

Evaluation of Serum Haematological and Electrolyte Changes in Wistar Rats Administered Some Polyherbal Preparations

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ABSTRACT

Coronavirus Disease-19 (COVID-19) has today become a major public health threat. Despite several ongoing clinical trials, there is as yet no specific treatment for this disease. This study evaluated the effects of 3 polyherbal mixtures (CoV-1, CoV Pla-2 and CoV Pla-3) on some electrolyte and haematological parameters in laboratory animals.

The parameters evaluated were PCV, HGB, MCV, MCH, MCHC, RBC, WBC, neutrophils and lymphocytes counts, including Na^+ , Cl^- , HCO_3^- , K^+ . Treatment was per oral with doses of 100, 200 and 400 mg/kg for the 3 polyherbal mixtures. Control group had 0.1 mL of distilled water per oral. The extract was safe up to 5000 mg/kg. An insignificant ($P>0.05$) decrease in RBC was observed at all doses except for 200 mg/kg for CoV Pla-3 which was significant ($P<0.05$) compared to control. When compared to control, an insignificant increase in the mean lymphocyte values in all extracts was observed. WBC in CoV Pla-1 and CoV Pla-3 increased, compared to control, as well as MCV, MCH for all polyherbal mixtures. Neutrophils increased in the rats administered the highest doses of CoV Pla-1 (200 and 400 mg/kg) and CoV Pla-3 (100 and 200 mg/kg) extracts. An insignificant difference ($P>0.05$) in serum levels of Na^+ , K^+ , Cl^- and HCO_3^- were observed in all test animals, compared to control. The results obtained in this study indicate that the extracts did not cause significant changes in haematological parameters evaluated, which implies it is non-toxic.

Keywords: Covid-19, Electrolytes, Haematological, Polyherbal.

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I. INTRODUCTION

The World Health Organization, recognizing the health impact the Coronavirus disease 19 (COVID-19) was having on populations, declared it a public health emergency early in 2020. This disease, caused by Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2), has been worsened by the lack or absence of a specific effective treatment. In order to bridge this gap, clinical trials are ongoing, in addition to research on herbal remedies.

Medicinal Plants have been playing an important role in traditional medicine healing since the beginning of time [1]. The oral ingestion of medicinal drugs has been reported to alter hematological parameters to either positive or negative [2].

The concept of polyherbal drug combination is a traditional herbal approach that allows several medicinal plants to be combined to achieve the desired therapeutic effectiveness. In order to attain the desired therapeutic effect, different phytochemicals are combined, as this may be advantageous over the active constituents of individual plants. Research has shown that when mixed, these drugs of variable potency can theoretically generate a better outcome compared to a single plant's activity [3].

The search for remedy on this ravaging pandemic saw Plateau State Government inaugurating a Research Team with the mandate to help the world discover the drugs for the treatment. The Team produced many formulations and eventually positioned 3 different polyherbal formulations (*CoV Pla-1*, *CoV Pla-2* and *CoV Pla-3*), while another formulation was used as a prophylactic.

The different plants that made up the three polyherbal combinations in this study have been reported by various researchers to increase levels of hematological parameters including Red Blood Cells, Packed Cell Volume and Haemoglobin. Various studies have been done on some of the plant materials, for instance *Salvia officinalis* [4]; *Cucumis metuliferus* [5]; *Securidata longepedunculata* [6]; *Garcinia kola* [7] [8]; *Nigella sativa* [9]; *Vernonia amygdalina* [10]; *Carissa edulis* [11]; *Zingiber officinale* [12]; *Artemisia annua* [13]; *Syzygium aromaticum* [14].

This study evaluated the effects of 3 polyherbal extracts (*CoV-1*, *CoV Pla-2* and *CoV Pla-3*) on some biochemical parameters of the liver and kidney of laboratory animals.

II. METHODS

A. Experimental Animals

Three-week old albino rats (140-200 g) and mice (20-30 g) of both sexes were obtained from the Animal Experimental Unit, Department of Pharmacology and Toxicology, University of Jos, Nigeria. The animals were kept at room temperature (27±2°C; 70-80 % humidity; 12 h light/dark cycle) in the Animal Experimental Unit for at least 48 h prior to the procedure. Commercial food pellets and water were

supplied *ad libitum*. Animal experimental protocols were in accordance with the current guidelines for the care of laboratory animals by the U.S. National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research. Approval to conduct the study was given by the Animal Ethics Committee of the Department of Pharmacology and Toxicology, University of Jos, Nigeria.

B. Collection/ Preparation of Plant Materials

The plants were identified by Mr. Joseph Agila of the Federal College of Forestry, Jos, Nigeria. Voucher specimens were deposited at the herbarium of the college. The various plant parts were air-dried in the laboratory. The dried parts of the different plants (as in the formulation of each-*CoV-1*, *CoV Pla-2* and *CoV Pla-3*) were then pulverized, mixed together and macerated in 10 L of hydroethanol (3 litres water to 7 liters ethanol) for 24 h. The mixture was thereafter decanted, filtered and the residue was remacerated in 1000 mL hydroethanol for 24 h to ensure exhaustive extraction. At the end of the extraction process, the combined filtrate was evaporated to dryness under reduced pressure at 40 °C. A dark brownish solid extract with a yield of 15.4 % was obtained. The solid extract was reconstituted in distilled water to give appropriate concentrations before administration to experimental animals.

C. Drug Administration

Twenty albino rats weighing 100-160 g were divided into 4 groups of 5 each and administered the three extracts (*CoV Pla-1*, *CoV Pla-2* and *CoV Pla-3*) respectively as follows: For *CoV Pla-1*, the test animals (groups 1-3) were administered 100, 200 and 400 mg/kg of the extract, while group 4 animals had 0.1 mL of distilled water *per oral*. For *CoV Pla-2*, animals in the test groups (1, 2, 3) received 100, 200 and 400 mg/kg of the extract respectively via the oral route, while animals in group 4 (control) had 0.1 mL distilled water throughout the period of the experiment. For *CoV Pla-3*, the test animals (groups 1-3) were administered 100, 200 and 400 mg/kg of the extract, while group 4 animals had 0.1 mL of distilled water orally.

D. Haematological Analysis

Haematological parameters (haemoglobin levels, mean cell volume, mean cell haemoglobin levels and mean cell haemoglobin concentrations) were measured using a Beckman-Coulter AcT 5 Diff. haematology analyser according to manufacturer's guidelines (Beckman-Coulter UK Ltd). Differential white cell counts were performed manually based on a blood smear assessment of 200 leucocytes.

Chloride was evaluated by the mercury chloride colorimetric method and total calcium by the orthocresolphthalein method. Sodium and potassium were measured by the flame photometric method (Flame Photometer - CL 26D - ELICO, UK).

E. Statistical Analysis

Data was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test using a commercially available statistics software package (SPSS for

Windows, V. 15.0) program. Results were presented as means $\pm$ SD. $P < 0.05$ were considered statistically significant.

III. RESULTS

TABLE 1: EFFECT OF ETHANOLIC EXTRACT ON SOME HAEMATOLOGICAL INDICES OF ALBINO RATS

Treatment	Dose (mg/kg)	WBC ($10^9/L$)	NEU ($10^9/L$)	T-LYM (%)	RBC ($10^{12}/L$)	HGB (g/dL)
CoV Pla-1	100	16720.00 $\pm$ 6930.51	26.20 $\pm$ 11.32	73.00 $\pm$ 11.25	3.24 $\pm$ 0.71	15.26 $\pm$ 1.51
	200	18300.00 $\pm$ 8169.05	17.25 $\pm$ 6.65	81.75 $\pm$ 7.27	2.78 $\pm$ 0.65	14.73 $\pm$ 1.58
	400	15333.33 $\pm$ 5749.20	30.00 $\pm$ 13.89	69.33 $\pm$ 14.15	2.27 $\pm$ 0.68	16.20 $\pm$ 1.71
CoV Pla-2	100	9600.00 $\pm$ 4036.50	22.00 $\pm$ 6.06	77.25 $\pm$ 7.14	2.60 $\pm$ 0.76	13.83 $\pm$ 1.81
	200	11967.00 $\pm$ 2743.53	25.40 $\pm$ 7.64	76.00 $\pm$ 8.16	3.14 $\pm$ 0.43	15.42 $\pm$ 1.85
	400	14900.00 $\pm$ 6076.18	21.75 $\pm$ 7.81	76.00 $\pm$ 10.86	2.30 $\pm$ 0.36	14.95 $\pm$ 2.25*
CoV Pla-3	100	15040.00 $\pm$ 4028.39	19.20 $\pm$ 8.14	79.40 $\pm$ 7.50	2.72 $\pm$ 0.50	13.18 $\pm$ 3.34
	200	19040.00 $\pm$ 12647.06	25.00 $\pm$ 8.89	74.60 $\pm$ 8.76	3.00 $\pm$ 0.44	13.18 $\pm$ 2.19
	400	21250.00 $\pm$ 10930.54	25.50 $\pm$ 10.85	74.00 $\pm$ 10.61	2.40 $\pm$ 0.48*	14.65 $\pm$ 2.28
Control		12120.00 $\pm$ 5452.71	23.95 $\pm$ 8.69	71.00 $\pm$ 7.65	3.96 $\pm$ 1.34	13.96 $\pm$ 1.69

All the values are expressed as mean $\pm$ SD ($n=5$ for each group); * $P < 0.05$.

Key: WBC= White Blood Cells; NEU=Neutrophils; T-LYM=T-Lymphocytes; RBC=Red Blood Cells; HGB= Haemoglobin.

TABLE 2: EFFECT OF ETHANOLIC EXTRACT ON SOME HAEMATOLOGICAL INDICES OF ALBINO RATS

Treatment	Dose (mg/kg)	PCV (L/L)	MCV (fL)	MCH (pg)	MCHC (g/dL)
CoV Pla-1	100	42.60 $\pm$ 2.61	137.00 $\pm$ 33.62	50.40 $\pm$ 13.59	37.40 $\pm$ 10.53
	200	38.75 $\pm$ 4.35	150.50 $\pm$ 30.49	55.75 $\pm$ 15.65	38.25 $\pm$ 1.89
	400	39.67 $\pm$ 0.58	188.33 $\pm$ 68.49	78.67 $\pm$ 36.69	40.67 $\pm$ 3.79
CoV Pla-2	100	42.75 $\pm$ 3.30	175.25 $\pm$ 52.00	55.75 $\pm$ 14.25	32.50 $\pm$ 3.87
	200	42.80 $\pm$ 5.50	137.60 $\pm$ 23.27	49.00 $\pm$ 2.92	36.80 $\pm$ 7.53
	400	44.00 $\pm$ 4.24	196.00 $\pm$ 40.92	56.25 $\pm$ 26.74	32.25 $\pm$ 6.13
CoV Pla-3	100	40.00 $\pm$ 3.61	151.80 $\pm$ 34.37	50.60 $\pm$ 19.09	33.60 $\pm$ 9.29
	200	38.20 $\pm$ 3.56	130.00 $\pm$ 27.50	44.80 $\pm$ 10.18	34.60 $\pm$ 5.60
	400	41.50 $\pm$ 5.07	181.75 $\pm$ 62.48	62.75 $\pm$ 15.52	35.75 $\pm$ 4.19
N/S (0.1 ml)		41.00 $\pm$ 4.18	110.80 $\pm$ 29.66	38.00 $\pm$ 10.49	34.20 $\pm$ 5.07

All the values are expressed as mean $\pm$ SD ($n=5$ for each group).

Key: PCV=Packed Cell Volume; MCV=Mean Cells Volume; MCH= Mean Cells Haemoglobin; MCHC= Mean Cells Haemoglobin concentration; D. water=Distilled water; Grp.=Group.

TABLE 3: EFFECT OF EXTRACTS ON ELECTROLYTES IN ALBINO RATS

Dose (mg/kg)	Na ⁺	K ⁺	Cl ⁻	HCO ₃ ⁻
CoV Pla-1	139.60 $\pm$ 1.82	5.22 $\pm$ 0.50	106.40 $\pm$ 3.85	23.60 $\pm$ 1.67
	141.80 $\pm$ 1.48	5.12 $\pm$ 0.33	107.40 $\pm$ 2.19	24.60 $\pm$ 0.89
	141.33 $\pm$ 1.53	5.10 $\pm$ 0.10	107.00 $\pm$ 3.46	25.00 $\pm$ 1.73
CoV Pla-2	141.33 $\pm$ 1.53	5.15 $\pm$ 0.44	106.75 $\pm$ 2.99	24.50 $\pm$ 1.00
	139.00 $\pm$ 2.55	5.18 $\pm$ 0.36	105.00 $\pm$ 3.00	23.80 $\pm$ 1.79
	138.50 $\pm$ 3.11	5.13 $\pm$ 0.54	105.00 $\pm$ 4.55	23.50 $\pm$ 3.00
CoV Pla-3	141.00 $\pm$ 2.24	4.66 $\pm$ 0.46	107.00 $\pm$ 2.55	25.00 $\pm$ 1.41
	140.00 $\pm$ 1.87	5.10 $\pm$ 0.26	108.00 $\pm$ 2.45	24.60 $\pm$ 0.55
	140.50 $\pm$ 4.12	5.00 $\pm$ 0.22	108.00 $\pm$ 2.16	24.00 $\pm$ 1.41
N/saline (0.1 ml)	139.60 $\pm$ 2.70	5.10 $\pm$ 0.37	106.20 $\pm$ 3.56	23.40 $\pm$ 0.89

All the values are expressed as mean $\pm$ SD ($n=5$ for each group).

IV. DISCUSSION

The haemotopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status for both animals and humans [15]. The haematological indices evaluated in this study are useful as they have higher predictive value for human toxicity, when data are translated from animal studies

[16]. MCH, MCHC and MCV relate to individual red blood cells, while Hb, RBC and PCV are associated with the total population of red blood cells [17]. In this present study, many of the haematological parameters did not change in a significant manner in the presence of the polyherbal extracts. Compared to the rats in the control group, the PCV of rats administered CoV Pla-2 was observed to have increased in a dose-dependent (100-400 mg/kg) manner. Similar study [18]

showed that an increase in the mean PCV can be attributed to increases in both red and white cell indices.

The insignificant decrease in levels of RBC observed in the test groups for all three polyherbal mixtures (except at the highest concentration for *CoV Pla-3* which was significant) compared to control might indicate an imbalance between the rate of production and destruction of the blood corpuscles as suggested by Patrick-Iwuanyanwu [19].

Heamoglobin concentration observed to increase in the test groups (*CoV Pla-1*, 2, 3) relative to the control, is indicative of increased oxygen carrying capacity of the blood. This is a pointer to increased tissue oxygenation which is corroborated by the reports of Wannang *et al.* [5]. An increase in the mean lymphocyte values, though insignificant compared to control in this present study, is associated with enhanced immunological status of the body [20] especially the cell-mediated immune response [21]. The WBC and lymphocytes are mediators of immunity and contribute to the immune protection against inflammation. The increase in the rate of T-lymphocytes in *CoV Pla-1* and *CoV Pla-3* (all doses) compared to control observed in this study might be due to the strengthening of the immune system by the extracts. This finding suggests the therapeutic importance of the polyherbal preparations in boosting the body's immunity against COVID-19.

Results showed an increase of mean MCV, MCH for all three polyherbal mixtures. MCHC, on the other hand, decreased in only some doses for *CoV Pla-2*. Reticulocytosis occur as the initial response to stimulation of the erythropoietic system [22] and is often characterized by macrocytic and hypochromic red blood cells with increased MCV and decreased MCHC values [22], which is comparable with the findings in the test rats in this present study.

The value of circulating neutrophils was increased in the rats administered the highest doses of *CoV Pla-1* and *CoV Pla-3* extracts in this study. This is in variance to the findings of Saba *et al.* [18]. Increased circulating neutrophils serve as an index of bacterial infection in the body [23].

Electrolytes such as sodium, potassium, chloride and bicarbonate ions are among the parameters that are useful in the determination of kidney function [24]. The elevation or depletion of the level of any of them may be an indicator for a kidney problem.

Serum levels of Na^+ , K^+ , Cl^- and HCO_3^- were measured and there was no significant difference ($P > 0.05$) observed in their levels in all test animals, compared to control. However, Na^+ increased in *CoV Pla-1* and *CoV Pla-3*, relative to control. This corresponds to the findings of other researchers [25]. The same authors reported a significant change ($P < 0.05$) in K^+ concentration which is at variance with findings from this present study where insignificant increase was observed in *CoV Pla-1* and *CoV Pla-2*, compared to control. This increase, though insignificant, is also an indication that membrane channels may possibly be affected by these two polyherbal combinations.

V. CONCLUSION

In conclusion, the extracts did not cause significant changes in electrolytes and haematological parameters evaluated and were found to be safe up to 5000 mg/kg, indicating their safety. The results similarly showed an immune boosting property of the polyherbal preparations, as evident in the increase in T-lymphocytes.

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