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Original Research Article

Evaluation of *in vivo* toxicity of selected Nigerian medicinal plants used against viral diseases

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Abstract

Purpose: *To evaluate the subchronic toxicity of four plants frequently used in Nigerian ethnomedicine to treat various viral infections.*

Methods: *Andrographis paniculata, Artemisia annua, Annona muricata, and Bryophyllum pinnatum extracts were assessed for their subchronic toxic effect in albino rats (Sprague-Dawley strain). Each extract was administered orally at 100, 200, and 400 mg/kg body weight once daily for 28 days, and the biochemical and haematological parameters of the animals were evaluated. The average body weights of the rats were taken before and after the treatment, after which they were sacrificed under chloroform anaesthesia. Blood samples were collected through cardiac puncture and were used to assess for toxicity markers (lipid profile, liver and renal function parameters, and haematological indices).*

Results: *The sub-chronic administration of these plants, especially Annona muricata and Bryophyllum pinnatum, caused a significant decrease in body weight, an increase in blood glucose and liver enzymes (Alkaline phosphatase and Alanine aminotransferase), and a decrease in hematocrit (HCT) and platelet (PLT) count ($p < 0.05$). A. paniculata extract was shown to tend to cause dislipidaemia due to the significant increase in serum levels of total cholesterol and low-density lipoprotein cholesterol ($p < 0.05$).*

Conclusion: *The findings from the study show that the extracts altered toxicological indices in a dose-dependent manner. Hence, caution is needed in the long-term use of these plant extracts.*

Keywords: *Antiviral, Biochemical parameters, Lipid profile, Medicinal plants, Sub-chronic Toxicity*

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INTRODUCTION

Medicinal plants have been used to treat various diseases since time immemorial and are sources of unique molecules currently used in modern medicine [1,2]. Due to the inherent side effects associated with most synthetic drugs, there is an increasing use of herbal medicines due to their perceived safety. However, some of these herbal remedies may be associated with severe cases of toxicity. In recent times, researchers have developed a keen interest in natural products, such as medicinal plants, and have investigated different plant materials used by the local

population for the treatment of numerous diseases [3,4]. Several of these medicinal plants, including *Allium sativum*, *Glycyrrhiza glabra*, *Ocimum sanctum*, *Ocimum kilim*, and *Solanum nigrum,* etc., have been tested and showed promising activity against viral infections [5-7]. Usually, some of these plants are believed to be safe and efficacious. *Andrographis paniculata,* which contains a unique diterpene lactone (andrographolide), has been used in the treatment of influenza, common cold, inflammation, infertility, and malaria [8]. Also, *Artemisia annua, Annona muricata,* and *Bryophyllum pinnatum* have been shown to contain phytochemicals with potent biological activities. Different parts of these plants have been employed in ethnomedicine for the treatment of malaria, infectious diseases, diabetes, inflammations, fevers, respiratory tract infections, and cancers [9-11]. The present study aimed to assess the sub-chronic toxic effects of these plants (*A. paniculata, A. annua, A. muricata,* and *B. pinnatum*), which are commonly used in the treatment of respiratory tract infections and related diseases.

EXPERIMENTAL

Animals

Healthy Sprague-Dawley rats were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The rats were kept in well-ventilated plastic cages with bedding of wood shavings and allowed to acclimatize to the laboratory conditions for two weeks. The rats were fed with dry rodent pelleted finisher feeds and had unrestricted access to drinking water. The bedding materials (wood shavings) of the cages were changed daily. Ethical approval with reference number: EC/FP/020/12 was obtained from the Ethics Committee, Faculty of Pharmacy, University of Benin, Benin City, and all the experiments were carried out following NIH guidelines [12].

Plant collection and authentication

Plant collection and identification are presented in Table 1.

Plant preparation and extraction

The plant materials (leaves, Table 1) were air-dried for 3 weeks, ground into powder, and stored in an air-tight container. The powdered plant materials (200 g each) were macerated separately in methanol (2 L) at room temperature for 1 week. The extracts were concentrated *in vacuo* using a rotary evaporator at 45℃ and then kept in a refrigerator at 4℃ until further use.

Preparation of plant extract doses

The extracts were dissolved in 10 mL of dimethylsulfoxide (DMSO) and diluted with distilled water up to volumes equivalent to three doses: 100, 200 and 400 mg/kg.

Animal grouping and treatment

The animals were randomly divided into thirteen (13) groups of 6 animals each, consisting of one (1) control group and twelve (12) test groups. Appropriate doses (100, 200, and 400 mg/kg body weight) of the extracts were administered orally using an Orogastric tube once daily for 28 days. The body weights of the animals were recorded before the administration of the extracts and at the end of the treatment period.

Measurement of biochemical and haematological parameters

At the end of the treatment period, the animals were anaesthetized using chloroform and sacrificed by cervical dislocation. Blood samples were collected through cardiac puncture into plain bottles and allowed to clot at room temperature before centrifugation (Rototix 32A, Germany) at 4000 rpm for 10 minutes. The sera samples were withdrawn carefully into plain containers using sterile syringes, and used to assess common toxicity markers such as lipid profile, liver function enzymes, renal function parameters, and haematological indices as previously described [13].

Table 1: Plant collection and identification

Plant name	Plant part	Place of collection	Date of collection	Plant ID*
Annona muricata	Leaves	Ekosodin, Edo State, Nigeria	Feb, 2021	UBH-A356
Artemisia annua	Leaves	Ibafo, Ogun State, Nigeria	April, 2021	FHI113652
Andrographis paniculata	Leaves	Ekosodin, Edo State, Nigeria	Feb, 2021	UBH-P290
Bryophyllum pinnatum	Leaves	Ugbowo, Edo State, Nigeria	Feb, 2021	UBH-B593

*UBH = University of Benin, Herbarium no.; FHI = Forestry Research Institute of Nigeria, Ibadan Voucher no.

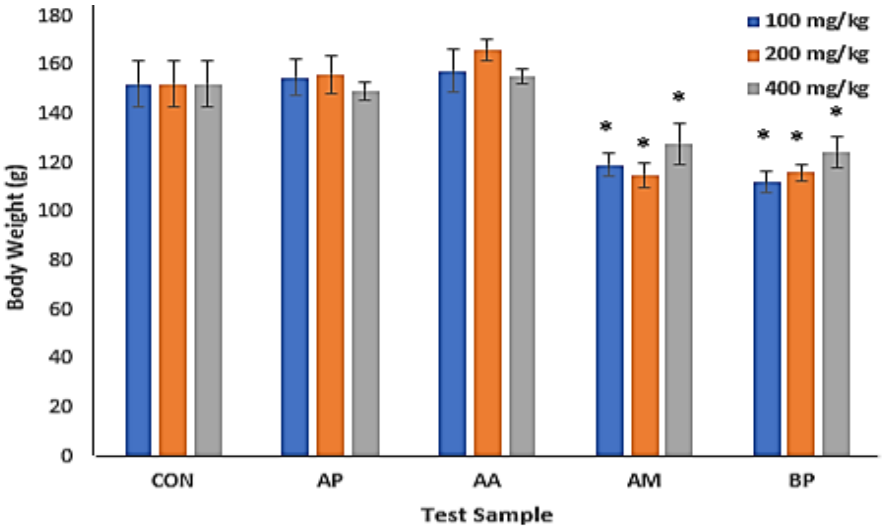

Figure 1: Changes in body weights of animals pre- and post-administration of the different plant extracts. Data represent mean ± standard deviation (SD), n = 6. '*' indicates significant changes in body weight compared to control (P < 0.05). *Andrographis paniculata* (AP), *Artemisia annua* (AA), *Annona muricata* (AM), *Bryophyllum pinnatum* (BP), Control (CON)

Essential biochemical parameters such as serum glucose, renal function indices (creatinine, sodium ion, potassium ion, urea, bicarbonate), liver function indices (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, conjugated bilirubin, total bilirubin, albumin, and total protein), and serum lipids (total cholesterol, low-density lipoprotein, high-density lipoprotein, and total triglycerides) were assessed using an automated Clinical System analyzer (VIS-7220 G, Biotech Engineering Management Company Limited, UK). Haematological indices were assayed using the automated haematological multichannel analyzer (ERMA PCE 210, Japan) as described [13].

Statistical analysis

Data were analyzed with GraphPad Prism Version 6, San Diego, USA. Data were presented as mean ± standard deviation (SD). Inferential statistics were applied using Analysis of Variance (ANOVA), Tukey's, and Fisher's post hoc tests. Statistical significance was set at $P \leq 0.05$.

RESULTS

Effect of extracts on body weight and blood glucose

There were significant ($p < 0.05$) decreases in body weight (Figure 1) for the animals treated with *A. muricata* and *B. pinnatum* extracts at all doses tested. For blood glucose, significant increases were observed in the animals treated with *A. muricata* and *B. pinnatum* extracts at 100 mg/kg, whereas, at 400 mg/kg, only *B. pinnatum* extract resulted in a significant increase in blood glucose. In contrast, *A. annua* extract resulted in a significant decrease in body weight at both 100 and 400 mg/kg doses, while *A. paniculata* extract did not cause any significant change in body weight at the doses tested (Figures 2 and 3).

Effect of extracts on biochemical and haematological parameters

The results of the *in vivo* sub-chronic toxicity screening of the methanol extracts of the four plants showed significant alterations in biochemical and haematological parameters of the animals (Tables 2 - 5). All the plant extracts significantly increased serum ALP and ALT levels, while only *B. pinnatum* decreased serum AST level (Table 2).

For the lipid profile, the levels of total cholesterol and LDL were significantly increased in rats treated with *A. paniculata* extract at 100 mg/kg, while the triglyceride level was significantly decreased in rats treated with *A. annua* extract at 100 mg/kg (Table 3). There were significant decreases in serum urea and sodium ion concentrations in rats treated with *A. muricata* and *A. annua* extracts, respectively, at 200 mg/kg. However, serum potassium ion concentration was increased in rats treated with *A. muricata* extract at 200 mg/kg.

Other renal function parameters (chloride, bicarbonate, and creatinine) were not significantly altered (Table 4). There was a significant increase in the percentage of monocytes at the lower doses of *Annona muricata* extracts, and an increase in RBC levels due to *A. paniculata* and *A. annua* extracts (Table 5).

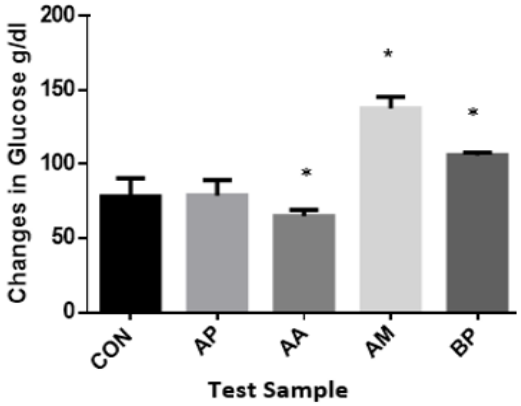

Figure 2: Changes in blood glucose levels due to treatment with plant extracts at 100 mg/kg. Data represent mean ± standard deviation (SD), n = 6. '*' indicates significant changes in blood glucose compared to control (P < 0.05). *Andrographis paniculata* (AP), *Artemisia annua* (AA), *Annona muricata* (AM), *Bryophyllum pinnatum* (BP), Control (CON)

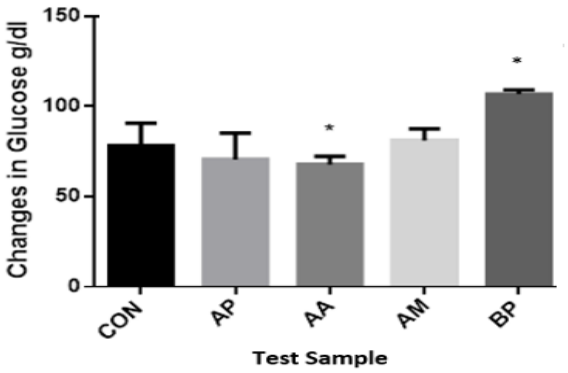

Figure 3: Changes in blood glucose levels due to treatment with plant extracts at 400 mg/kg. Data represent mean ± standard deviation (SD), n = 6. '*' indicates significant changes in blood glucose compared to control (P < 0.05). *Andrographis paniculata* (AP), *Artemisia annua* (AA), *Annona muricata* (AM), *Bryophyllum pinnatum* (BP), Control (CON).

Table 2: Serum levels of liver function enzymes pre- and post-administration of plant extracts

Extract dose (mg/kg)	CON	AP	AA	AM	BP
			ALP (U/L)		
100	110.00 ± 12.95	131.20 ± 11.61	117.80 ± 9.81	138.50 ± 9.51*	152.00 ± 15.33*
200	110.00 ± 12.95	148.80 ± 6.00*	127.50 ± 6.95	164.70 ± 18.50*	145.70 ± 6.21*
400	110.00 ± 12.95	151.00 ± 7.41*	147.00 ± 8.08*	152.20 ± 5.40*	144.70 ± 2.74*
			AST (U/L)		
100	90.17 ± 4.57	83.83 ± 5.94	93.17 ± 10.57	97.00 ± 11.82	77.33 ± 4.13*
200	90.17 ± 4.57	85.00 ± 7.64	98.00 ± 6.41	80.00 ± 3.42	83.17 ± 2.41
400	90.17 ± 4.57	115.7 ± 10.85*	102.70 ± 8.63	87.67 ± 2.04	84.67 ± 3.32
			ALT (U/L)		
100	20.00 ± 1.29	22.33 ± 2.38	25.83 ± 2.31	30.50 ± 1.80*	32.00 ± 2.71#
200	20.00 ± 1.29	25.17 ± 2.63	26.17 ± 2.04	30.67 ± 2.72*	32.17 ± 2.36*
400	20.00 ± 1.29	25.50 ± 2.17	27.17 ± 2.29	25.83 ± 2.57	29.33 ± 1.67*
			TOTAL BILIRUBIN (mg/dL)		
100	0.25 ± 0.02	0.32 ± 0.03	0.32 ± 0.03	0.32 ± 0.03	0.30 ± 0.03
200	0.25 ± 0.02	0.30 ± 0.03	0.33 ± 0.03	0.28 ± 0.03	0.25 ± 0.03
400	0.25 ± 0.02	0.30 ± 0.03	0.30 ± 0.03	0.27 ± 0.02	0.30 ± 0.03
			CONJUGATED BILIRUBIN (mg/dL)		
100	0.12 ± 0.02	0.17 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.15 ± 0.02
200	0.12 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	0.15 ± 0.02
400	0.12 ± 0.02	0.12 ± 0.02	0.13 ± 0.02	0.12 ± 0.02	0.15 ± 0.02
			TOTAL PROTEIN (mg/dL)		
100	6.53 ± 0.40	6.63 ± 0.31	7.78 ± 0.77	8.37 ± 0.47*	7.95 ± 0.24*
200	6.53 ± 0.40	6.53 ± 0.25	10.15 ± 0.60*	7.30 ± 0.60	8.23 ± 0.60*
400	6.53 ± 0.40	7.90 ± 0.84	8.82 ± 0.35*	9.00 ± 0.43*	8.78 ± 0.40*
			ALBUMIN (mg/dL)		
100	3.42 ± 0.22	3.72 ± 0.11	3.57 ± 0.24	3.68 ± 0.17	3.97 ± 0.12
200	3.42 ± 0.22	3.58 ± 0.22	3.92 ± 0.06	3.60 ± 0.27	3.73 ± 0.13
400	3.42 ± 0.22	3.55 ± 0.16	3.92 ± 0.15	3.87 ± 0.09	3.92 ± 0.09

Values are mean ± SD, n = 6. *P < 0.05 vs control, **ALP:** Alkaline phosphatase, **AST:** Aspartate aminotransferase, **ALT:** Alanine aminotransferase, **AP:** *Andrographis paniculata*, **AA:** *Artemisia annua*, **AM:** *Annona muricata*, **BP:** *Bryophyllum pinnatum*, **CON:** Control

Table 3: Serum lipid profile of animals after 28 days of extract administration

Extract dose (mg/kg)	CON	AP	AA	AM	BP
	CHOLESTEROL (mg/dL)				
100	66.17 ± 4.62	86.50 ± 5.19*	69.33 ± 3.52	77.67 ± 3.24*	67.00 ± 4.31
200	66.17 ± 4.622	72.67 ± 4.38	61.17 ± 2.17	75.83 ± 6.15	69.17 ± 6.61
400	66.17 ± 4.622	73.50 ± 2.95	66.50 ± 3.77	62.33 ± 3.16	75.00 ± 4.28
	TRIGLYCERIDE (mg/dL)				
100	70.17 ± 6.40	66.50 ± 5.70	56.33 ± 2.79*	75.83 ± 5.15	85.17 ± 8.84
200	70.17 ± 6.40	67.00 ± 5.40	81.17 ± 8.14	89.50 ± 10.40	72.33 ± 5.30
400	70.17 ± 6.40	65.67 ± 2.88	69.67 ± 8.15	94.67 ± 12.31*	67.83 ± 2.89
	HIGH-DENSITY LIPOPROTEIN (mg/dL)				
100	18.50 ± 1.43	18.67 ± 1.45	23.17 ± 1.14	20.83 ± 1.56	20.17 ± 1.97
200	18.50 ± 1.43	18.33 ± 1.26	16.50 ± 0.85	19.50 ± 2.62	20.33 ± 1.84
400	18.50 ± 1.43	18.17 ± 1.58	17.67 ± 1.91	20.33 ± 1.93	22.17 ± 1.62
	LOW-DENSITY LIPOPROTEIN (mg/dL)				
100	33.50 ± 4.51	54.17 ± 6.07*	34.50 ± 2.64	41.17 ± 4.17	29.50 ± 4.75
200	33.50 ± 4.51	40.83 ± 4.87	28.17 ± 4.20	38.17 ± 6.04	34.00 ± 7.09
400	33.50 ± 4.51	42.00 ± 3.81	34.67 ± 2.88	26.00 ± 3.63	36.50 ± 5.66

Values are mean ± SD, n = 6. *P < 0.05 vs control, **AP:** *Andrographis paniculata*, **AA:** *Artemisia annua*, **AM:** *Annona muricata*, **BP:** *Bryophyllum pinnatum*, **CON:** Control

Table 4: Renal indices pre- and post-administration of plant extracts

Extract dose (mg/kg)	CON	AP	AA	AM	BP
	UREA (mg/dL)				
100	30.67 ± 0.99	30.83 ± 1.14	29.67 ± 1.33	37.17 ± 2.04*	27.33 ± 2.03
200	30.67 ± 0.99	32.33 ± 1.15	34.50 ± 1.09	23.17 ± 1.14*	32.67 ± 2.11
400	30.67 ± 0.99	35.17 ± 1.40	34.00 ± 3.18	30.33 ± 1.73	32.00 ± 1.27
	SODIUM ION (mEq/L)				
100	140.70 ± 1.26	140.30 ± 1.61	141.80 ± 1.35	141.20 ± 1.11	139.80 ± 0.70
200	140.70 ± 1.26	142.30 ± 1.12	137.30 ± 1.12	141.50 ± 1.18	143.20 ± 1.30
400	140.70 ± 1.26	138.70 ± 0.80	141.30 ± 0.72	141.70 ± 1.45	140.50 ± 0.89
	POTASSIUM ION (mEq/L)				
100	6.38 ± 0.29	6.05 ± 0.25	6.35 ± 0.39	6.83 ± 0.33	6.58 ± 0.79
200	6.38 ± 0.29	5.93 ± 0.19	6.32 ± 0.27	8.07 ± 0.17*	6.82 ± 0.45
400	6.38 ± 0.29	6.60 ± 0.18	6.52 ± 0.63	7.40 ± 0.38	6.47 ± 0.24
	BICARBONATE ION (mEq/L)				
100	20.17 ± 0.54	18.83 ± 0.60	17.67 ± 0.76	19.67 ± 0.42	19.33 ± 0.49
200	20.17 ± 0.54	19.00 ± 0.89	18.83 ± 0.83	19.00 ± 0.37	19.83 ± 0.65
400	20.17 ± 0.54	19.67 ± 0.67	19.17 ± 0.40	18.83 ± 0.87	19.33 ± 0.33
	CHLORIDE ION (mEq/L)				
100	100.30 ± 0.62	99.83 ± 1.08	99.17 ± 1.08	100.80 ± 1.42	97.17 ± 1.08
200	100.30 ± 0.62	100.20 ± 1.01	100.30 ± 1.28	99.00 ± 1.08	99.83 ± 1.14
400	100.30 ± 0.62	98.17 ± 2.07	98.83 ± 0.75	98.83 ± 0.95	97.50 ± 0.62
	CREATININE (mg/dL)				
100	0.35 ± 0.02	0.37 ± 0.03	0.35 ± 0.02	0.35 ± 0.02	0.37 ± 0.02
200	0.35 ± 0.02	0.38 ± 0.03	0.43 ± 0.02*	0.35 ± 0.03	0.32 ± 0.03
400	0.35 ± 0.02	0.37 ± 0.03	0.43 ± 0.30*	0.37 ± 0.04	0.32 ± 0.02

Values are mean ± SD, n = 6. *P < 0.05 vs control. **AP:** *Andrographis paniculata*, **AA:** *Artemisia annua*, **AM:** *Annona muricata*, **BP:** *Bryophyllum pinnatum*, **CON:** Control

Table 5a: Changes in the hematological parameters of animals pre- and post-extract administration

Extract dose (mg/kg)	CON	AP	AA	AM	BP
			WBC (x10^3/µL)		
100	13.03 ± 0.29	14.53 ± 1.21	14.28 ± 3.00	12.28 ± 1.11	14.28 ± 1.35
200	13.03 ± 0.29	16.90 ± 0.89*	15.02 ± 1.35	16.20 ± 1.64*	13.97 ± 0.66
400	13.03 ± 0.29	13.20 ± 1.56	13.22 ± 1.74	15.68 ± 3.60	16.30 ± 2.43
			LYM (%)		
100	79.52 ± 2.48	77.35 ± 1.28	73.62 ± 3.94	79.33 ± 1.77	76.37 ± 2.14
200	79.52 ± 2.48	75.48 ± 2.42	74.55 ± 2.03	75.62 ± 3.94	83.32 ± 1.37
400	79.52 ± 2.48	71.48 ± 1.97*	78.33 ± 1.72	71.72 ± 1.97*	74.77 ± 3.02
			MON (%)		
100	3.23 ± 0.31	3.50 ± 0.55	5.95 ± 1.00*	7.12 ± 1.37*	5.92 ± 0.82*
200	3.23 ± 0.31	6.90 ± 1.97*	5.18 ± 0.60*	6.57 ± 0.20*	5.58 ± 1.04*
400	3.23 ± 0.31	5.50 ± 0.28*	4.58 ± 0.84	6.07 ± 1.81*	7.07 ± 1.17*
			NEU (%)		
100	11.48 ± 1.28	12.40 ± 0.90	12.33 ± 2.19	8.05 ± 1.31	10.78 ± 1.31
200	11.48 ± 1.28	9.43 ± 0.74	11.00 ± 1.22	11.02 ± 2.78	6.90 ± 0.83*
400	11.48 ± 1.28	12.47 ± 1.57	8.92 ± 1.00	12.42 ± 1.05	10.52 ± 1.22
			EOS (%)		
100	0.32 ± 0.05	0.42 ± 0.09	0.63 ± 0.05*	0.42 ± 0.16	0.47 ± 0.08
200	0.32 ± 0.05	0.27 ± 0.06	0.28 ± 0.05	0.38 ± 0.09	0.23 ± 0.04
400	0.32 ± 0.05	0.70 ± 0.15*	0.50 ± 0.11	0.30 ± 0.10	0.47 ± 0.07
			BAS (%)		
100	6.48 ± 0.98	6.33 ± 0.52	7.47 ± 1.03	5.08 ± 0.74	6.47 ± 0.78*
200	6.48 ± 0.98	7.92 ± 0.94	8.98 ± 0.83	6.42 ± 1.12	3.97 ± 0.55*
400	6.48 ± 0.98	9.85 ± 0.68*	7.67 ± 0.73	9.50 ± 0.82*	7.18 ± 1.50
			RBC (x10^6/µL)		
100	10.15 ± 0.68	9.75 ± 0.17	11.46 ± 0.6$^{\#}$	8.33 ± 0.26*	8.05 ± 0.29*
200	10.15 ± 0.68	10.34 ± 0.35	11.48 ± 0.6$^{\#}$	8.07 ± 0.42*	8.30 ± 0.30*
400	10.15 ± 0.68	12.03 ± 0.93*	10.83 ± 1.10	8.17 ± 0.59*	8.03 ± 0.45*
			HGB (g/dL)		
100	16.57 ± 0.10	15.75 ± 0.32	18.45 ± 1.30*	13.83 ± 0.26*	13.32 ± 0.38*
200	16.57 ± 0.10	16.40 ± 0.55	18.48 ± 1.00*	13.32 ± 0.37*	13.65 ± 0.34*
400	16.57 ± 0.10	18.42 ± 1.77	16.70 ± 2.47	13.68 ± 0.80*	13.67 ± 0.54*

Values are mean ± SD, n = 6. *P < 0.05 vs control. **WBC:** White blood cells, **LYM:** Lymphocytes, **MON:** Monocytes, **NEU:** Neutrophils, **EOS:** Eosinophils, **BAS:** Basophils, **RBC:** Red blood cells, **HGB:** Haemoglobin, *Andrographis paniculata* (**AP**), *Artemisia annua* (**AA**), *Annona muricata* (**AM**), *Bryophyllum pinnatum* (**BP**), Control (**CON**)

DISCUSSION

Findings from the *in vivo* sub-chronic toxicity screening of the extracts of four medicinal plants (*Andrographis paniculata*, *Artemisia annua*, *Annona muricata*, and *Bryophyllum pinnatum*) used in the treatment of viral infections indicated that the sub-chronic administration of these plants, especially *Annona muricata* and *Bryophyllum pinnatum,* caused significant changes in body weight, blood glucose, biochemical and haematological parameters of animals. Body weight decreased, while blood glucose increased with the administration of *A. muricata* and *B. pinnatum* extracts. Also, with the administration of these two extracts (*A. muricata* and *B. pinnatum*), liver enzymes ALP and ALT levels increased significantly, which is indicative of liver toxicity. On the other hand, *A. paniculata* extract has the tendency to cause dyslipidaemia due to the significant increase in serum levels of total cholesterol and LDL with the administration of this extract.

or the renal function parameters, all the extracts seem not to pose a significant risk to the renal system, except for the increase in potassium ion seen with the administration of *A. muricata* extract (Table 4). Generally, haematological indices were not significantly altered, except for decrease in hematocrit (HCT) and platelet (PLT) count with the administration of *A. muricata* and *B. pinnatum* extracts. These findings suggest that the long-term use of these plants may pose toxicity issues to individuals using the herbals for treatment, especially dose-sensitive persons, who may be more at risk. The pattern of herbal use has been reported in some regions, and this risk may get worse with the concomitant use of conventional drugs, especially those with the same pharmacodynamic fate as the herbal preparation [14]. Interactions that are common with specific conventional drugs may be complicated by the use of herbal drugs with a complex composition [15-18].

Table 5b: Changes in the hematological parameters of animals pre- and post-extract administration – (continued)

Extract dose (mg/kg)	CON	AP	AA	AM	BP
			HCT (%)		
100	50.08 ± 2.88	46.95 ± 1.08	47.20 ± 5.63	41.47 ± 0.80*	39.58 ± 1.03*
200	50.08 ± 2.88	48.55 ± 1.62	54.70 ± 2.85	39.67 ± 1.13*	40.52 ± 1.17*
400	50.08 ± 2.88	54.22 ± 5.42	55.13 ± 5.38	41.05 ± 1.85*	40.12 ± 1.28*
			MCV (fL)		
100	49.45 ± 0.49	48.15 ± 0.50	47.35 ± 0.81	49.88 ± 0.99	49.42 ± 0.99
200	49.45 ± 0.49	46.97 ± 0.64	47.75 ± 0.61	49.57 ± 1.62	49.22 ± 0.83
400	49.45 ± 0.49	48.58 ± 0.80	48.53 ± 1.41	50.92 ± 1.92	48.77 ± 1.27
			MCH (pg)		
100	16.37 ± 0.12	16.17 ± 0/12	16.05 ± 0.28	16.63 ± 0.32	16.62 ± 0.27
200	16.37 ± 0.12	15.85 ± 0.28	16.12 ± 0.15	16.63 ± 0.51	16.60 ± 0.33
400	16.37 ± 0.12	16.52 ± 0.26	16.33 ± 0.48	16.90 ± 0.37	16.57 ± 0.34
			MCHC (g/dL)		
100	33.07 ± 0.09	33.58 ± 0.15	33.75 ± 0.20	33.38 ± 0.25	33.63 ± 0.21
200	33.07 ± 0.09*	33.80 ± 0.18	33.75 ± 0.15	33.57 ± 0.19	34.03 ± 0.36
400	33.07 ± 0.09	34.03 ± 0.29	33.67 ± 0.19	33.22 ± 0.61	34.03 ± 0.36
			RDW-CV (%)		
100	17.35 ± 0.40	18.10 ± 0.26	18.55 ± 0.31	18.68 ± 1.09	16.70 ± 0.59
200	17.35 ± 0.40	16.50 ± 0.33	18.18 ± 0.44	19.50 ± 0.51*	17.17 ± 0.50
400	17.35 ± 0.40	15.60 ± 0.30	15.88 ± 0.36	19.47 ± 1.61*	18.08 ± 0.52
			RDW-SD (fL)		
100	26.92 ± 0.75	26.45 ± 0.64	27.18 ± 1.16	29.88 ± 1.94	26.78 ± 1.90
200	26.92 ± 0.75	23.75 ± 0.61*	26.57 ± 0.54	30.32 ± 2.13	26.95 ± 0.76
400	26.92 ± 0.75	24.07 ± 0.36	24.47 ± 0.76	32.75 ± 4.42	27.37 ± 1.15
			PLT ($\times 10^3$/µL)		
100	778.70 ± 46.05	795.20 ± 23.02	702.30 ± 19.40*	715.3 ± 19.32	656.2 ± 39.93*
200	778.70 ± 46.05	827.20 ± 20.43	814.20 ± 22.60	636.8 ± 63.5*	717.5 ± 26.23
400	778.70 ± 46.05	754.00 ± 51.12	727.50 ± 25.70	756.7 ± 44.69	643.2 ± 35.57*

Values are mean ± SD, n = 6. *P < 0.05 vs control. **HCT:** Hematocrit, **MCV:** Mean cell volume, **MCH:** Mean cell haemoglobin, **MCHC:** Mean cell haemoglobin concentration, **RDW-CV:** Red cell distribution width-Coefficient of variation, **RDW-SD:** Red cell distribution width-Standard deviation, **PLT:** Platelets, *Andrographis paniculata* (**AP**), *Artemisia annua* (**AA**), *Annona muricata* (**AM**), *Bryophyllum pinnatum* (**BP**), Control (**CON**)

Table 5c: Changes in the hematological parameters of animals pre- and post-extract administration – (continued)

Extract dose (mg/kg)	CON	AP	AA	AM	BP
			MPV (fL)		
100	6.90 ± 0.09	6.92 ± 0.09	7.05 ± 0.10	7.30 ± 0.12	6.98 ± 0.16
200	6.90 ± 0.09	6.88 ± 0.09	7.02 ± 0.07	7.17 ± 0.06	6.78 ± 0.03
400	6.90 ± 0.09	7.08 ± 0.12	6.85 ± 0.11	6.95 ± 0.16	6.75 ± 0.04
			PCT (ng/mL)		
100	0.55 ± 0.02	0.55 ± 0.02	0.50 ± 0.03	0.52 ± 0.02	0.52 ± 0.04
200	0.55 ± 0.02	0.58 ± 0.02	0.58 ± 0.02	0.48 ± 0.05	0.50 ± 0.03
400	0.55 ± 0.02	0.53 ± 0.03	0.50 ± 0.03	0.52 ± 0.05	0.43 ± 0.02*
			PDW (%)		
100	16.60 ± 1.57	17.73 ± 0.95	22.00 ± 1.00*	16.48 ± 1.40	15.43 ± 1.48
200	16.60 ± 1.57	17.65 ± 1.12	19.52 ± 1.91	18.72 ± 1.99	14.90 ± 0.85
400	16.60 ± 1.57	16.97 ± 1.58	14.28 ± 1.67	15.63 ± 0.67	18.15 ± 1.86
			PLCR (%)		
100	4.98 ± 0.42	5.15 ± 0.47	5.58 ± 0.59	7.48 ± 0.64*	5.32 ± 0.66
200	4.98 ± 0.42	5.57 ± 0.51	6.35 ± 0.44	6.60 ± 0.45	4.52 ± 0.18
400	4.98 ± 0.42	6.70 ± 0.79	6.35 ± 0.85	5.70 ± 0.88	4.80 ± 0.27

Values are mean ± SD, n = 6. *P < 0.05 vs control. **MPV:** Mean platelet volume, **PCT:** Procalcitonin, **PDW:** Platelet distribution width, **PLCR:** Platelet large cell ratio. *Andrographis paniculata* (**AP**), *Artemisia annua* (**AA**), *Annona muricata* (**AM**), *Bryophyllum pinnatum* (**BP**), Control (**CON**)

It is worth mentioning that the combination of herbs with orthodox drugs may lead to toxicity even when they individually have an inherent safety profile. The significant reduction in body weight in the groups treated with *Annona muricata* and *Bryophyllum pinnatum* may be of benefit in obese individuals or those with other morbid conditions with concomitant increase in body weight [16]. The weight reduction effects of the extract may also be due to poor feed intake and suppressed appetite, which were noticed in the rats. On the other hand, *Artemisia annua*

extract caused an increase in body weight. This weight increase could be clinically relevant in patients whose condition may be complicated due to weight loss [16]. *Annona muricata* and *Bryophyllum pinnatum* increased blood glucose levels significantly at the doses tested, meaning that these extracts could worsen the clinical condition of patients with diabetes mellitus. It may also mask the activity of conventional anti-diabetic agents. In another vein, a higher reduction of serum glucose was observed with *Artemisia annua* extract; as such, patients may have a high risk of hypoglycemia on long-term use of this plant extract. However, the decreased blood glucose levels may benefit patients with diabetes [13,16,17]. The increase in serum ALP and ALT levels could pose a risk of liver toxicity, especially in subjects with existing liver disease [19]. On the contrary, the decrease in AST activity caused by *B. pinnatum* extract suggests that the plant may have hepatoprotective potential [13,16,17]. From the results, serum levels of all lipid parameters except HDL were significantly increased at the lowest dose (100 mg/kg) of the extract. For cholesterol level in particular, there was an increase from 66.17 ± 4.62 mg/dL in the control group to 86.50 ± 5.17 mg/dL in *A. paniculata* extract-treated group at 100 mg/kg, representing about 30.72% increase, which was the highest among all the extracts. Similarly, at 100 mg/kg of *A. paniculata* extract, LDL level increased from 33.50 ± 4.51 mg/dL in the control group to 54.17 ± 6.07 mg/dL in the extract-treated group, representing 61.70% increase. However, at higher doses of the extracts, there were no significant changes in lipid parameters, meaning that higher doses of the plant extracts may not significantly affect lipid profile. It is important to note that LDL is positively correlated with cardiovascular disease risk, while HDL is negatively correlated with risk of cardiovascular diseases [20,21]. From the above observation, the uncontrolled consumption of *A. paniculata* may cause hyperlipidaemia, with increased risk of cardiovascular diseases [22]. For the renal function parameters, the increase in serum level of potassium ion due to *Annona muricata* extract may cause a classical hyperkalaemic effect and possible electrolyte imbalance in patients on diuretics [16,17]. Although there were no significant changes in serum levels of creatinine and chloride ions, there is a need to exercise caution in using these plant extracts for therapeutic purposes. Increased creatinine levels have been confirmed to reflect the extent of kidney damage [17]. The significant increase in the percentage of monocytes at lower doses of *Annona muricata* extracts may worsen inflammatory changes during infection. Hence, individuals taking extracts of this plant may experience a marked elevation of monocytes, as seen in most cases of viral infection. An increase in RBC levels with the administration of *A. paniculata* and *A. annua* extracts may benefit anemic patients. Based on the results of the present study, the occurrence of adverse effects or drug interactions with the use of these plants cannot be foreclosed. Therefore, close monitoring is strongly recommended when using any of the plants.

CONCLUSION

The toxicological investigation of the crude methanol extracts of *Andrographis paniculata, Artemisia annua, Annona muricata,* and *Bryophyllum pinnatum* in the sub-chronic toxicity screening showed varied safety patterns. The findings indicated that all the plant extracts significantly increased serum ALP and ALT activities, which suggests that these plants may be contraindicated in patients with compromised liver function. It is important to note that the reduction in serum glucose levels associated with *A. annua* intake may be of concern in patients on antidiabetic medications. This study highlighted the importance of long-term intake of herbal medicines to treat various diseases, with alterations in different biomarkers.

DECLARATIONS

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Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. A Falodun conceived and designed the study. S Aghahowa, I Akhigbe, I Nahandoo, BE Olowoeyo, and PC Akubuiro performed the experiment, S Aghahowa, VO Imieje, O Erharuyi, KO Ogbeide, E Igbinosa, and JO Igoli collected and analyzed the data, and S Aghahowa, VO Imieje, and O Erharuyi wrote the manuscript. All authors read and approved the manuscript for publication.

Ethical approval

Ethical approval (EC/FP/020/12) was obtained from the Animal Ethics Committee of the Faculty of Pharmacy, University of Benin, Nigeria, before the commencement of the study.

Availability of data and materials

Data used during the current study are available from the corresponding author.

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