Haematological indices of *Salmonella Gallinarum* (Gr. D1-1, 9, 12) infected broiler chickens treated with ethanolic leaf extract of *Chrysophyllum albidum* (G. Don)

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Abstract

This experiment was conducted for eight weeks to evaluate the haematological and serum biochemical indices of broiler chickens infected with *Salmonella Gallinarum* (Gr. D1-1, 9, 12) and treated with ethanolic leaf extract of *Chrysophyllum albidum* (G. Don). The experimental design was a factorial arrangement in a completely randomised design (CRD) involving one hundred and sixty two (162) unsexed day-old Arbor Acre broiler chicks. The chickens were allotted to nine (9) groups of three (3) replicates with each replicate comprising six (6) birds of infected, non-infected, treated and untreated group. Phytochemical screening of *C. albidum* revealed the presence of tannins, saponins, cardiac glycosides, steroids, terpenoids and flavonoids. The extract and the standard drug were administered at 7th day post infection. There were significant differences ($P < 0.05$) in Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV), Red Blood Cell (RBC), Haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Lymphocyte (LYM) and none in Heterophil (HET), Mean Corpuscular Haemoglobin Concentration (MCHC), Monocytes, Basophils and Eosinophils at all levels. The highest values of PCV (33.67 ± 1.86^a), RBC (3.02 ± 0.4^a) and Hb (11.23 ± 0.62^a) though normal were observed in the standard drug (Doxxygen®) group. Most of the serum biochemical parameters were found to be within range usually seen in avian species. It was concluded that ethanolic leaf extract of *C. albidum* had no deleterious effect on haematological and biochemical indices of broiler chickens and hence can be further explored pharmaceutically for its usefulness in treating fowl typhoid.

Keywords: *C. albidum*, haematology, serum bio-chemicals, *Salmonella Gallinarum*, broiler chickens

Introduction

Animals are coexisting with a tremendous number of micro-organisms such as bacteria, fungi, mycoplasma, chlamydia, parasites (helminths, protozoa and insects) and viruses. A number of these are strict pathogens that lead to lethal infectious diseases (Ian, 2016). Salmonellosis is a **bacterial** disease condition in poultry causing heavy economic loss due to mortality and reduced production. Pullorum disease (PD) and fowl typhoid (FT) are great problem in poultry farming (WHO, 2018) and the presence of *Salmonella* organism in poultry or poultry products can hinder international trade due

to its zoonotic nature (Lotte, 2018). Livestock farmers are generally faced with the challenge of improving livestock performance through disease control so as to secure more reliable net returns (Pervez, 1992). A lot of research and production strategies have been employed, including the use of antibiotics to achieve this aim. Antibiotic growth promoters (AGP) have made a tremendous contribution to the profitability of the poultry industry in time past (Kehinde *et al.*, 2010). Recently, it was reported that the use of antibiotics as a growth promoter in chickens has caused some unwanted results (Botsoglou and Fletouris, 2001). Although antibiotics

achieved good performance, their potential side effects became a real public health concern globally (Donoghue, 2003) and eventually led to the ban of the products especially in the western world (Nweze and Nwankwagu, 2010). Most antibacterial performance promoters were banned not only because of cross-resistance but also due to multiple resistances. Residues of conventional drugs in food of animal origin as a result of the abuse of these medicines can also limit the development of sustainable livestock production by resource poor farmers (Neu, 1992). Therefore, poultry farmers are being challenged to develop an alternative for AGP and this has triggered an explosion of interest in the use of herbs and spices and their products as supplements in animal rations (Owen, 2011). Considerable attention has been paid to medicinal herbs as replacements for AGP (Ibrahim *et al.*, 2005). Plants remain an inexhaustible source of antimicrobials and more than 200 plants were used in the treatment of animal diseases such as foot and mouth disease, mange, tuberculosis, etc. Some of these plants are: *Acacia nilotica*, *Gardenia erubescens*, *Vernonia amygdalina*, *Azadirachta indica* among others (Mahomoodally, 2013; Saganuwan, 2017; Rajesh *et al.*, 2018). The methanol leaf extract of *Spondias mombin* was reported to be safe and hepatoprotective against acetaminophen-induced liver injury (Aba *et al.*, 2018). It was reported that livestock farmers use the fruit of *Lagenaria brevisflora* in the treatment of Newcastle disease and coccidiosis in poultry (Adepegba and Abu, 2018). The most active compounds of plant extracts are absorbed in the intestine by the enterocytes, readily metabolized by the body and have a short half-life. The metabolic products are transformed into polar compounds by conjugation with glucuronate and excreted in the urine. *C.*

albidum G. Don. (*Sapotaceae*) is a tree with fruit of high commercial values and common throughout the tropical Central, East and West Africa (Amusa *et al.*, 2003). It is commonly called African star apple and it is a very useful medicinal plant common in the tropical and sub-tropical regions of the world (Emudainohwo *et al.*, 2015). The plant has an edible tropical fruit called *Utieagadava* in Urhobo, *agbalumo* in Yoruba, *udara* in Ibo, *Efik* and *Ibibio*, *ehya* in Igala and *agwaluma* in Hausa tribes of Nigeria (Laurent *et al.*, 2012). The fruit is very common both in rural and urban areas especially during the months of December to April. Most of the time, the fruits are not usually harvested from the trees, but left to drop naturally to the ground where they are picked up (Vade, 2010). The fruit has been reported to contain the highest content of ascorbic acid per 100g of edible fruit or about 100 times that of oranges and 10 times of that of guava or cashew (Okoli and Okere, 2010). The fruit is equally an excellent source of vitamins, iron and flavour to diets (Adisa, 2000). The pharmacological activities of *C. albidum* include; antioxidant, anti-microbial, antiplasmodia, anti-inflammatory, analgesic and anti-diabetic properties. This justifies the usage of the plant in ethnomedicine in the treatment of numerous diseases. The rich sources of natural antioxidants in *C. albidum* have been harnessed and established to promote health by acting against oxidative stress related disease such as; diabetics, cancer and coronary heart diseases (Bruits and Bucar, 2002). The extracts of the seeds and roots of *C. albidum* have good potentials as anti-inflammatory and anti-diarrhoea compound. The extracts of the roots, barks and leaves are commonly used in southern Nigeria as an application to sprains, bruises and wounds. The seeds and roots extracts are also used to arrest bleeding from fresh

wounds, inhibit microbial growth and accelerate the process of wound healing (Okoli and Okere, 2010). According to Duyilemi and Lawal (2009), *C. albidum* leaf is a potential source of antimicrobial against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Shigella* spp. Medicinal plants are ubiquitous and their usage have little or no deleterious effect on human as well as in the treatment of animals' infectious diseases. They pose minimal risk of tissue accumulation unlike the synthetic antibiotics (Kohlert *et al.*, 2000; Huyghebaert, 2003). Therefore, this experiment was designed and conducted to examine the haematological and serum biochemical indices of broiler chickens infected with *Salmonella enterica* serovar *Gallinarum* (D. E. Salmon) and treated with ethanolic leaf extract of *Chrysophyllum albidum* (G. Don).

Materials and methods

Experimental site

The experiment was carried out at the Animal Parasitology and Microbiology Unit of the Department of animal Production and Health of the Federal University of Technology, Akure. The

geographical location of the Federal University of Technology, Akure is (5° 07' E, 7° 19' N), (5° 09' E, 7° 19' N), (7° 17' N, 5° 07'E), (7° 17' N, 5° 09' E) and has a tropical climate of average annual temperature of 26.7°C and average annual rainfall of 2378mm (Kareem 1997).

Experimental materials

A total of one hundred and sixty-two (162), day-old Arbor Acre broiler chicks breed were obtained from a reputable commercial hatchery at Ibadan, Oyo State Nigeria for the experiment. Typed bacterial organism, *Salmonella enterica* serovar *Gallinarum* Gr. D1-1, 9, 12 was sourced from National Veterinary Research Institute, Vom (NVRI in Jos, Nigeria).

Management practices

The pen was properly swept, cleaned and disinfected with Diskol®. (The active ingredients in Diskol® are; Quaternary ammonium compounds, Glutaraldehyde & Formaldehyde). The chicks were fed *ad-libitum* using reputable commercial feed with the nutritional composition shown in Table 1. Clean and cool water was supplied regularly through-out the period of the experiment. Normal vaccination schedule and bio-security measures were scrupulously applied.

Table 1: Nutritional composition of the experimental diets at starter and finisher phases

Parameters	Broiler starter	Broiler finisher
Crude protein (%)	22.00	18.00
Metabolizable energy (Kcal/Kg)	2900.00	2900.00
Fat/oil (%)	6.00	6.00
Crude fibre (%)	5.00	5.00
Calcium (%)	1.00	1.00
Phosphorus (%)	0.45	0.40
Lysine (%)	1.20	0.85
Methionine (%)	0.55	0.35
Salt (%)	0.30	0.30

Experimental design

The experimental design was a factorial arrangement in a completely randomised design (CRD). The chickens were allotted to nine (9) groups of three (3) replicates with each replicate comprising six (6) birds. Group 1: Infected birds but not treated

(negative control). Group 2: Infected and treated with standard drug (Doxygen®) and thus served as positive control. Group 3: Not infected and not treated with any drug or extract. Group 4: Infected and treated with dose 1 of the prepared extract. Group 5: Infected and treated with dose 2. Group

6: Infected and treated with dose 3. Group 7: Not infected but given dose 1. Group 8: Not infected but given dose 2. Group 9: Not infected but given dose 3 of the extract.

Ethanol extraction of Chrysophyllum albidum leaf extract

Fresh leaves of *C. albidum* were harvested right in its natural habitat of the environment of the Federal University of Technology Akure, Ondo State, Nigeria. The plant was identified in the Department of Forestry and Wood Technology Federal University of Technology Akure. The leaves were gently rinsed in clean water in order to get rid of the dust particles on them and thereafter air-dried under shade for three weeks until well dried and crispy. The air-dried leaves were then pulverized into fine powder using a pulverizing machine (Thomas-Willey milling machine) and stored in airtight container pending further procedure. 100g of the sample was measured and soaked in 500 mL of ethanol for 72 hours. Filtration exercise was perfected using a clean laboratory flask, muslin cloth and finally through Whatman No 1 (125mm) filter paper. After filtration, the wet extract was then concentrated by using rotary evaporator and finally the water was sublimated into a trap at low temperature (freeze-dried) in a lyophilizer (lyophilizing machine). The extract was kept in the fridge until needed for the experiment (Adeoluwa *et al.*, 2015).

Phytochemical screening of ethanolic leaf extract of C. albidum

The phytochemical screening (qualitative and quantitative) of the ethanolic leaf extract of *C. albidum* was carried out to confirm the presence of tannin, saponin, phlobatannin, cardiac glycosides (Salkowski's test and Keller-killiani's test), steroid, terpenoid, anthraquinone and flavonoids as described by Trease and

Evans (1989); Parekh and Chanda, (2007).

Infection of the experimental chickens

The organism (*Salmonella enterica serovar Gallinarum Gr. D1-1, 9, 12*) was cultured on nutrient agar at 37°C for 24 hours. With the aid of sterile inoculating loop, colonies were picked unto broth culture to obtain suspension of different bacterial concentration as determined by the McFarland scale. The broth culture of the organism was later prepared and serially diluted to obtain 1×10^6 CfU/mL that was adopted to inoculate the chickens at 0.5 mL per bird orally. The extract and the standard drug were administered 7th day post infection.

Administration of ethanolic leaf extract of C. albidum

Therapeutic dose of the extract was given to the broiler chickens in milligram per kilogram (mg/kg/day) of body weight for five days following Lorke's method (1983) and guidelines of OECD (2001). Group 1: Infected birds but not treated (negative control). Group 2: Infected and treated with standard drug Doxygen® (positive control) at the manufacturer's recommendation of 1g/3litres of water for 5days. Group 3: Not infected and not treated. Group 4: Infected and treated with 100mg/kg/day. Group 5: Infected and treated with 250mg/kg/day. Group 6: Infected and treated with 625mg/kg/day. Group 7: Not infected but given 100mg/kg/day. Group 8: Not infected but treated with 250mg/kg/day. Group 9: Not infected but given 625mg/kg/day.

Blood samples collection

At the end of the experiment 5 mL of blood sample was collected from the jugular vein of each of the two broiler chickens per replicate totalling fifty four (54) samples. The blood samples were introduced into plain bottles for serum analysis and EDTA (Ethylene diamine tetra-acetic acid) bottles for haematology.

Determination of haematological and serum biochemical parameters

Haematological parameters such as Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR), Red Blood Cells (RBC), White Blood Cells (WBC), and Haemoglobin (Hb) concentration were determined according to the standard procedure by Heshu (2016) while biochemical parameters of the serum were determined in line with the laboratory manual (Ochei and Kolhatkar, 2007).

Statistical analysis

Collated data were analysed by using General Linear Model of Statistical Analysis System (SAS) software version 9.2 of 2009 and compared between groups by Two-way ANOVA. Means were separated by the Duncan's Multiple Range Test of the same package. Probability values of less than 0.05 ($p < 0.05$) were considered significant.

Results

The results of the qualitative phytochemical screening of *C. albidum*, haematological and serum biochemical parameters of broiler chickens infected with *Salmonella gallinarum* and treated with ethanolic leaf extract of *C. albidum* are shown in Tables 2, 3 and 4 respectively. In the leaf extract, tannins, saponins, cardiac glycosides, steroids, terpenoids and flavonoids were detected; while Phlobatannins and anthraquinones were absent. There were significant differences ($P < 0.05$) in eight out of the twelve parameters considered viz:

ESR, PCV, RBC, Hb, MCH, MCV, LYM and HET. There were no significant differences ($P > 0.05$) in MCHC, monocytes, basophils and eosinophils at all levels. The PCV, RBC and Hb values were observed to be highest in the groups of broiler chickens that were administered standard drug (positive control, PC). Serum biochemical parameters determined include; Total serum protein, albumin, globulin, albumin-globulin ratio, total bilirubin, direct bilirubin, alkaline phosphatase, creatinine and urea. Significant differences occurred in total protein in the serum obtained from the experimental birds ($P < 0.05$). The standard drug group (positive control, PC), 100 mg/mL group and 625 mg/mL group had similar statistical mean while the 0mg/mL group (negative control, NC) and the group treated with 250 mg/ml of the extract equally had similar statistical mean. There were no significant differences in the serum albumin, serum globulin and albumin globulin ratio ($P > 0.05$), but significant differences occurred in serum alkaline phosphatase and creatinine ($P < 0.05$) at different levels of ethanolic leaf extract of *C. albidum* administration. There was no significant difference ($P > 0.05$) in the serum urea, serum total bilirubin and serum direct bilirubin both at the level of infected and non-infected birds as well as the level of various doses of the administered extract.

Table 2: Phytochemical constituents of ethanolic leaf extract of *C. albidum*

Phytochemicals	Qualitative	Quantitative (mg/g)
Tannins	+	5.17
Saponins	+	13.91
Terpenoids	+	8.52
Steroids	+	9.01
Flavonoids	+	9.45
Cardiac glycosides	+	30.34
Phlobatannins	-	-
Anthraquinones	-	-

Where positive sign (+) represents present and Negative sign (-) means absent

Haematological indices of *Salmonella Gallinarum* (Gr. D1-1, 9, 12)

Table 3: Haematological indices of broiler chickens infected with *S. Gallinarum* and treated with ethanolic leaf extract of *C. albidum*

INFECTION	LEVEL	ESR (mm/hr)	PCV (%)	RBC ($\times 10^6/\text{mm}^3$)	HB (g/dL)	MCH (pg)	MCV (fL)	MCHC (g/dL)	LYM (%)	HET (%)	MON (%)	BAS (%)	EOS (%)
Not infected	.	2.33 $\pm$ 0.19	29.75 $\pm$ 0.7	2.29 $\pm$ 0.16	9.91 $\pm$ 0.23	44.55 $\pm$ 1.65	133.77 $\pm$ 4.98	33.33 $\pm$ 0.03	62.25 $\pm$ 0.6	22.08 $\pm$ 0.92	12 $\pm$ 0.58	2.92 $\pm$ 0.19	0.75 $\pm$ 0.13
Infected	.	2.2 $\pm$ 0.39	31.4 $\pm$ 0.94	2.71 $\pm$ 0.18	10.47 $\pm$ 0.31	40.35 $\pm$ 1.8	121.04 $\pm$ 5.39	33.33 $\pm$ 0.02	60.67 $\pm$ 0.67	24.27 $\pm$ 0.98	11.4 $\pm$ 0.46	2.93 $\pm$ 0.15	0.73 $\pm$ 0.12
NC	NC	1.83 $\pm$ 0.31 ^b	31.83 $\pm$ 1.4a ^b	2.76 $\pm$ 0.3 ^{ab}	10.6 $\pm$ 0.47 ^{ab}	39.9 $\pm$ 2.81 ^{ab}	119.83 $\pm$ 8.42 ^{ab}	33.3 $\pm$ 0.04	60.5 $\pm$ 1.23 ^{ab}	23.5 $\pm$ 1.67 ^{ab}	12.5 $\pm$ 0.92	2.67 $\pm$ 0.21	0.83 $\pm$ 0.17
	100	3.33 $\pm$ 0.56 ^a	28.17 $\pm$ 1.05 ^b	2.04 $\pm$ 0.2 ^b	9.38 $\pm$ 0.35 ^b	47.16 $\pm$ 2.49 ^a	141.59 $\pm$ 7.55 ^a	33.31 $\pm$ 0.05	63 $\pm$ 0.73 ^a	21.17 $\pm$ 1.25 ^b	12 $\pm$ 0.58	3.17 $\pm$ 0.31	0.67 $\pm$ 0.21
	250	2.67 $\pm$ 0.56 ^{ab}	29.67 $\pm$ 1.12 ^b	2.36 $\pm$ 0.25 ^{ab}	9.88 $\pm$ 0.37 ^b	43.45 $\pm$ 2.88 ^{ab}	130.43 $\pm$ 8.69 ^{ab}	33.31 $\pm$ 0.05	62.33 $\pm$ 0.8 ^{ab}	22.17 $\pm$ 1.58 ^{ab}	12.17 $\pm$ 0.87	2.67 $\pm$ 0.21	0.67 $\pm$ 0.21
	625	1.67 $\pm$ 0.21 ^b	31.5 $\pm$ 1.09 ^{ab}	2.67 $\pm$ 0.26 ^{ab}	10.5 $\pm$ 0.36 ^{ab}	40.43 $\pm$ 2.28 ^{ab}	121.26 $\pm$ 6.78 ^{ab}	33.34 $\pm$ 0.04	60.67 $\pm$ 0.92 ^{ab}	25 $\pm$ 1.24 ^{ab}	10.5 $\pm$ 0.56	3 $\pm$ 0.26	0.83 $\pm$ 0.17
PC	PC	1.33 $\pm$ 0.33 ^b	33.67 $\pm$ 1.86 ^a	3.02 $\pm$ 0.4 ^a	11.23 $\pm$ 0.62 ^a	38.05 $\pm$ 3.4 ^b	114.05 $\pm$ 10.24 ^b	33.37 $\pm$ 0.03	59.33 $\pm$ 1.33 ^b	26 $\pm$ 1.73 ^a	10.67 $\pm$ 0.88	3.33 $\pm$ 0.33	0.67 $\pm$ 0.33
	NC	2.33 $\pm$ 0.33	29.33 $\pm$ 0.88	2.19 $\pm$ 0.22	9.77 $\pm$ 0.29	45.15 $\pm$ 3.07	135.59 $\pm$ 9.08	33.3 $\pm$ 0.08	62.67 $\pm$ 0.88	20.33 $\pm$ 0.88	13.33 $\pm$ 1.67	2.67 $\pm$ 0.33	1 $\pm$ 0
	Not infected	2.67 $\pm$ 0.33	29.33 $\pm$ 1.33	2.22 $\pm$ 0.32	9.77 $\pm$ 0.47	45.15 $\pm$ 4.01	135.68 $\pm$ 12.3	33.29 $\pm$ 0.07	62.33 $\pm$ 1.20	22.67 $\pm$ 2.19	11.33 $\pm$ 0.67	3 $\pm$ 0.58	0.67 $\pm$ 0.33
	Not infected	2.67 $\pm$ 0.33	29.00 $\pm$ 1.00	2.09 $\pm$ 0.19	9.63 $\pm$ 0.33	46.48 $\pm$ 2.48	139.92 $\pm$ 7.47	33.22 $\pm$ 0	63.33 $\pm$ 0.67	20.00 $\pm$ 0.58	13 $\pm$ 1	3 $\pm$ 0	0.67 $\pm$ 0.33
Infected	Not infected	1.67 $\pm$ 0.33	31.33 $\pm$ 2.33	2.65 $\pm$ 0.54	10.47 $\pm$ 0.77	41.41 $\pm$ 4.59	123.9 $\pm$ 13.61	33.41 $\pm$ 0.04	60.67 $\pm$ 1.86	25.33 $\pm$ 1.86	10.33 $\pm$ 0.33	3 $\pm$ 0.58	0.67 $\pm$ 0.33
	NC	1.33 $\pm$ 0.33	34.33 $\pm$ 1.67	3.33 $\pm$ 0.30	11.43 $\pm$ 0.57	34.65 $\pm$ 1.58	104.08 $\pm$ 4.85	33.3 $\pm$ 0.04	58.33 $\pm$ 1.45	26.67 $\pm$ 1.76	11.67 $\pm$ 0.88	2.67 $\pm$ 0.33	0.67 $\pm$ 0.33
	100	4.00 $\pm$ 1.00	27.00 $\pm$ 1.53	1.86 $\pm$ 0.24	9.00 $\pm$ 0.51	49.16 $\pm$ 3.31	147.51 $\pm$ 9.93	33.33 $\pm$ 0.08	63.67 $\pm$ 0.88	19.67 $\pm$ 0.88	12.67 $\pm$ 0.88	3.33 $\pm$ 0.33	0.67 $\pm$ 0.33
	Infected	2.67 $\pm$ 1.2	30.33 $\pm$ 2.19	2.62 $\pm$ 0.46	10.13 $\pm$ 0.72	40.41 $\pm$ 5.12	120.94 $\pm$ 15.21	33.41 $\pm$ 0.04	61.33 $\pm$ 1.33	24.33 $\pm$ 2.73	11.33 $\pm$ 1.45	2.33 $\pm$ 0.33	0.67 $\pm$ 0.33
Infected	Infected	1.67 $\pm$ 0.33	31.67 $\pm$ 0.67	2.69 $\pm$ 0.20	10.53 $\pm$ 0.23	39.45 $\pm$ 2.00	118.62 $\pm$ 6.13	33.26 $\pm$ 0.04	60.67 $\pm$ 0.88	24.67 $\pm$ 2.03	10.67 $\pm$ 1.2	3 $\pm$ 0	1 $\pm$ 0
	625	1.33 $\pm$ 0.33	33.67 $\pm$ 1.86	3.02 $\pm$ 0.40	11.23 $\pm$ 0.62	38.05 $\pm$ 3.4	114.05 $\pm$ 10.24	33.37 $\pm$ 0.03	59.33 $\pm$ 1.33	26.00 $\pm$ 1.73	10.67 $\pm$ 0.88	3.33 $\pm$ 0.33	0.67 $\pm$ 0.33
	PC												
	PC												
Status		0.8461	0.3483	0.1770	0.3428	0.1560	0.1521	0.5485	0.1636	0.1760	0.5871	0.7528	1.000
Level		0.0039	0.0092	0.0020	0.0092	0.0023	0.0023	0.8938	0.0150	0.0205	0.3511	0.4054	0.9475
Status *Level		0.3113	0.1796	0.1853	0.1807	0.2265	0.2236	0.0299	0.1415	0.0563	0.4196	0.5945	0.7350

Means with different superscripts within the same column are significantly different from each other at $P < 0.05$. Key: ESR= Erythrocyte sedimentation rate, PCV= Packed cell Volume, RBC = Red blood cells, HB= Haemoglobin, MCH=Mean corpuscular haemoglobin, MCV= Mean corpuscular volume, MCHC= Mean corpuscular haemoglobin concentration, LYM= Lymphocyte, HET= Heterophil, MON= Monocyte, BAS= Basophil and EOS= Eosinophil, NC= Negative control, PC = Positive control and SEM = Standard error of means.

Table 4: Serum biochemistry of broiler chickens infected with *S. Gallinarum* and treated with ethanolic leaf extract of *C. albidum*

INFECTION	LEVEL	TP (g/L)	ALB (g/L)	GLOB (g/L)	AGRA	ALP (iu/L)	CREAT (μmol/L)	URE (μmol/L)	TOBIL (μmol/L)	DIBIL (μmol/L)
Not infected	.	59.92±2.46	30.67±2.47	29.25±1.45	1.09±0.1	32±5.08	49.17±7.42	2.73±0.87	7.41±1.49	2.14±0.42
Infected	.	57.33±2.2	28±1.76	29.33±1.54	1.01±0.1	26.07±2.11	48.33±5.25	2.42±0.34	7.35±1.17	2.01±0.29
NC		50.17±3.81 ^b	23.17±3.11	27±2.61	0.9±0.13	31.5±3.39 ^{ab}	51.5±9.46 ^{ab}	3.8±1.67	7±2.37	1.73±0.44
100		63.33±3.32 ^a	31.67±3.75	31.67±1.56	1.03±0.15	26.83±4.42 ^{bc}	32.17±7.74 ^b	1.53±0.24	8.77±1.97	2.93±0.57
250		56.83±2.96 ^b	29.33±2.55	27.5±3.06	1.17±0.22	17.67±2.6 ^c	46.5±10.56 ^{ab}	2.55±0.73	5.4±1.48	1.28±0.27
625		61±2.18 ^a	31.33±2.79	29.67±1.09	1.08±0.12	39.67±7.97 ^a	57.33±4.97 ^{ab}	2.05±0.28	7.23±1.91	2.02±0.58
PC		63.67±0.33 ^a	31.67±3.18	32±3.51	1.04±0.24	27±1.53 ^{bc}	63.33±16.76 ^a	3.17±0.49	9.57±3.34	2.7±0.76
Not infected	NC	54±3.21	23.67±5.9	30.33±3.48	0.84±0.28	29±3	61±16.82	5.73±3.15	5.2±2.43	1.4±0.61
Not infected	100	69.33±1.33	36.67±3.33	32.67±2.91	1.16±0.19	31.33±4.26	20.67±5.17	1.2±0.15	8.17±4.34	2.83±1.21
Not infected	250	51±1	25±2.52	26±3.51	1.02±0.21	12.33±0.33	64.33±15.43	2.4±0.26	7.53±2.29	1.73±0.38
Not infected	625	65.33±2.03	37.33±1.2	28±1	1.34±0.04	55.33±8.41	50.67±8.41	1.6±0.31	8.73±3.87	2.6±1.1
Infected	NC	46.33±6.89	22.67±3.67	23.67±3.28	0.95±0.05	34±6.51	42±8.62	1.87±0.57	8.8±4.35	2.07±0.69
Infected	100	57.33±4.18	26.67±5.84	30.67±1.67	0.89±0.23	22.33±7.69	43.67±11.87	1.87±0.38	9.37±0.43	3.03±0.38
Infected	250	62.67±2.96	33.67±2.73	29±5.69	1.33±0.43	23±2.31	28.67±1.33	2.7±1.6	3.27±1.07	0.83±0.15
Infected	625	56.67±0.88	25.33±1.2	31.33±1.45	0.82±0.07	24±1	64±2.89	2.5±0.31	5.73±1.02	1.43±0.38
Infected	PC	63.67±0.33	31.67±3.18	32±3.51	1.04±0.24	27±1.53	63.33±16.76	3.17±0.49	9.57±3.34	2.7±0.76
Status		0.0815	0.1825	0.8016	0.5778	0.0864	0.5692	0.5707	0.7696	0.5593
Level		0.0027	0.1287	0.5002	0.8066	0.0046	0.0145	0.4049	0.7290	0.1892
Status*Level		0.0067	0.0421	0.3867	0.2821	0.0018	0.0574	0.2026	0.5221	0.5352

Means with different superscripts within the same column are significantly different from each other at P<0.05

Key: TP = Total protein, ALB =Albumin, GLO B= Globulin, AGRA=Albumin globulin ratio, ALP= Alkaline phosphatase, CREAT= Creatinine, URE=Urea, TOBIL= Total bilirubin and DIBIL= Direct bilirubin, NC= Negative control and PC = Positive control.

Discussion

The phytochemical screening of ethanolic leaf extract of *C. albidum* showed the presence of tannins, saponins, cardiac glycosides, steroids, terpenoids and flavonoids in appreciable quantities. According to Mercy *et al.* (2017) phytochemicals are found in plants and they are non-nutritive chemicals that are protective or efficacious in diseases prevention. Plants produce these chemicals as protective measures but it has been discovered that they can protect against varieties of diseases in man or animals. The responsible therapeutic phytochemicals in medicinal plants are mainly alkaloids, tannins, saponins, glycosides, flavonoids, phenols, minerals and vitamins (Saganuwan and Gulumbe, 2005; Saganuwan, 2017). Laudato and Capasso (2013) reported that natural products are often used as antibacterials, antimycotics, antiparasitics, disinfectants and immunostimulants. The ethanolic and methanolic extracts from the stem bark and leaves of *Ficus sycomorus* also showed remarkable antimicrobial activity against *Staphylococcus aureus* and *Salmonella Typhi* (Adamu *et al.*, 2016). The anti-inflammatory efficacy of ethanolic leaf extract of *Carica papaya* was equally demonstrated in Rats orogastrically dosed with *Salmonella typhi* and *Staphylococcus aureus* by Oladunmoye and Osho (2007). Haematological examination contributes immensely to detection of some changes in health status of birds that may not be obvious at the time of physical examination but undoubtedly affect the fitness of the birds (Gavett and Wakely, 1986; Lakurbe *et al.*, 2018). According to Isaac *et al.* (2013) haematological parameters are diagnostic tools for monitoring toxicity that can affect the blood and health condition of livestock. It was reported by Ukorebi *et al.* (2019) in an *in-vivo* study that *G. latifolia* leaf extract

enhanced blood building capacity in broiler chickens. The erythrocyte sedimentation rate (ESR) of the experimental broiler chickens was in the range of 1 - 3mm/hr and this agrees with Ross *et al.* (1978), who recommends 2 – 4 mm/hr for broiler chickens. The study of Ukorebi *et al.* (2019) on broiler chickens also conformed to this range. The ESR values though normal were significantly different ($p < 0.05$) from one another. According to Nanbol *et al.* (2016), haematological parameters of 8 weeks old broiler were: PCV 32 - 45 (%), Haemoglobin concentration 9.0 - 12.0 (g/L), eosinophils 0-2 (%) and basophils 0 - 2 (%). The PCV of this experimental broiler chickens ranged between 28.17 ± 1.05 - 33.67 ± 1.86 which was lower than the recommendation of Nanbol *et al.* (2016) but in line with that of Ross *et al.* (1978) recommendation of 25 – 40%. Jain, (1993) recommended the PCV for avian species to be 22 – 35% which actually agreed with the PCV result from this study and that of Lakurbe *et al.* (2018) and Sunmola *et al.* (2019). Ilo *et al.* (2019) arrived at the PCV of 28.67 – 30.33% for broiler chickens in their study, which also followed the same pattern with the values from this study. However, there were significant differences ($p < 0.05$) in the mean values of the PCV at dose level with the positive control group having the highest mean value of 33% and lowest value of 28% in 100 mg/mL group. The values for RBC were within the range of 2.04 ± 0.20 – 3.02 ± 0.4 ($\times 10^6 \text{mm}^3$) and falls within the range reported by Banerjee (2012), Lakurbe *et al.* (2018) and Sunmola *et al.* (2019). Haemoglobin values were significant ($p < 0.05$) with the values ranging from 9 – 11g/dL and this was in line with the recommendation of Ross *et al.* (1978) and Sunmola *et al.* (2019) that the Hb of avian blood ranged from 7 – 13g/dL. The Hb values in this experiment were also in agreement with those obtained by

Lakurbe *et al.* (2018) and Ilo *et al.* (2019) from their experiments (9.70 - 10.27g/dL). Worthy of note is the highest values of PCV, RBC and Hb observed in the standard drug (Doxygen®) group. Probably the *Salmonella* organism induced erythrocytosis in the chickens since drugs like gentamicin and methyldopa have been reported to cause elevated values in RBC (Heshu, 2016). However, neither erythrocytosis (higher numbers) nor erythrocytopenia (lower numbers) was noticed in all the groups as the blood parameters were in line with the report of Lakurbe *et al.* (2018) and Sunmola *et al.* (2019). MCH values ranged from 38 – 47pg and significant differences were observed at the level of extract's dosage administration. These values were at par with Bounous and Stedman (2000), Sunmola *et al.* (2019), who reported 23 – 47pg for avian species. MCV was significant ($p < 0.05$) and the values were within the range recommended 90 – 140fL and 104 – 140fL by Mirtuka and Rawnsley (1997), Bounous and Stedman (2000), respectively, indicating normocytic condition of the birds. The MCH values were in the range of 38.05 – 47.16 pg and it agreed with Sunmola *et al.* (2019) whose study presented 34.10 – 52.46 pg which can be described as normochromic. There was no significant difference in the MCHC values. The values ranged from 33.30 – 33.37g/dL and it conformed to the range of 30.20 – 36.2g/dL reported by Gulland and Hawkey (1990) of MCHC for avian species. It also conformed to the values obtained by Lakurbe *et al.* (2018), Ilo *et al.* (2019) and Sunmola *et al.* (2019). These results also presented normochromic and healthy condition of the birds. The range for the lymphocytes of the experimental broiler chickens was 59.33 – 63.00% and this agrees with 45 – 75% and 58.10 – 71.70% reported by Gylstorff (1983) and Nemi, (1993) respectively. There was significant

difference ($p < 0.05$) in the analysed result of the lymphocytes. Heterophils result showed a significant difference ($p < 0.05$) with the values of 21 – 26%. The range agrees with Ross *et al.* (1978) who reported 23.5 – 35.1% and Gylstorff (1983) who recommended 19.80 – 32.60% as heterophils value range for broiler chickens. There were no significant differences ($p > 0.05$) in the values of monocytes (10.67 – 12.50%). Monocytes value of broiler chickens was an average of 16% as reported by Sebastian *et al.* (2012) and, 8.10 – 16.10% by Gylstorff, (1983). The range values obtained for basophils was 2.67 – 3.33% and that of eosinophils was 0.67 – 0.83. According to Nemi (1993), the normal range for basophils was 1.5 – 2.5% and eosinophils 1.2 – 3.1%. The basophils and eosinophils were within the range reported by Nanbol *et al.* (2016). The normal range values of the haematological parameters obtained from this study pointed to the normal health condition of the experimental broiler chickens. Total serum protein and albumin is a measure of the biosynthetic function of the liver because the liver is the primary site of synthesis of most plasma proteins. The measurement is a pointer to chronic liver disease. According to Oladele and Ayo, (1999) serum albumin is a strong predictor of health. When serum albumin is low, it is a sign of bad health. The higher the value of serum albumin the higher the clotting ability of the blood; this singular quality will prevent haemorrhage. Alkaline phosphatase level in the serum serves as a marker of biliary obstruction. Total Serum protein values of broilers according to Harr *et al.* (2002) is most of the time lower than that of the mammals and values range from 25.00 to 45.00 g/L. The determination of serum albumin in broilers by Ross *et al.* (1978) presented 10.80 to 16.00g/L while that of serum globulin by Rezende *et al.*

Haematological indices of Salmonella Gallinarum (Gr. D1-1, 9, 12)

(2017) for non-vaccinated broilers against Newcastle disease and Infectious bursal disease was 10.00 to 11.9g/L. That of vaccinated broilers was found to be 16.30 to 19.20 g/L. The total serum protein of this experimental broiler chickens was significant at the level of doses administration ($p < 0.05$) and the values ranged from 57.33 ± 2.2 – 63.67 ± 0.33 , serum albumin 28 ± 1.76 – 31.67 ± 3.18 , alkaline phosphatase 17.67 ± 2.6 – 39.67 ± 7.97 . These three parameters values were in line with the recommendation of Nanbol *et al.* (2016) for broiler's total serum protein of 40 – 65 g/L, serum albumin 22.00 – 40.00 g/L and alkaline phosphatase 30 – 68 IU/L. Urea and creatinine are excreted in the urine; their serum concentrations can be used as markers of renal function because the serum concentration increases as renal function deteriorate. According to Rezende *et al.* (2017), serum urea in broilers ranges from 6.5 and 7.76 mg/dL. The serum creatinine of the experimental broiler chickens differed significantly ($p < 0.05$) at varying level of extract dose administration with the mean values ranging from 32.17 ± 7.74 to 63.33 ± 16.76 . These values were slightly higher than those values of 40.2 ± 2.9 – 52.3 ± 5.7 obtained by Victoria *et al.* (1989) but on the average still at par with those values obtained in this study. According to Sandhu *et al.* (1998), creatinine is directly related to increased muscle activity and that blood creatinine used to be low in young and old broiler chickens. There were no significant differences ($p > 0.05$) in the values of the serum urea, total bilirubin (unconjugated) and direct bilirubin (conjugated). In all, most of the serum biochemical parameters were found to be within the range usually seen in avian species. According to Victoria *et al.* (1989) one of the diseases associated with broiler production is sudden death syndrome (SDS) which was not seen in this

study attested to the normal biochemical indices of the experimental broiler chickens.

Conclusion

It was concluded that ethanolic leaf extract of *C. albidum* had no deleterious effect on haematological and serum biochemical parameters of the experimental broiler chickens infected with *Salmonella enterica* serovar *Gallinarum* Gr. D1-1, 9, 12.

Recommendations

Ethanolic leaf extract of *C. albidum* is safe as potential source of herbal antibiotic and hence it is a candidate medicinal plant that can be explored for usage in salmonella infection in poultry production. Its incorporation into feed may be a good prophylaxis against salmonellosis as well. However, further study should be carried out in order to identify, isolate, characterize and elucidate the structures of the bioactive compounds in the leaf extract of *C. albidum* and enhance their potentials for industrial and phyto-pharmaceutical utilization.

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