family popularly known as fluted pumpkin is highly reputed in traditional medicine practice in Nigeria [17]. It is commonly called *Ugu* in most parts of Nigeria with different parts of the plant being employed for various diseased conditions ranging from convulsion, malaria and anaemia [18]. The leaves are rich in essential and non-essential amino acids, vitamins and minerals [21]. Aqueous extracts of *T. occidentalis* leaves is used locally in Nigeria as antimalaria and haematinic agent [22].

The aqueous extract of the leaves have been reported to possess significant enhancement of haematological parameters (erythrocyte count, packed cell volume, heamoglobin concentration and white blood cell count), antiparasitic effect on *P. berghei* infected mice as well as hypoglycaemic activity [23, 24, 25]. In view of the increasing rate at which herbal preparations are used with orthodox medicines, there is the need to evaluate the clinical implications of co-administration of herbal preparations with orthodox medicines.

Earlier reports on the enhancement of heamatological parameters as well as antiparasitic activity of aqueous extract of the leaves of *T. occidentalis* prompted this study. The study was aimed at evaluating the possible clinical implications of the co-administration of *T. occidentalis* with artesunate in order to proffer support or question the feasibility of developing a cost–effective and efficacious management for malaria using a combination of herbs and antimalarials especially in poor resourced economies.

This present investigation evaluated the effect of the co-administration of crude aqueous extract of *T. occidentalis* leaves; at an earlier reported antiparasitic concentration of 200mg/Kg body weight and artesunate at 2mg/Kg body weight on the antiplasmodial activity and haematological parameters using *P. berghei* animal model.

Methods

Plant material

Fresh leaves of *T. occidentalis* (fluted pumpkin) were collected from a local farm within the University of Ibadan, the plant was identified and authenticated by Mr. D. Esimekhwai of the Department of Botany, University of Ibadan. A voucher specimen was deposited in the Herbarium of the Department with voucher number UIH 22357.

Preparation of aqueous extract

The extracts were prepared as earlier described by Salman, et al., 2008 [25]. Briefly, fresh leaves were air-dried at room temperature (28 – 30^{o}C). The dry leaves were reduced to coarse powder by grinding. 200g of the powdered leaves was soaked in 400L of distilled water for 48hrs with mixing at intervals, after which it was filtered using muslin cloth. The filtrate was concentrated using rotary evaporator followed by drying in vacuum oven at 50^{o}C. The dry thick slurry was transferred to dry sample bottle and stored at -5^{o}C until use.

Identification and assay of artesunate powder

The melting point, thin layer chromatography and chemical content determination was carried out according to the official method [26].

Animals and Parasite

Male and female albino mice weighing 20.2 ± 2.1g (18 – 22g) obtained from Central Animal House, College of Medicine, University of Ibadan were use for the study. The mice were separated into male and female, maintained under standard laboratory conditions [Temperature: 25 – 30^{o}C, 12 hour light and 12hour darkness cycles] and fed with mice pellet diet (Ladokun Farms, Nigeria) with water *ad libitum*. The animal study was in accordance with the National Institute of Health Guidelines for Care of Laboratory animals. The animals were allowed to acclimatize for a week prior to random distribution into experimental groups.

Plasmodium berghei, chloroquine sensitive ANKA strain was obtained from Prof. O. G. Ademowo of the Institute for Advanced Medical Research and Training (IAMRAT), University of Ibadan and maintained by passage in mice.

In vivo antimalarial studies

Inoculums preparation and parasitaemia load determination

Standard inoculum was prepared from a donor mouse with Chloroquine–sensitive NK-65 strain of *P. berghei* parasitized erythrocytes. The donor mouse was previously infected with the parasite intraperitoneally through standard procedure, and was kept under standard laboratory conditions [Temperature: 25 – 30^{o}C, 12 hour light and 12hour darkness cycles] with food and water "ad libitum" until the desired level of parasitemia is achieved.

Infected blood from the donor mouse was obtained by cardiac puncture after anaesthesia with chloroform. Microscopic examination of the thin blood film was used to establish parasitaemia.

Each mouse to be used for the test was infected with a standard inoculum of 10^6 parasitized erythrocyte suspension in normal saline (0.2ml) from a donor mouse. The inoculum was prepared based on the percentage parasitaemia and the number of erythrocytes counted per microlitre.

Curative treatment (Rane test)

This was carried out according to an earlier reported method [27]. The study design used sixty-four albino mice which were randomized into eight groups of eight animals per group (male and female).

The parasitized groups were intraperitoneally infected with 10^6 parasitized erythrocytes with oral treatment commencing on day 4 post inoculations and daily till day 7.

The animals were grouped as follows;

ATocP - Artesunate + Aqueous Extract of *T. occidentalis* in *P. berghei* infected mice, TocP - Aqueous Extract of *T. occidentalis* only in *P. berghei* infected mice,

ATOc - Artesunate + Aqueous Extract of *T. occidentalis* in healthy mice, Toc - Aqueous Extract of *T. occidentalis* in healthy mice, CA - Artesunate in healthy mice, CN - Healthy mice given only water.

CAP - Artesunate alone in *P. berghei* infected mice,

CNP - *P. berghei* infected mice given only water,

Animal in ATocP were given the extract at 200mg/kg body weight in combination with artesunate at 2mg/kg body weight, while TocP were given the extract at 200mg/kg only. CAP were given artesunate at 2mg/kg only, while CNP were given water only and served as positive control.

The other four groups which were healthy animals were similarly treated; ATOc were administered the extract and artesunate, Toc were administered only extract alone, CA were administered artesunate alone, while CN were given only water serving as negative control.

Tail snips was used to prepare thin blood films starting from day 4 post inoculation on a daily basis till day 7 (3rd day of treatment) and then on the 14th day post inoculation (10th day of treatment). Tail blood films were prepared, fixed in methanol, stained with Giemsa stain (4%w/v) for 20 minutes and examined microscopically under oil immersion.

Percentage reduction in parasitemia was calculated using the equation below;

$$\%\ Reduction\ in\ Parasitemia = \frac{Initial\ Load\ -\ Load\ at\ Time\ T}{Initial\ Load} \times \frac{100}{1}$$

Haematological parameters

Blood samples were collected from the mice through the retro-orbital puncture into heparinised sample bottles at the end of the study i.e. four mice per group at 3rd day of treatment and the remaining four mice at the 10th day of treatment. Haematological parameters; packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), neutrophils, eosinophils and Haemoglobin level (Hb) were evaluated in accordance with standard procedures. Haemoglobin levels were measured using the cyanomethaemoglobin method, red blood and white blood cell counts were done using haemocytometer, while PCV was measured by the conventional method of filling capillary tubes with blood and centrifuging using microhaematocrit centrifuge.

Statistical analysis

Data were expressed as mean ±S.E.M. Statistical significance was determined using Student t-test, $p < 0.05$ was considered significant.

Results

Phytochemical screening of the aqueous extract of *T. occidentalis* leaves showed the presence of alkaloids, cardenolides, anthraquinones, saponins, flavonoids and tannins. Identification and assay of the pure artesunate powder confirmed the purity of the drug with a percentage chemical content of 106.24 ± 0.36 %w/v.

The effect of the aqueous extract of *T. occidentalis* on the ability of artesunate to clear parasiteamia due *P. berghei* is presented in Figure 1.

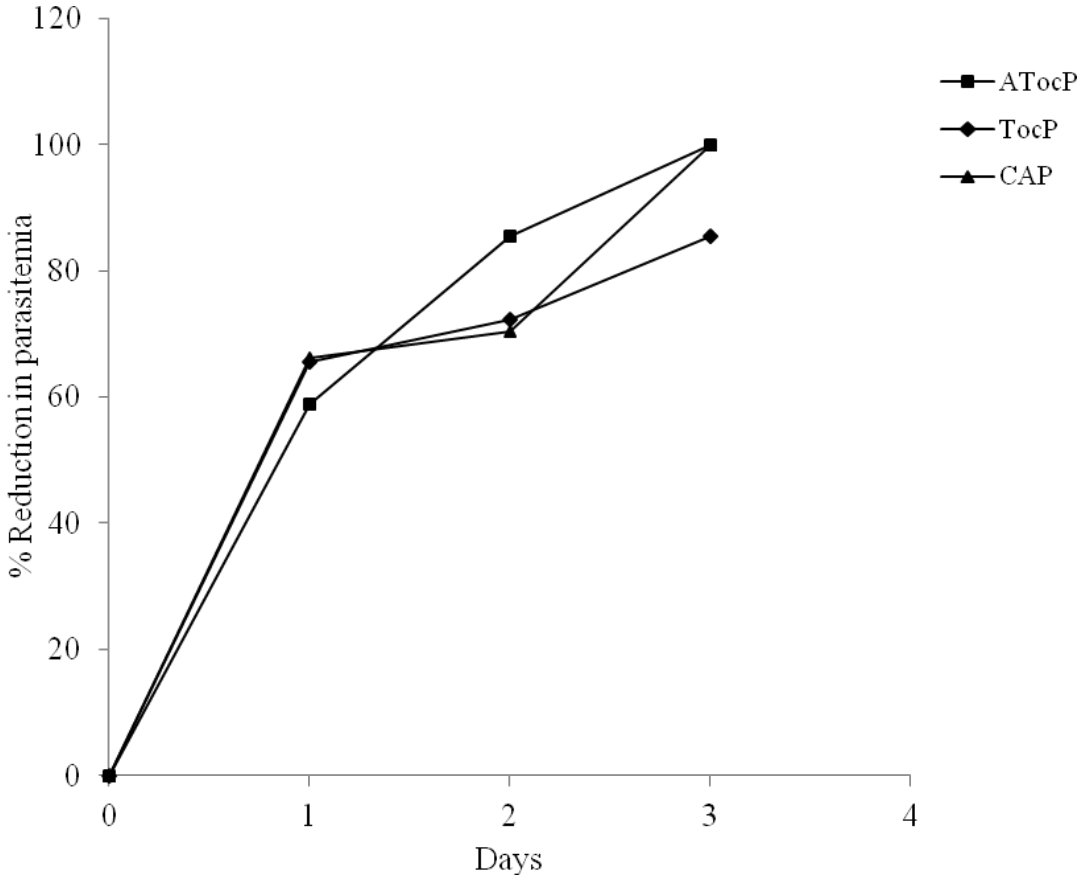

Figure 1: % Reduction in parasiteamia showing the effect of aqueous extract of T. occidentalis **on artesunate in** P.berghei berghei **infected mice.**

Aqueous extract of *T. occidentalis* and artesunate gave 72.17± 4.07% and 70.43± 4.27% reduction in parasitemia by the 2nd day of administration respectively, while the combination gave 85.43± 3.65% reduction. The co-administration of aqueous extract of *T. occidentalis* with artesunate and artesunate alone gave a total clearance of parasitemia (100%) by the 3rd day of administration, while the aqueous extract of *T. occidentalis* alone gave 85.52± 3.62% reduction in parasitemia. All the treatments gave 100% clearance by the 10th day of administration.

Evaluation of the haematological parameters showed an insignificant enhancement of the PCV by *T. occidentalis* on the 3rd day of administration in the diseased state. PCV of 33.3± 1.45% was obtained for the control group in the presence of infection (CNP), while 37 ± 0.58% was obtained for the aqueous extract of *T. occidentalis* (TocP). Similarly, 42.5 ± 0.96% was obtained in the absence of infection (CN) while 44.5 ± 0.65% was obtained after the administration of the extract (Toc) (Table 1).

Table 1: Some haematological parameters obtained after administration of aqueous extract of T. occidentalis **with Artesunate for 3 and 10 days to** P.berghei **infected and healthy mice. (Data expressed as Mean± S.E.M).**

Animal Groups	PCV (%)		Hb. Concentration (g/dL)		RBC count (1012/L)	
	3 Days	10 Days	3 Days	10 Days	3 Days	10 Days
ATocP	42.0 ± 2.31a	24.0 ± 2.00b	13.3 ± 0.87	7.3 ± 0.50	6.1 ± 0.22	3.4 ± 0.13
TocP	37.0± 0.58 a	40.3 ± 1.69b, c	11.6 ± 0.12	12.9 ± 0.67	6.1 ± 0.12	6.7 ± 0.24
CAP	34.0 ± 1.08 a	21.5 ± 6.50c	10.6 ± 0.41	6.7 ± 2.0	5.5 ± 0.27	2.6 ± 1.56
CNP	33.3 ± 1.45	23.0 ± 5.51	10.7 ± 0.48	7.1 ± 2.10	5.3 ± 0.24	3.3 ± 1.21
AToc	46.0 ± 1.08	46.0 ± 1.41	14.6 ± 0.35	14.8 ± 0.42	7.5 ± 0.44	7.6 ± 0.31
Toc	44.5 ± 0.65	46.5 ± 2.50	14.2± 0.32	14.6 ± 0.95	7.3 ± 0.14	8.0 ± 0.42
CA	46.0 ± 1.23	35.0 ± 13.00	14.8 ± 0.51	11.2 ± 4.40	7.6 ± 0.18	5.6 ±2.31
CN	42.5 ± 0.96	46.5 ± 1.50	13.4± 0.15	15.2 ± 0.40	7.0 ± 0.15	7.5 ± 0.18

Values are expressed as the mean ± SEM, with significance at $p < 0.01$ for a, while b and c was $p < 0.05$ compared with the control; $n = 4$.

Note: ATocP: Artesunate + Aqueous Extract of *T. occidentalis* in *P. berghei* infected mice, TocP: Aqueous Extract of *T. occidentalis* only in *P. berghei* infected mice, CAP: Artesunate alone in *P. berghei* infected mice, CNP: *P. berghei* infected mice given only water,

AToc: Artesunate + Aqueous Extract of *T. occidentalis* in healthy mice, Toc: Aqueous Extract of *T. occidentalis* in healthy mice, CA: Artesunate in healthy mice, CN: Healthy mice given only water.

However, the co-administration of artesunate with the aqueous extract (ATocP) gave a significant enhancement of the PCV when compared with artesunate (CAP) and extract alone (TocP) at day 3 ($p < 0.01$) (Table 1).

Similar trend was also observed with Haemoglobin concentration, RBC, WBC, platelet count, neutrophils and eosinophils (Tables 1 and 2).

The results obtained by the 10th day of administration gave a PCV of 40.3 ± 1.69, 21.5± 6.5 and 24.0 ± 2.0% for aqueous extract of *T. occidentalis,* artesunate and combination of *T. occidentalis* and artesunate respectively in the diseased state. This indicates a significant enhancement of the PCV with extract alone ($p < 0.05$) and a non-significant increase with the combination of *T. occidentalis* with artesunate ($p > 0.05$) (Table 1) when compared with the control diseased state (CNP).

On the other hand, the absence of infection showed a non-significant difference in the haematological parameters for the extract alone, combination of artesunate and extract and the control group, while artesunate alone gave lower values. Similar trends were observed for Heamoglobin level, RBC, and WBC (Table 2).

Table 2: Other haematological parameters obtained after administration of aqueous extract of T. occidentalis **with Artesunate for 3 and 10 days to** P.berghei **infected and healthy mice. (Data expressed as Mean± S.E.M).**

	3 Days	10 Days	3 Days	10 Days	3 Days	10 Days	3 Days	10 Days	3 Days	10 Days	3 Days	10 Days
ATocP	6,866 ± 1,301	6,775 ± 425	108,333 ± 29,946	112,000 ± 11,000	68.33 ± 2.73	57.50 ± 10.50	27.70 ± 3.18	39.50 ± 9.50	3.30 ± 0.33	2.00 ± 1.00	0.67 ± 0.33	0.50 ± 0.50
TocP	8,667 ±1,203	9,867 ± 2,097	109,666 ± 10,477	151,000 ± 20,253	68.67 ± 1.86	63.80 ± 5.94	27.30 ± 1.86	31.75 ± 5.89	3.30 ± 0.67	3.00 ± 1.23	0.67 ± 0.33	1.00 ± 0.41
CAP	8,387 ± 1,137	8,150 ± 550	97,000 ± 7,593	157,000 ± 37,000	74.75 ± 4.79	42.00 ± 1.00	22.30 ± 4.66	50.00 ± 4.00	2.50 ± 0.29	4.00 ± 2.00	1.00 ± 0.41	0.50 ± 0.50
CNP	10,867 ± 573	11,283 ± 1,487	106,666 ± 3,527	224,000 ± 57,003	73..00 ± 5.86	56.70 ± 2.67	22.70 ± 4.91	37.30 ± 1.86	3.70 ± 0.88	1.30 ± 0.88	0.67 ± 0.67	0.33 ± 0.33
AToc	8,050 ± 883	8,575 ± 895	91,000 ± 9,755	108,000 ± 5,033	64.75 ± 7.65	58.50 ± 14.68	29.75 ± 7.75	37.75 ± 7.64	5.50 ± 0.29	2.75 ± 0.25	0.00	1.00 ± 0.58
Toc	7,312 ± 1,281	9,325 ± 125	118, 250 ± 6,860	124,000 ± 12,000	74.00 ± 1.68	52.00 ± 8.00	22.25 ± 0.85	45.00 ± 7.00	2.00 ± 0.71	2.50 ± 0.50	1.00 ± 0.41	0.50 ± 0.50
CA	6,787 ± 876	9,825 ± 425	95,750 ± 6,169	153,000 ±13,000	54.00 ± 2.48	48.50 ± 14.50	42.75 ± 3.15	49.00 ± 13.00	3.00 ± 0.91	2.50 ± 1.50	0.25 ± 0.25	0.00
CN	6,262 ± 159	4,975 ± 75	128,000 ± 18,722	78,000 ± 4,000	51.25 ± 4.49	54.00 ± 2.00	44.78 ± 3.17	43.50 ± 0.50	3.50 ± 1.66	1.50 ± 0.50	0.25 ± 0.25	1.00 ± 1.00

Values are expressed as the mean ± SEM, with significance at $p < 0.01$ for a, while b and c was $p < 0.05$ compared with the control; $n = 4$.

ATocP: Artesunate + Aqueous Extract of *T. occidentalis* in *P. berghei* infected mice, TocP: Aqueous Extract of *T. occidentalis* only in *P. berghei* infected mice,

CAP: Artesunate alone in *P. berghei* infected mice, CNP: *P. berghei* infected mice given only water,

AToc: Artesunate + Aqueous Extract of *T. occidentalis* in healthy mice, Toc: Aqueous Extract of *T. occidentalis* in healthy mice, CA: Artesunate in healthy mice,

CN: Healthy mice given only water.

Discussion

This investigation further confirms that *T. occidentalis* leaves contain secondary metabolites like alkaloids, saponins, tannins anthraquinones which is in agreement with earlier reports on the plant agent [22]. Also, chemical content of the pure artesunate used was 106.24 ± 0.36%w/v, which is line with the expected official specification for artesunate [26].

The prevention of severe malaria induced anaemia necessitates rapid treatment of symptomatic high density parasitaemia, as well as reduction of asymptomatic parasite prevalence and to provide recovery period for the replacement of infected of erythrocytes [28]. The need for appropriate drug management to achieve this is crucial as anaemia associated with malaria represents a major cause of childhood mortality in sub-Saharan Africa [29]. This investigation reported a reduction in the percentage parasitemia following administration of aqueous extract of T. occidentalis, artesunate and the co-administration of the two by the 48hours of administration. This indicates that *T. occidentalis* and artesunate are antiplasmodic while the co-administration of the two agents gave a synergistic antiplasmodic effect on *P. berghei* infection in mice. This result is comparable to the significant enhancement of antimalarial effect reported when *Vernonia amygdalina* and *Khaya grandifolia* were co-administered with other antimalarial drugs such as chloroquine and halofantrine respectively [28, 30].

It is worthy noting that there was an enhancement of haematological indices by aqueous extract of *T. occidentalis* both on haematological parameters in healthy rats as showed in (Table 1), this is in agreement with earlier

report [23]. Similar enhancement of haematological indices was observed with the co-administration of *T. occidentalis* with artesunate as it reversed the observed anaemia induced by plasmodial infection. This was evident by significant higher packed cell volume (PCV), haemoglobin level (Hb) and red blood cell (RBC) count in the *T. occidentalis*-artesunate combination than those obtained for the artesunate (CAP) and *T. occidentalis* aqueous extract alone (TocP) ($p < 0.05$, ANOVA) in the infected animals.

This showed that the co-administration of aqueous extract of *T. occidentalis* and artesunate have a synergistic effect on the rate of parasite clearance of *P. berghei* infection in mice with a significant enhancement of haematological parameters within three days of administration. This indicates an advantage of rapid rate of recovery from plasmodial infections when the two are co-administered.

This observed significant enhancement of haematological parameters in the diseased state by the third day of the aqueous extract of *T. occidentalis* showed the possible prospect of the combination in the treatment of acute malaria in children.

Furthermore, administration of *T. occidentalis* aqueous extract alone in the diseased state for ten days revealed a significant enhancement of haematological indices when compared with artesunate alone. *T. occidentalis* in combination artesunate is beneficial in the management of plasmodial infection, in poor resourced economies.

Conclusion

Thus, it can be concluded that the combined effect of artesunate and aqueous extract of *T. occidentalis* enhanced rate of parasitemia clearance and enhanced haematological indices in anaemia induced by plasmodial infection. The combination is beneficial in the management of the infection as it will ensure rapid recovery especially in poor resourced economies.

Acknowledgements

The authors acknowledge Bond Chemical Industries, Ltd. Awe, Oyo – State, Nigeria for the provision of pure artesunate used for the study. Similar thanks also go to Prof. O G. Ademowo of Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University College Hospital, Ibadan, Nigeria, for the Chloroquine–sensitive NK-65 strain of *P. berghei*.

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