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Hematological and Liver Function of Plasmodium Berghei Positive Wister Treated With Herbs and Acts

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ABSTRACT

Eradication of malaria in Africa continues to be one of the greatest challenges in the health sector. All the drugs developed thus far have their limitations and are generally expensive. In Africa and Nigeria the use of herbs in treating sicknesses dated as far back as the existence of man and is still in use today by many Nigerians in rural areas. Here we evaluated the therapeutic efficacy of ethanolic extract for three of these herbs (lemon grass, lime and turmeric) used singly and in combination on Plasmodium berghei infected rats. Results reveals that there was an 81.54% reduction in parasite level in groups treated with herbs and an 88.9% reduction in those treated with ACTs. The time frame of this study wasn't enough to determine if there will be a resurgence in the parasitemia level. Results from the liver function test also reveals that the herbs also reduced the levels of liver enzymes in the serum but the results from the liver histology from the onset shows little or no damage to the liver; This helps us to understand that the plasmodium parasites does not cause much damage to the liver cells or requires more time to do so. Hence the study concludes that potent herbs like turmeric, lemon grass and lime although not as effective as ACTs but if harnessed properly can be substituted for ACTs in treating malaria in low income rural areas of Nigeria.

KEYWORDS: *Plasmodium Berghei, liver function, haematology*

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INTRODUCTION

Malaria is a deadly infection caused by *plasmodium* parasites that are transmitted to people through the bite of infected female anopheles mosquito. Malaria has been a major concern as half of the world's population is at risk of being infected with the disease [18].

In Nigeria is the number one public health problem, a 2013 study on the economic burden of malaria on households and the health system in Enugu state southeast Nigeria by Onwujekwu et al reports that over 57.6% of the households had an episode of malaria within one month. And the average household expenditure per case was 12.57US$ and 23.20US$ for OPD and IPD visits respectively. Indirect consumer costs of treatment were higher than direct consumer medical costs. From a health system perspective, the recurrent provider cost per case was 30.42 US$ and 48.02 US$ for OPD and IPD while non recurrent provider costs were 133.07US$ and 1857.15US$. It will only make sense that these figures have since then gone higher that because of the continuous inflations happening in the country.

A community based study carried out in kano state, Nigeria, to investigate the prevalence and risk factor of malaria and to evaluate the knowledge, attitudes and practices (KAP) regarding malaria among rural Hausa communities in the state reports that malaria is still higly prevalent among rural communities in Nigeria. Despite high levels of knowledge and attitudes in the study area, significant gaps persist in appropriate preventive practices, particularly the use of ITNs [3]. In southwestern Nigeria, fifty different

medicinal plants have bben identified that are used in treatment of malaria by the locals [12].

Cymbopogon Citratus

*CymbopogonCitratus*widely known as lemon grass and commonly found in Asia, Africa, Australia and tropical islands. It belongs to the poaceae family and is commonly cultivated as a culinary and medicinal herb because of its scent. Over the years several research has shown that lemon grass extract has so many health benefits ranging from reducing cholesterol level to its use in treatment of Type 2 diabetes [1].

Citrus Aurantifolia

Citrus aurantifolia generally known as lime is grown across the world and in Nigeria. Due to its aroma and delicious taste, citrus may be called a miracle fruit. Several research on the traditional use of citrus has shown that it has antibacterial, antidiabetic, anti-lipidemia, antioxidant , anti-parasitic, and anti-platelet activities.[8,11,2,7,6,16,5,4,15]

Curcuma Longa

Curcuma Longa, which is commonly known as turmeric is known to be one of the oldest spices that have been used in western and southern parts of India. Several medicinal properties have been attributed to Curcuma Longa. It has been used by medical practioners as anti-inflammatory [17], anti-diarrhoeal [14].These various plants are usually boiled together and the extract taken in the treatment of malaria disease in Nigeria.

METHODS

Experimental animals

Resource equation method was used to determine the amount of test animals required for the study. A total of 80 wister rats was used for this study, they were allowed to acclimatize before commencing the study on them. The experimental design divided the animals into a group of five as shown below.

Plant materials

Fresh lime fruits, leaves of lemon grass and rhizomes of turmeric were obtained from the local market at NkwoNnewi. Samples of the plants were taken to the department of botany, NnamdiAzikiwe University for identification.

PREPARATION OF SAMPLES AND CONFIRMATION OF PARASITE LOAD

After 4 days of inoculation before commencement of tretreatment, three animals from each group were anaesthesized using diethy ether. Blood sample was drawn via ocular puncture. Blood samples were collected in Ethylene diamine tetra-acetate acid (EDTA) bottle and mixed properly for the analyses of haematological parameters and parasite load.

PREPARATION OF PLANT EXTRACTS

Preparation of turmeric and lemon grass extract

Fresh rhizomes of turmeric were collected and dried in the oven at 60^{o}C for a period of 45 hours. Stalks of lemon grass were also dried at ambient temperature for a period of two weeks. The dried turmeric and lemon grass were grounded to fine powder. 250g each of the powdered turmeric and lemon grass were dissolved separately in 1000ml of 98% ethanol and allowed for 48hours at room temperature after which it was sieved using porcelain clothe and was further filtered using a filter paper. The filtrate was concentrated using digital rotary evaporator (TT-52 technel and technel USA) and dried using thermostat oven into a gel like substance and stored in a refrigerator. The turmeric extract was be stored in a dark or amber bottle.

Preparation of lime extract

Fresh lime fruits was used for this study, the green bark of the fruit was peeled off and discarded, the remaining part of the fruit were blended in a blender and the juice filtered out. The residue were be discarded and the filtrate was collected and stored in a refrigerator.

TREATMENT

Animals in different groups were administered with 500mg/kg of their respective extracts and drugs at the appropriate dosage for a period of two weeks with exception to animals in groups A and B which served as positive and negative controls respectively. Groups with two combinations were administered with 250mg/kg of each extract while groups with three combinations received 200mg/kg of each extract. After this two week period, the animals were sacrificed and blood samples were collected. The livers were also harvested for the liver histology.

RESULTS AND DISCUSSION

Results obtained after the treatment for assessment of the parasite clearance level shows that there was an average of 81.54% clearance in groups treated with herbs and an 88.9% clearance in groups treated with ACTs.

TABLE 1.1A: Shows the Plasmodium count amongst different treatment groups after inoculation of malaria parasite (day 4).

		MEAN	±SEM	P-value	F-value
Plasmodium count day 4	Group A (Positive control)	0.00	±0.00		
	Group B (Negative control)	27.66	±1.45	0.00*	
	Group C (Lime Extract Only)	29.33	±0.33	0.00*	
	Group D (Lemon grass extract only)	30.00	±0.00	0.00*	
	Group E (Turmeric Extract Only)	26.00	±0.57	0.00*	76.80
	Group F (Lime extract + Lemon grass extract)	28.66	±2.02	0.00*	
	Group G (Lime extract + Turmeric extract)	30.33	±0.33	0.00*	
	Group H (Turmeric extract + Lemon grass)	29.00	±0.57	0.00*	
	Group I (Lime extract + Lemon grass extract + Turmeric extract)	30.66	±0.88	0.00*	
	Group J (Artmether + Lumifethrian)	27.66	±1.76	0.00*	
	Group K (DHA + Pipraquine)	29.33	±0.33	0.00*	

Data was analyzed using ANOVA followed by post HOC Turkey HSD multiple comparism and values were considered significant at $p<0.05$.

Table 4.1A result on day 0 showed a significant increase ($p<0.05$) in Plasmodium count in-group B, C, D, E, F, G, H, I, J and K when compared to group A.

TABLE 1.1B: Shows the Plasmodium count amongst different treatment groups after 14 days of treatment (day 14).

		MEAN	±SEM	P-value	F-value
Plasmodium count day 14	Group A (Positive control)	0.00	±0.00		
	Group B (Negative control)	19.00	±0.57	0.00*	
	Group C (Lime Extract Only)	5.00	±0.57	0.00*	
	Group D (Lemon grass extract only)	2.33	±0.33	0.45	
	Group E (Turmeric Extract Only)	6.33	±1.45	0.00*	47.99
	Group F (Lime extract + Lemon grass extract)	4.33	±0.33	0.01*	
	Group G (Lime extract + Turmeric extract)	5.00	±0.00	0.00*	
	Group H (Turmeric extract + Lemon grass)	7.00	±0.57	0.00*	
	Group I (Lime extract + Lemon grass extract + Turmeric extract)	7.66	±1.45	0.00*	
	Group J (Artmether + Lumifethrian)	3.00	±0.00	0.16	
	Group K (DHA + Pipraquine)	3.33	±0.33	0.09	

Data was analyzed using ANOVA followed by post HOC Turkey HSD multiple comparism and values were considered significant at $p<0.05$.

Table 4.1B showed the Plasmodium count result on day 14 had a decrease in-group B, C, E, F, G, H and I but are still significantly high ($p<0.05$) when compared to group A, while group D, J and K showed a significant decrease ($p>0.05$)when compared to group A.

TABLE 1.2A: Shows the Effect of malaria parasite on Red blood cell three days after inoculation

		MEAN	±SEM	P-value	F-value
Red blood cell () Day 0	Group A (Positive control)	7.20	±0.12		
	Group B (Negative control)	5.56	±0.29	0.00*	
	Group C (Lime Extract Only)	6.41	±0.16	0.36	
	Group D (Lemon grass extract only)	6.27	±0.42	0.18	
	Group E (Turmeric Extract Only)	6.37	±0.15	0.30	3.77
	Group F (Lime extract + Lemon grass extract)	6.69	±0.23	0.87	
	Group G (Lime extract + Turmeric extract)	6.59	±0.12	0.69	
	Group H (Turmeric extract + Lemon grass)	6.59	±0.16	0.70	
	Group I (Lime extract + Lemon grass extract + Turmeric extract)	6.21	±0.33	0.12	

	Group J (Artmether + Lumifethrian)	7.00	±0.09	1.00	
	Group K (DHA + Pipraquine)	6.81	±0.03	0.97	

Data was analyzed using ANOVA followed by post HOC Turkey HSD multiple comparism and values were considered significant at $p<0.05$.

Table 4.2A result showed a significant ($p<0.05$) decrease in red blood cell on day 0 in-group B while group C, D, E, F, G, H, I, J and K had a non-significant ($p>0.05$) decrease when compared to group A.

TABLE 1.2B Red blood cell of treatment groups infected with plasmodium parasite after 14 days of treatment.

		MEAN	±SEM	P-value	F-value
Red blood cell () Day 14	Group B (Negative control)	6.14	±0.27		
	Group C (Lime Extract Only)	7.40	±0.29	0.11	
	Group D (Lemon grass extract only)	8.94	±0.53	0.00*	
	Group E (Turmeric Extract Only)	8.00	±0.18	0.00*	
	Group F (Lime extract + Lemon grass extract)	8.23	±0.38	0.00*	9.70
	Group G (Lime extract + Turmeric extract)	8.44	±0.06	0.00*	
	Group H (Turmeric extract + Lemon grass)	7.92	±0.30	0.00*	
	Group I (Lime extract + Lemon grass extract + Turmeric extract)	7.11	±0.72	0.00*	
	Group J (Artmether + Lumifethrian)	8.89	±0.40	0.00*	
	Group K (DHA + Pipraquine)	8.81	±0.93	0.00*	

Data was analyzed using ANOVA followed by post HOC Turkey HSD multiple comparism and values were considered significant at $p<0.05$.

Table 4.2B showed a significant ($p<0.05$) increase in red blood cell on day 14 in-group D, E, F, G, H, I, J and K while group C had a non-significant ($p>0.05$) increase when compared to group B.

TABLE 1.3A: Shows the Effect of malaria parasite on the liver enzymes, AspartateTransaminase (AST), three days after inoculation

		MEAN	±SEM	P-value	F-value
Aspartatetransaminase (IU/L) Day 0	Group A (Positive control)	18.33	±1.76		
	Group B (Negative control)	172.00	±0.57	0.00*	
	Group C (Lime Extract Only)	170.33	±0.33	0.00*	
	Group D (Lemon grass extract only)	171.00	±1.15	0.00*	
	Group E (Turmeric Extract Only)	169.66	±2.60	0.00*	125.94
	Group F (Lime extract + Lemon grass extract)	167.33	±2.18	0.00*	
	Group G (Lime extract + Turmeric extract)	164.66	±4.05	0.00*	
	Group H (Turmeric extract + Lemon grass)	156.66	±3.38	0.00*	
	Group I (Lime extract + Lemon grass extract + Turmeric extract)	131.33	±0.33	0.00*	
	Group J (Artmether + Lumifethrian)	160.00	±10.59	0.00*	
	Group K (DHA + Pipraquine)	168.33	±4.63	0.00*	

Data was analyzed using ANOVA followed by post HOC Turkey HSD multiple comparism and values were considered significant at $p<0.05$.

Table 4.7A result on day 0 showed a significant increase ($p<0.05$) in Aspartatetransaminase level in-group B, C, D, E, F, G, H, I, J and K when compared to group A.

TABLE 1.3B Aspartatetransansaminse of different treatment groups infected with plasmodium parasite after 14 days of treatment.

		MEAN	±SEM	P-value	F-value
Aspartatetransaminase (IU/L) Day 14	Group B (Negative control)	99.67	±0.33		
	Group C (Lime Extract Only)	92.33	±0.33	0.07	
	Group D (Lemon grass extract only)	94.00	±0.00	0.27	
	Group E (Turmeric Extract Only)	98.33	±0.33	1.00	
	Group F (Lime extract + Lemon grass extract)	95.00	±0.58	0.51	109.61
	Group G (Lime extract + Turmeric extract)	94.00	±1.15	0.27	
	Group H (Turmeric extract + Lemon grass)	94.33	±0.67	0.34	
	Group I (Lime extract + Lemon grass extract + Turmeric extract)	84.67	±4.48	0.00*	
	Group J (Artmether + Lumifethrian)	55.00	±0.58	0.00*	
	Group K (DHA + Pipraquine)	60.00	±0.58	0.00*	

Data was analyzed using ANOVA followed by post HOC Turkey HSD multiple comparism and values were considered significant at $p<0.05$.

Table 4.7B result on day 14 showed a non-significant decrease ($p>0.05$) in Aspartatetransaminase level in-group C, D, E, F, G and H while group I, J and K had a significant decrease ($p>0.05$) when compared to group B

TABLE 1.4A: Shows the Effect of malaria parasite on the liver enzymes AlanineTransaminase (ALT), three days after inoculation

		MEAN	±SEM	P-value	F-value
Alaninetransaminase (IU/L) Day 0	Group A (Positive control)	24.33	±1.20		
	Group B (Negative control)	42.66	±1.45	0.00*	
	Group C (Lime Extract Only)	43.00	±2.08	0.00*	
	Group D (Lemon grass extract only)	42.33	±1.76	0.00*	
	Group E (Turmeric Extract Only)	42.33	±1.85	0.00*	10.99
	Group F (Lime extract + Lemon grass extract)	40.33	±1.20	0.00*	
	Group G (Lime extract + Turmeric extract)	41.00	±2.08	0.00*	
	Group H (Turmeric extract + Lemon grass)	38.33	±1.20	0.00*	
	Group I (Lime extract + Lemon grass extract + Turmeric extract)	44.33	±0.33	0.00*	
	Group J (Artmether + Lumifethrian)	40.66	±2.18	0.00*	
	Group K (DHA + Pipraquine)	40.00	±1.73	0.00*	

Data was analyzed using ANOVA followed by post HOC Turkey HSD multiple comparism and values were considered significant at $p<0.05$.

TABLE 1.4B Alaninetransaminase different treatment groups infected with plasmodium parasite after 14 days of treatment.

		MEAN	±SEM	P-value	F-value
Alaninetransaminase (IU/L) Day 14	Group B (Negative control)	69.00	±0.58		
	Group C (Lime Extract Only)	17.00	±0.58	0.00*	
	Group D (Lemon grass extract only)	19.33	±0.33	0.00*	
	Group E (Turmeric Extract Only)	24.00	±0.58	0.00*	
	Group F (Lime extract + Lemon grass extract)	18.33	±0.88	0.00*	830.67
	Group G (Lime extract + Turmeric extract)	18.67	±0.88	0.00*	
	Group H (Turmeric extract + Lemon grass)	16.67	±0.33	0.00*	
	Group I (Lime extract + Lemon grass extract + Turmeric extract)	15.67	±0.33	0.00*	
	Group J (Artmether + Lumifethrian)	19.67	±0.33	0.00*	
	Group K (DHA + Pipraquine)	18.67	±0.33	0.00*	

Data was analyzed using ANOVA followed by post HOC Turkey HSD multiple comparism and values were considered significant at $p<0.05$.

TABLE 1.5A: Shows the Effect of malaria parasite on the liver enzymes, Alkaline Phosphatase (ALP), three days after inoculation.

		MEAN	±SEM	P-value	F-value
Alkaline Phosphatse (IU/) Day 0	Group A (Positive control)	109.33	±3.48		
	Group B (Negative control)	453.00	±1.73	0.00*	
	Group C (Lime Extract Only)	445.66	±3.38	0.00*	
	Group D (Lemon grass extract only)	421.33	±11.60	0.00*	
	Group E (Turmeric Extract Only)	424.66	±6.88	0.00*	175.54
	Group F (Lime extract + Lemon grass extract)	438.33	±9.59	0.00*	
	Group G (Lime extract + Turmeric extract)	434.33	±6.76	0.00*	
	Group H (Turmeric extract + Lemon grass)	429.33	±13.64	0.00*	
	Group I (Lime extract + Lemon grass extract + Turmeric extract)	439.66	±5.92	0.00*	
	Group J (Artmether + Lumifethrian)	437.00	±6.08	0.00*	
	Group K (DHA + Pipraquine)	430.33	±2.40	0.00*	

Data was analyzed using ANOVA followed by post HOC Turkey HSD multiple comparism and values were considered significant at $p<0.05$.

TABLE 1.5B: Shows the Alkaline phosphate of different treatment groups infected with plasmodium parasite after 14 days of treatment.

		MEAN	±SEM	P-value	F-value
Alkaline Phosphatse (IU/) Day 14	Group B (Negative control)	465.33	±3.18		
	Group C (Lime Extract Only)	149.00	±0.58	0.00*	
	Group D (Lemon grass extract only)	151.00	±0.58	0.00*	
	Group E (Turmeric Extract Only)	163.33	±0.88	0.00*	
	Group F (Lime extract + Lemon grass extract)	154.67	±3.18	0.00*	3491.67
	Group G (Lime extract + Turmeric extract)	159.67	±0.88	0.00*	
	Group H (Turmeric extract + Lemon grass)	148.33	±0.33	0.00*	
	Group I (Lime extract + Lemon grass extract + Turmeric extract)	144.00	±2.31	0.00*	
	Group J (Artmether + Lumifethrian)	129.67	±0.33	0.00*	
	Group K (DHA + Pipraquine)	147.33	±0.88	0.00*	

Data was analyzed using ANOVA followed by post HOC Turkey HSD multiple comparism and values were considered significant at $p<0.05$.

DISCUSSION

Plasmodium parasite infection has shown to be associated with cases such as anaemia and thrombocytopenia [9]. Haematological results obtained from patients with plasmodium infection indicates that virtually all haematological parameters showed a decline when compared to those who are plasmodium negative [10]. Results obtained from this study after the experimental animals were inoculated indicates that plasmodium berghei have a damaging effect on red blood cells and the liver cells; this is evident from the low levels of RBC in the test grops as compared to the control group and also the high levels of liver enzymes (AST, ALP, ALT) in the serum samples of test groups. The herbs used for the treatment proved to be effective, as the data was compared to that of those treated with known ACTs. But it is not known how long the therapeutic effects of the herbs will last because the parasite clearance level was not zero so there is likely to be a surge in the parasitemia level after a period of time. Hence it is suggested that futher studies will be carried out to determine the lasting duration of these herbs and also explore the possibility of vaccine for malaria.

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