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HAEMATOLOGICAL AND ELECTROLYTE PROFILE OF ALBINO RATS TREATED WITH ETHANOL EXTRACT OF *CLEOME CILIATA* (*CLEOMACEAE*) IN A CHRONIC TOXICITY STUDY

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ABSTRACT

Cleome Ciliata is a famous medicinal plant from the Cleomaceae family and is widely used in traditional medicine to treat various ailments. However, there are no toxicological data on safety after repeated exposure and long-term use; therefore, the present study was designed to evaluate the 90-day chronic toxicity of ethanol (80%) *Cleome ciliata* extract in adult Wistar. A chronic toxicity experiment was conducted by oral administration of graded doses (250 mg/kg, 500mg/kg and 1000 mg/kg) of test extract daily for 90 days. Signs of toxicity and body weight were evaluated. The toxic effects were also assessed using haematological and electrolyte data. All data collected were expressed as mean ± standard deviation. ANOVA followed by post hoc Turkey's test was used for data interpretation and $p<0.05$ was considered significant. There were no treatment-related differences in haematological and electrolyte indices. Moreover, no gross abnormalities or histological alterations were observed. No deaths or evident of toxic signs were found during the experimental period. There were no significant differences in body weight between the control and the treated groups. The ethanol extract of *cleome ciliata* leaves was nontoxic in chronic intake at the dosages tested. Thus, this study

demonstrated potential safe clinical applications and warrant further clinical studies.

1. INTRODUCTION

The 21st century has ushered in a new era, receptive to Eastern philosophy of health through positive attitudes [7]. Plants, animals and humans have had intimate biological relationships since the distant past and have evolved along parallel lines cooperating and depending on each other for existence. In the current scenario, more emphasis is placed on the traditional knowledge of the ethnic people by imparting to them the use of knowledge in the bio-prospecting of biological resources as a new source of medicines, foodstuffs and other industrial raw materials and pharmaceuticals [9].

Herbal medicine plays a significant role in the health of millions of people worldwide through direct utilization and conventional medicine development [32]. Available literature indicates that 60% of the world's population depends on traditional medicine and 80% of the people in developing countries depend entirely on traditional medicine practices and herbal medicines for their primary health care needs [6].

Traditionally considered non-toxic, herbs have been used by the general public and practitioners of traditional medicine around the world to treat various conditions [27]. Although the literature has on many occasions documented various toxicities resulting from the use of herbs, the potential toxicity of herbs has not been recognized by the general public or professional groups in traditional medicine [26]. Poisonous plants are those that cause serious problems or even death if a small amount of their stems, leaves, seeds, fruits, and roots are ingested. Some other plants are normally harmless but can become toxic if the supplements are taken in excess, in high doses, or over a long period of time (Khajja et al, 2011). Toxicity in phytotherapy can be caused by: accidents due to incorrect botanical identification, accidental ingestion of cardiotonic plants, poisoning from folk remedies and plants interfering with traditional pharmacological therapy (Rates, 2019).

Toxicology studies help in deciding whether or not a new drug should be accepted for clinical use [2]. Depending on the duration of exposure of the animals to the drug, toxicological studies can be of three types, namely acute, subacute and chronic [4]. Toxicity depends not only on the dose of the substance, but also on the toxic properties of the substance. The relationship between these two factors is important in evaluating therapeutic dosage in pharmacology and herbalism [15].

Blood which forms the main medium of transport in the body is a very important tissue. It serves to transport many drugs and xenobiotic. Since all foreign compounds are distributed via the bloodstream, the various components, cellular and non-cellular, are initially exposed to significant concentrations of toxic compounds (Timbrel, 2009).

Haematological status is one of the important ways for the diagnosis of root cause of disease [3]. Haematological disorders include a wide range of abnormal conditions indicating the profile of blood parameters, due to changes in metabolism. The bone marrow is a major target for many toxic substances [3]. As a result of failure of generation of new cells, there may be failure of the red cell system (anaemia) and failure of the white cell system, causing both overwhelming infection due to absence of granulocytes and

failure of the immune system from a total reduction on the white cell count [3]. Alterations in blood parameters may be due to changes in cellular integrity, membrane permeability of cells or even due to exposure to toxic chemicals [16].

Electrolyte imbalance in the definition of [24] is the serum concentrations of an electrolyte that are either higher or lower than normal. Electrolyte imbalance can be caused by numerous factors which according to [25] include; kidney disease, vomiting for a prolonged period, severe dehydrations, congestive heart failure, cancer treatment, some drugs such as diuretics or ACE inhibitors, acid/base (pH) imbalance, severe and persistent vomiting and nausea during pregnancy.

Aim of Study
Haematological and electrolyte profile of albino rats treated with ethanol extract of *cleome ciliata* in a chronic toxicity study

2. Materials and Methods

2.1 Procurement of Animals
Albino rats of either sex were procured from Department of Veterinary Medicine, University of Nigeria Nnsukka. They were kept in the animal house, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu and were given access to water and pelletized vital grower feed *ad libitum* under 12:12 hours light and dark cycle. Animals were handled in conformity with the National Institute of Health Guidelines for the care and use of laboratory animals for research purpose (Pub No. 85-23, revised 1985).

2.2 Plant Collection and Authentication
Fresh leaves of *cleome ciliata* were collected between 6:00 and 7:30 am in the month of February, 2019 in Irri community Isoko South L.G.A, Delta State, Nigeria. Plant sample was validated by plant taxonomist from the Department of Botany, Faculty of Life Sciences, Nnamdi Azikiwe University, Nigeria. It was deposited in the herbarium of the department with voucher number "NAU45679"

2.3 Preparation of Plant Extract
The extract was prepared using cold maceration method as described by [17]. Fresh leaves of *cleome ciliata* were washed with tap water and air dried at room temperature for two weeks. Dried leaves were pulverized using mechanical grinder and a total of 7.2 kg was extract by cold-macerated using 80 % aqueous ethanol for a period of 48 hours, with occasional agitation. Filtrate was recovered with the aid of a muslin clothe. Final filtrate recovered was concentrated with an evaporator.

2.4 Acute toxicity test
Lorks method of 1983, toxicity determination was employed in this study. Three Doses (10, 100 and 1000 mg/kg) were administered in the first phase. The animals were observed for 24 hours. At end of the 24 hours there was no death or signs of toxicity. This led to the second phase. In the second stage, four dose ranges were used 2000, 3000, 4000 and 5000 mg/kg body weight this also lasted for another 24 hours.

2.5 Chronic toxicological studies
In this study, the protocol described by [18] was used with some modifications. A total of forty (40) male albino rats with a body weight between 100 and 120) were randomized into four groups of ten animals each as follows: control group (10 ml/kg, distilled water) and test groups (250, 500 and 1000 mg/kg of the extract).

The extract was reconstituted in distilled water to form different concentrations, 100 mg/ml, 50 mg/ml and 25 mg/ml for 1000, 500 and 250 mg/kg, respectively. After the initial assessment, the animals received a daily dose of the extract for a period of 90 days

2.6 Recovery studies

After 90 days of chronic toxicity studies, the animals received food and water ad libitum without extract given for 28 days. At the end of the 28th days, blood samples taken from the animals' retroorbital plexus were used to determine hematological and electrolyte parameters. Body weights was also recorded.

2.7 Collection of Blood Sample

Blood samples were taken from the retroorbital plexus on days 31, 61 and 91 in tubes with EDTA and smooth tubes for the determination of haematological and electrolyte parameters, respectively, using similar protocols as described by [18]. The blood or haematological parameters were analysed using an automated haematology analyser (Procan Electronics, Model PE6800). The parameters included hemoglobin (HGB), red blood cell (RBC) count, hematocrit (HCT), RBC distribution width, mean cell volume, mean cell HGB concentration (MCHC), white blood cell count, lymphocytes, granulocytes, platelet (PLT), PLT distribution width, mean PLT volume, and PLT large cell ratio etc.

2.8 Electrolyte Test

Blood serum was used for this test, using the following adopted methods: Modified method of [21] was used for the determination of serum sodium, The complex metric procedure of [13] was used in the determination of calcium level in serum Calcium, Serum chloride determination was based on the modified colorimetric method of [29] and Bicarbonate ion was estimated using the titrimetric method in which a known amount of concentratd HCL was added to a fresh serum sample. Shaking the sample expels CO2 liberated. H+ was back titrated with NaOH using phenol indicator (Chees brough, 1992).

2.9 Method of data analyses

Results obtained from this study were presented as mean ± Standard error of mean (SEM) of sample replicates (n=10). Raw data were analysed using one-way analyses of variance (ANOVA), followed by post hoc Turkey's test using Statistical Package for Social Science (SPSS, version 25). $p<0.05$ was established to be statistically significant.

3. Results

Table 1: Results of Phytochemical screening of cleome ciliate

Phytochemicals	Present
Flavonoids Shinoda test Alkaline reagent test	+++
Alkaloids Wagner's test	+++
Steroids Liebermann-burchard test	+++

Phenols Ferric chloride test Lead acetate test	+++
Terpenoids Salkowski test	+++
Anthroquinone Borntrager s test	+
Saponin Frothing test	+++
Tannins Gelatin test	+++
Carbohydrates Iodine test	-
Proteins & Amino acids Ninhydrin test Millon's test	+
Resins	++
Cardiac Glycosides Keller- killani test Liebermann's test	+++

(-) => Not Present, (+) => Faintly Present,
(++) => moderately present, (+++) => Highly present

Table 1: Acute Toxicity of Cleome ciliata

Groups	Dose (Mg/Kg)	Mortality
1 (Low dose)	10	0/3
	100	0/3
	1000	0/3
2 (High dose)	2000	0/3
	3000	0/3
	4000	0/3
	5000	0/3

From above result, no deaths were recorded after 24 hours of administration varying doses of the extract in phase 1 (10, 100 and 1000 mg/kg body weight) and phase 2 (2000, 3000, 4000 and 5000 mg/kg body weight). The LD_{50} was therefore above 5000mg/kg.

There were no statistically significant differences in body weight changes (Table 2, figure 1)

Table 2: Effect of Extract on Body weight gain (%)

Treatment	Initial body weight (g)	Day 31	Day 61	Day 91	Mean body weight increase (g)	Weight gain (%)
Control	120.90±1.75	161.14 ± 0.56	189.38±1.66	239.65±0.35	118.75±1.43**	49.55**
250 mg/kg	125.32± 0.45	176.65 ± 1.11	204.76±0.47	242.71 ± 0.18	117.39± 0.27**	48.37**
500 mg/kg	124.61±0.37	165.54 ± 0.32	213.56±6.48	240.99± 0.29	116.38 ±0.10**	48.29**
1000 mg/kg	125.33±1.56	173.72 ± 0.39	218.82±6.07	244.14 ± 1.39	118.81 ± 0.17**	48.66**

Values are expressed as Mean ± SEM for (n =10/group). Values with the same superscript across treatments are not significantly different from each other at p>0.05 (Analysed by ANOVA followed by Tukey's test).

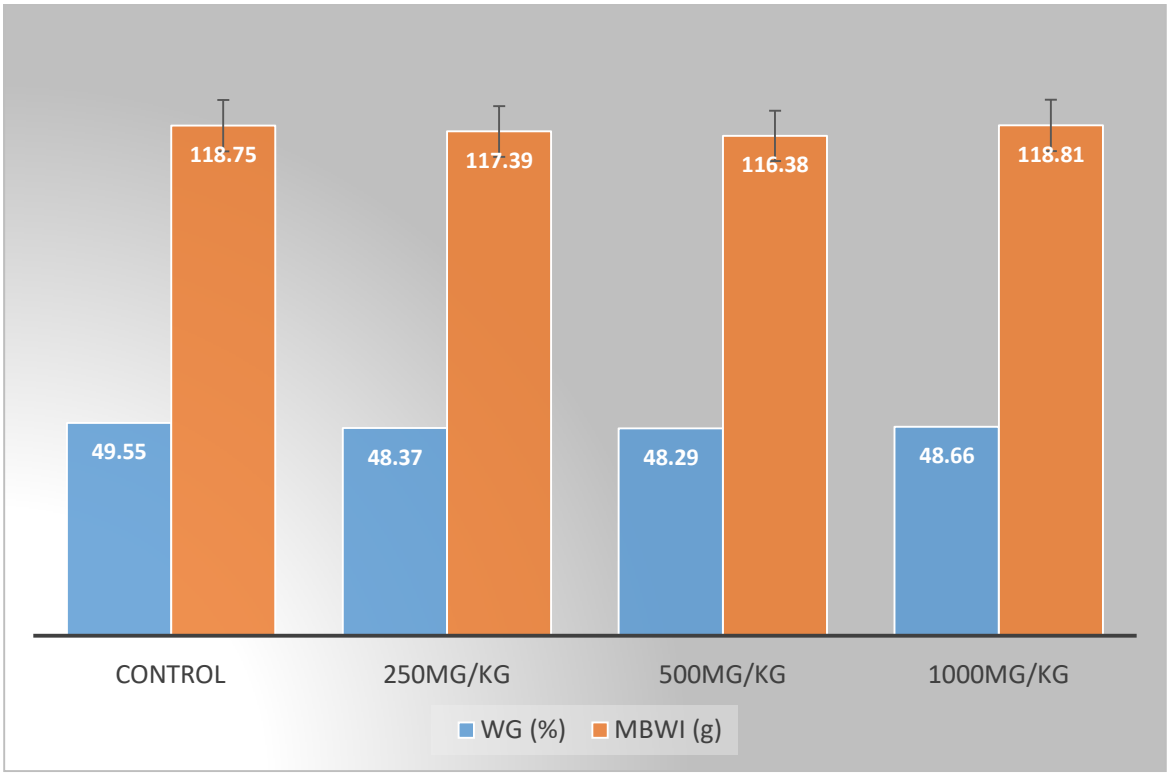

Fig: Effect of cleome ciliate on Body weight
WG= Weight gain
MBWI= Mean body weight increase

Table 3: Table 4: Effect of *cleome ciliata* on haematological parameters of the treated and untreated rats

TIME	TREATMENT	PCV (%)	RBC (10^6/PL)	HEMOGLOBIN (G/DL)	PLAT (10^3/UL)	PCT (%)	WBC (10^3/ML)

BASE LINE	**Control**	45.66±1.00	7.91±1.32	15.22 ±0.21	882.12±0.55	0.81±0.08	8.23±0.65
	250 mg/kg	46.78±0.74	7.69±0.27	14.12 ±0.77	890.33±1.43	0.89±0.03	8.37±0.87
	500 mg/kg	47.46±1.50	8.11±1.65	15.10 ±0.65	888.26±1.11	0.88±0.02	8.34±1.27
	1000mg/kg	45.56±0.93	7.76±0.50	15.11 ±0.66	868.99±0.42	0.78±0.03	7.84±1.44
DAY 31	**Control**	45.63±0.41	7.72±0.71	15.31±1.11	889.67±0.48	0.78±0.09	8.40±0.24
	250 mg/kg	46.23±0.27	8.21±0.26	14.94±0.60	892.32±0.51	0.89±0.07	7.93±1.39
	500 mg/kg	45.56±0.56	7.69±0.44	15.29±0.48	839.52±3.33	0.79±0.04	7.86±0.25
	1000mg/kg	48.12±0.78	7.81±0.67	14.87±1.26	875.31±1.22	0.87±0.09	7.74±2.45
DAY 61	**Control**	47.90±0.25	7.78±0.33	14.99±0.24	865.41±1.61	0.86±0.07	7.67±1.56
	250 mg/kg	45.37±1.45	8.07±0.49	15.35±0.24	884.79±1.44	0.81±0.02	7.94±0.37
	500 mg/kg	46.51±0.27	8.12±0.21	14.79±1.30	888.55±2.99	0.83±0.04	7.99±1.48
	1000mg/kg	46.79±0.47	7.74±0.58	15.38±1.41	861.43±1.88	0.86±0.02	8.16±2.00
DAY 91	**Control**	45.45±0.76	7.87±0.65	15.43±0.56	884.90±0.67	0.81±0.05	7.87±0.75
	250 mg/kg	47.88±0.73	8.13±0.53	15.05±0.21	897.67±0.67	0.89±0.08	7.82±0.43
	500 mg/kg	46.56±0.85	8.05±0.27	14.99±0.65	867.79±0.43	0.64±0.06	7.62±0.65
	1000mg/kg	47.76±0.45	7.90±0.45	15.23±0.89	834.43±0.62	0.80±0.05	8.35±0.33
RECOVERY PERIOD	**Control**	45.54±0.20	7.83±1.11	14.95±0.20	839.47±1.22	0.83±0.02	7.72±1.45
	250 mg/kg	45.31±0.96	8.15±1.00	15.22±0.58	854.23±0.64	0.85±0.08	7.78±0.69
	500 mg/kg	44.49±0.58	7.81±0.45	14.88±0.11	898.32±2.77	0.89±0.05	7.99±0.58
	1000mg/kg	45.68±0.55	7.99±1.32	15.25±1.67	889.32±1.47	0.88±0.04	7.89±1.66

Values are expressed as Mean ± SEM for (n =10/group). Values across treatments groups are not significantly different from each other at $p<0.05$ (Analyzed by ANOVA followed by Tukey's test).

PCV= (packed cell volume), RBC= (Red Blood Cell), platelet= (PLAT) and percentage (PCT), WBC= (white blood cell)

Table 4: Effect of cleome ciliata on haematological parameters of the treated and untreated rats

BASELINE	**Control**	65.52±1.39	4.88±0.12	12.43±0.79	39.87±0.49	21.81±0.44	38.60±0.21

	250 mg/kg	65.73±1.34	4.69±0.41	12.11±0.12	39.18±0.79	22.18±1.32	38.65±0.23
	500 mg/kg	65.53±0.43	5.03±0.25	12.15±0.57	38.76±0.55	22.08±0.41	39.00±1.24
	1000mg/kg	64.54±0.21	5.14±1.24	12.05±1.33	39.17±2.09	21.52±0.34	38.76±0.13
DAY 31	**Control**	65.25±1.49	4.92±1.34	12.22±0.23	38.56±1.88	21.56±1.26	38.84±0.68
	250 mg/kg	66.47±0.64	4.57±2.00	12.23±1.88	39.24±1.44	22.11±1.69	38.66±0.24
	500 mg/kg	63.77±0.28	5.06±1.66	12.35±0.67	38.90±1.67	21.73±0.35	39.09±1.77
	1000mg/kg	65.33±0.27	5.13±1.23	12.16±1.55	39.11±2.11	21.87±1.55	38.96±2.00
DAY 61	**Control**	66.09±0.76	5.03±0.54	11.65±0.98	39.22±1.09	20.65±0.43	38.65±0.65
	250 mg/kg	65.84±0.54	4.98±0.59	12.17±0.54	39.10±0.88	21.77±0.65	39.09±0.23
	500 mg/kg	66.30±0.21	5.05±0.65	11.78±0.54	38.99±0.65	21.99±0.45	38.52±0.33
	1000mg/kg	64.48±0.48	5.12±0.54	12.22±1.00	39.66±0.61	22.44±0.56	38.64±0.11
DAY 91	**Control**	67.12±1.35	5.22±1.47	12.33±0.79	38.85±0.45	21.90±1.88	39.16±0.89
	250 mg/kg	65.69±0.41	4.86±1.38	12.34±0.47	39.40±1.23	21.56±0.65	39.24±0.11
	500 mg/kg	63.67±1.12	5.34±1.37	12.10±3.12	38.99±1.11	21.78±1.69	39.33±0.78
	1000mg/kg	66.45±1.35	5.11±0.68	12.20±1.44	38.87±2.34	21.62±1.44	38.57±0.38
RECOVERY PERIOD	**Control**	67.38±0.56	5.09±1.67	12.14±1.55	39.33±1.77	20.89±0.27	38.87±1.61
	250 mg/kg	66.09±0.58	5.24±0.46	11.59±1.51	38.77±2.44	22.34±1.69	39.17±0.56
	500 mg/kg	65.21±1.11	4.98±0.28	12.44±0.47	39.00±0.76	21.87±0.97	38.69±0.45
	1000mg/kg	63.94±0.93	4.85±0.65	12.12±1.20	38.69±2.55	22.34±1.55	38.59±1.44

Values are expressed as Mean ± SEM for (n =10/group). Values across treatments groups are not significantly different from each other at $p<0.05$ (Analyzed by ANOVA followed by Tukey's test). Lymphocyte (Lymp), granulocyte (Gran), medium size cell counts (MID), haematocrit (HCT), mean corpuscular haemoglobin (MCH), MCH concentration (MCHC)

Table 5: Effect of cleome ciliata on haematological parameters of the treated and untreated rats

TIME	TREATMENT	MCV (FL)	MPV(FL)	MO (10^3/µL)	RCDW (%)	EOSIN (%)	BASO (%)
BASELINE	**Control**	56.60±0.41	7.67±0.80	2.13 ±1.42	17.23±0.56	1.09 ±0.75	0.40±1.40

	250mg/kg	55.55±0.43	7.39±0.78	2.09 ±0.61	17.25±0.78	1.25 ±0.26	0.40±0.26
	500mg/kg	56.29±0.86	7.11±0.18	2.45 ±0.15	17.43±0.43	1.16 ±0.76	0.40±0.20
	1000mg/kg	57.45±1.36	7.26±0.19	2.30±2.87	17.12±1.88	1.44±0.88	0.43±0.40
DAY 31	**Control**	55.63±0.76	7.37±0.65	2.16±0.33	17.28±0.44	1.43±0.43	0.45±0.43
	250mg/kg	55.65±0.43	7.43±0.87	2.45±0.87	17.18±2.55	1.22±1.52	0.45±0.46
	500mg/kg	56.68±1.77	7.06±0.54	2.44±1.44	17.40±0.33	1.12±3.66	0.43±0.23
	1000mg/kg	56.76±0.76	7.15±0.43	2.10±1.99	17.25±1.00	1.34±0.43	0.48±0.98
DAY 61	**Control**	55.54±2.98	7.32±0.19	2.26±3.66	17.36±1.66	1.39±0.65	0.44±0.67
	250mg/kg	57.87±0.62	6.90±0.53	2.54±1.00	17.21±0.89	1.44±2.00	0.43±0.32
	500mg/kg	56.48±0.75	7.26±0.33	2.32±0.76	17.22±0.53	1.27±2.88	0.48±0.32
	1000mg/kg	55.98±0.21	7.21±0.78	2.18±0.70	17.11±1.32	1.34±0.43	0.40±0.21
DAY 91	**Control**	55.90±0.54	7.18±0.12	2.22±0.54	17.40±0.41	1.16±0.22	0.45±0.29
	250mg/kg	56.33±0.32	7.17±0.43	3.08±0.43	17.09±0.76	1.48±2.00	0.48±0.28
	500mg/kg	54.32±0.76	6.96±0.10	2.45±1.00	17.31±0.62	1.45±0.49	0.46±0.44
	1000mg/kg	56.98±0.66	7.43±0.45	2.21±0.87	17.23±0.52	1.21±0.54	0.45±0.54
RECOVERY PERIOD	**Control**	54.89±0.87	7.37±2.90	2.45±0.65	17.44±0.32	1.21±2.66	0.40±0.34
	250mg/kg	53.98±0.68	7.25±0.67	2.05±3.88	17.23±0.43	1.45±1.98	0.44±0.87
	500mg/kg	56.90±0.43	6.89±0.56	2.54±1.00	17.33±1.43	1.43±0.22	0.42±0.31
	1000mg/kg	58.76±0.54	7.34±0.32	2.12±1.90	17.47±2.54	1.28±0.18	0.43±0.65

Values are expressed as Mean ± SEM for (n =10/group). Values across treatments groups are not significantly different from each other at $p<0.05$ (Analyzed by ANOVA followed by Tukey's test).

MPV= Mean platelet volume, MCV= mean corpuscular volume, MO= Monocytes, RCDW= Red cell distribution width, Eosin= Eosinophils and Baso= Basophils

Table 6: Effect of cleome ciliata on haematological parameters of the treated and untreated rats

BASELINE	**Control**	25.80 ± 1.18	14.46±0.34	13.34±0.45	20.58±0.89	39.02±0.91	18.30±0.44
	250mg/kg	24.60 ± 0.03	14.38±0.67	13.18±0.12	20.93±0.39	38.90±1.07	18.23±0.35
	500mg/kg	24.85±0.44	14.08±0.62	13.11±1.13	20.80±1.11	39.10±0.71	17.98±0.87

	1000mg/kg	25.17±0.21	14.23±0.90	13.45±0.54	21.30±0.16	38.77±2.09	17.50±0.67
DAY 31	**Control**	24.65±1.34	13.76±0.32	13.12±1.47	21.44±1.00	38.54±0.50	18.22±0.26
*	**250mg/kg**	24.85±1.00	13.87±0.43	13.43±0.21	20.52±0.65	38.76±0.76	18.33±0.49
	500mg/kg	25.44±2.87	14.25±1.87	13.18±1.00	21.09±0.53	39.26±0.44	18.45±0.76
	1000mg/kg	24.56±0.89	14.18±0.32	13.22±2.09	21.18±1.88	37.99±1.00	18.22±1.33
DAY 61	**Control**	24.58±0.65	14.32±1.38	13.27±0.65	20.99±0.52	38.76±0.65	17.92±1.88
	250mg/kg	24.90±2.23	14.35±0.32	13.47±2.98	21.47±0.77	39.43±1.00	18.16±2.00
	500mg/kg	24.89±0.43	14.21±0.54	13.44±0.21	21.09±1.54	38.77±0.54	18.15±0.67
	1000mg/kg	25.13±0.51	13.65±0.39	13.44±0.93	20.66±1.65	38.90±3.00	18.11±0.36
DAY 91	**Control**	25.19±0.65	13.87±0.29	13.17±0.55	21.30±1.56	37.87±1.00	17.76±0.24
	250mg/kg	24.87±0.77	13.90±0.69	13.19±0.74	21.09±0.76	38.73±2.65	18.18±1.43
	500mg/kg	24.89±0.54	14.11±0.70	13.33±0.32	21.14±0.65	39.46±0.89	18.43±2.65
	1000mg/kg	24.67±0.99	13.99±1.88	13.70±2.99	20.86±1.43	37.90±1.66	17.79±0.88
RECOVERY PERIOD	**Control**	24.78±0.65	14.67±0.20	13.76±0.12	21.87±1.88	38.44±0.12	17.90±2.44
	250mg/kg	25.09±0.43	14.34±1.00	14.44±0.68	21.61±0.11	39.22±0.18	18.44±0.23
	500mg/kg	25.31±0.11	13.74±0.56	13.43±3.33	20.92±0.54	38.29±0.78	17.90±0.44
	1000mg/kg	24.55±0.54	13.99±0.22	13.59±0.54	20.68±0.59	39.09±0.54	17.75±0.78

Values are expressed as Mean ± SEM for (n =10/group). Values across treatments groups are not significantly different from each other at $p<0.05$ (Analyzed by ANOVA followed by Tukey's test).

Neutro= Neutrophils, PDW= platelet distribution width, ESR= Erythrocyte sedimentation rat, P-LCR = Platelet Larger Cell Ratio, RDW-SD = Standard Deviation in Red Cell Distribution Width; RDW-CV = Coefficient of Variation in Red Cell Distribution Width; PDW =Platelet Distribution Width.

Table 7: Effects of leaves extract on Serum electrolyte levels of experimental rats of ciliate extract

TIME	TREATMENT	SODIUM (MEQ/L)	POTASSIUM (MEQ/L)	CHLORIDE (MEQ/L)	CALCIUM (MG/DL)
BASE LINE	**Control**	145.31 ±0.34	4.09 ± 0.13	93.21 ±0.87	9.70 ±0.44
	250 mg/kg	144.87 ±0.52	4.44 ±0.41	94.54 ±0.54	9.17±0.56
	500 mg/kg	145.75 ±0.12	4.29 ±0.62	92.11 ±3.04	9.76 ± 1.76

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	1000 mg/kg	144.87 ±0.41	4.16 ±0.80	94.24 ±2.72	9.41 ±0.49
DAY 31	**Control**	144.64 ±0.21	4.12 ± 0.54	92.50 ±0.32	9.90 ± 0.27
	250 mg/kg	146.43± 0.77	3.77 ±0.31	91.86 ± 0.43	9.80 ±0.23
	500mg/kg	144.52 ±0.63	4.20 ± 0.49	94.16 ± 0.55	9.79 ±0.12
	1000 mg/kg	144.90 ±0.31	4.19 ±0.13	95.00 ± 0.67	9.54 ±0.76
DAY 61	**Control**	145.54 ± 0.54	4.36 ±0.33	93.12 ± 0.77	9.54 ±0.15
	250 mg/kg	144.54 ± 0.43	4.32 ±0.51	92.88 ±0.43	9.35 ±0.13
	500 mg/kg	145.76 ± 0.49	4.37 ± 0.60	94.44 ±1.10	9.27 ±0.72
	500 mg/kg	144.76 ± 0.47	5.22±0.12	93.90 ± 0.55	9.45 ±0.21
DAY 91	**Control**	144.72±0.32	3.80 ± 0.65	95.46 ±0.23	9.34 ±0.43
	250 mg/kg	145.43±0.67	3.74 ±0.69	93.88 ± 0.84	9.45 ±0.52
	500 mg/kg	144.88±0.98	4.38 ±0.40	92.65 ± 0.42	9.51 ±0.29
	1000 mg/kg	145.12±0.37	4.09 ±0.54	94.09 ± 0.51	9.44 ±0.38
RECOVERY PERIOD	**Control**	144.3 ±1.83	4.00 ± 0.21	93.77 ± 0.69	9.19 ±0.87
	250 mg/kg	146.26 ±0.11	3.65 ±0.42	94.79 ± 0.55	9.78 ± 0.13
	500 mg/kg	145.02 ±0.80	4.28 ±0.48	95.32 ± 0.32	9.65 ± 0.21
	1000 mg/kg	145.98 ± 1.75	3.87 ± 0.67	94.85 ±0.25	9.70 ±0.14

Values are expressed as Mean ± SEM for (n =10/group). Values across treatments groups are not significantly different from each other at $p<0.05$ (Analyzed by ANOVA followed by Tukey's, test)

3.1 Effects of cleome ciliata extract on haematological parameters:

The ethanol leaf extract of *cleome ciliata* investigated at all treatment dose levels (250mg/kg, 500mg/kg and 1000mg/kg) had a good hematological tolerance from day31 to day91 of the study. Hematological parameters of extract treated rats were not significantly ($p <0.05$) different from those of the control group. Hematological parameters of the test groups and the control group are presented in Table 3-6.

3.2 Effect of extract on Electrolytes

Administration of *cleome ciliata* extract did not cause any alterations ($p <0.05$) in sodium, potassium, chloride, calcium, chloride, bicarbonate and pH of rats treated with extract on days 31, 61 and 91 when compared with control group (Table 7-8)

Table 8: Effects of leaves extract on Serum electrolyte levels of experimental rats of ciliate extract

TIME	**TREATMENT**	**HCO^{-}_{3} (MMOL L^{-1})**	**BICARBONATE (MEQ/L)**	**PH**
BASELINE	**Control**	22.8±1.47	34.85±1.44	7.36±1.14
	250mg/kg	23.8±1.72	35.28± 2.29	7.45±2.23
	500mg/kg	23.2±1.94	34.95±0.66	7.44±0.99
	1000mg/kg	24.5±1.87	35.16±0.49	7.35±0.43
DAY 31	**control**	23.08±0.09	35.30±0.71	7.23±0.13
	250mg/kg	24.76±1.00	34.69±0.22	7.34±0.50
	500mg/kg	23.98±0.54	34.52±0.13	7.45±0.22
	1000mg/kg	23.54±0.65	35.88±0.65	7.54±0.32
DAY 61	**control**	24.32±0.43	34.87±0.13	7.36±0.13
	250mg/kg	23.65±0.57	35.43±0.12	7.35±0.76
	500mg/kg	24.77±0.21	34.82±0.11	7.46±0.43
	1000mg/kg	24.33±0.67	34.77±0.38	7.38±0.78
DAY 91	**control**	23.09±0.67	35.29±0.27	7.39±0.56
	250mg/kg	23.67±0.88	34.92±0.29	7.45±0.53
	500mg/kg	23.90±0.21	35.21±0.24	7.37±0.27
	1000mg/kg	24.78±0.72	36.77±0.17	7.49±0.94

RECOVERY PERIOD	**control**	23.34±0.33	35.69±0.14	7.45±0.32
	250mg/kg	23.69±0.21	35.76±0.48	7.32±0.13
	500mg/kg	23.54±0.37	34.79±0.58	7.45±0.51
	1000mg/kg	24.56±0.31	34.64±0.57	7.48±0.28

Values are expressed as Mean ± SEM for (n =10/group). Values across treatments groups are not significantly different from each other at $p<0.05$ (Analyzed by ANOVA followed by Tukey's, test).

4. Discussion

Toxicological testing is used to examine products such as individual compounds, mixtures of compounds, crude extract, pesticides, pharmaceuticals, food additives, packaging materials or their chemical ingredients [8]. The World Health Organization (WHO) recommends that herbal medicine would be the dominant source for a variety of medicines. Therefore, such medicinal plants should be studied to better understand their medicinal properties, safety and efficacy [8]. Phytochemical studies of *cleome ciliate* revealed tannins, saponins, alkaloids, flavonoids, anthraquinones, phenols, terpenoids, cardiac glycosides, resins, steroids and amino acids in the leaves [19]. These bioactive components are responsible for various pharmacological activities and some toxicity. The contained phytochemicals agree with other studies by [19], [31].

Acute toxicity tests with LD50 estimation are essential for conducting toxicological studies on chemicals, including plant extracts. In the present study, acute oral administration of a single dose of whole leaf extracts up to 5000 mg/kg did not result in deaths or behavioural changes in the animals. Therefore, the LD_{50} was above 5000 mg/kg. [5] reported that plant extracts with an LD_{50} greater than 5000 mg/kg are considered safe. [17] also showed that LD_{50} values greater than 5000 mg/kg can be classified as practically non-toxic.

The hematopoietic system is more sensitive to the effects of toxic compounds [22]. Therefore, evaluation of hematological parameters is essential to assess the effect of plant extracts on the animal's blood system and [28]. In this study, the examined hematological parameters showed no significant variations compared to the control. Estimation of hematological parameters can be used to verify the magnitude of the harmful effects of Compounds/extracts in the blood of the analyzed animals. Furthermore, such research is relevant for risk assessment, since changes in hematological parameters have significant prognostic importance for human toxicity when interpreting data from animal studies [23]. According to a previous report, the hematopoietic system is one of the most sensitive targets for toxic substances, hematological tests easily detect abnormalities in the body's metabolic processes and provide important information about the body's response to injury, deprivation and/or stress (Fischman 2019). However, in the present study, the mean value for each parameter was within normal limits, further supporting the non-toxic nature of the extract. After hematological analysis, it was found that *Cleoma ciliata* did not cause significant changes in the level of all measured parameters. A normal hematological profile observed in the *Cleoma ciliata* treated groups compared to the control group further justified the non-toxic nature of *Cleoma ciliata* [14]. Studies show that Abrus precatorius L. seed extract did not cause treatment-related dose-dependent hematological differences in hematological parameters [30]. A study by Erhirhie and Ilodigwe showed a non-significant difference in hematological

parameters in the chronic treatment of rats with *Dryopteris filixmas* [10]. All in support of this present study. Electrolytes play an essential role in maintaining electrical neutrality, generating and conducting electrical impulses inside the cell and other cells. The kidneys keep working hard to maintain the electrolyte concentration fairly constant despite any changes in the body system [28]. In this study, the level of electrolytes did not reveal significant difference in comparison to control. Chloride, sodium, potassium and bicarbonate values were within the normal ranges of these parameters, which ruled out the possibility of precipitated abnormalities. Thus, these findings suggest that the extract does not cause electrolyte imbalance.

The level of electrolytes in the blood is the result of a fine regulation mechanism of ionic charges and osmotic balance. This homeostasis is achieved through an interaction involving the kidney, lungs and endocrine system (Florence et al., 2020). Electrolyte imbalance can be caused by numerous factors, which according to [25] include: kidney disease, prolonged vomiting, severe dehydration, congestive heart failure, cancer treatment, some medications such as diuretics or ACE inhibitors, acid-base imbalance (pH -value), severe and persistent vomiting and nausea during pregnancy. Altered levels of magnesium, sodium, potassium, or calcium can cause one or more of the following symptoms: irregular heartbeat, weakness, fatigue, muscle spasms, numbness, bone disorders, convulsions, changes in blood pressure, confusion, seizures, and nervous system disorders (Nordqvist 2016). In the current study, the serum electrolyte levels of rats treated with different doses of *ciliata cleome* extract showed no significant difference from the control. This is an indication that the extract may not have a significant effect on water, electrolyte, and acid-base balance. Normal serum electrolytes have also been reported from animals treated with *M. balsamina* extract [12], [1]. Studies of *Acacia nilotica* leaf extract also shows a non-significant effect on the electrolytes tested.

5. Conclusion

Following chronic treatment, the ethanol (80%) leave extract of *cleome ciliata* was well tolerated and produced no toxicity signs and change in organ weights. The extract also produced no signs of hematotoxicity or detectable abnormalities in the electrolyte profile. In general, all values remained within normal limits and were not indicative of toxic effects with chronic treatment. The present study therefore demonstrates the non-toxic nature of the test extract and therefore the doses of 250, 500 and 1000 mg/kg may be used in phytomedicinal formulations with a low risk of side effects. However, further research needs to be done to determine, for example, its effect on pregnant animals, as well as drug development trials of on other animals to prove its supposed traditional therapeutic value.

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