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Six months chronic toxicity of *Dryopteris filix –mas* (L.) Schott ethanol leaf extract on Wistar rats

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ABSTRACT

Background & Aim: *Dryopteris filix-mas* (*D. filix*-mas) is used among the Southern Nigerian populace in the management of rheumatoid arthritis, treatment of wounds, worm infestations, among other diseases. We evaluated the 6 months chronic exposure effects of its ethanol leaf extract in Wistar rat.

Experimental: A total of 48 rats were randomized into four groups of 12 each as follows; group A (control) and the test groups B-D received 31.25, 62.5 and 125 mg/kg of the leaf extract, respectively. Blood samples were collected via retro-orbital puncture for baseline determination of haematological and biochemical parameters. Thereafter, rats were dosed orally (p.o) for 180 days (6 months) and blood samples were collected for the determination of haematological, biochemical parameters on the 181st day. Liver and kidneys were harvested for histopathology analyses. A 28 - day recovery study was also conducted to determine reversibility in toxicological effects.

Results: There was no significant alteration (P>0.05) in heamatological, lipid profile and electrolyte parameters as well as body weight gain and relative organ weights of animals that were exposed to the extract when compared with control group. However, there was significant (P<0.005) reductions in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as elevation in urea and creatinine levels of extract treated groups. Histological sections did not reveal toxicity of liver architecture on day 181st, except dose dependent kidney toxicity, which was reversed following the recovery study.

Recommended applications/industries: The leaf extract of *Dryopteris filix-mas* may be nephrotoxic following 6 months exposure.

1. Introduction

Dryopteris filix-mas, (Dryoperidaceae), commonly known as male fern (filix-mas), is an evergreen medicinal plant that grows between 60–150 cm in stream and waterlogged locations (Bafor *et al.*, 2017). It originated from Europe, American and North American (Bafor *et al.*, 2017). Its bipinnated leaves tapered with the basal pinnae, about half the length of the middle pinnae are blunt and equally lobed all around. The stalks are covered with orange-brown scales. Five to six sori develop in two rows on the abaxial surface of the mature blade (Sekendar *et al.*, 2012).

D. filix-mas common names in the Southern Nigeria include; Akpaka (Igbos), Eraketa (Urhobos), Imu (Ondos), In various parts of the world it is serves as an ancient remedy for tapeworm and flatworm infestation

in human and animals. Its anthelminthic activity is due to one of its active components, filicin which aids in the detachment of the scolex from the intestinal mucosa (Laudato and Capasso, 2013; Valentyna *et al.*, 2017).

Besides the worth of its leaves as vegetables, infusion of its leave is highly utilized used in the management of rheumatic disorders, treatment of topical wounds, abscesses, malaria, fever, menstrual bleeding, postpartum haemorrhage, gastrointestinal disorders especially diarrhea and low male sexual drive. It also serves as a natural intestinal cleanser and a revitalizer of normal liver function (Tagarelli *et al.*, 2010; Bafor *et al.*, 2017; Nwosu *et al.*, 2002).

In earlier studies have found that the leaf *D. filix-mas* possessed various biological activities including Insecticidal (Shukla and Tiwari, 2011), antihelmintic (Urban *et al.*, 2014), antimicrobial (Mandal and Mondal, 2011), anti-diarrheal (Uwumarongi *et al.*, 2016), antioxidant and cytotoxic (Sekendar *et al.*, 2012) and teratogenic (Erhirhie *et al.*, 2018) activities.

Although, medicinal plants are the most patronized form of alternative medicine, they are usually assumed to be natural and without deleterious effects (Uma *et al.*, 2013). This misconception does not guarantee their safety until they are subjected to scientific validations (Obi *et al.*, 2012). One of these approaches is when the long term and cumulative toxicity effects of medicinal plants are explored using animal species (Saganuwan, 2016; Nazari *et al.*, 2017).

In chronic toxicity test, the maximum tolerable dose (MTD) and its fractions are exposed to animals for a long duration of time, usually between six months and two years in rodents (Goodman *et al.*, 2006; Jacobs and Hatfield, 2012). The outcome of chronic toxicity test aids in safety criteria for human exposure during clinical trials of prospective agents used in the management of chronic diseases such as diabetes, hypertension and rheumatoid arthritis (Jaijoy *et al.*, 2010; Parasuraman, 2011).

Chronic toxicity study is necessary in establishing the "no-observed-adverse-effect-level" (NOAEL), the dose at which there is no obvious toxicity. This is also important for providing acceptable daily intake and setting exposure limit dose in humans for a particular duration (Chanda *et al.*, 2015).

Earlier studies have found that higher doses, 250 and 500 mg/kg of *D. filix mas* produced toxicities in 3 months repeated administration (Erhirhie and Ilodigwe, 2019).

In this present study, 6 months systemic chronic toxicity test was conducted on *D. filix mas* in other to establish its no-observed-adverse-effect-level (NOAEL) that should be established prior to clinical trials.

2. Materials and Methods

2.1. Chemicals and reagents

Ethanol (JHD, Guangdong GuanghuaSchi-Tech), Formaldehyde (May and Baker Ltd, Dagenham England), Biochemical reagents for lipid profile, hepatic and renal function assessment were procured from Randox Laboratories Limited, Country Atrium, United kingdom as well as Teco diagnostics, California U.S.A.

2.2. Experimental animals

Albino rats of the Wistar stain used in the study were acquired from University of Nigeria Nnsukka, Faculty of Veterinary Medicine. They were acclimatized in the animal facility, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu. During the study, they were given access to Palletized grower feed (Vital feed Ltd, Jos, Nigeria) and water *ad libitum* under 12:12 hours light and dark cycle. Animal handling procedure was in line with the National Institute of Health Guidelines for laboratory animals' use in experiments (Pub No. 85-23, revised 1985).

2.3. Plant collection and authentication and extraction

Leaves of *Dryopteris filix-mas* were obtained fresh from a swampy area beside Horticulture botanical garden Amawbia, Awka South L.G.A, Anambra State, Nigeria, in the month of March, 2016. Plant sample was authenticated by Dr. Akinnibosun H.A, from the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Nigeria, with voucher reference number "UBH$_d$285A". Extract was prepared using cold maceration method earlier reported (Erhirhie *et al.*, 2018).

2.4. Chronic toxicity test

2.4.1. Dosage selection

The maximum tolerable dose (MTD) obtained from the outcome of earlier reported 3 month sub-chronic

toxicity test result was chosen for this chronic toxicity test. Thus, MTD (125 mg/kg), 1/2 of MTD (62.5 mg/kg) and 1/4th of MTD (31.25 mg/kg) of sub-chronic toxicity test were selected for the chronic toxicity study.

2.4.2. Animal grouping and dosing

A total of 48 rats of either sex (66.15 ± 1.41 g body weight) were randomized into four groups of twelve animals each. Control group (A) received 10 mL/kg of distilled water while the test groups B-D received 31.25, 62.5 and 125 mg/kg of the leaf extract, respectively. Blood samples were collected from rats' retro-orbital plexus for determination of baseline hematological and biochemical parameters. Thereafter, animals were dosed daily for 6 months (180-days). Body weights were recorded during and after the administration period. At the end of 180 day (day 181st), blood samples were collected from retro-orbital plexus of animals for the determination of heamatological and biochemical parameters. Organs, liver, kidney, heart, spleen and lungs were isolated and weighed while liver and kidney were fixed in 10 % formal saline for histopathological analyses. Stomach of each animal was removed, cut open along the greater curvature, washed with tap water and observed for ulceration using standard method described by Moke *et al.*, (2015).

2.4.3. Recovery study

After 6 months of extract administration, animals were placed on feed and water *ad-libitum* for 4 weeks without extracts administration, to observe for reversibility in toxicity. At the end of the 4 weeks (day 29th), blood samples were collected for heamatological, biochemical and histopathology analyses of liver and kidney.Body weights, organs weights and presence of gastric lesion were also recorded using similar method above-mentioned.

2.5. Determination of haematological, biochemical and histological parameters

Blood samples collected into EDTA tube were subjected to hematological parameters analyses using automated haematology analyzer (Diatron Abacus 380, Hungary). Blood samples withdrawn into plain tubes were centrifuged at 3500 rpm for 10 minutes and serum were aspirated into separated tubes and were diluted 5-fold with normal saline for the determination of biochemical parameters; total cholesterol, triglyceride, high density lipoprotein cholesterol, total protein, albumin, alkaline phosphatase (ALP), alanine aminotransferases (ALT), aspartate transaminases (AST), sodium, potassium, chloride, urea and creatinine. ALT, AST, Triglyceride, Total cholesterol, and High density lipoprotein cholesterol (HDL-c) reagents were of the product of Randox Laboratories Limited, United kingdom, while Sodium, Chloride, Potassium, Urea, Creatinine, Total protein, Albumin and Alkaline phosphatase (ALP), were of the product Teco diagnostics, California U.S.A. Normal saline was added to reagent blank and the resulting absorbance of sample was multiplied by five. Biochemical parameters were analyzed using manufactures' reagent leaflets procedures. Absorbance of final reaction mixture was read using Spectrophotometer (721G, Zhejiang Top Cloud-Agri Technology Co., Ltd., China). Histological sections were prepared from organs fixed in 10% formal saline using the method described by Bancroft and Gamble (2002). Photomicrographs were presented as × 400 magnifications.

2.6. Statistical analysis

Data were analyzed with one way analyses of variance (ANOVA) statistical tool followed by post hoc dunnet's test using Statistical Package for Social Science (SPSS, version 20). Results were expressed as mean ± Standard error of mean (SEM), n = 5, and $P<0.05$ was established to be statistically significant.

3. Results and discussion

In this study we investigated 6 months chronic systemic toxicological effects of three doses, 31.25, 62.5 and 125 mg/kg of *Dryopteris filix* mas, a popular herbal remedy used in various disease conditions in Southern parts of Nigeria. The study was prompted by the need to establish the NOAEL of *D. filix –mas* following chronic uses.

3.1. Effects on heamatological parameters

Chronic administration of various doses of *D. filix-mass* extract did not produce significant alterations ($P>0.05$) heamatological indices, PCV, RBC, hemoglobin, platelet, WBC, Lymphocyte, Granulocyte, MID, PCT, MPV, MCV, MCH and MCHC levels of albino rats (Tables 1 and 2). Heamatological indices are useful parameters to assess the potential of xenobiotics

to alter red blood cell production or cause bone marrow toxicity (Uma *et al.*, 2013). From the outcome of the study, there were no significant differences in haematology parameters, suggesting that the extract constituents did not cause perturbation on red blood cell production as well as immune function at the tested doses and duration.

Table 1. Effects of chronic administration of extract on heamatological parameters, PCV, RBC, hemoglobin and PLAT.

	Group	PCV (%)	RBC (10^6/µL)	Hemoglobin (g/dL)	PLAT (10^3/uL)
Baseline	**A**	44.35 ± 0.83	7.10 ± 0.15	14.58 ± 0.34	856.00 ± 40.42
	B	42.56 ± 0.98	6.68 ± 0.23	14.90 ± 0.49	842.20 ± 38.91
	C	43.77 ± 0.59	6.55 ± 0.17	15.32 ± 0.47	806.80 ± 41.09
	D	43.66 ± 0.70	6.77 ± 0.11	14.58 ± 0.34	781.60 ± 35.05
Day 181st	**A**	40.29 ± 1.49	6.95 ± 0.46	13.87 ± 0.73	848.23 ± 70.82
	B	42.39 ± 0.57	7.01 ± 0.17	14.22 ± 0.52	831.81 ± 30.93
	C	41.68 ± 0.57	6.73 ± 0.08	13.69 ± 0.17	855.85 ± 36.47
	D	42.51 ± 0.57	6.95 ± 0.14	14.32 ± 0.19	907.73 ± 36.23
Recovery	**A**	42.64 ± 0.74	7.18 ± 0.18	13.40 ± 0.16	852.04 ± 52.91
	B	41.06 ± 0.66	6.66 ± 0.15	12.68 ± 0.16	704.00 ± 36.38
	C	40.30 ± 0.47	6.45 ± 0.10	12.32 ± 0.13	804.68 ± 67.74
	D	40.73 ± 2.21	6.69 ± 0.25	12.60 ± 0.62	880.36 ± 64.07
		WBC (103/µL)	**Lymp (%)**	**Gran (%)**	**MID (%)**
Baseline	**A**	6.24 ± 0.34	66.62 ± 0.48	20.74 ± 0.31	12.64 ± 0.71
	B	6.31 ± 0.41	67.22 ± 1.22	20.08 ± 1.30	12.70 ± 0.46
	C	6.08 ± 0.42	65.16 ± 0.61	21.10 ± 0.51	13.74 ± 0.57
	D	5.56 ± 0.16	64.92 ± 1.18	22.48 ± 0.95	12.60 ± 0.62
Day 181st	**A**	7.01 ± 0.28	65.18 ± 1.00	20.14 ± 1.33	14.68 ± 1.01
	B	6.34 ± 0.32	61.88 ± 0.74	22.78 ± 0.26	15.34 ± 0.67
	C	5.65 ± 0.21	64.02 ± 1.65	20.44 ± 1.21	15.55 ± 0.66
	D	6.05 ± 0.46	63.62 ± 1.45	22.08 ± 0.76	14.30 ± 0.82
Recovery	**A**	6.50 ± 0.50	67.12 ± 0.65	20.42 ± 0.55	12.46 ± 0.36
	B	6.10 ± 0.42	65.30 ± 1.37	21.82 ± 1.02	12.88 ± 0.67
	C	5.23 ± 0.64	64.68 ± 0.60	21.90 ± 0.60	13.42 ± 0.50
	D	6.02 ± 0.60	66.78 ± 1.71	19.72 ± 1.00	13.50 ± 1.10

Values are presented as mean ± Standard error of mean (n =5). P>0.05: Not significantly different from control group. PCV (packed cell volume), RBC (red blood cell) PLAT (platelet), WBC: White blood cell count, LYMP: Lymphocytes, Gran: Granulocytes, MID: medium size cell counts. **A** (control), **B** (31.25 mg/kg), **C** (62.5 mg/kg) **D** (125 mg/kg).

Table 2. Effects of chronic administration of extract on hematological parameters, PCT, MPV, MCV, MCH and MCHC.

	Group	PCT (%)	MPV (fL)	MCV (fL)	MCH (pg)	MCHC (g/dl)
Baseline	**A**	0.75 ± 0.02	8.60 ± 0.23	62.60 ± 0.51	20.96 ± 0.30	31.40 ± 0.50
	B	0.66 ± 0.02	8.40 ± 0.05	63.00 ± 1.14	20.90 ± 0.47	32.04 ± 0.60
	C	0.66 ± 0.03	8.34 ± 0.14	62.40 ± 0.93	20.96 ± 0.29	32.30 ± 0.61
	D	0.66 ± 0.03	8.52 ± 0.09	61.40 ± 0.51	20.52 ± 0.49	32.02 ± 0.38
Day 181st	**A**	0.72 ± 0.06	8.64 ± 0.17	62.99 ± 1.32	20.29 ± 0.50	31.91 ± 0.51
	B	0.64 ± 0.03	8.10 ± 0.15	62.25 ± 0.99	19.70 ± 0.34	31.26 ± 0.09
	C	0.66 ± 0.03	8.30 ± 0.13	64.23 ± 0.72	19.64 ± 0.22	31.38 ± 0.30
	D	0.73 ± 0.02	8.16 ± 0.13	63.24 ± 0.88	19.97 ± 0.26	31.33 ± 0.14
Recovery	**A**	0.67 ± 0.04	8.56 ± 0.11	59.52 ± 1.24	19.48 ± 0.46	31.44 ± 0.35
	B	0.55 ± 0.02	8.47 ± 0.22	61.44 ± 0.80	19.85 ± 0.27	30.94 ± 0.18
	C	0.62 ± 0.05	8.36 ± 0.06	62.40 ± 0.76	19.90 ± 0.25	30.56 ± 0.32
	D	0.68 ± 0.04	8.47 ± 0.10	60.48 ± 1.64	19.58 ± 0.39	30.92 ± 0.30

Values are presented as mean ± Standard error of mean (n =5). P>0.05: Not significantly different from control group. PCT (platelet percentage), MPV (mean platelet volume), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration). **A** (control), **B** (31.25 mg/kg), **C** (62.5 mg/kg) **D** (125 mg/kg).

3.2. Effects on lipid profile

Chronic administration of various doses of *D. filix-mass* extract did produce significant alterations (P>0.05) in total cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterollevels of albino rats (Table 3). Non-significant alteration in lipid profile parameters indicates that the extract may not alter lipid function at the tested doses and duration.

Table 3. Effects of chronic administration of extract on lipid profile.

	Group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
Baseline	**A**	147.37 ± 3.86	113.65 ± 2.42	50.10 ± 2.04	74.54 ± 3.41
	B	147.85 ± 1.90	112.46 ± 3.45	51.71 ± 2.47	73.64 ± 3.81
	C	139.76 ± 1.20	112.39 ± 2.66	48.35 ± 3.38	68.94 ± 3.98
	D	143.89 ± 3.61	111.02 ± 1.98	48.43 ± 4.44	73.26 ± 5.49
Day 181st	**A**	144.96 ± 3.14	113.30 ± 4.18	53.86 ± 0.92	68.44 ± 2.99
	B	144.66 ± 2.36	118.08 ± 2.22	55.90 ± 0.62	65.15 ± 2.45
	C	146.73 ± 3.29	114.73 ± 3.62	57.39 ± 2.19	66.39 ± 4.10
	D	144.66 ± 1.51	114.25 ± 3.50	55.76 ± 1.71	66.05 ± 2.42
Recovery	**A**	151.70 ± 3.50	106.26 ± 6.82	58.43 ± 2.33	72.02 ± 1.81
	B	148.80 ± 4.98	101.09 ± 3.06	56.58 ± 2.06	72.00 ± 3.55
	C	152.86 ± 2.13	109.24 ± 5.22	56.99 ± 0.70	74.02 ± 2.68
	D	151.70 ± 1.96	105.63 ± 5.69	56.17 ± 1.83	74.40 ± 1.09

Values are presented as mean ± Standard error of mean (n =5). P>0.05: Not significantly different from control group. HDL (High density liporprotein), LDL (Low density lipoprotein). **A** (control), **B** (31.25 mg/kg), **C** (62.5 mg/kg) **D** (125 mg/kg).

3.3. Effects on liver and kidney parameters

Chronic administration of various doses of *D. filix-mass* extract caused significant reduction (*P<0.05) in ALT and AST levels as well as significant (P<0.05) increase in urea and creatinine levels when compared with control group on the 181st day. However, these changes were reversible in recovery studies (Table 4).

Table 4. Effects of chronic administration of extract on liver enzymes and kidney parameters.

	Group	ALT (U/L)	AST (U/L)	ALP (IU/L)	Albumin (g/dl)	Total protein (g/dl)
Baseline	**A**	15.59 ± 0.57	37.51 ± 0.68	46.29 ± 1.37	3.06 ± 0.05	4.61 ± 0.22
	B	16.53 ± 0.49	34.18 ± 0.87	48.09 ± 1.49	3.04 ± 0.06	4.76 ± 0.32
	C	15.39 ± 0.40	36.85 ± 0.92	46.81 ± 1.68	3.03 ± 0.12	4.54 ± 0.58
	D	15.63 ± 0.28	35.27 ± 0.88	46.36 ± 1.87	3.03 ± 0.05	4.06 ± 0.04
Day 181st	**A**	17.10 ± 0.83	40.30 ± 2.71	46.95 ± 4.31	3.11 ± 0.04	4.73 ± 0.29
	B	14.72 ± 0.36*	33.70 ± 0.46*	51.43 ± 5.83	3.08 ± 0.04	5.33 ± 0.14
	C	14.90 ± 0.33*	33.00 ± 0.91*	49.69 ± 3.98	2.98 ± 0.18	5.18 ± 0.43
	D	14.03 ± 0.16*	31.10 ± 0.37*	46.76 ± 3.45	2.46 ± 0.62	4.99 ± 0.26
Recovery	**A**	15.82 ± 1.57	31.36 ± 3.54	47.82 ± 5.50	4.06 ± 0.10	5.24 ± 0.32
	B	16.71 ± 0.58	26.75 ± 2.45	46.17 ± 7.43	3.84 ± 0.11	5.40 ± 0.17
	C	15.86 ± 1.40	31.56 ± 1.67	46.45 ± 3.76	4.15 ± 0.12	5.24 ± 0.33
	D	16.53 ± 1.68	28.32 ± 3.39	40.81 ± 8.38	4.11 ± 0.14	5.43 ± 0.15

		Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)	Urea (mg/dl)	Creatinine (mg/dl)
Baseline	**A**	142.94 ± 2.46	4.04 ± 0.08	105.87 ± 2.07	20.19 ± 0.90	3.79 ±0.21
	B	145.00 ± 5.35	3.96 ± 0.09	102.00 ± 1.44	21.31 ± 0.77	3.73 ± 0.15
	C	140.25 ± 1.65	4.04 ± 0.17	102.97 ± 3.15	20.01 ± 0.29	3.43 ± 0.14
	D	143.43 ± 6.21	4.30 ± 0.14	104.04 ± 2.51	20.68 ± 0.51	3.47 ± 0.15
Day 181st	**A**	132.94 ± 1.63	3.72 ± 0.19	102.91 ± 2.00	18.90 ± 0.42	4.34 ± 0.16
	B	130.81 ± 1.04	4.60 ± 0.09	101.43 ± 2.18	24.00 ± 1.46*	4.86 ± 0.20
	C	131.68 ± 1.31	3.56 ± 0.39	103.71 ± 2.32	32.55 ± 1.39*	6.77 ± 0.49*
	D	131.37 ± 0.70	3.77 ± 0.47	102.44 ± 2.13	35.14 ± 0.95*	8.07 ± 0.39*
Recovery	**A**	126.87 ± 3.03	3.53 ± 0.11	94.61 ± 2.85	19.78 ± 0.18	4.27 ± 0.11
	B	132.40 ± 1.96	3.53 ± 0.11	101.39 ± 3.77	19.85 ± 0.25	4.34 ± 0.11
	C	128.94 ± 3.30	3.53 ± 0.09	98.37 ± 2.88	20.00 ± 0.20	4.16 ± 0.17
	D	131.02 ± 2.02	3.46 ± 0.11	97.06 ± 3.57	20.07 ± 0.31	4.43 ± 0.17

Values are presented as mean ± Standard error of mean (n =5). *P<0.05: Significantly different from control group. ALT (alanine aminotransferase), AST (aspertate aminotransferase), ALP (alkaline phosphatase). **A** (control), **B** (31.25 mg/kg), **C** (62.5 mg/kg) **D** (125 mg/kg).

3.4. Effects on liver and kidney histology

There was no distortion in liver architecture following the administration of 31.25, 62.5 and 125 mg/kg doses of extract to rats for a period of 6 months (Figure 1). However, there were dose dependent distortions in kidney architecture characterized by glomerulonephritis of the glomerulus and tubular necrosis of the renal tubules in all dose levels, 31.25, 62.5 and 125 mg/kg (Figure 2). Following withdrawal

of the various doses of extract from animals for 28 days, there was reversibility in distorted kidney architecture to normal (Figure 3).

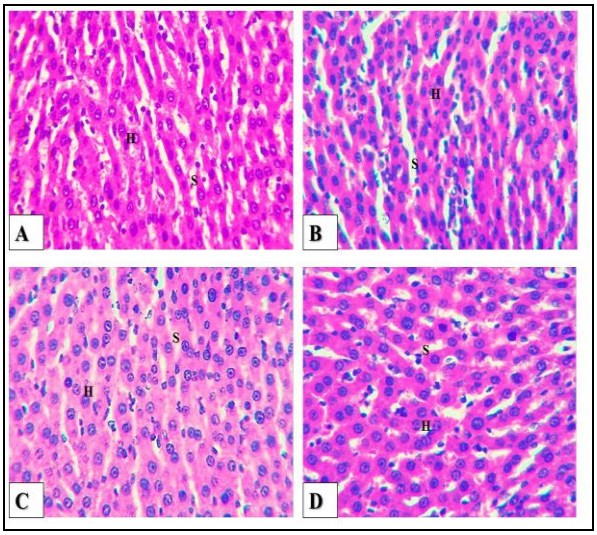

Figure 1. Chronic toxicity liver sections photomicrographs. H and E x 400. **Plate A** (Control), **Plate B** (31.25 mg/kg), **Plate C** (62.5 mg/kg), **Plate D** (125 mg/kg). **H:** Hepatocytes disposed in sheet. **S:** sinusoids. These are features of a normal liver histoarchitecture.

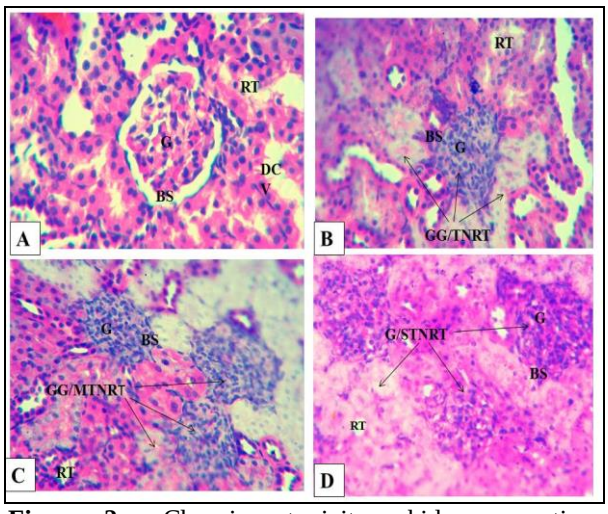

Figure 2. Chronic toxicity kidney sections photomicrographs. H and E x 400. **Plate A** (Control), **Plate B** (31.25 mg/kg), **Plate C** (62.5 mg/kg), **Plate D** (125 mg/kg). **G:** Glomeruli, **BS: B**owman's space, **RT:** Renal tubule. **GG/TNRT:** Glomerulonephritis of the glomerulus with tubular necrosis of the renal tubules. **GG/MTNRT:** Moderate glomerulonephritis of the glomerulus with moderate tubular necrosis of the renal tubules. **GG/STNRT:** Severe glomerulonephritis of the glomerulus with severe tubular necrosis of the renal tubules.

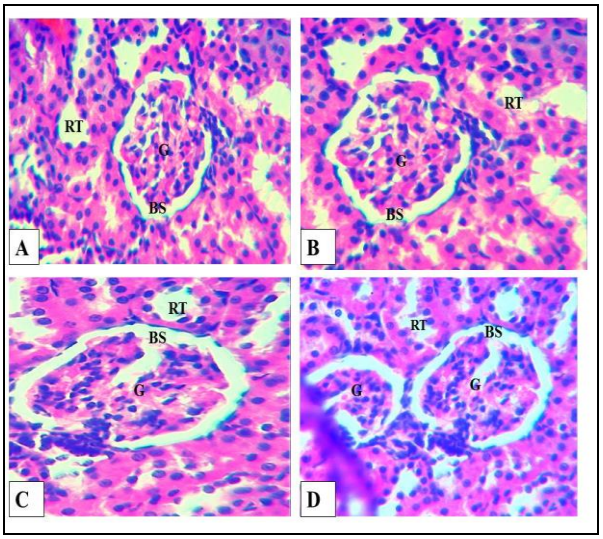

Figure 3. Chronic toxicity recovery's kidney photomicrographs. H and E x 400. **Plate A** (Control), **Plate B** (31.25 mg/kg), **Plate C** (62.5 mg/kg), **Plate D** (125 mg/kg). **G:** Glomeruli, **BS:** Bowman's space, **RT:** Renal tubule. These are features of a normal kidney histoarchitecture.

The liver and kidneys are major target organs of investigation following exposure of test animals to xenobiotics, including medicinal plants (Muhammad *et al.*, 2015). The liver serves in metabolizing foreign agent while the kidneys aid in eliminating of waste products as well as metabolites originating from the liver (Obidike and Salawu, 2013). This prompted the selection of the liver and kidneys as target organs in this study.

Studies on other medicinal plants for potential toxicities revealed that kidney toxicity manifests due to elevation in renal functions parameters, usually urea and creatinine (Chanda *et al.*, 2015). From this study, significant increase in urea and creatinine as well as kidney toxicity characterized by glomerulonephritis of the glomerulus with tubular necrosis of the renal tubules suggests that the extract could be nephrotoxic when used for a longer duration of 6 months at all the tested doses. This may be due to impairment of animals' kidney in eliminating bio-accumulated toxic metabolites of the *D. filix mas* metabolized by the liver. Absence of kidney toxicity as well as non-alterations in urea and creatinine levels of animals following stoppage of the extract for 4 weeks suggests that the toxicity posed by the extract was reversible.

Elevation in blood ALT and AST have been reported to occur due to cytoplasmic membrane damage, an indication of liver damage, which most times are reflected by histological alterations (Chanda *et al.*, 2015; Otunola and Afolayan, 2017). The observed significant (P<0.05) reduction in liver biomarkers, ALT and AST suggests that the extract was not toxic to the liver when used up to 125 mg/kg for 6 months. This is substantiated by normal liver architecture on day 181st. The liver has been reported to have high continuous regeneration and proliferative capacity with high resistant to toxicant substances when compared to the kidney (Kristine, 2010; Irina and Konstantin, 2017). This may account for the non-toxic effect of the extract on animals' liver.

3.5. Effects on body weight gain

Body weight gain of rats in control and extract treated groups were not significantly different (P>0.05) through the 1st and 6th month as well as in recovery study (Table 5). There was progressive body weight gain from the first month through the fifth and sixth months in control and treated groups, which suggests that water and feed intake were not adversely affected by the extract's phytoconstituents.

Table 5. Effects of chronic administration of extract on body weight.

Duration	Body weight gain (%)			
	A	B	C	D
1st month	51.54 ± 3.44	44.90 ± 2.37	47.29 ± 3.20	45.21 ± 2.78
2nd month	63.51 ± 2.05	57.27 ± 3.13	60.15 ± 2.14	64.63 ± 1.59
3rd month	68.55 ± 1.28	61.85 ± 2.79	62.92 ± 2.14	69.01 ± 1.17
4th month	70.24 ± 1.17	65.54 ± 2.44	66.51 ± 1.90	71.13 ± 1.12
5th month	73.22 ± 0.74	69.92 ± 1.74	70.04 ± 1.99	73.41 ± 1.08
6th month	73.74 ± 0.80	69.24 ± 1.41	70.77 ± 2.98	73.90 ± 1.28
Recovery	72.06 ± 1.78	66.14 ± 2.63	74.93 ± 2.14	73.99 ± 3.55

Values are presented as mean ± Standard error of mean (n =5). P>0.05: Not significantly different from control group. **A** (control), **B** (31.25 mg/kg), **C** (62.5 mg/kg) **D** (125 mg/kg).

3.6. Effects on relative organs weights

Similarly, no significant alteration (P>0.05) was observed in relative organs, liver, kidney, heart, lung, spleen weights of animals in control and extract treated groups (Table 6). This substantiated the body weight results, where weight gain was not adversely affected by the extract.

Table 6. Effects of chronic administration of extract on relative organs weights.

		Liver (%)	Kidney (%)	Heart (%)	Spleen (%)	Lung (%)
Day 91st	**A**	2.86 ± 0.06	0.44 ± 0.02	0.27 ± 0.01	0.23 ± 0.01	0.54 ± 0.03
	B	2.96 ± 0.08	0.54 ± 0.05	0.27 ± 0.01	0.22 ± 0.01	0.63 ± 0.04
	C	2.74 ± 0.08	0.54 ± 0.02	0.32 ± 0.04	0.30 ± 0.04	0.59 ± 0.02
	D	2.63 ± 0.12	0.54 ± 0.02	0.34 ± 0.01	0.31 ± 0.05	0.74 ± 0.09
Recovery	**A**	3.19 ± 0.22	0.55 ± 0.03	0.31 ± 0.01	0.30 ± 0.05	0.69 ± 0.04
	B	3.25 ± 0.19	0.57 ± 0.04	0.32 ± 0.02	0.30 ± 0.02	0.75 ± 0.08
	C	3.18 ± 0.17	0.53 ± 0.02	0.29 ± 0.01	0.25 ± 0.01	0.67 ± 0.03
	D	3.20 ± 0.15	0.54 ± 0.03	0.30 ± 0.01	0.23 ± 0.01	0.74 ± 0.05

Values are presented as mean ± Standard error of mean (n =5). P>0.05: Not statistically significantly different from control group. **A** (control), **B** (31.25 mg/kg), **C** (62.5 mg/kg) **D** (125 mg/kg).

3.7. Effects on stomach mucosa

From macroscopic observation, there was no ulceration recorded among the various groups of rats treated with extract for at the end of 6 months. Absence of lesion on the stomach mucosa of animals suggests that the extract did not have the potential to produce ulceration at the tested doses and duration.

The observed kidney toxicity could be attributed to secondary metabolites, saponins, tannins, flavonoids, alkaloids, cardiac glycosides present in the extract of *Dryopteris filix mas* as reported on this plant (Erhirhie *et al.*, 2018). Studies have shown that overuse of cardiac glycosides in medicinal plants could cause renal toxicity (Haden *et al.*, 2011; Chikezie *et al.*, 2015). This is also similar with most alkaloids which trigger nitrogen, urea and uric acid secretion, resulting in kidney toxicity (Olivoto *et al.*, 2017). Studies have revealed that excessive consumption of flavonoids (aside their beneficial effects) was associated with renal toxicity in humans and animals (Lee *et al.*, 2006; Hasanvand *et al.*, 2018). Study by Eweka and Enogieru

(2011) also revealed that long term intake flavonoids enriched medicinal plant could cause auto-oxidation of reactive oxygen species (ROS) liver kidney toxicities. Therefore, high level of flavonoid earlier found to be present in the leaf extract of *D. filix* mas (Erhirhie *et al.*, 2018) may be associated with the observed toxicities.

4. Conclusion

From this study, 6 months chronic administration of *Dryopteris filix mas* extract at 31.25, 62.5 and 125 mg/kg produced dose dependent selective renal toxicity in Wistar rats. Thus, its NOAEL is below 31.25 mg/kg following chronic utilization. This calls for its discouragement in the treatment of chronic diseases among users.

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