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Research Article

Effect of Methanol Extract and Fractions of *Chrysophylum albidum* Bark on Gut Morphology in bled and Plasmodium-Infected Mice

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Abstract

The gut stimulating effect of methanolic extract of *Chrysophyllum albidum* bark (MeCaB) and its' chromatographic fractions (CFr 1, 2 and 3) was investigated in mice using two separate models of experimental anaemia. Male Swiss mice (19-22g) were inoculated with *Plasmodium berghei bergehi* and treated with distilled water, *Chrysophylum albidum (1000mg/Kg), CFr-1(250mg/Kg), CFr-2 (250mg/Kg), CFr-3 (250mg/Kg) and* Chloroquine (30mg/kg). A control group of mice were not parasitized. In another experiment, mice were made anaemic by bleeding out blood via the retro-orbital plexus and thereafter treated with Normal saline, *Chrysophylum albidum (1000mg/Kg) or* Ferrous sulphate (100mg/Kg*).* An un-bled group served as control. Body weights and parasitaemia counts were monitored daily. Blood was collected for complete blood and parasitaemia counts by days 3 and 7 of experiment and were later sacrificed (by day 7) with the duodenal and jejunal segments of the small intestine excised from each animal for histological analysis. Data were expressed as Mean±SEM, analyzed using one-way ANOVA and $p \leq 0.05$ was statistically significant. Significant reductions in body weight and blood cell counts were observed in the untreated inoculated and bled animals. Treatment with *C. albidum* bark and fractions *CFr-1 and CFr-3* significantly reduced parasitaemia levels in the inoculated mice and increased the body weights and blood cell counts towards control values in both models of anaemia. Chloroquine totally cleared the parasitaemia. Duodenal cryptal depths, surface area, villi height/cryptal depth ratio were significantly higher in treated when compared with the untreated parasitized animals. Mice either in the parasitized or bled models treated with *MeCaB* and fractions had significantly higher jejunal heights, cryptal depth, surface area and width by day 7. Significant decreases were observed in the duodenal and jejunal villi height/width ratio of parasitized treated with fractions 1, 2 and 3 by day 7 compared with PUn. Bled mice treated with *MeCaB* had significantly lower duodenal villi height/width ratio. Observations from this study suggest that *Chrysophyllum albidum* and its fractions (1 and 3) promote and help maintain normal homeostatic condition which boosts growth performance via increased absorptive capacity.

Key Words: *C. albidum and fractions, anaemia, gut morphology*

INTRODUCTION

Growth promoters are substances that help improve feed efficiency, weight gain, carcass quality or egg and milk production and could either be in form of implants placed at specific site on animals, feed additives, antibiotics i.e antibiotic growth promoters (AGP) (Barton 2000; Gaskin *et al* 2002; Dibner and Richards, 2005 and Niewold, 2007) . However, ban on antibiotics (Wegener, 2006, Castanon 2007) became apparent when WHO suggested that it can cause and or suffice as a reservoir of antibiotic resistant bacteria population (primarily enterococci) which could be transferred to humans from animal foods (World Health Organisation, 2000; Falcao –e-Cunha *et al* 2007). Consequently, safer alternatives of growth promoters were being sourced for. The use of natural probiotics (Hamilton *et al*., 2003, Amber *et al*., 2004), prebiotics (Forchielli and Walker, 2005, Lan *et al*., 2005, Kim *et al*., 2011; Bozkurt *et al*., 2014, Baurhoo *et al*., 2007b; Pourabedin *et al*., 2014) or both (Sohail *et al*., 2012, Mookiah *et al*., 2014) which can directly stimulate the gut immune system as well as providing beneficial microbes to the gut floral of such animals were explored for possible growth stimulating activities. Probiotics, Awad *et al*., (2009) are 'good' or safe micro-organisms found in the gut which assist in maintaining a homeostatic balance during health and in disease conditions (Cho and Blaser 2012). They assist in getting rid of toxins during diseases cases within the gut and ameliorating inflammation or inflammatory processes (Sartor, 2004; Di Giacinto *et al*., 2005). Probiotics also improves nutrient digestibility (Nahashon *et al*., 1994, 1996 and Jin *et*

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al., 1997), increase growth and body performance like body weight (Awad *et al*., 2009, Zhang *et al*., 2013), inhibition of pathogen growth (Jin *et al*., 1997), improve gut mucosal immunity (Koenen *et al*., 2004, Yu *et al*., 2008) as well as improved antioxidant status (Anwar *et al*., 2012, Inatomi and Konosuke Otomaru 2018). Probiotics are mostly dietary (Granato *et al*., 2010) and can be diary (Ewe *et al*., 2010) in nature. Recently, reports of *Chrysophyllum albidum* G.Don_Holl. (Sapotaceae) (Dalziel,1955; Pearson, 1976; Burits and Bucar 2002; Amusa *et al*, 2003; Akaneme *et al*, 2008; Okoli and Okere 2010) bark has been documented to enhance the activities of the good microflora during the inflammatory phase of colitis healing (Salami *et al*., 2018). This probably classified *C. albidum* as a probiotic, besides these, several ameliorative activities of varied plant parts (leaves, fruits, barks) of *Chrysophyllum albidum* (Adisa, 2000; Idowu *et al* 2006, Olorunnisola *et al*., 2008). Adebayo *et al* 2010, Onyeka *et al*., 2012) (but consistently stem bark) during experimental conditions. Few of these activities of the stem bark include antiplasmodial (Adewoye *et. al.*, 2010), haemtological (Adewoye *et al*., 2012), Attenuation of plasmodium induced bone marrow suppression (Adewoye *et al*., 2013), Erythrocyte membrane stability (Adewoye *et al*., 2013), In-vitro antihelmintic and kill kinetics activities (Salami *et al*., 2015), gastric ulcer healing activities via modulated blood inflammatory markers (Salami and Famurewa 2017), modulatory activities of *C. albidum* on microflora and colonic pump activities (Salami *et al*., 2018). However, the stem bark researches have observed a consistent increase in the body weights during experimentally induced diseased conditions (Adewoye *et al* 2010, Salami and Famurewa 2017 and Salami *et al*., 2018) in different experimental animal models.

In spite of the rich component of the fruits, vast local and documented ameliorative activities of *Chrysophyllum albidum* plant parts, there is dearth of information on its effect as a growth promoter. This study was thus designed to investigate the effect of *Chrysophyllum albidum* bark on the body weight and gut morphology in Swiss male mice using two different anaemic conditions.

MATERIALS AND METHODS

Reagents: All reagents are of analytical grade and were obtained from BDH Chemicals LTD, Poole England.

Plant materials, collection and identification: The fresh bark of *C. albidum* was collected during the fruiting seasons (between the months of November and April) from its natural habitat at Igbo Owe cash crop farm at Moniya, Akinyele Local Government Area of Oyo State, South-West of Nigeria. The plant was identified and voucher number FHI 107514 given at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria was retained. The plant materials (barks) were dusted and air dried at room temperature before it was milled into a coarse powdery form using a dry electric milling machine. This was thereafter firmly stored in an air tight condition to avoid moisture absorption.

Preparation of plant sequential extraction or partitioning and column fractionation: Previous methods according to Salami *et al*., 2015 were employed but briefly, *Chrysophyllum albidum* bark (1.5kg) was well macerated in 2.5 litres of n – hexane for 72 hours in a glass extraction chamber and the filtrate was collected as hexane partition. The marc (residue) was spread out evenly and completely air-dried for 24 hours. To the dried residue on turning into the glass chamber, 2.5 litres of dichloromethane was added and was macerated for 72 hours after which the filtrate (dichloromethane partition) was decanted; the marc (residue) was again spread out to air dry for 24 hours. Methanol (2.5litres) was added to the dried residue in the glass chamber and left for 72 hours, after which the whole mixture was filtered and the filtrate was labelled methanol partition. On each maceration, the marc and solvent were thoroughly stirred daily, the mixture was always filtered with What-man's filter paper (No. 1). All the 3 different filtrates were evaporated to dryness *in-vacuo* and stored at 4◦C until use.

The methanol partition (*MeCaB*) was then allowed to pass through a silica gel column chromatography (Salami *et al*., 2015) using different solvent mixture of n-hexane, dichloromethane, ethylacetate and methanol (in varying polarity gradient) to obtain 3 fractions *(CFr1,2 and 3).*

Parasites and Inoculation: Chloroquine-sensitive *P. berghei berghei* (NK 65) was obtained from the National Institute of Malaria Research (NIMAR) Yaba, Lagos State. The parasite was maintained in mice by serial passage of infected blood to uninfected mice in the animal house. Parasitized red blood cells used for inoculation in the experiment were obtained by cardiac puncture from an infected donor mouse using the method of Adewoye *et al*., 2010. The blood was diluted to desired parasite density in 0.9% NaCl solution (Kendall McGraw, Laboratories, Inc, USA). Each mouse was inoculated with 1.0 x 10_6 parasitized red blood cells contained in 0.2 mLs of the NaCl solution.

In this study, the day of inoculation was defined as day zero (D_0) while subsequent days were named D_1, D_2, D_3 etc.

Animal model, housing and feeding: Sixty six (66) Swiss male mice (19-22 g) in total were used in this experiment. They were obtained from the Animal House of the College of Medicine, University of Ibadan, Nigeria. the animals were habituated in a germ-free plastic cages with a solid base at the Departmental Animal House. They were first acclimatised for two weeks and then maintained under standard conditions of 26_oC-28_oC room temperature, 70-80% relative humidity, environmental light and dark cycles of 12/12 hours each with daily free access to mice chow and clean water.

Extract administration: Drugs and extracts were administered orally using orogastric tube in the two separate anaemic condition studies

Parasite-induced anaemia: The activities of *C.albidum* bark extract and its 3 fractions *(CFr 1, 2 and 3)* on the body weight and gut morphormetry of *Plasmodium bergehi berghei* infected mice. This study (curative) contained six groups of 7 mice each which were sacrificed by day 4 of treatment.

- Group 1 animals were Control (normal, not infected and not treated)
- Group 2 animals were infected and untreated, (*PUn*).
- Group 3 mice were infected and treated with 30mg/kg *b.w.* Chloroquine for 3 consecutive days, (*PCQ*).

- Group 4 consist of mice infected and treated with 1000mg/kg *b.w* of *C. albidum for 3 consecutive days*, (P*MeCaB*).
- Group 5 mice were infected and treated with 250mg/kg *b.w* of fraction 1 for 3 consecutive days, *(PCFr1)*.
- Group 6 mice were infected and treated with 250mg/kg/day *b.w* of fraction 2 for 3 consecutive days, (*PCFr2*).
- Group 7 consists of mice infected and treated with 250mg/kg *b.w* of fraction 3 for 3 consecutive days, (*PCFr3*).

Bleeding induced anaemia: The effect of methanol extract of *C.albidum (MeCaB)* on gut morphormetry in anaemia-induced mice. The study contained five groups of 6 animals each which were all sacrificed by day 7 post bleeding and of treatment. Bleeding out method was followed by method of Redondo *et al*., 1995 and modified by Adewoye *et al.*, 2012 in which anaemia was induced to achieve packed cell volume of about 35 - 40%. Drugs and extract administrations were given orally.

- Group 1 consists of *Nor*mal mice that were not bled and did not receive *C albidum* extract, (control).
- Group 2 consists of mice not bled but received methanolic extract of *C.albidum* (1000mg/kg b.w), (*MeCaB*).
- Group 3 animals were bled (0.25 mLs of blood) and administered ferrous sulphate (100mg/kg), (*BHaem*).
- Group 4 animals were bled (0.25 mLs of blood) and administered *MeCaB* (1000mg/kg b.w), (*BMeCaB*).
- Group 5 animals were bled (0.25 mLs of blood) and untreated, (*Bla*).

Histomorphometry: Intestinal sections (duodenum and jejunum) of animals treated with *C. albidum* were routinely prepared in Histopathology Laboratory of Department of Veterinary Pathology, University of Ibadan, Nigeria. Measurements of different intestinal segments (duodenum and jejunum) cryptal depth, villi length and width were taken using microscope with a micrometer rule as described by Yu *et al*., (1988); Spadoni *et al.* (2005); and Eyarefe *et al*. (2008). Five different villi were measured in each slide per parameter, recorded and an average values calculated.

Statistical analysis

Experimental data were analyzed using one way analysis of variance (ANOVA) and multiple range tests to determine significant differences between means. Pearson's Correlation coefficient was calculated using the GraphPad Prism version 7 software package Difference between Means were regarded as significant at $p<0.05$

RESULTS

Effect of Methanolic extract of C. *albidum* bark (*MeCaB*) and its' fractions administration on body weight of parasitized animals: Body weights of the parasitized animals were significantly lower than those of controls. However, treatments with C. *albidum* (PMeCaB), its fractions and chloroquine significantly increased body weight towards normal values by day 7 of experiment. (Fig.1).

Effect of Methanolic extract of C.*albidum* bark (*MeCaB)* administration on body weight of bled animals: There was a significant increase in the body weight of C. *albidum* animals treated after bleeding when compared with the bled animals that were not given the extract (Fig. 2).

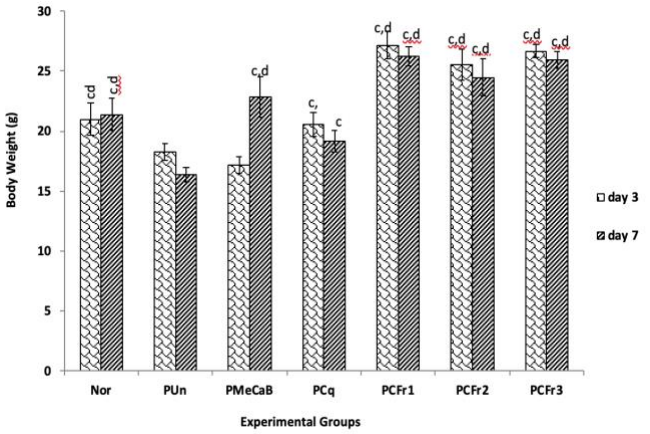

Figure 1:
Effect of methanolic extract of C.*albidum* bark (*MeCaB)* and its' fractions administration on body weight of parasitized animals by days 3 and 7.
Values are Mean ± S.E.M.; ^{c,d} means significant ($p< 0.05$) when compared with parasitized untreated (*PUn*), and parasitized treated with chloroquine (*PCQ*) by days 3 and 7 respectively; n= *10*.
PUn – Parasitized Untreated animals, P*MeCaB* – Parasitized animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum*, *PCFr 1* – parasitized animals treated with 250mg/kg b.w of fraction 1, *PCFr 2* – parasitized animals treated with 250mg/kg b.w of fraction 2, *PCFr 3* – parasitized animals treated with 250mg/kg b.w of fraction 3, *PCQ* – parasitized animals treated with 30mg/kg b.w of chloroquine

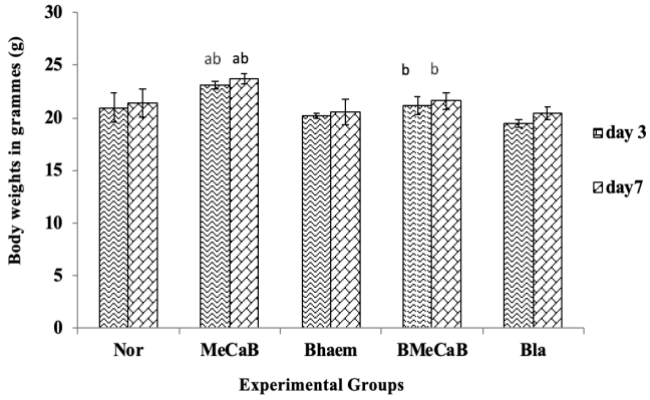

Figure 2:
Effect of methanolic extract of C.*albidum* bark (*MeCaB)* administration on body weight of bled animals by days 3 and 7.
Values are Mean ± S.E.M.; ^{a,b} means significant ($p< 0.05$) when compared with Normal (Nor) and bled untreated animals (Bla) respectively by days 3 and 7, n= 10.
Nor – Normal Control animals, *MeCaB* – animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum*, *BHaem* – animals bled and administered with ferrous sulphate, *BMeCaB* – animals bled and administered with 1000mg/kg of methanol extract of *C.albidum*, *Bla* – animals bled untreated.

Effect of Methanolic extract of C.*albidum* bark (*MeCaB*) and its fractions on percentage parasitaemia clearance after 3 and 7 days post inoculation: Treatment with *C. albidum* bark and fractions *CFr-1 and CFr-3* significantly reduced parasitaemia levels in the inoculated mice. Chloroquine totally cleared the parasitaemia. (Fig. 3).

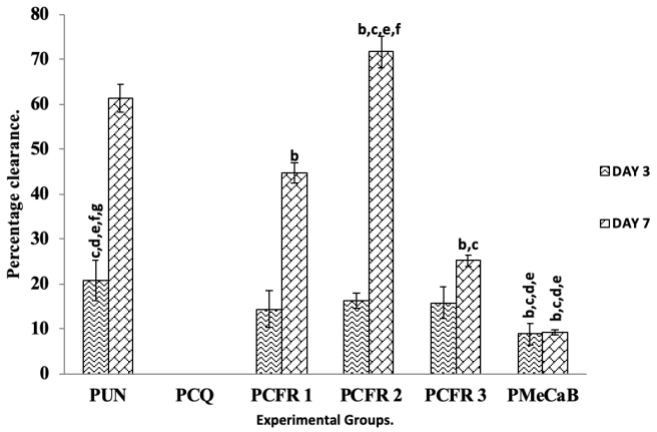

Figure 3:
Effect of methanolic extract of C.*albidum* bark (*MeCaB*) and its fractions on percentage parasitaemia clearance by days 3 and 7 post inoculation.
Values are Mean ± S.E.M.; *significant compared* $_a$ *with Nor,* $_b$ *with PUN,* $_c$ *with PFr1,* $_d$ *with PFr2,* $_e$ *with PFr3,* $_f$ *with PMeCaB,* $_g$ *with PCQ.*
PUn – Parasitized Untreated animals, P*MeCaB* – Parasitized animals + 1000mg/kg b.w *C.albidum, PCFr 1* – parasitized animals + 250mg/kg of fraction 1, *PCFr 2* – parasitized animals + 250mg/kg of fraction 2, *PCFr 3* – parasitized animals + 250mg/kg of fraction 3, *PCQ* – parasitized animals + 30mg/kg of chloroquine.

Effect of Methanolic extract of C.*albidum* bark (*MeCaB*) and fractions on Haematological indicies in parasitized and bled animals by days 3 and 7 post inoculation: The untreated parasitized (PUn) animals had a significantly lower PCV, RBC and Hb content compared with all other experimental groups both on days 3 and 7. Treatment with the extracts of *C. albidum* and its fractions (but not Chloroquine) increased the values towards normal (Table 1a). Conversely, parasitemia significantly increased platelet and leucocyte counts while treatment with *C. albibum* and its fractions decreased the values towards the control values

Effect of Methanolic extract of C.*albidum* bark (*MeCaB*) and fractions on Haematological indicies in bled animals by days 3 and 7 post bleeding out: As shown in Table 1b, significant decreases in blood cell counts (RBC, WBC and platelets), haemoglobin content and PCV, were observed in the untreated, bled animals when compared with control. Treatment with C. *albidum* (*BMeCaB*) significantly increased the values towards the normal by day 7 (but not day 3) post-bleeding..

Table 1a:
Effect of methanolic extract of C.*albidum* (*MeCaB)* bark and fractions on Haematological indices in parasitized animals by days 3 and 7 post inoculation.

Groups	RBC (million/mm$_3$)		Hb (mg/dL)		PCV (%)		Reticulocytes		Platelets (10$_3$/mm$_3$)		WBC (10$_3$/mm$_3$)	
	Day 3	Day 7	Day 3	Day 7	Day 3	Day 7	Day 3	Day 7	Day 3	Day 7	Day 3	Day 7
Nor	7.28 ± 0.55	6.91 ± 0.14	13.92 ± 0.22	13.5 ± 0.33	43.6 ± 0.4	42.4 ± 1.12	-	-	114.600 ± 9.097	105.400 ± 7.089	8.80 ± 0.2	9.60 ± 1.48
Pun	4.58 ±0.9$_{abcde}$	3.31 ±0.4$_{abcde}$	9.22 ±1.4$_{abcde}$	6.43 ±0.7$_{abcde}$	29.2 ±3.12$_{abcde}$	20.00 ±2.0$_{abcde}$	1.60 ±0.25	1.80 ±0.20	188.400 ±6.11$_{abcde}$	159.666 ±743.03$_{abcde}$	12.63 ± 1.8$_{abcde}$	16.377 ±3.60 $_{abcde}$
PCFr1	7.05 ± 0.13	5.56 ± 0.39	13.54 ± 0.53	10.78 ± 0.53$_{abcde}$	42.6 ± 1.33	34.6 ± 1.83	1.60 ±0.25	2.20 ±0.37	131.600 ± 6.07	229.000 ± 49.246	9.58 ± 1.09 $_{abcde}$	17.10 ± 1.87
PCFr2	8.00 ± 0.23	4.10 ± 0.38$_{abcde}$	16.36 ± 0.35	7.8 ± 1.27$_{abcde}$	49.4 ± 2.42	25.00 ± 3.22$_{abcde}$	1.00 ±0.00	2.00 ±0.45	116.80 ± 13.49	186.333 ± 9.038 $_{abcde}$	8.35 ± 0.47 $_{abcde}$	14.12 ± 1.10
PCFr3	7.56 ± 0.11	5.37 ± 0.46	15.78 ± 0.58	10.23 ± 0.64	46.6 ± 0.68	32.67 ± 2.19	1.20 ±0.20	2.20 ±0.45	108.00 ± 3.146	141.000 ± 30.238	7.38 ± 1.09	10.05 ± 2.22
PMeCaB	5.33 ± 0.36	4.69 ± 0.54$_{abcde}$	10.72 ± 0.67	9.6 ± 0.97$_{abcde}$	33.2 ± 1.99	30.00 ± 3.05	1.20 ±0.20	2.20 ±0.45	141.40 ± 13.7$_{abcde}$	205.000 ± 30.6 $_{abcde}$	12.4 ±1.9 $_{abcde}$	14.16 ± 0.86
PCQ	4.85 ± 1.21$_{abcde}$	6.03 ± 0.41	9.14 ± 1.74$_{abcde}$	11.48 ± 0.75	30.00 ± 0.86	36.75 ± 2.14	2.00 ±0.32	1.60 ±0.25	169.200 ±43.17$_{abcde}$	110.500 ± 19.551 $_{bcde}$	12.20 ± 4.00	40.13 ±3.70 $_{abcde}$

Values are Mean ± S.E.M $_a$ *significant compared with Nor,* $_b$ *with PUN,* $_c$ *with PFr1,* $_d$ *with PFr2,* $_e$ *with PFr3,* $_f$ *with PMeCaB,* $_g$ *with PCQ.*
PUn – Parasitized Untreated animals, P*MeCaB* – Parasitized animals + 1000mg/kg b.w *C.albidum, PCFr 1* – parasitized s + 250mg/kg of fraction 1, *PCFr 2* – parasitized animals + 250mg/kg of fraction 2, *PCFr 3* – parasitized animals + 250mg/kg of fraction 3, *PCQ*– parasitized animals + 30mg/kg of chloroquine. Nor – Normal Control animals.

Table 1b:
Effect of methanolic extract of C.*albidum* (*MeCaB) bark* and fractions on Haematological indices in bled animals by days 3 and 7 post bleeding out.

Groups	RBC (million/mm$_3$)		Hb (mg/dL)		PCV (%)		Platelets(thousand/mm$_3$)		WBC (10$_3$/mm$_3$)	
	Day 3	Day 7	Day 3	Day 7	Day 3	Day 7	Day 3	Day 7	Day 3	Day 7
Nor	7.28 ± 0.55	6.91 ± 0.14	13.92 ± 0.22	13.5 ± 0.33	43.6 ± 0.4	42.4 ± 1.12	114.600 ± 9.097	105.400 ± 7.089	8770 ± 2301.11	9600 ± 1484.99
Bla	6.26 ± 0.27	6.37 ± 0.51	11.94 ± 0.49	11.48 ± 0.93	38.2 ± 1.43	36.5 ± 2.93$_{ahi}$	82.400 ± 4.445$_{ahik}$	88.167 ± 9.583$_a$	4830 ± 420.59$_{hk}$	4110 ± 406.94$_a$
MeCaB	7.16 ± 0.19	7.04 ± 0.26	13.64 ± 0.29	14.02 ± 0.87	43.2 ± 0.97	43.00 ± 2.17	123.400 ± 16.869	96.800 ± 10.136	6270 ± 696.9	5580 ± 1179.3
BMeCaB	5.69 ± 0.49$_a$	7.04 ± 0.09	11.98 ± 1.18$_a$	13.8 ± 0.34	37.6 ± 3.44$_{ai}$	42.5 ± 1.32	121.800 ± 10.988	96.000 ± 6.364	9150 ± 2224	6850 ± 785.33
Bhaem	6.68 ± 0.18	7.04 ± 0.09	12.68 ± 0.34	12.56 ± 0.44	40.6 ± 1.07	39.2 ± 0.49$_i$	133.400 ± 8003.75	92.400 ± 5.474	9610 ± 1548.58	6950 ± 1187.86

Values are Mean ± S.E.M $_a$ *significant compared with Nor,* $_h$ *with BMeCaB,* $_i$ *with MeCaB,* $_j$ *with Bla* $_k$ *with BHae,*. Nor – Normal Control animals, *MeCaB* – animals administered 1000mg/kg *C.albidum, BHaem* – animals bled and administered with ferrous sulphate, *BMeCaB* – animals bled +1000mg/kg *C.albidum, Bla* – animals bled untreated.

Effect of *MeCaB* and its' fractions on duodenal morphometry of parasitized treated mice by day 7 post inoculation: Duodenal villi height in the parasitized treated with chromatographic fraction 1 (*PCFr 1)* treated groups were significantly decreased compared with other experimental groups (Table 2a). Duodenal villi width in the parasitized treated with C.*albidum* (*PMeCaB)* group was significantly increased compared with other experimental groups except the parasitized chloroquine (PCQ) treated group, (Table 2a). Duodenal villi cryptal depth in both parasitized untreated (*PUn)* and chromatographic fraction 2 (*PCFr 2)* groups were significantly decreased compared with other experimental groups, (Table 2a). Duodenal villi surface area of parasitized treated with chloroquine (*PCQ)* chromatographic fractions 1 and 3 (*PCFr* 1 and 3) groups were significantly increased compared with other experimental groups, (Table 2a). Duodenal villi height/cryptal depth ratio in normal (*Nor), parasitized untreated (PUn) and* chromatographic fraction 1 (*PCFr 1)* experimental group was significantly decreased compared with other experimental groups, (Table 2a). Duodenal villi height/width ratio in parasitized treated with *C.albidum (PMeCaB),* chromatographic fractions 2 and 3 (*PCFr 2 and 3)* groups were significantly decreased compared with other experimental groups, (Table 2a).

Effect of *MeCaB* and its' fractions on duodenal morphometry of bled mice by day 7 post bleeding out: Duodenal villi height in the bled (*Bla) alone* group were significantly increased compared with other experimental groups (Table 2b). Duodenal villi cryptal depth in both C.*albidum* treated alone (*MeCaB)* and bled treated with C.*albidum* (*BMeCaB)* groups were significantly increased compared with other experimental groups, (Table 2b). The Duodenal villi surface area of bled treated with C.*albidum* (*BMeCaB)* treated group were significantly decreased compared with other experimental groups (Table 2b). Duodenal villi height/cryptal depth and height/width ratios in bled treated with C.*albidum* (*BMeCaB)* treated group was significantly decreased compared with other experimental groups (Table 2b).

Table 2a:
Effect of methanolic extract of C.*albidum* (*MeCaB*) bark and its' fractions on duodenal morphometry of parasitized mice by day 7 post inoculation

GROUP	Nor	PUn	PCFr1	PCFr2	PCFr3	PMeCaB	PCQ
Height (µm)	26.0±2.1$_{bgi}$	37.33 ± 1.20	23.33± 0.88$_{bdefig}$	32.0 ± 1.53	37.67 ± 0.67	33.0 ± 1.53	33.0 ± 1.53
Width (µm)	8.0 ± 0.6$_{f}$	7.0 ± 0.58$_{fjk}$	8.667 ± 0.67$_{f}$	7.667± 0.33$_{fk}$	6.0± 0.58$_{fgj}$	13.0 ± 1	11.67± 0.88
Cryptal Depth (µm)	12.67±0.3 $_{aghi}$	9.33± 1.33$_{efhij}$	12.67 ± 0.88$_{ehij}$	10.33±0.88$_{ehijk}$	18.67 ± 0.67	15.67± 1.45$_{i}$	14.67±0.33$_{g}$
Surface Area (µm2)	218.0±10.3$_{cek}$	215.7±3.0$_{cefijk}$	400 ± 17.32	212.3 ± 2.33$_{eg}$	306.7±36.6$_{eg}$	250.0 ± 10$_{k}$	399.3 ± 5.2
Villi Height/ Cryptal Depth	1.63 ± 0.34$_{eh}$	1.63 ± 0.11$_{eh}$	1.83 ± 0.091$_{h}$	2.46 ± 0.12$_{h}$	2.59 ± 0.11	2.36 ± 0.13$_{k}$	3.43± 0.23
Villi Height/Width	4.23 ± 0.30$_{e}$	5.00 ± 0.43$_{e}$	2.92 ± 0.11$_{beh}$	2.42± 0.08$_{abeh}$	6.51 ± 0.19	2.71±0.1$_{abeh}$	4.69 ± 0.5$_{e}$

Values are Mean ± S.E.M.; *$_{a}$ significant compared with Nor, $_{b}$ with PUN, $_{c}$ with PCFr1, $_{d}$ with PCFr2, $_{e}$ with PCFr3, $_{f}$ with PMeCaB, $_{g}$ with PCQ.*
PUn – Parasitized Untreated animals, P*MeCaB* – Parasitized animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum, PCFr 1* – parasitized animals treated with 250mg/kg b.w of fraction 1, *PCFr 2* – parasitized animals treated with 250mg/kg b.w of fraction 2, *PCFr 3* – parasitized animals treated with 250mg/kg b.w of fraction 3, *PCq* – parasitized animals treated with 30mg/kg b.w of chloroquine

Table 2b:
Effect of methanolic extract of C.*albidum* (*MeCaB*) bark and its' fractions on duodenal morphometry of bled mice by day 7 post bleeding out.

GROUP	Nor	Bla	BMeCaB	MeCaB	BHaem
Height (µm)	26 ± 2.08$_{j}$	33.33 ± 0.88	22.33 ± 1.45$_{j}$	26 ± 1.53$_{j}$	25.67 ± 0.67$_{j}$
Width (µm)	8 ± 0.58	9 ± 0.58	10.33 ± 0.33	9 ± 0.58	11 ± 1
Cryptal Depth (µm)	12.67 ± 0.33 $_{ahi}$	18.33 ± 0.88	20.33 ± 0.88	22.67 ± 1.76	17 ± 0.58$_{i}$
Surface Area (µm2)	218 ± 10.26$_{ijk}$	279.3 ± 5.207	129.7 ± 10.17$_{aijk}$	263 ± 10.15	231.7 ± 9.28
Villi Height/Cryptal Depth	1.63 ± 0.34$_{h}$	1.82 ± 0.039$_{k}$	0.94 ± 0.033$_{ik}$	1.77 ± 0.13$_{k}$	1.47 ± 0.13$_{h}$
Villi Height/Width	4.23 ± 0.30	3.51 ± 0.11	1.79 ± 0.15$_{ahj}$	3.14 ± 0.099$_{h}$	2.49 ± 0.17$_{h}$

Values are Mean ± S.E.M.; *$_{a}$ significant compared with Nor, $_{h}$ with BMeCaB, $_{i}$ with MeCaB, $_{j}$ with Bla $_{k}$ with BHae,.*
Nor – Normal Control animals, *MeCaB* – animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum, BHaem* – animals bled and administered with ferrous sulphate, *BMeCaB* – animals bled and administered with 1000mg/kg of methanol extract of *C.albidum, Bla* – animals bled untreated.

Table 3a: Effect of methanolic extract of C.albidum bark (*MeCaB)* and its fractions on intestinal jejunal morphometry of parasitized mice by day 7 post inoculation**.**

GROUP	Nor	PUN	PCFr1	PCFr2	PCFr3	PMeCaB	PCQ
Height (µm)	23 ± 1.53$_{cf}$	20 ± 1.16$_{c}$	32.67 ± 1.20	24.67 ± 1.20$_{c}$	20.33 ±0.88$_{c}$	16.3± 0.7$_{cdg}$	19.3 ±0.7$_{c}$
Width (µm)	10.67 ±0.88	5.33 ±0.33$_{ad}$	8 ± 0.58	11 ± 1	9 ± 0.58	8.67 ± 0.67	12.33± 0.33
Cryptal Depth (µm)	16.33 ± 0.67	11 ± 0.58$_{a}$	11.67 ± 0.33$_{a}$	14.33 ± 0.33	11.67 ± 0.89$_{a}$	10.33±0.33$_{a}$	12 ± 1.53$_{g}$
Surface Area (µm2)	245.3 ± 27.36	117.3±7.3$_{acd}$	252 ± 2.31	221 ± 9.85$_{c}$	136 ± 4.62$_{ad}$	135.3±8.7$_{acd}$	252 ± 26.41
Villi Height/Cryptal Depth	1.48 ±0.15$_{cg}$	1.67 ± 0.09$_{c}$	3.267 ± 0.12	1.47± 0.092$_{cg}$	2.05 ± 0.10$_{c}$	1.64± 0.047$_{c}$	2.15 ± 0.15$_{c}$
Jejuna Villi Height/Width	2.009±0.096$_{bcd}$	4.27 ± 0.13	4.054 ± 0.12	3.73 ± 0.15	2.15±0.076$_{bcd}$	1.75±0.13$_{bcd}$	1.1±0.016$_{bcd}$

Values are Mean ± S.E.M *$_{a}$ significant compared with Nor, $_{b}$ with PUN, $_{c}$ with PCFr1, $_{d}$ with PCFr2, $_{e}$ with PCFr3, $_{f}$ with PMeCaB, $_{g}$ with PCQ*
PUn – Parasitized Untreated animals, P*MeCaB* – Parasitized animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum, PCFr 1* – parasitized animals treated with 250mg/kg b.w of fraction 1, *PCFr 2* – parasitized animals treated with 250mg/kg b.w of fraction 2, *PCFr 3* – parasitized animals treated with 250mg/kg b.w of fraction 3, *PCq* – parasitized animals treated with 30mg/kg b.w of chloroquine. Nor – Normal Control animals

Table 3b:
Effect of methanolic extract of C.albidum bark (*MeCaB)* and its fractions on intestinal jejunal morphometry of bled mice by day 7 post bleeding out.

GROUP	Nor	BMeCaB	MeCaB	Bla	BHaem
Height (µm)	23 ± 1.53	25.33 ± 1.20	24 ± 1.155	21 ± 0.58	25 ± 1.16
Width (µm)	10.67 ±0.88	7 ± 0.58$_{ik}$	12.33 ± 0.67	10.67± 0.67	11.33± 1.33
Cryptal Depth (µm)	16.33 ± 0.67	19.67 ± 0.33	11.33 ± 0.33$_{ahj}$	20.33 ± 1.45	15.67 ± 0.67$_{hj}$
Surface Area (µm2)	245.3 ± 27.36	151.7 ± 6.98$_{k}$	250 ± 15.28	202.7 ± 3.71	257.3 ± 6.36
Villi Height/Cryptal Depth	1.48 ±0.15$_{i}$	1.57 ± 0.099$_{i}$	2.34 ± 0.19	1.045 ± 0.092$_{hk}$	1.9 ± 0.035
Jejuna Villi Height/Width	2.009 ± 0.096$_{h}$	3.63 ±0.13	1.73 ±0.098$_{hk}$	2.06 ± 0.031$_{h}$	2.48 ± 0.13$_{h}$

Values are Mean ± S.E.M $_{a}$ *significant compared with Nor,* $_{h}$ *with BMeCaB,* $_{i}$ *with MeCaB,* $_{j}$ *with Bla* $_{k}$ *with BHae.*
Nor – Normal Control animals, *MeCaB* – animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum, BHaem* – animals bled and administered with ferrous sulphate, *BMeCaB* – animals bled and administered with 1000mg/kg of methanol extract of *C.albidum, Bla* – animals bled untreated.

Table 4a:
Effect of methanolic extract of C.*albidum* bark (*MeCaB)* and its fractions on correlation of intestinal jejunal surface area and body weight of parasitized mice by day 7 post inoculation.

GROUP	Pearson coefficient value	P-value
Nor	0.8447	0.0718
PUn	0.7145	0.1750
PCFr 1	0.7438	0.1496
PCFr 2	0.9041*	0.0351
PCFr 3	0.7690	o.1286
PMeCaB	0.9324*	0.0209
PCQ	0.9581*	0.0102

Values are correlation coefficient * *significant*
PUn – Parasitized Untreated animals, P*MeCaB* – Parasitized animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum, PCFr 1* – parasitized animals treated with 250mg/kg b.w of fraction 1, *PCFr 2* – parasitized animals treated with 250mg/kg b.w of fraction 2, *PCFr 3* – parasitized animals treated with 250mg/kg b.w of fraction 3, *PCq* – parasitized animals treated with 30mg/kg b.w of chloroquine. Nor – Normal Control animals,

Table 4b: Effect of methanolic extract of C.albidum bark (*MeCaB)* and its fractions on correlation of intestinal jejunal surface area and body weight of bled mice by day 7 post bleeding out.

GROUP	Pearson coefficient	P value
Nor	0.8447	0.0718
BMeCaB	0.9434*	0.0160
MeCaB	0.8684	0.0562
Bla	0.8614	0.0606
BHaem	0.8710	0.0545

Values are correlation coefficient * *significant*
Nor – Normal Control animals, *MeCaB* – animals administered 1000mg/kg b.w methanol extract of *Chrysophyllum albidum, BHaem* – animals bled and administered with ferrous sulphate, *BMeCaB* – animals bled and administered with 1000mg/kg of methanol extract of *C.albidum, Bla* – animals bled untreated.

Effect of methanolic extract of C.albidum bark (*MeCaB)* and its fractions on intestinal jejunal morphometry of parasitized mice by day 7 post inoculation.

There was a significant increase in the jejunum villi height of the parasitized treated with chromatographic fraction 1 (*PCFr* 1) group compared with all other groups while the villi height of the parasitized C.*albidum* (*PMeCaB)* was significantly decreased compared with all other groups. However, the jejunum villi height of the parasitized chloroquine (*PCQ)* was significantly decreased compared with parasitized C.*albidum* (*PMeCaB)* treated groups, (Table 3a). Jejunum villi width of the parasitized untreated (PUn) group was significantly decreased compared with all other groups (Table 3a). Jejunum cryptal depth of the parasitized untreated (PUn), parasitized treated with and C.*albidum* (*PMeCaB),* chromatographic fraction 1 and 3 (*PCFr 1*, 3) respectively, chloroquine (PCQ) groups, were significantly decreased compared with the normal (Nor) experimental groups, (Table 3a). Jejunum villi surface area of the parasitized untreated (*PUn)* group was significantly decreased compared with all other experimental groups, (Table 3a).

The jejunum villi Height/Cryptal depth was significantly increased in the parasitized treated with chromatographic fraction 1 (*PCFr* 1) group compared with all other experimental groups, (Table 3a). The jejunum villi Height/Width ratios were significantly increased in the parasitized untreated (*PUn)* and treated with chromatographic fraction 1 (*PCFr* 1) groups compared with other experimental groups. In the parasitized C.*albidum* (*PMeCaB)* treated group the jejunum villi height/width ratio were significantly decreased compared with other experimental groups, (Table 3a).

Effect of *MeCaB* and its fractions on intestinal jejunal morphometry of bled mice by day 7 post bleeding out.

The jejunum villi width of the bled treated with C.*albidum* (*BMeCaB)* group was significantly decreased compared with the C.*albidum* (*MeCaB)* alone, bled treated with haematinic (*BHaem)* groups, (Table 3b). The jejunum cryptal depth of both the bled treated with C.*albidum* (*BMeCaB)* and bled alone (*Bla)* groups were significantly increased compared with all other groups (Table 3b). The bled treated with C.*albidum* (*BMeCaB*) had a significantly lower jejunal surface area compared with all the other experimental groups.

The jejunum villi Height/Width ratio was significantly increased in the bled treated with C.*albidum* (*BMeCaB)* group compared with other experimental groups. In the C.*albidum* (*MeCaB)* treated group the jejunum villi height/width ratio was significantly decreased compared with bled treated with C.*albidum* (*BMeCaB*) and bled treated with haematinic (BHaem) treated groups, (Table 3b).

Effect of methanolic extract of C.*albidum* bark (*MeCaB)* and its fractions on correlation of intestinal jejunal surface area and body weight of parasitized mice by day 7 post inoculation.

The jejunal surface area and body weights of C.*albidum* (*MeCaB*) alone and parasitized treated with chromatographic fraction 2 (PCFr 2) were significantly and positively correlated compared with other experimental groups, (Table 4a)

Effect of methanolic extract of C.albidum bark (*MeCaB)* and its fractions on correlation of intestinal jejunal surface area and body weight of bled mice by day 7 post bleeding out.

The jejunal surface area and body weight of the bled treated with C.*albidum* (*BMeCaB*) was significantly and positively correlated compared with other experimental groups, (Table 4b)

DISCUSSION

There has been reports on decreases in body weight during haemolytic anaemia which was mimicked in this study by *Plasmodium berghei berghei* parasite infection and blood loss through bleeding out (via the orbital sinus). This decreases might be as a result of obstruction in the intestinal and might result in severe cases to death. However, mild or moderate parasitaemia count might lead to intestinal mucosa cell inflammation (Negrao 2001, Silveira *et al*., 2002). In this study, there was weight gain in both the parasitized mice and bled animals treated with C.albidum and its fractions. This is in consonant with previous studies in which C.albidum bark extracts have been used in ameliorating diseased conditions (Adewoye *et al*.,2012, 2013,2013 ; Salami *et al*. 2017, 2018).

Groups treated with *C. albidum* and fractions also had lower parasitaemia count by days 3 and 7 post inoculation and treatment. This antiplasmodic activity is in agreement with previous studies (Adewoye *et al*., 2010) in which *C.albidum* supressed *Plasmodium berghei berghei* parasite. The antiplasmodic, antimicrobial and kill kinetic property of MeCaB however has been attributed to the presence of a bioactive component alkaloids (Adewoye *et al*, 2010 and 2011) inherent in *C.albidum*.

The haematological variables obtained were also similar to earlier reports in which there were no signs of anaemia but erythropoietic activities (Adewoye *et al*., 2012) in the C.albidum treated animals in both haemolytic models used. This increase in weight gain might have also been due to the potential activities of *C.albidum* begin a probiotic (Salami *et al* 2018) which might have been conferred to the animals used in this study. Probiotics have been reported to thrive in the guts of host and modulates digestive processes (Kalavathy *et al*., 2008, Shokryazdan *et al*., 2017). This enhances their growth and production of microbial enzymes which invariably assist in maintaining microflora homeostatsis in the gut (Mohapatra *et al*., 2012). Probiotics can directly provide nutrients or stimulate nutrient absorption thus increasing body weight (Ghazalah *et al*., 2010; Aderolu *et al*., 2013).

The duodenum is saddled with the task of final digestion and initial absorptive processes of chyme exiting the stomach through the pyloric sphincter. The jejunum functions majorly in motility and absorptive processes of nutrients from chyme (ingested and digested food from the duodenum) while the ileum functions with passive food absorption. In this study much emphasis was placed on both the duodenal and jejununal portions of the small intestine. Food absorption (is made possible as a result of the presence of fingerlike projections) is also very vital to growth which is reflective in body weight (Awad *et al*., 2001) during diseased conditions. Presences of parasite in the gastrointestinal tract have been documented to cause decreases in the villi and microvilli which ultimately leads to reduced absorption and malnutrition in the host (Batista *et al*, 2015)..

The Gut morphometry (Villi height, width, cryptal depth and surface area) has been found to be a very useful tool in the evaluation and absorption of nutrients in animals (Bello *et al*., 2012) which correlates positively mostly with growth reflected in body weight gain. The villi height has been documented to be a good representation of the function as well as activation of intestinal villi (Shamoto and Yamauchi 2000). *C. albidum* treatment increased in the jejunal villi height, width and surface area (in both models of haemolytic anaemia used in this study). A concomitant increase both the villi height and width has been suggestive to increases in the absorptive surface area of the gut (Samanya and Yamauchi, 2002; Pappenheimer and Michel 2003; Zhou *et al* 2003; Bowen 2011). This has also been reported to correspond to increase in body weight (Bello *et al*., 2012) which have been observed in this study in groups treated with *C.albidum* and its fractions especially PCFr1. The surface areas of the parasitized animals treated with *C. albidum* and the chromatographic fractions 1 and 3 were increased, which might be indicative of increased nutrient absorptive area.

The villi height width ratio Cryptal depth has been used to indicate higher mucosal proliferation activity (ie regeneration) and greater intestinal glandular activity. It has also been noted to be responsible for secretion of electrolytes thus enhancing water secretion into intestinal lumen for the purpose of digestion (Bowen, 2011). The cryptal depth is responsible for the production of new epithelial and enterocytes or cells found along the villi. However, a decreased cryptal depth has been documented to promote villi renewal (Sobolewska *et al*., 2017). In the groups treated with C.albidum and fractions (except PFr2) the jejunal cryptal depth were decreased this was also observed in the parasitized untreated group as well. This is suggestive of adequate villi cell or tissue turnover and optimum gut conditions (Sobolewska *et al*., 2017) as there was no intense demand for villi cell renewal due to pathological conditions. A different scenario was observed in the bled alone and bled treated with haematinics which had very high cryptal depth indices thus indicating renewal of the villi cells in these groups during this study. This was the trend in the duodenal as all the C.albidum and fractions treated groups had increases in the cryptal depth which is also indicative of increased cell renewal. Xu *et al*., (2003) stated that there is poor absorption of nutrients with consequent low performance when there is a reduced villi height but increased cryptal depth. This might be the case of animals in the bled alone and bled but haematinic treated groups however, the duoedenal villi heights in the *C.albidum* and fractions treated groups were all increased thus negating the case of poor nutrient absorption. There were increased villi height cryptal ratios in the jejunum of the parasitized animals treated with *C. albidum* and the chromatographic fractions 1 and 3groups but increases in the duodenum of all the *C.albidum* treated groups in this study. The villi height cryptal depth ratio is also indicative of enhanced nutrient digestibility as it can cause release of digestive enzymes from the villi tip (Nabila *et al*., 2017), absorptive capacity (Montage *et al*., 2003), cell mitosis (Khambualai *et al*., 2009), and epithelial turnover with little geared towards maintaining intestinal homeostasis (Montagne *et al*., 2003; Pelicano *et al*., 2005; Incharoen *et al*., 2010). This probably means that *C.albidum* enhanced optimum intestinal homeostasis and nutrient uptake irrespective of disease

condition. It is also possible that the bioactive components found in *MeCaB* were acting as prebiotics within the animal's gut. This in turn stabilizes and maintains the gut microfloral without interfering with the intestinal absorptive capacity. These observed results could suggest *C. albidum* as a good source of natural growth promoters with greater health benefits. The observed significant correlation between the increasing body weights of the chromatographic fractions and its surface area in the parasitized animals has further buttressed it as a plant with growth promoting activity in livestock production.

Results of this study suggests that *C. albidum* and its fractions have beneficial potentials in enhancing, stabilizing and maintaining the integrity of intestinal mucosa especially at the jejunum.

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