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Effect of nutriment from monoculture fermentation of Adasonia digitata seeds by Penicillium chrysogenum on haematology and functional endpoints of intra and extrahepatic tissues in rats

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ABSTRACT

This current study is to identify the most appropriate dose against intra and extrahepatic toxic agents as well as hematosuppressive agent in rats. The animals were divided into six groups (n = 10). Group 1 was placed on basal diet. Group II, III and IV were placed on a basal diets supplemented with 9.9%, 19.8% and 30%, respectively of MCF-ADSPC. Group V was placed on basal diet fed with unfermented *Adasonia digitata* seeds (UF-ADS) for 56 days. The results revealed 9.9% MCF-ADSPC as better dose to augment weight growth and total protein in rats. Similarly, MCF-ADSPC generated slight increase of WBC and centrophils count at lowest dose (9.9%) and highest dose (30%), respectively better than UF-ADS. The levels of PCV, RBC, haemoglobin, neutrophil, lymphocyte, eosinophils, basophils and monocytes showed no significant difference. Whereas, 19.8% MCF-ADSPC showed a better protection against hepatotoxicity, nephrotoxicity, intestinal mucosa, abnormal cholesterol level and splenomegaly in relation to 9.9%, 30% MCF-ADSPC and UF-ADS. Conclusively, MCF-ADSPC nutriment at 19.8% dose protected functional endpoints of intra and extrahepatic tissues than UF-ADS in a rat model.

1. Introduction

Nutrition containing high quantity of secondary metabolites and minerals have been reported to play a crucial role in delaying the development of cancer, cardiovascular diseases, and diabetes [1,2]. Intake of foods with low protein levels in mammals may exert quite number of adverse metabolic alterations in the liver and extra hepatic tissues [3,4]. A link between malnutrition, liver cirrhosis, dyslipidaemia, myocardial infarction, and hypertension has been demonstrated [5–7]. However, applying innumerable biotechnology techniques to optimize the quantity and quality of nutriment remain a laudable dietary therapy [5]. Among the conventional techniques is fermentation of foods or seeds to produce safer and better products for human consumption [7]. Additionally, the liver and kidney are predisposes to starvation in humans resulting from low in-take of nutritive foods [8].

The positive effects of fermentation have been ageless reported as product preservation, flavour development and reduction of anti-nutrients [9]. Also, it has been discovered that fermentation could enhance the nutrients, vitamins, essential amino acids and protein [10]. Several studies have suggested that fermentation is one of the primordial methods to improve seeds of legumes and cereals including sorghum and millets [11,12]. It was also reported that fermentation could decrease trypsin inhibitory activity (TIA), amylase inhibitor activity, phytic acid and tannins contents [13,14]. Recently, it was shown that cassava products are detoxified by fermentation by increasing protein and some amino acids including lysine, luecine, and methionine [15]. Experimentally, our laboratory reported that monoculture fermentation of *Moringa oleifera* seed by *Rhizopus stolonifer* could elevate haematological parameters [7]. Also, fermentation process could chelate transition metals [16], preserve food by enhancing its nutritional value [9] for medicinal purposes [12].

Adansonia digitata seed (ADS) has been utilized in the preparation of local condiments and a good coffee substitute [17]. It is an excellent source of pectins, calcium, vitamin C, iron, copper, magnesium, potassium, thiamine, niacin and carbohydrate [18]. Exactly, *A. digitata* is used as antioxidant, which prevents oxidative stress and related diseases

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including cancer, aging, inflammation and cardiovascular diseases [19]. Further studies also reported that bioactive compounds of the seed possess countless medicinal properties including analgesic, anti-inflammatory, antimicrobial and antipyretic activities among people infected with malaria parasites [20,21]. In the same vein, blanching of ADS may impact on the phenolic composition, antioxidant activity, and glucose hydrolytic enzymes [22]. Currently, it is interesting to hypothesize that monoculture fermentation of plant seeds by fungi is novel and may be used as food therapy. Hence, this study aimed at identifying the most effective dose of monoculture fermentation of AD seeds by *Penicillium chrysogenum* (MCF-ADSPC) on haematological parameters, hepatorenal function and endpoints of cholesterol in rat.

2. Materials and methods

2.1. Sample selection and preparation

The fresh seeds of AD collected from the premises of Nigerian Stored Products Research Institute (NSPRI) Kano, Nigeria and authenticated from the Department of Biology, University of Ilorin with the voucher number-UIH 1048. The seeds were dried at 60 °C in the oven to maintain the moisture content. Thereafter, seeds were powdered with an electrical grinder to particle size of about 2 mm and stored in air tight container for further use.

2.2. Quantification of compounds by HPLC-DAD

Phenolic compounds by HPLC-DAD reverse phase chromatographic analyses were carried out under gradient conditions [23].

2.3. Natural fermentation of ***Adasonia digitata*** powder

The fermentation of the powdered *Adasonia digitata* seeds was done according to the method described by Kayode and Sani [24]. Briefly, 5 g of powdered seeds was added to 5 ml of sterile distilled water. The mixture was stirred properly for a uniform mash to be obtained. The fermenter was covered and the content was allowed to ferment at room temperature (28 ± 2 °C) for seven (7) days. Also, the anti-nutrients for both fermented and non-fermented AD seeds were determined. Similarly, amino acid analyzer was used to determine the essential and non-essential amino acids.

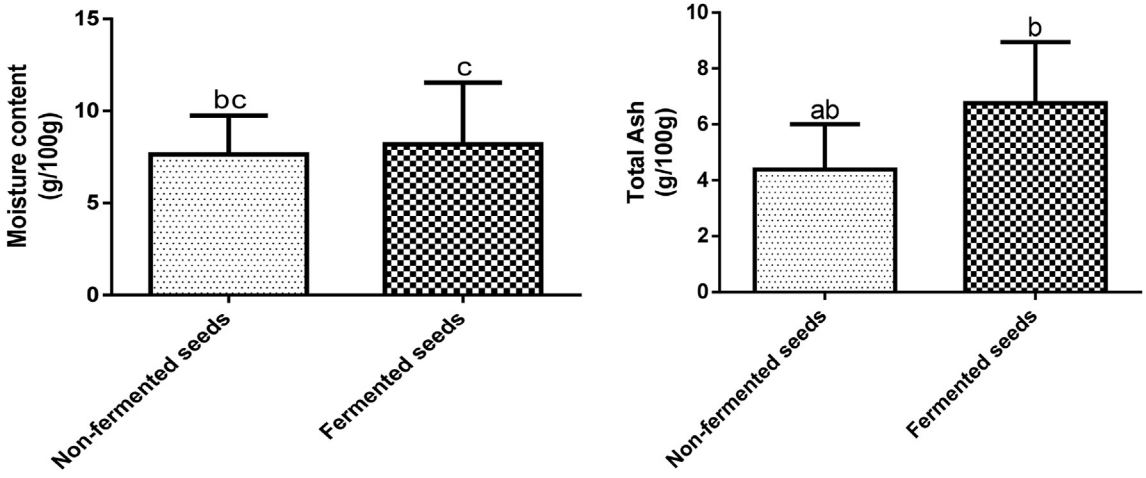


Fig. 1. Effect of monoculture fungal fermentation of *Adasonia digitata* seed by *Penicillium chrysogenum* on moisture content and total ash (g/100 g) for both non-fermented seeds and fermented seeds. Bars with different letters on the same column are statistically different ($p < 0.05$). The results are expressed as mean ± SD of three determinations.

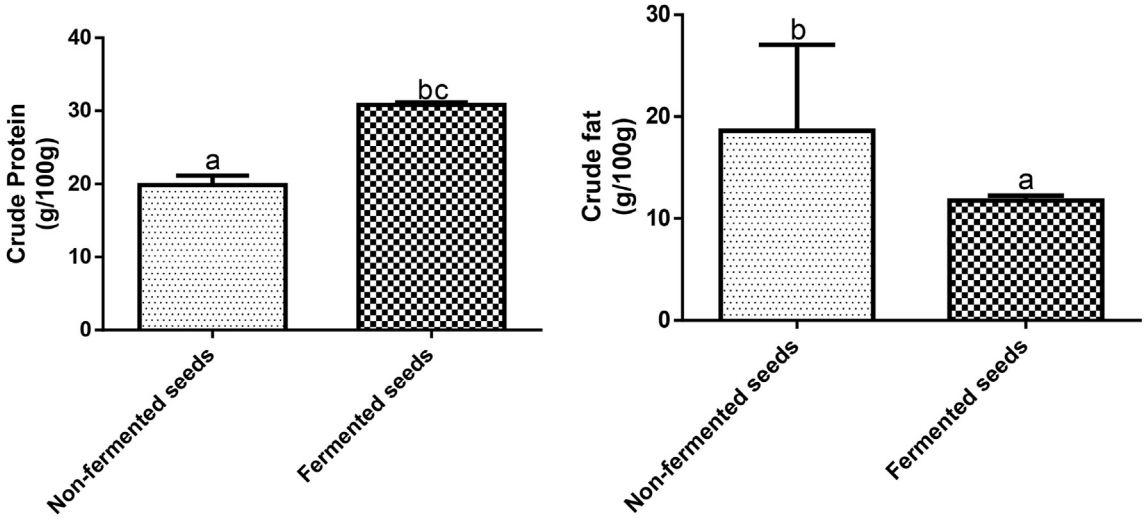


Fig. 2. Effect of monoculture fungal fermentation of *Adasonia digitata* seed by *Penicillium chrysogenum* on crude protein and crude fat (g/100 g) for both non-fermented seeds and fermented seeds. Bars with different letters on the same column are statistically different ($p < 0.05$). The results are expressed as mean ± SD of three determinations.

2.4. Isolation of fungal organisms from naturally fermented Adasonia digitata seeds

Fungi were isolated from the naturally fermented seeds through serial dilution and pour plate methods using Potato Dextrose Agar (PDA) into which 10% Streptomycin solution was added to inhibit bacteria growth. Culturing was done in duplicates. The plates were incubated at room temperature (28 ± 2 °C) for 48 h. Isolated visible fungal colonies were further sub-cultured to obtain pure cultures. Morphological and microscopic analysis to identify the isolates were carried out and compared with literature [25]. The pure isolates were maintained on agar slant and kept in the refrigerator at 4 °C for further use.

2.5. Macroscopic characterization of fungi isolates from Adasonia digitata seeds

The plates were examined macroscopically for morphological characteristics. Characteristics considered were colour of the colony, production and release of pigment into the medium, rate of growth (fast or slow) during incubation, diameter of colony, spreading of the colony and the mycelia. Characteristics observed were in line with literature for the identification of the isolates [25].

2.6. Microscopic characterization of fungi isolates from Adasonia digitata seeds

The isolates were observed in wet mount preparation under X10 and under X40 objective lens. They were observed before staining with cotton-blue in Lactophenol and observed again under X10 and under X40 objective lens. Indexes of observation were hyphae length, type (sporangiophore or conidiophores), breath, size, colour, presence or absence of septa, rising or spreading, branching type, spore shape, and texture of the cell wall (smooth or rough). The observations were identical with literature [25].

2.7. Monoculture fermentation of Adasonia digitata seeds by Penicillium chrysogenum (MCF-ADSPC)

Penicillium chrysogenum spore suspension of actively growing mid log phase culture was prepared according to the method described by Sani et al. [26]. Briefly, an agar slant of four-day-old pure culture of *Penicillium chrysogenum* was used. About 10 ml sterile distilled water was added to the slant and shaken to wash the spores. The spore suspension was counted using the Neubauer counting chamber. A spore suspension of about 5×10^4 spore suspension/mL was used for inoculation. Exactly, 20 g of powdered *Adasonia digitata* seed sample was added into 250 ml Ehlenmeyer flask, plugged with cotton wool, wrapped with aluminium foil and sterilized in the autoclave at 121 °C for 15 min. The sterile sample was mixed with 20 ml of sterile distilled water and stirred properly until uniform mashes were obtained. Thereafter, 2 ml of *Penicillium chrysogenum* monoculture suspension was used as fermentation starter to inoculate *Adasonia digitata* sample in the fermenter. The mixture was allowed to ferment for 72 h at 28 ± 2 °C [24,27]. Fermented sample was dried at 60 °C in the oven for 4 h. The residue was used for diet formulation and the proximate composition was analyzed. The time point of 72 h was selected because fermentation process using monoculture fungi is completely done within 3 days. This was supported by our previous study [24]. Using fermented product before 72 h suggests the fermentation process is still ongoing while the use of fermented product after 72 h will not cause a significant change in proximate composition because significant reaction may have been stopped.

2.8. Determination of proximate analysis and anti-nutrients

Proximate analysis of the MCF-ADSPC and UF-ADS such as total ash, moisture content, crude fiber, crude protein, crude fat and carbohydrate were analyzed [28]. Specifically, total saponins of the MCF-ADSPC and UF-ADS was determined according to the method of Makkar et al. [29]. Oxalate content was evaluated using a method reported by Wuoha and Kalu [30]. Tannin content was quantified according to the method described by Padmaja [31]. Afterward, phytate content was examined following the procedure of Lorenz et al. [32].

2.9. Determination of essential amino acids (EAAs) and non-essential amino acids (NEAAs)

Total essential amino acids (TEAAS) and non-essential amino acids (NEAAS) of the MCF-ADSPC and UF-ADS were determined according to the method of Dai et al. [33].

2.10. Diet formulation

Supplemented diets having three doses (9.9%, 19.8% and 30%) of mono-culture fermentation of *Adasonia digitata* seeds by *Penicillium chrysogenum* (MCF-ADSPC) for 72 h were formulated according to a modified method of Yussuf et al. [34] (Table 3) . Soya bean was used as the source of protein. This was prepared by adding 30%, 20.1%, and 10.2% of soya bean meal to obtain 100% formulation (Table 3). Thereafter, the proximate quantity of the diet used to feed the animals in each

Table 1
Effect of monoculture fungal fermentation of *Adasonia digitata* seed by *Penicillium chrysogenum* on proximate composition (g/100 g) between 0 and 120 h.

Fermentation Period (hours)	MC	TA	CP	CF	CFB	CHO
0	7.64 ± 2.11^{a}	4.39 ± 1.61^{a}	19.86 ± 1.26^{a}	18.6 ± 8.46^{b}	8.87 ± 5.31^{b}	39.50 ± 4.88^{b}
24	8.02 ± 2.94^{a}	5.43 ± 1.94^{ab}	30.52 ± 3.68^{b}	12.84 ± 0.35^{a}	3.81 ± 2.16^{a}	39.45 ± 6.68^{b}
48	8.22 ± 3.20^{a}	6.64 ± 1.75^{b}	30.37 ± 3.45^{b}	12.59 ± 0.87^{a}	3.84 ± 2.11^{a}	38.35 ± 7.16^{b}
72	8.20 ± 3.33^{a}	6.75 ± 2.02^{b}	30.83 ± 3.42^{b}	11.78 ± 0.46^{a}	3.74 ± 1.90^{a}	38.76 ± 7.40^{b}
96	8.32 ± 3.22^{a}	6.65 ± 2.14^{b}	31.23 ± 2.68^{b}	11.16 ± 1.22^{a}	3.43 ± 1.47^{a}	44.73 ± 0.02^{c}
120	10.61 ± 0.00^{a}	8.14 ± 0.00^{c}	32.93 ± 0.00^{b}	12.06 ± 0.00^{a}	2.37 ± 0.00^{a}	33.29 ± 0.00^{a}

MC = moisture content, CP = crude protein, CF = crude fat, CFB = crude fibre, TA = total ash, CHO = carbohydrate. Bars with different letters on the same column are statistically different ($p < 0.05$). The results are expressed as mean ± SD of three determinations.

Table 2
Effect of monoculture fungal fermentation of *Adasonia digitata* seed by *Penicillium chrysogenum* on proximate composition (g/100 g) at 72 h.

Phytochemical	Non-fermented (mg/g)	Fermented (mg/g)
MC	7.64 ± 2.11^{bc}	8.20 ± 3.34^{c}
TA	4.39 ± 1.61^{ab}	6.75 ± 2.20^{b}
CP	19.86 ± 1.30^{a}	30.83 ± 0.34^{bc}
CF	18.60 ± 8.46^{b}	11.77 ± 0.46^{a}
CFB	8.87 ± 5.31^{b}	3.74 ± 1.90^{a}
CHO	40.01 ± 1.35^{b}	38.76 ± 7.40^{a}

Bars with different letters on the same row are statistically different ($p < 0.05$). MC = moisture content, CP = crude protein, CF = crude fat, CFB = crude fibre, TA = total ash. Bars with different letters on the same column are statistically different ($p < 0.05$). The results are expressed as mean ± SD of three determinations.

Table 3
Diet formulation for basal and supplemented diets for control and test groups.

Treatment	Group 1	Group 2	Group 3	Group 4	Group 5
Corn meal	50.6	50.6	50.6	50.6	50.6
Cellulose	3.0	3.0	3.0	3.0	3.0
Sucrose	8.0	8.0	8.0	8.0	8.0
Premix	3.0	3.0	3.0	3.0	3.0
D-methionine	0.4	0.4	0.4	0.4	0.4
Oil	5.0	5.0	5.0	5.0	5.0
Soya bean meal	30.0	20.1	10.2		
MCF-ADSPC	–	9.9	19.8	30.0	–
UF-ADS		–	–		30.0
Total (g)	100	100	100	100	100

MCF-ADSPC: Monoculture fermentation of *Adansonia digitata* seed base diet by *Penicillium chrysogenum*; UF-ADS: Unfermented *Adansonia digitata* seed based diet.

Note: The vitamin premix (mg or IU/g) has the following composition; 3200 IU vitamin A, 600 IU vitamin D3, 2.8 mg vitamin E, 0.6 mg vitamin K3, 0.8 mg vitamin B1, 1 mg vitamin B2, 6 mg niacin, 2.2 mg pantothenic acid, 0.8 mg vitamin B6, 0.004 mg vitamin B12, 0.2 mg folic acid, 0.1 mg biotin H2, 70 mg choline chloride, 0.08 mg cobalt, 1.2 mg copper, 0.4 mg iodine, 8.4 mg iron, 16 mg manganese, 0.08 mg selenium, 12.4 mg zinc, 0.5 mg antioxidant. Group 1: serve as the control group placed on a basal diet; Group 2: serve as the group placed on a basal diet supplemented with 9.9% of mono-culture fermented pulverized seeds of *Adansonia digitata* Group 3: serve as the group placed on a basal diet supplemented with 19.8% of mono-culture fermented pulverized seeds of *Adansonia digitata*; Group 4: serve as the group placed on a basal diet supplemented with 30% of mono-culture fermented pulverized seeds of *Adansonia digitata*; Group 5: (Negative control) serve as the normal group placed on a basal diet supplemented with 30% of unfermented pulverized seeds of *Adansonia digitata*.

group was measured as shown in Table 3. The unfermented *Adasonia digitata* seed was designated as UF-ADS.

2.11. Animal handling

Wistar rats (41.3 ± 2.5 g) from the Central Animal House of the University of Ilorin, were used in this experiment. The animals were maintained at a constant temperature (22 ± 2 °C) on a 12 h light/dark cycle with free access to food and water.

Table 4
Effect of monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* on anti-nutrients.

Phytochemical	Non-fermented (mg/g)	Fermented (mg/g)
Saponin	6.80	1.2595
Oxalate	4.86	1.9505
Tannins	1.92	0.2605
Phytate	1.82	0.4950

2.12. Experimental protocol

The rats were acclimatized for two weeks and randomly divided into five groups (n = 10). Care and Use of Laboratory Animals prepared by the National Academy of Science. The guidelines were followed to ensure the protection of the animals' wellbeing during the experiment. The experiment was approved by Departmental examination committee, with number of the researcher- 91/028133. Group 1 serve as the control placed on basal diet; Group 2 was placed on basal diet supplemented with 9.9% MCF-ADSPC. Group 3 was placed on basal diet supplemented with 19.8% MCF-ADSPC. Group 4 was placed on basal diet supplemented with 30% MCF-ADSPC. Group 5 (Negative control) was placed on basal diet supplemented with 30% UF-ADS. The treatment of the animals with supplement lasted for 56 days (sub-chronic exposure). The doses were selected following the rationale of Yussuf et al., [34]. The animals were fasted overnight, weighed and euthanized 24 h after the last treatment and blood was collected by cardiac puncture. The blood was allowed to clot and centrifuged at low speed (3000 g) at room temperature for 15 min. The supernatant (serum) was removed and used for the determination of biochemical parameters.

2.13. Chemicals and reagents

All the kits used for the bioassay were gotten from Randox Laboratories Ltd. (Crumlin, Dublin, Northern Ireland, UK). All other reagents used were of analytical grade.

2.14. Haematological estimation

Haemoglobin concentration (Hb %) was measured by Drabkin's

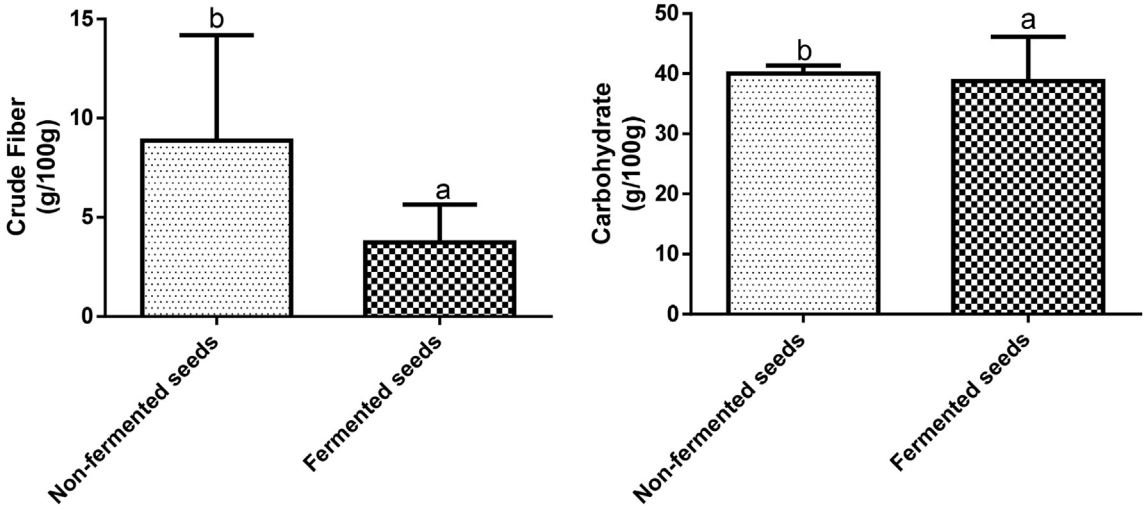


Fig. 3. Effect of monoculture fungal fermentation of *Adasonia digitata* seed by *Penicillium chrysogenum* on crude fibre and carbohydrate (g/100 g) for both non-fermented seeds and fermented seeds. Bars with different letters on the same column are statistically different ($p < 0.05$). The results are expressed as mean ± SD of three determinations.

Table 5
Effect of monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* on essential amino acids composition as compared to dietary reference intake (DRI).

Element	RDI (g)	UF-ADS (g)	MCF-ADPC (g)
Isoleucine	3.20	3.77	3.77
Leucine	7.00	8.55	8.24
Lysine	6.50	7.69	8.22
Methionine	3.20	1.29	2.75
Phenylalanine	6.00	5.52	5.11
Valine	4.10	5.18	5.87
Histidine	2.30	2.86	3.72
Threonine	3.50	3.54	2.98
Arginine	6.10	6.28	5.58
TEAAS	41.90	44.60	46.20

RDI; Recommended dietary intake; MCF-ADPC: Monoculture fermented pulverized seeds of *Adansonia digitatai* by *Penicillium chrysogenum*; UF-ADS: Unfermented *Adansonia digitata* seed. TEAAS: Total essential amino acids.

Table 6
Effect of monoculture fungal fermented *Adansonia digitata* seed by *Penicillium chrysogenum* on non-essential amino acids composition (g).

Element	UF-ADS (g)	MCF-ADPC (g)
Glycine	3.29	3.19
Alanine	5.82	5.13
Serine	4.96	4.76
Proline	4.46	4.11
Aspartate	10.72	8.46
Glutamate	16.14	16.21
Cysteine	8.17	5.49
TNEAAS	53.56	47.35

MCF-ADPC: Mono-culture fermented pulverized seeds of *Adansonia digitata* by *Penicillium chrysogenum*; UF-ADS: Unfermented *Adansonia digitata* seed. TNEAAS: Total non-essential amino acids.

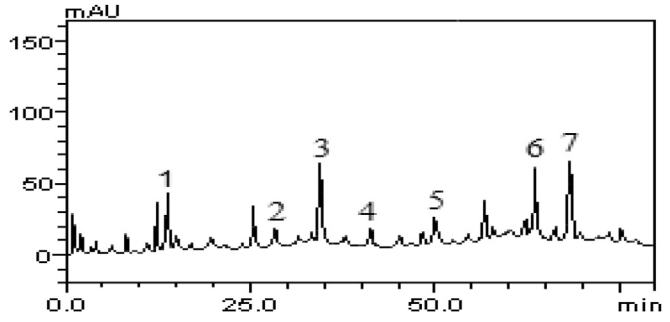


Fig. 4. Representative high performance liquid chromatography profile of black seed aqueous extract. Gallic acid (peak 1), caffeic acid (peak 2), *p*-coumaric acid (peak 3), Rutin (peak 4), quercetin (peak 5), luteolin (peak 6) and apigenin (peak 7).

colorimetric method (cyanomethemolgobin formation) and packed cell volume (PCV) by scale of microhameatocrit reader [35]. The concentration of red blood cells (RBC) and white blood cells (WBC) were measured and expressed as volume of cells [36]. Additionally, neutrophils, lymphocytes, monocytes, eosinophils, basophils and centrophils were determined using conventional method [37].

2.15. Determination of HDL, LDL and cholesterol

Some lipid markers; high density lipoprotein (HDL), low density lipoprotein (LDL) and cholesterol were measured using commercially available kits (Randox Laboratories Kits, St Louis, MO, USA) according to the manufacturer's guideline.

Table 7
Composition of *Adansonia digitata* aqueous extract.

Compounds	A. digitata (mg/g)	LOD (μg/mL)	LOQ (μg/mL)
Gallic acid	4.17 ± 0.01a	0.021	0.068
Caffeic acid	1.45 ± 0.02 b	0.018	0.059
P-coumaric acid	6.28 ± 0.01c	0.009	0.030
Rutin	1.47 ± 0.01 b	0.025	0.082
Quercetin	2.06 ± 0.04 d	0.009	0.030
Luteolin	6.21 ± 0.02c	0.027	0.089
Apigenin	6.30 ± 0.03c	0.015	0.049

Note: The results are expressed as mean ± SD of three determinations. Comparing various groups, different letters indicate statistically significant findings. i.e. $p < 0.05^a$, $p < 0.05^b$, $p < 0.05^c$ and $p < 0.05^d$ are significantly different.

Table 8
Effect of monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* on the weight of treated rat.

Parameters (g)	Animal treatment				
	Group 1	Group2	Group 3	Group 4	Group 5
Initial weight	$41.3 \pm 2.5_b$	$40.5 \pm 0.5_{ab}$	$40.5 \pm 2.5_{ab}$	$40.5 \pm 0.5_{ab}$	$42.5 \pm 2.5_b$
Final weight	$90.8 \pm 3.5_b$	$101.1 \pm 4.7_b$	$99.8 \pm 1.4_a$	$98.5 \pm 1.5_{ab}$	$78.4 \pm 0.5_a$
Weight gain	$49.5.0 \pm 1.0_b$	$60.6 \pm 4.2_c$	$59.3 \pm 1.1_c$	$58.0 \pm 2.0_c$	$35.9 \pm 3.0_a$
Daily feed intake	$26.3 \pm 2.0_a$	$33.9 \pm 0.3_a$	$30.5 \pm 4.7_a$	$27.8 \pm 2.9_a$	$24.7 \pm 1.7_a$
Daily feed retention	$21.2 \pm 0.6_a$	27.3 ± 0.7^b	$22.6 \pm 6.3_a$	$20.4 \pm 3.2_a$	$18.2 \pm 2.7_a$
Protein retention	25.0	23.9	24.8	23.7	13.9
Mortality	0	0	0	0	0

Data are presented as mean ± SD (n = 10). Rows with different letters are statistically significant; p < 0.05.i.e. $p < 0.05^a$, $p < 0.05^b$ and $p < 0.05^{ab}$ are significantly different.

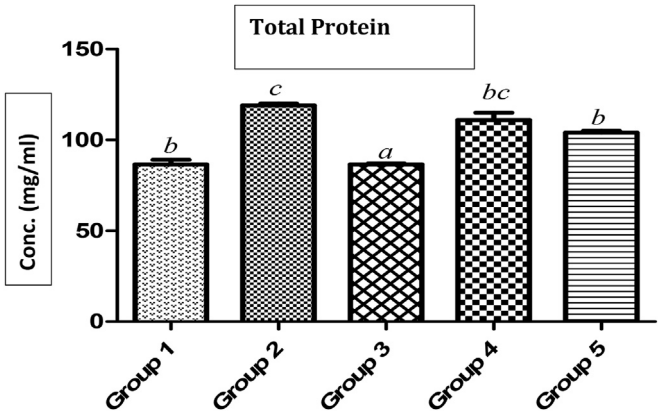


Fig. 5. Effect of monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* on total protein level in rat. Data are presented as mean ± SD (n = 10). Bars with different letters are statistically different. i.e. $p < 0.205^a$, $p < 0.05^b$, $p < 0.05^c$ and $p < 0.05^{bc}$ are significantly different.

2.16. Estimation of globulin and albumin levels-liver function

Serum globulin and albumin were quantified using the method of [38].

Table 9
Effect of monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* on haematological parameters in treated rat.

Nutriment group	PCV (%)	CENTROPHIL	WBC ($X10^9$/L)	RBC ($X10^{12}$/L	Hb (g/dL)	NEUTROPHIL (%)	LYMPHOCYTE (%)
Group 1	32.5 ± 0.5^{a}	17.5 ± 1.5^{b}	80.5 ± 0.5^{b}	23.5 ± 0.5^{a}	6.4 ± 0.1^{a}	65.5 ± 0.5^{a}	34.5 ± 0.5^{a}
Group 2	$34.0 \pm 5.0_{a}$	$11.5 \pm 3.5_{ab}$	$75.0 \pm 0.0_{ab}$	$24.0 \pm 5.0_{a}$	$6.8 \pm 1.0_{a}$	$60.5 \pm 2.5_{a}$	$38.5 \pm 2.5_{a}$
Group 3	$35.5 \pm 0.5_{a}$	$7.5 \pm 1.5_{ab}$	$70.5 \pm 0.5_{ab}$	$24.0 \pm 1.0_{a}$	$7.1 \pm 0.15_{a}$	$66.5 \pm 1.5_{a}$	$33.0 \pm 2.0_{a}$
Group 4	$36.0 \pm 9.0_{a}$	$15.5 \pm 1.5_{ab}$	$70.0 \pm 1.0_{ab}$	$24.5 \pm 4.5_{a}$	$7.2 \pm 1.95_{a}$	$64.5 \pm 3.5_{a}$	$33.5 \pm 1.5_{a}$
Group 5	$34.5 \pm 2.5_{a}$	$6.0 \pm 4.0_{a}$	$59.5 \pm 0.5_{a}$	$25.5 \pm 4.5_{a}$	$8.2 \pm 0.75_{a}$	$62.5 \pm 8.5_{a}$	$37.0 \pm 9.0_{a}$

Data are presented as mean ± SEM (n = 10). Bars with different letters are statistically different. i.e. $p < 0.05^{a}$, $p < 0.05^{b}$ and $p < 0.05^{ab}$ are significantly different.

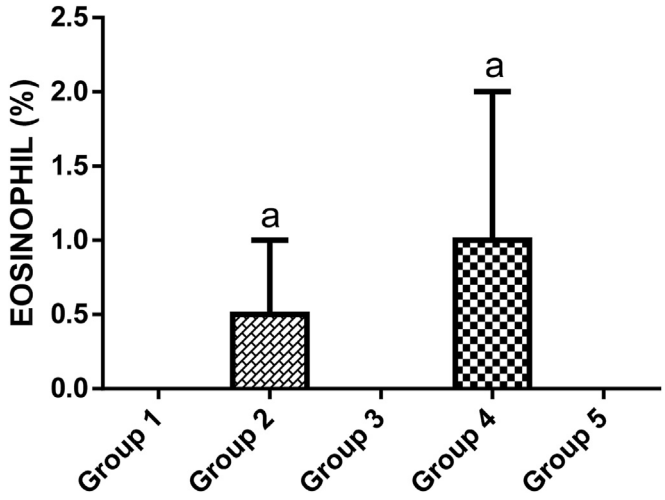


Fig. 6. Effect of monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* on eosinophil in treated rat. Data are presented as mean ± SD (n = 10). Bars with different letters are significantly different.

2.17. Estimation of urea and creatinine levels-kidney function

Serum blood urea nitrogen (BUN) was measured by the method of Kanter [39]. Serum creatinine was evaluated by the method of Hare [40].

2.18. Histological examination

Liver, kidney, small intestine and spleen specimen were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned. After deparaffinization and dehydration, the paraffin blocks were stained with haematoxylin and eosin for microscopic examination.

2.19. Statistical analysis

The data in each group were expressed as mean ± standard deviation. A one-way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple test was used for the post hoc (Zar, [41]. Statistical package for Social Science (SPSS) 17.0 for windows was used for the analysis and the least significance difference (LSD) was accepted at $P < 0.05$.

3. Results

3.1. Effect of monoculture fungal fermentation of Adasonia digitata seed by Penicillium chrysogenum on proximate composition

Aside crude fat, crude fibre and carbohydrate, the proximate composition including total ash and crude protein were significantly ($p < 0.05$) higher at the various hours of fermentation by *Penicillium chrysogenum* (Figs. 1 and 2). Crude fat and crude fibre were significantly ($p < 0.05$) depleted at different hours of fermentation while, moisture content was not increase for zero hour (Table 1) . Specifically, at 72 h of fermentation by *Penicillium chrysogenum*, moisture content, total ash and crude protein in MCF-ADSPC were remarkably ($p < 0.05$) higher than the UF-ADS (Table 2). Crude fat, crude fibre and carbohydrate in MCF-ADSPC were significantly ($p < 0.05$) lower than the UF-ADS (Figs. 1, 2 and 3).

3.2. Effect of monoculture fungal fermentation of Adansonia digitata seed (ADS) by Penicillium chrysogenum on anti-nutrient levels, essential and non-essential amino acids

As represented in Table 4, the fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* at 72 h significantly ($p < 0.05$) depressed the anti-nutrients including saponin, oxalate, tannin and phytate when compared with non-fermented ADS. Table 5 shows the effect of monoculture fungal fermentation of ADS by *Penicillium chrysogenum* on essential amino acid contents. With the exclusion of methionine and phenylalanine, the essential amino acids such as isoleucine, leucine, lysine, valine and histidine were greater in MCF-ADSPC and UF-ADS than recommended dietary intake (RDI). Absolutely, the total essential amino acid (TEAAS) was higher in MCF-ADSPC (46.20 g/100 mg), UF-ADS (44.60 g/100 mg) than RDI (41.90 g/100 mg) by 4% and 9%, respectively (Table 5). Non-essential amino acids particularly glycine, alanine, serine, proline, aspartate, glutamate and cysteine were down-regulated in MCF-ADSPC in relation to UF-ADS (Table 6). The total non-essential amino acid (TNEAAS) was reduced in MCF-ADSPC than UF-ADS by 12% (Table 6).

3.3. Phenolic composition of Adansonia digitata seed (ADS)

HPLC-DAD was used to determine the phenolic composition of *Adansonia digitata* seed. The chromatogram of the extract is presented in Fig. 4. The phenolic constituents of the extract were related to the retention time and mass spectra of single compound with the reference standard. The identified compounds were gallic, caffeic and *p*-coumaric acids, rutin, quercetin, luteolin, and apigenin. Moreover, Table 7 revealed high levels of gallic acid (4.17 mg/g), *p*-coumaric acid (6.28 mg/g), luteolin (6.21 mg/g) and apigenin (6.30 mg/g) when compared to caffeic acid (1.45 mg/g), rutin (1.47 mg/g) and quercetin (2.06 mg/g).

3.4. Effect of monoculture fungal fermentation of Adansonia digitata seed (ADS) by Penicillium chrysogenum on body weight growth and protein in rat

As depicts in Table 8, upon treatment with MCF-ADSPC (9.9%, 19.8% and 30%), there was a significant ($p < 0.05$) increase in the weight gain by 69%, 65% and 62%, respectively in relation to UF-ADS. All the treated animals showed no mortality while 9.9% of MCF-ADSPC exhibited highest weight growth rate (Table 8). Also, protein was reserved better in rats treated with 9.9%, 19.8% and 30% MCF-ADSPC than UF-ADS by 72%, 78% and 71%, respectively. Interestingly, the animals treated with

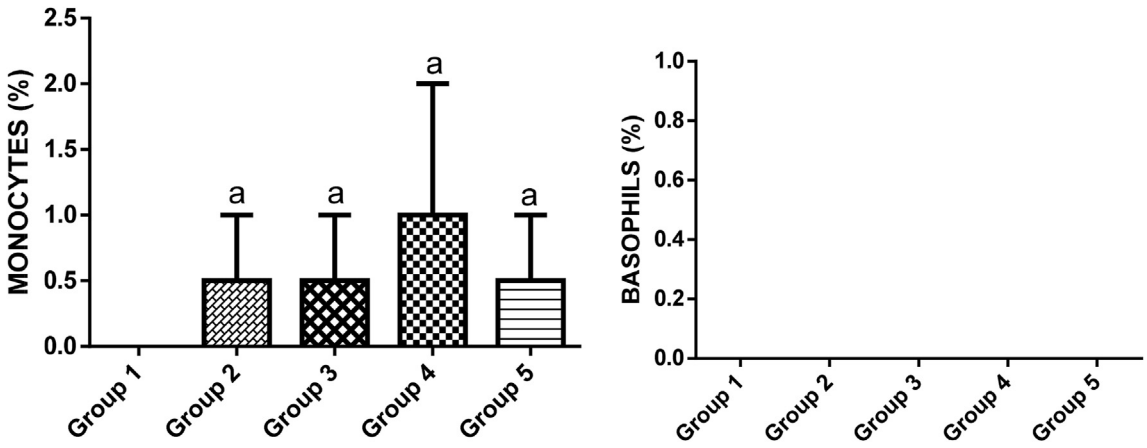


Fig. 7. Effect of monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* on monocytes and basophils in treated rat. Data are presented as mean ± SD (n = 10). Bars with different letters are significantly different.

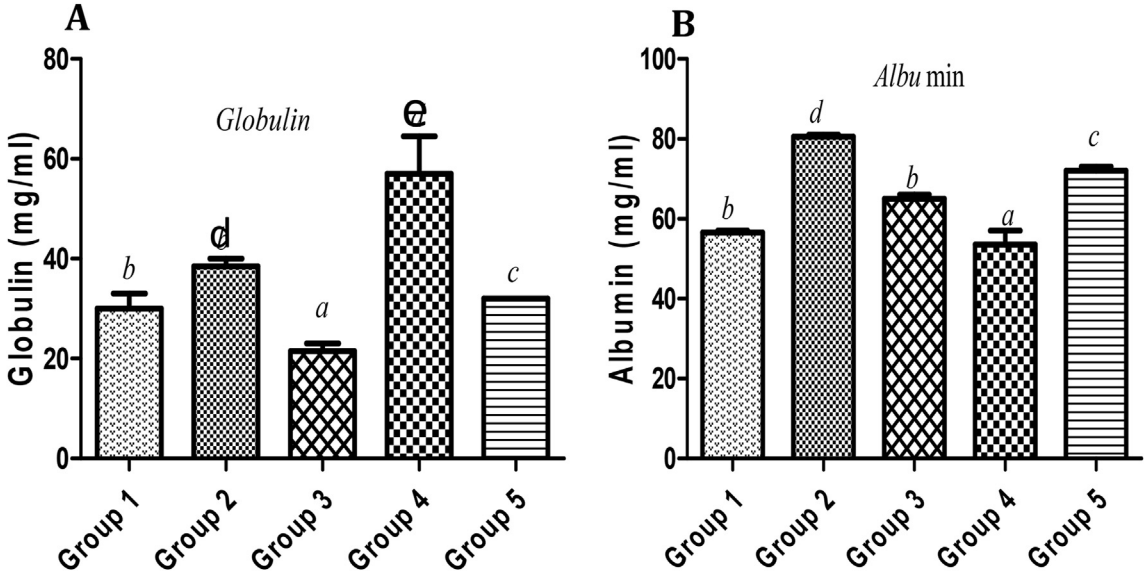


Fig. 8. Effect of monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* on two endpoints of liver functions (globulin-graph A and albumin-graph B) in treated rat. Data are presented as mean ± SD (n = 10). Bars with different letters are statistically different. i.e. $p < 0.05^a$, $p < 0.05^b$, $p < 0.05^c$, $p < 0.05^d$ and $p < 0.05^e$ are significantly different.

9.9% MCF-ADSPC remarkably ($p < 0.05$) up-surged the total protein level in the body (Fig. 5) than UF-ADS.

3.5. Effect of monoculture fungal fermentation of Adansonia digitata seed (ADS) by Penicillium chrysogenum on haematological parameters in rat

The haematological changes of rat treated with MCF-ADSPC at various doses (9.9%, 19.8% and 30%) and UF-ADS (30%) are presented in Table 9. No significant changes ($p > 0.05$) in the blood quantity and quality of haematology in animals fed with MCF-ADSPC when compared with UF-ADS and control groups. The control group of WBC was significantly ($p < 0.05$) higher than UF-ADS (Table 9). Whereas, no significant difference ($p > 0.05$) between MCF-ADSPC and UF-ADS (Table 9). However, 9.9%, 19.8% and 30% MCF-ADSPC augmented WBC count better than UF-ADS by 26%, 19% and 18%, respectively. Whereas, 9.9% MCF-ADSPC produced highest level of WBC (Table 9). Similarly, 9.9%, 19.8% and 30% MCF-ADSPC increased the metabolic production of centrophil better than UF-ADS by 92%, 25% and 158%, respectively. Whereas, 30% MCF-ADSPC produced highest amount of centrophil (Table 9). The control rats significantly ($p < 0.05$) increased centrophil than UF-ADS (Table 9). While, no significant difference ($p > 0.05$) between MCF-ADSPC and UF-ADS (Table 9). Finally, rats treated with MCF-ADSPC at all doses showed no significant difference in eosinophils, monocytes and basophils in relation to UF-ADS and the control (Figs. 6 and 7).

3.6. Effect of monoculture fungal fermentation of Adansonia digitata seed (ADS) by Penicillium chrysogenum on some endpoints of liver functions

Fig. 8A and B provide the account of serum levels of globulin and albumin in rats treated with MCF-ADSPC and UF-ADS for 56 days. The treatment with 19.8% MCF-ADSPC significantly decreased ($p < 0.05$) the serum globulin level than UF-ADS and control by 33% and 28%, respectively (Fig. 8A). Whereas, the treatment with 30% MCF-ADSPC significantly ($p < 0.05$) lowered the level of serum albumin better than UF-ADS by 10% and 26%, respectively (Fig. 8B). ALT and AST were not

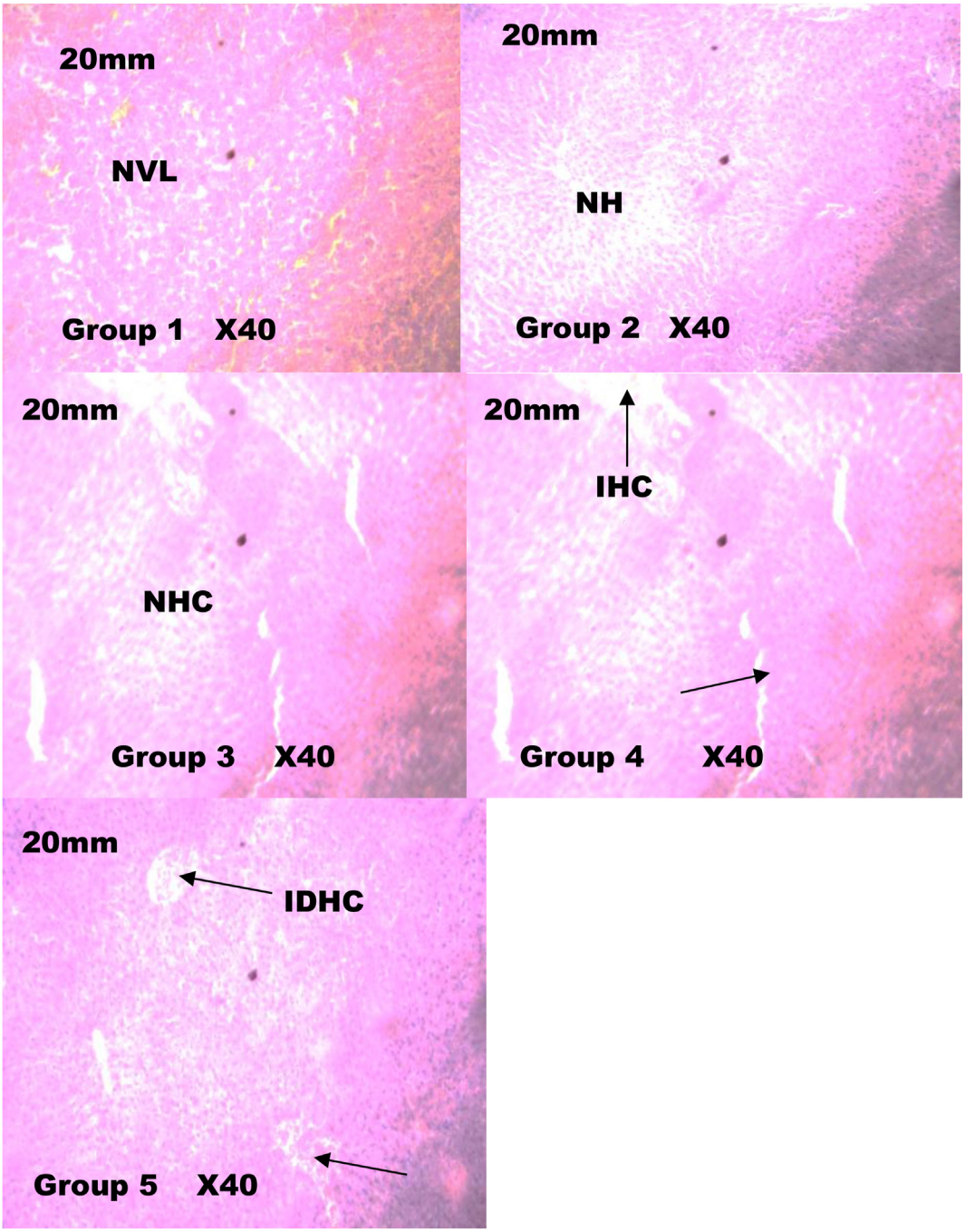


Fig. 9. Liver histological examination (X 40) in rat supplemented with monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* (X40) **(Group 1):** Rats showed no visible lesions to the hepatocytes (NVL) **(Group 2):** Rats showed normal hepatocytes (NH) **(Group 3):** Rats depicted normal hepatic cells (NHC) **(Group 4):** This group of animals demonstrated inflammation of the hepatic cells (IHC) **(Group 5):** The group of animals showed inflammation as well as degeneration hepatic cells (IDHC).

detected because of their similarity in metabolic activities with globulin and albumin. Moreover, cytotoxicity study showed that 9.9% and 19.8% MCF-ADSPC depicts no visible lesion to the hepatocytes, indicating no hepatic inflammation (Fig. 9). Whereas, 30% MCF-ADSPC and UF-ADS showed inflammation with degeneration of hepatic cells.

3.7. Effect of monoculture fungal fermentation of Adansonia digitata seed (ADS) by Penicillium chrysogenum on two endpoints of kidney functions

There is a significant ($p < 0.05$) difference in the level of urea among 9.9%, 19.8%, 30% MCF-ADSPC, UF-ADS and the control group (Fig. 10A). Whereas, UF-ADS significantly ($p > 0.05$) down-regulated the serum urea better than MCF-ADSPC (Fig. 10A). Additionally, animals fed with 9.9% MCF-ADSPC significantly ($p < 0.05$) up-regulated the serum creatinine level than the control and UF-ADS (Fig. 10B). Remarkably, UF-ADS protected kidney better because of its lowest creatinine level (Fig. 10B). Histologically, 9.9% and 19.8% MCF-ADSPC prohibit renal inflammation with no visible lesion to the nephrocytes (Fig. 11). Whereas, 30% MCF-ADSPC and UF-ADS showed renal inflammation with the degeneration of nephrons (Fig. 11).

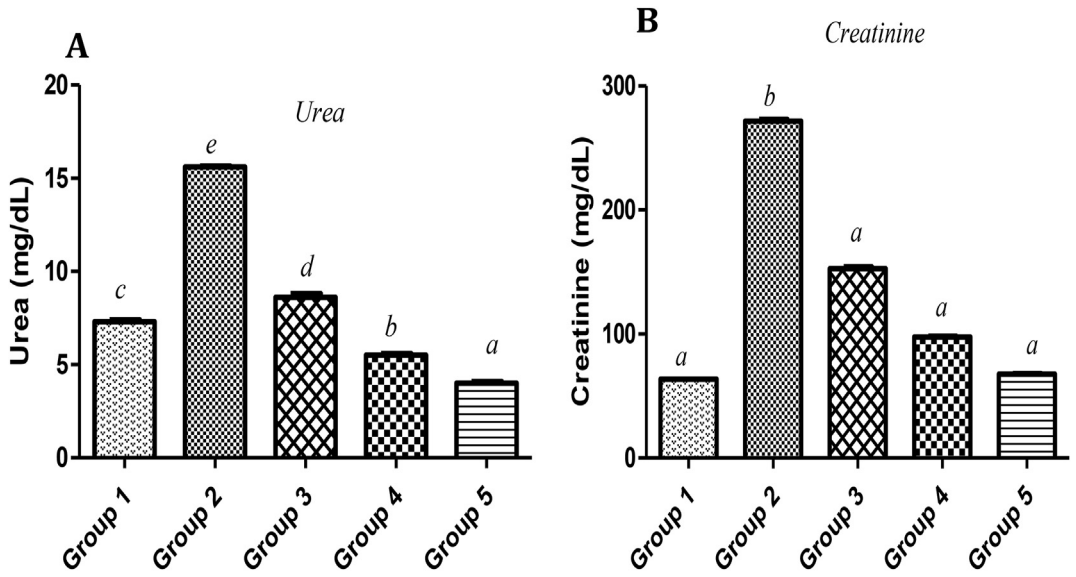


Fig. 10. Effect of monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* on two endpoints of kidney functions (urea-graph A and creatinine-graph B) in treated rat. Data are presented as mean ± SD (n = 10). Bars with different letters are statistically different. i.e. $p < 0.05^{a}$, $p < 0.05^{b}$, $p < 0.05^{c}$, $p < 0.05^{d}$ and $p < 0.05^{e}$ are significantly different.

3.8. Effect of monoculture fungal fermentation of Adansonia digitata seed (ADS) by Penicillium chrysogenum on some endpoints of cholesterol

For HDL (Fig. 12A), 9.9% MCF-ADSPC significantly ($p < 0.05$) increased the level of good lipid better than UF-ADS and control by 7% and 39%, respectively. Treatment with 9.9% and 30% MCF-ADSPC doses significantly ($p < 0.05$) lowered LDL level than UF-ADS (Fig. 12B). Whereas, 19.8%, 30% MCF-ADSPC and UF-ADS significantly reduced ($p < 0.05$) the cholesterol level than control and 9.9% MCF-ADSPC groups (Fig. 12C). Finally, histological examination discovered that rats administered with 9.9% and 19.8% MCF-ADSPC for 56 days depicts no visible lesion to the architectural structures of the intestinal membrane, which is an indication of normal intestinal mucosa and low cholesterol levels (Fig. 13). Whereas, 30% MCF-ADSPC and UF-ADS showed focal loss of the overlaying mucosa (Fig. 13). Similarly, 9.9% and 19.8% MCF-ADSPC portray no visible lesion to the spleen cells, indication of spleen cells distortions (Fig. 14). Whereas, 30% MCF-ADSPC and UF-ADS showed degeneration of splenocytes (Fig. 14).

4. Discussion

The current study examined the most effective dose of fermented AD seed on nutritional values and haematology as well as extrahepatic functions in rats. To achieve this, we formulated nutriment from monoculture fermentation of AD seeds by *Penicillium chrysogenum* at 72 h. It was evidently seen that the crude protein in MCF-ADSPC was higher than the UF-ADS at 72 h during fermentation. The increased protein as a result of monoculture fermentation with *Penicillium chrysogenum* may be responsible for the detoxification of toxicants in the liver and extrahepatic tissues. It has been reported that increased dietary protein could facilitate detoxification of toxicants in rats [4]. Although, this is not a confirmatory indicator but suggestive indicator. Our future study will demonstrate whether changes in the contents of proteins after fermentation by keeping other variables constant can improve detoxification of toxicants in the tissues. The hike level of carbohydrate due to fermentation of AD seed with P. *chrysogenum* could also show strong tissue metabolism and protection via production of cellular ATP in the body [42]. Also, significant elevation of protein in renal and hepatic tissues was linked to normal metabolic processes [4]. Anti-nutrients including saponin, oxalate, tannin and phytate are secondary metabolites. They have been implicated in organ toxicity.

[43,44]. In the study, remarkable depletion of these anti-nutrients following the fermentation AD seeds by P. *chrysogenum* suggests their chelation [9]. Also, excessive ingestion of these secondary compounds have been reported to trigger IgG and IgM antibodies to provoke abnormal immune responses known as hypersensitivity [9]. They bind to erythrocytes to produce hemagglutination and anaemia [45]. Consistent with this study, fermentation of *Pakia biglobosa* seed was reported to increase the protein quantity while the anti-nutrient levels were reduced [46]. Moreover, the total essential amino acids (TEAAS) was high and the total non-essential amino acids (TNEAAS) was low in MCF-ADSPC than UF-ADS when compared with the recommended dietary intake (RDI) [46]. This suggests that fermentation of MCF-ADSPC by P. *chrysogenum* may optimize TEASS load [47]. Previous study has shown that the fermentation of baobab fruit pulp flour could produce essential and non-essential amino acids as phenylalanine and aspartic acid, respectively, with highest values after 24 h fermentation [48]. Similarly, the accumulation of phenolic acids and flavonoids in this study suggests that fermentation can trigger their assembly [49]. It has also been reported that accrued flavonoids during fermentation can scavenge free radicals and act as antioxidative agent against oxidized proteins and DNA damage [50] in rats. Studies further suggested that luteolin as a product of fermentation is safe and may be used as anti-inflammatory, anti-tumor, anti-ulcer, immune modulator, and vasodilator [51,52]. It is interesting to report here that phenolic compounds produced from fungi fermentation may be used as therapy against some metabolic diseases [53–55].

The body growth and total protein level were increased in MCF-ADSPC better than the animals treated with UF-ADS. The increased weight gain and retention of total protein speak about the protection on the total blood quantity and quality [56]. We therefore report here that body weight gain is proportional to the packed cell volume (PCV) and erythrocyte counts [57] while depleted leucocyte counts may initiate anemia in rats [58]. Also, monoculture fermentation by P. *chrysogenum* may trigger bioavailability of total proteins in the liver and extrahepatic tissues [57]. This finding corresponds with the previous study, which suggested that low level protein could cause red blood cells malformation

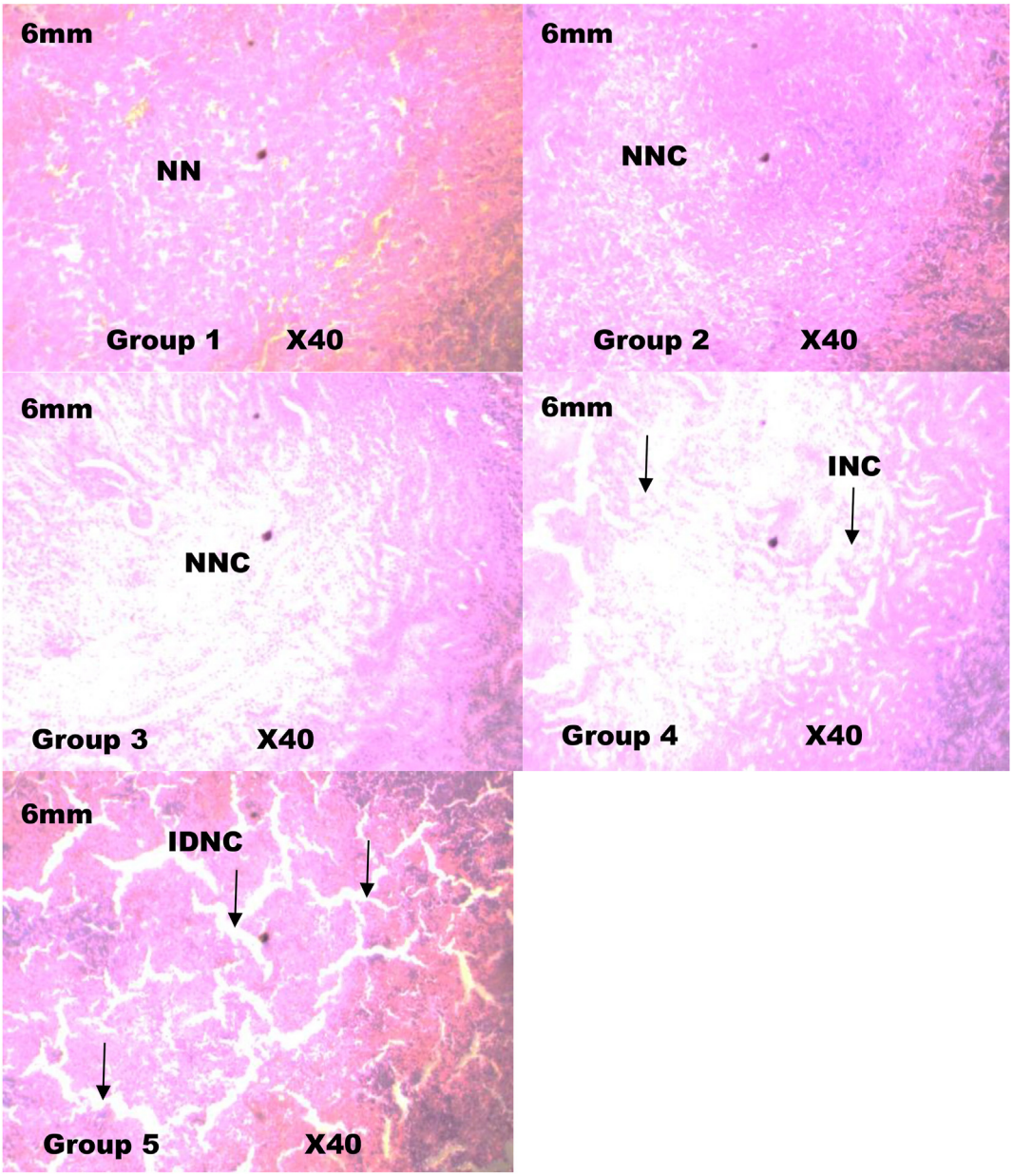


Fig. 11. Kidney histology (X 40) in rat supplemented with monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* **(Group 1):** This group showed normal nephrons (NN) **(Group 2):** The group showed normal architecture of the kidney as well as normal nephritic cells (NNC) **(Group 3):** Rats depicted normal nephritic cells (NNC) **(Group 4):** This group of animals demonstrated inflammation of the nephritic cells (INC) **(Group 5):** The group of animals showed inflammation as well as degeneration nephritic cells (IDNC).

or death [58]. Similarly, grounded fermented AD seeds was incorporated in the diets of one-week old chicks at 12% dietary level [59]. It was discovered that AD seed meal showed no effect on the chicks' feed intake, final body weight, feed conversion ratio and protein intake whereas the body weight gain and protein efficiency ratio of the chicks fed for 96 h was higher than the chicks fed for 48 h and 72 h. Also, the chicks placed on fermented AD seeds for 72 h improved nutrient digestibility in relation to 96 h fed chicks [59]. It is interest to also report that previous studies on fermentation of AD seed for 120 h can serve as additional plant protein food source in feed formulation for the livestocks [60]. Also, mixed-culture of *Alternaria tenuis* and *Mucor racemosus* used for the fermentation of AD was able to produce amylase, hydrolytic enzyme of carbohydrate [61]. This further suggests that fermented AD seed by P. *chrysogenum* may be a fundamental method for the industrial production of amylase.

The erythrocytes, leucocyte, haemoglobin and lymphocytes are generated at a turnover rate of about 1–3 million per second in a healthy human [62]. It has been reported that their abnormal rates can initiate some pathological conditions as hemolytic anemia and leukemia [56]. Here, MCF-ADSPC slightly increased WBC and centrophils count at 9.9% and 30% doses, respectively better than UF-ADS. This suggests haematopoiesis in rats liver and kidney stem cells [62]. Whereas, reduction in WBC level has been connected to leukemia [62]. Also, increase WBC can prevent infection of the liver and kidney [63,64]. It is pertinent to hypothesize here that MCF-ADSPC may be a potent phagocytic agent and inflammatory defence mediator [65]. EAAs has been reported to mediate

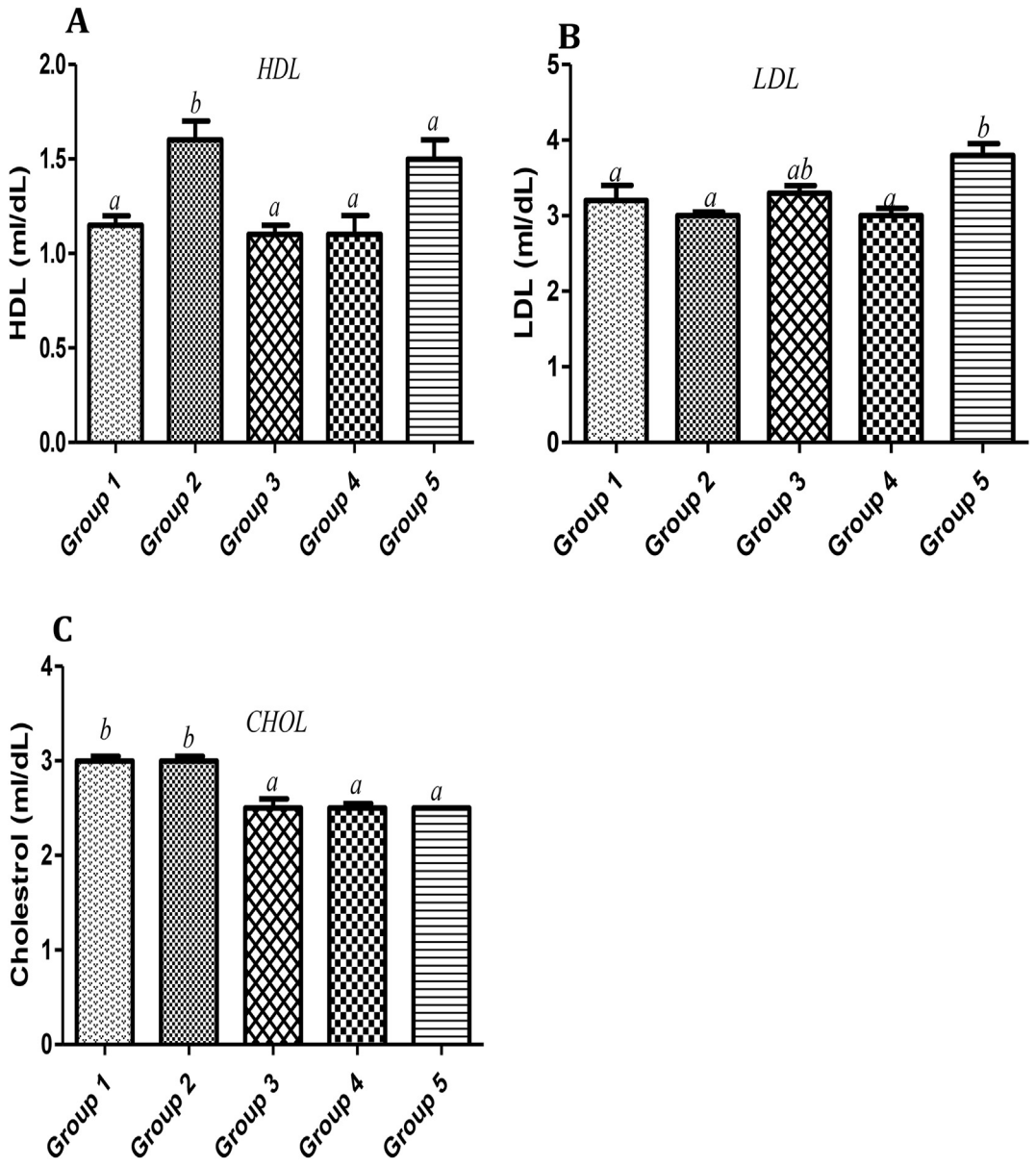


Fig. 12. Effect of monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* on some endpoints of cholesterol (HDL-graph A, LDL –graph B and cholesterol-graph C) in treated rat. Data are presented as mean ± SD (n = 10). Bars with different letters are statistically different. i.e. $p < 0.05^{a}$, $p < 0.05^{b}$ and $p < 0.05^{ab}$ are significantly different.

the immune cells against pathogens, and cancerous cell development [1]. Apparently, centrophils produced following fermented AD seed by P. *chrysogenum* suggests MCF-ADSPC as stimulator of the growth factors, T-lymphocytes and natural killer cells (NKs) [66,67]. It's no significant difference in lymphocyte and neutrophil may suggests that administration of MCF-ADSPC and UF-ADS has no effect on cell-mediated and innate/adaptive immunity [67]. It further suggests that fermented AD seed by P. *chrysogenum* reverts neutropenia by the creation of antibodies (T and B lymphocytes) [68]. Previous studies [68,69] have reported that flavonoids and phenolic acids maintain strong T and B lymphocytes (phagocytes) to reduce vulnerability of the body to infectious diseases through the depletion of angiogenesis in cancer cells [70]. Similarly, no significant difference of RBC and Hb suggested that MCF-ADSPC and UF-ADS has no effect on RBCs and (Hb) [71]. Additionally, little or no production of eosinophils after fermented AD seed by P. *chrysogenum* indicated no cytotoxicity was induced by MCF-ADSPC and UF-ADS in rats [72] at 9.9% dose. None detection of basophils signifies that MCF-ADSPC and UF-ADS could inhibit basophils. Expression of basophils is a risk factor of asthma, allergic rhinitis and anaphylaxis [73]. Also, no expression of monocytes in animals fed with MCF-ADSPC and UF-ADS may indicate the inhibition of monocytosis, pericarditis, or joint effusions and ascites in rats [74].

The two endpoints of liver functions-globulin and albumin were decreased on treatment with 19.8% and 30% MCF-ADSPC, respectively than UF-ADS. Also, the cytotoxicity examination showed that the rat exposed to 9.9% and 19.8% MCF-ADSPC mitigated the inflammation and degeneration of hepatic cells than UF-ADS, thus revealing the hepatoprotective nature of MCF-DASPC in relation to UF-ADS. Serum urea and creatinine are measured as imperative, dependable, well-recognized indicators of renal function in animal and man [75]. In line with the

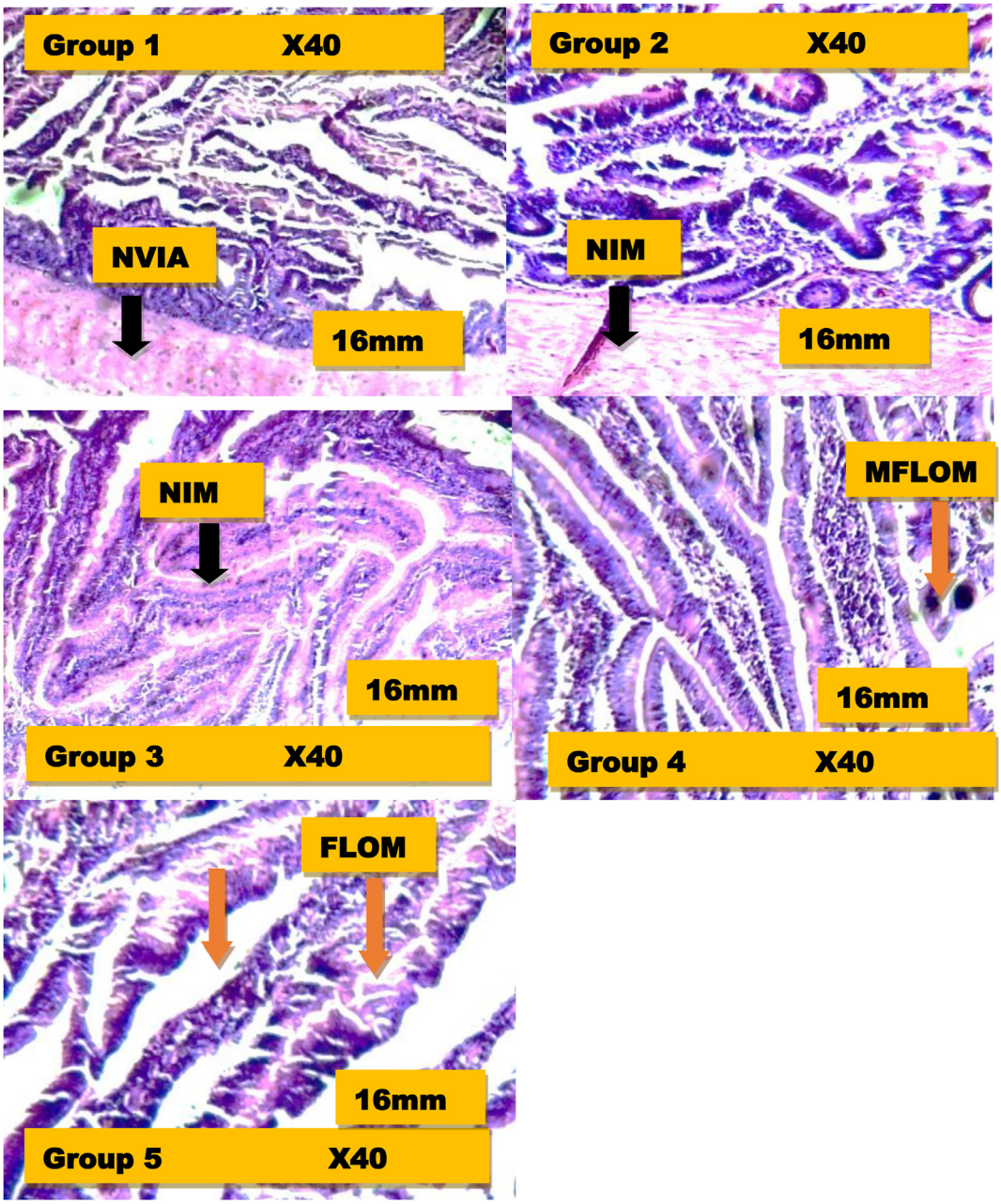


Fig. 13. Histology of small intestine in rat supplemented with monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum*. **(Group 1):** showed normal and visible intestinal architecture (NVIA) i.e. the villi were intact and normal architectural structures of the intestinal membrane were observed **(Group 2):** showed normal intestinal mucosa (NIM) **(Group 3):** depicted normal intestinal mucosa (NIM) **(Group 4):** showed mild focal loss of the overlaying mucosa (MFLOM) **(Group 5):** showed focal loss of the overlaying mucosa (FLOM).

previous study [42], higher doses of MCF-ADSPC (19.8% and 30%) strongly reduced urea and creatinine in our study, showing its protection against extra hepatic tissues. Similarly, 9.9% and 19.8% MCF-ADSPC abrogated the renal inflammation and degeneration of nephrons. It is imperative to report here that 19.8% MCF-ADSPC could prevent hepatotoxicity and nephrotoxicity in rat [76,77] while, depleted RBC and WBC could predispose liver and extrahepatic tissues to severe damage [62].

HDL, LDL and cholesterol are involved in the assembly of atherosclerosis, hypercholesterolemia as well as impairment of leucocyte, macrophage and liver dysfunction [78]. Treatment of animals with 9.9% MCF-ADSPC showed better protection against lipid alteration by increasing beneficial HDL than UF-ADS. Likewise, 9.9% and 30% MCF-ADSPC down-regulated the bad LDL better than UF-ADS. Whereas, 19.8%, 30% MCF-ADSPC and UF-ADS showed protection against dyslipidemia in rats. This predicts that 9.9%, 19.8% and 30% after fermented AD seed by P. *chrysogenum* may be a regimen for hyperlipidemia, hypolipoproteinemia and hypocholesterolemia [79 in a rat model. However, histopathology study showed that 9.9% and 19.8% MCF-ADSPC picked up excess cholesterol in the blood to inhibit its deposition in the duodenum (small intestine) [79] and by maintaining the integrity of intestinal mucosa [80]. It is therefore reported here that only 19.8% MCF-ADSPC could prevent hyperlipidemia in rats. This action was connected to essential amino acids and flavonoids in MCF-ADSPC, which has the capacity to down-regulate the cholesterol levels [81] and delay convoluted atherosclerosis [80].

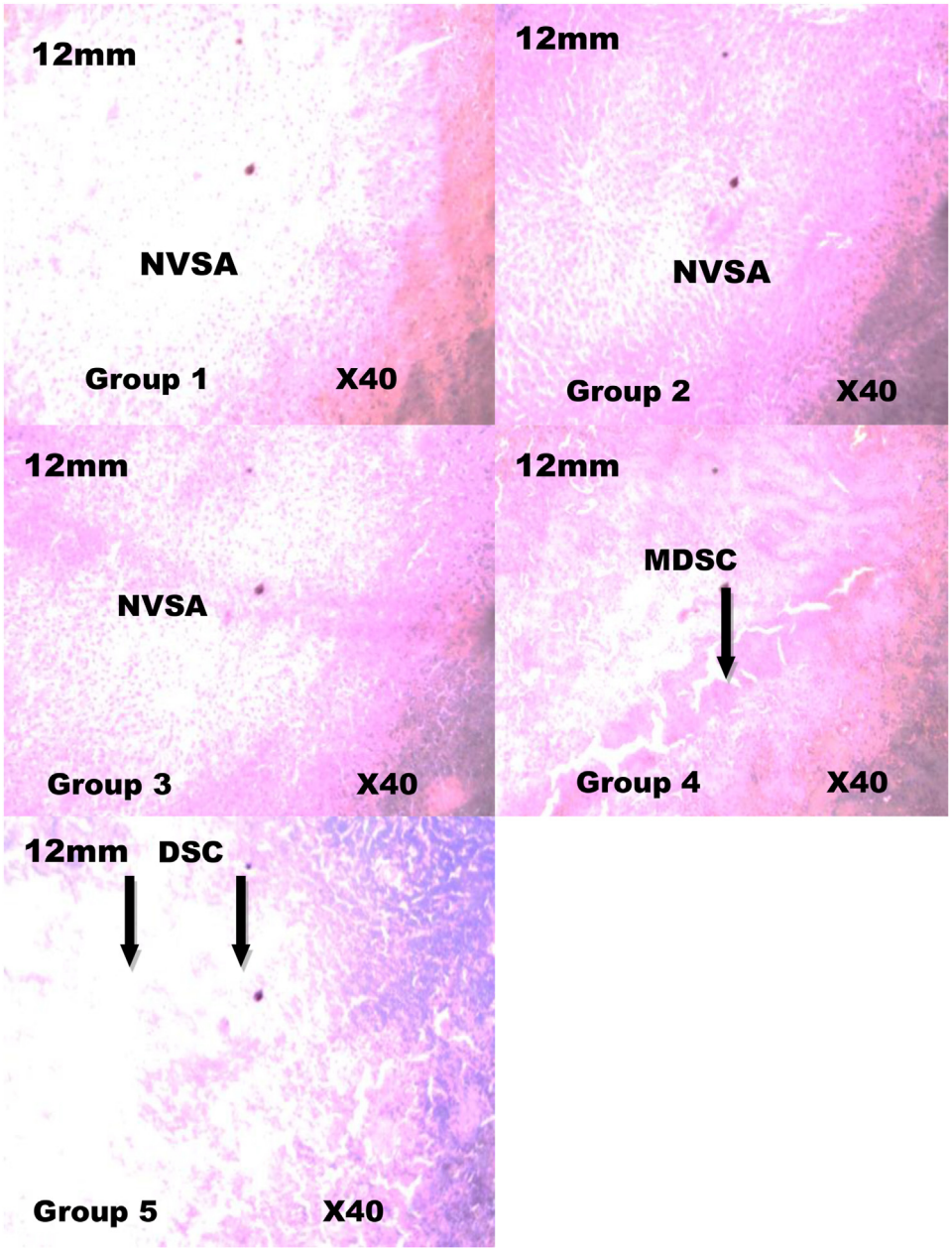


Fig. 14. Spleen histology (X 40) in rat supplemented with monoculture fungal fermentation of *Adansonia digitata* seed by *Penicillium chrysogenum* **(Group 1):** showed normal and visible spleen architecture (NVSA) **(Group 2):** showed normal and visible spleen architecture (NVSA) **(Group 3):** depicted normal and visible spleen architecture (NVSA) **(Group 4):** showed mild distorted spleen cells (MDSC) **(Group 5):** showed degeneration of spleen cells (DSC).

Additionally, spleen is a vital extrahepatic tissue which produces useful antibodies [71]. In this study, rats' spleen showed that 9.9% and 19.8% MCF-ADSPC prevented the degeneration of the spleen cells. This implies that fermented AD seed by P. *chrysogenum* could protect against splenocyte injury. Study has shown that fermented product may activate the rejoining of immune systems via humoral and cell-mediated pathways to inhibit splenomegaly, blood-based leukemia and asplenia [71]. Also, previous study has shown that the nutritional values obtained from boiled, roasted or fermented AD seed were higher compared to the control [82]. Also, fat, carbohydrate, minerals, moisture and ash contents of fermented seed of AD was found to be higher in percentage composition than boiled and roasted samples [82]. Furthermore, alkaloids, saponins and protease inhibitors signified higher compositions in unfermented than fermented seeds [82] whereas, steroids and flavonoids were highly expressed in fermented seed [82]. Generally, following the present and previous studies, it is interesting to report here that fermented AD seed may be helpful in diet's preparation for patients with reduced proteins and metabolic diseases.

Author contribution

Adedayo M.R: Conceptualization, Methodology, Investigation, Akintunde J.K: HPLC Characterization, Software analysis, Writing - original draft, Writing - review and editing. Sani A: Supervision Boligon A: HPLC Characterization.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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