Original Article

Short-Term Administration of Thaumatococcus daniellii (Benn.) Benth Fruit Pulp Leads to Dyslipidaemia and Liver Dysfunction in Male Wistar Rats

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Abstract

Background: Thaumatin extraction from Thaumatococcus daniellii fruit leads to the generation of substantial waste, largely made up of the fruit's pulp. This study's objective was to determine the short-term administrative effect of T. daniellii fruit pulp ethanol extract (TDFPE) in male Wistar rats.

Methods: Thirty animals were designated into five groups. Group 1 was administered distilled water (1 mL/kg bw), while groups 2 – 5 were administered vitamin C (10 mg/kg bw), 500, 1000 and 1500 mg/kg bw TDFPE for 14 days, respectively. Liver function, antioxidant, lipid profiles and liver histology were assessed using standard methods.

Results: TDFPE treatment significantly increased ($p<0.05$) hepatic SOD activity, GSH and MDA concentration compared to vitamin C and normal control groups. An increase ($p<0.05$) was seen in plasma TRIG and LDL concentration while HDL level was reduced ($p<0.05$) in TDFPE-treated rats compared to vitamin C-treated rats and those in the normal control group. Plasma activities of ALT, AST and BIL concentration were significantly ($p<0.05$) increased in TDFPE-treated groups compared with the normal control group. Liver histology showed periportal infiltration by inflammatory cells and oedematous sinusoids after TDFPE treatment.

Conclusion: Thus, T. daniellii fruit pulp administration at the studied dosages and time frame may exacerbate oxidative stress, dyslipidaemia and induce hepatic injury.

Keywords: Thaumatococcus daniellii, oxidative stress, dyslipidaemia, histology, hepatic injury

INTRODUCTION

Thaumatococcus daniellii is a source of thaumatin, found in the rainforest of West African countries particularly Nigeria, Cote d'Ivoire and Ghana.[1,2] It has a slender stalk of about three meters high with crimson or bright red pyramidal fruits when fully ripe.[3] The fruit usually contains one to three seeds. The seeds are hard, impervious and black, surrounded by a gel, capped with a membranous sac of sticky transparent gel. The jelly-like arils are soft, fleshy and juicy caps surrounding the seeds and contain a sweet protein called thaumatin.[4] In Nigeria, it is one of the neglected, underutilised plants that grow widely in cocoa plantations.[5] The presence of T. daniellii vegetation indicates the soil is rich and fertile for agricultural practices.[6,7] The domestic and folkloric application of T.

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daniellii fruit cuts across a lot of use, such as sweetening the sour taste of old palm wine and other fruit beverages and rubbing on nursing mothers' nipples to stimulate newborns to feed.[8] It is also used as a laxative, while the seeds are an emetic for treating pulmonary problems.[8] Decoctions of the fruit pulp are used to treat dystocia and childbirth complications in women undergoing labour.[9] The fruit contains flavonoids, alkaloids, terpenoids, calcium, phosphorous, magnesium and carbohydrate. Nonyl-5-tetradecene, cyclopropane and 7-tetradecene are the major bioactive compounds in the essential oil abstracted from the fruit, while glucuronic acid, arabinose, xylose, and 4-O-methyl glucuronic acid were the major polysaccharide present.[10,11]

Thaumatin is an amino acid of 22.2 kDa molecular mass, which is about 3000 times more intense than sucrose. It is a high-intensity sweetener used as a flavour enhancer with GRAS status.[6] Also, due to the non-caloric value of thaumatin, the fruit is an ideal sweetener for managing diabetes mellitus.[12] This sweetener is traded globally with a current value of about $ 36 M and is projected to rise to about $ 42 M by 2023. Hence, T. daniellii contributes to the rural, urban and international economies, as local farmers and collectors harvest the fruits from its natural habitat in large quantities for sale to thaumatin processing companies.[13] Extraction of thaumatin from the arils of T. daniellii leads to the generation of substantial waste, largely made up of the fruit's seed and pulp, which constitute over 97% of the entire fruit weight.[3,6] There is also a paucity of information on the pharmacological and biological activities of the fruit pulp, as the majority of the study has been focused on the leaves and seed.[6,7,14-16] The study objective was to determine the short-term administrative effect of Thaumatococcus daniellii fruit pulp ethanol extract (TDFPE) in male Wistar rats.

Material And Methods

Collection of plant samples

Healthy-looking T. daniellii (TD) fruits were bought from rural farmers in Ekiti, Ekiti State. Dr. J.O. Popoola from Covenant University, Ota, identified the fruits and deposited them in the herbarium.

Preparation of crude extract

The T. daniellii fruits were washed thoroughly with distilled water, the pulp was separated, weighed and sundried (TDFP). The dried pulp was ground and extract prepared by macerating 150 g of ground TDFP in 1 L of 80 % ethanol for 3 days. The mixture was filtered using 125 mm paper and concentrated in a rotary machine to yield a semi-solid honey-like ethanol extract (TDFPE) which was soluble in water.[6]

Experimental animals

Thirty healthy male Wistar rats (150 ± 20 g; 4 – 6 weeks old) from the Nigerian Institute of Medical Research, Yaba were purchased for this study and cared for under standard experimental surroundings with access to feed and water. Experimental rats were acclimatised for 2 weeks prior to the experiments. The animal handling was conducted in compliance with Covenant University Biological Sciences Research Ethics Committee (CU/BIOSCRECU/BIO/2015/007) following the ARRIVE and National Institutes of Health (NIH) guidelines.

Experimental Design

The animals were shared into 5 groups (n=6) randomly, treated for 14 days, sacrificed and samples prepared following a previous experiment in the study of T. daniellii seed and leaves as follows:[6,7]

Normal: Rats administered distilled water (1 mL/kg bw)

Vit C: Rats administered vitamin C (10 mg/kg bw)

TDFPE500: Rats administered TDFPE (500 mg/kg bw)

TDFPE1000: Rats administered TDFPE (1000 mg/kg bw)

TDFPE1500: Rats administered TDFPE (1500 mg/kg bw)

The animals' weight was monitored in the course of the experiment. After 14 days, the animals were fasted and sacrificed under mild euthanasia with a xylazine-ketamine mixture (1:10 v/v) through cardiac puncture. Blood collected was dispensed in heparin bottles and centrifuged to obtain the plasma, while a portion of excised liver was homogenised and centrifuged to obtain the homogenate. Another part of the excised liver was placed in formalin for histology.

Biochemical Assessments

Aspartate transaminase (AST) activity, alanine transaminase (ALT) activity, bilirubin, triglycerides, cholesterol, high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) concentration were assayed in the plasma using Randox kits following directives placed by the manufacturers. Hepatic and renal superoxide dismutase (SOD) activity was determined as the inhibition of pyrogallol autooxidation rate.[17] Reduced glutathione (GSH) concentration in the liver and kidney was estimated according to Ellman's method described by Sedlak and Lindsay.[18] Lipid peroxidation was determined in the liver and kidney as the concentration of thiobarbituric acid and malondialdehyde (MDA) conjugate at 532 nm.[19]

Histology

Excised liver tissues stored in formal saline were sectioned, parched in alcohol (graded) and placed inside paraffin wax before staining with haematoxylin and eosin (H&E). Prepared sections were then evaluated for histopathological

changes to hepatic microarchitecture.[20]

Statistical analysis

All results were statistically analysed using one-way analysis of variance supplemented by the Duncan multiple range test at a 95 % significance level. They were presented as mean ± standard error of the mean.

Results

Animal and organs weight

In Figure 1, TDFPE treatment had no significant effect on animal and organ weights compared with the normal and vitamin C-administered group.

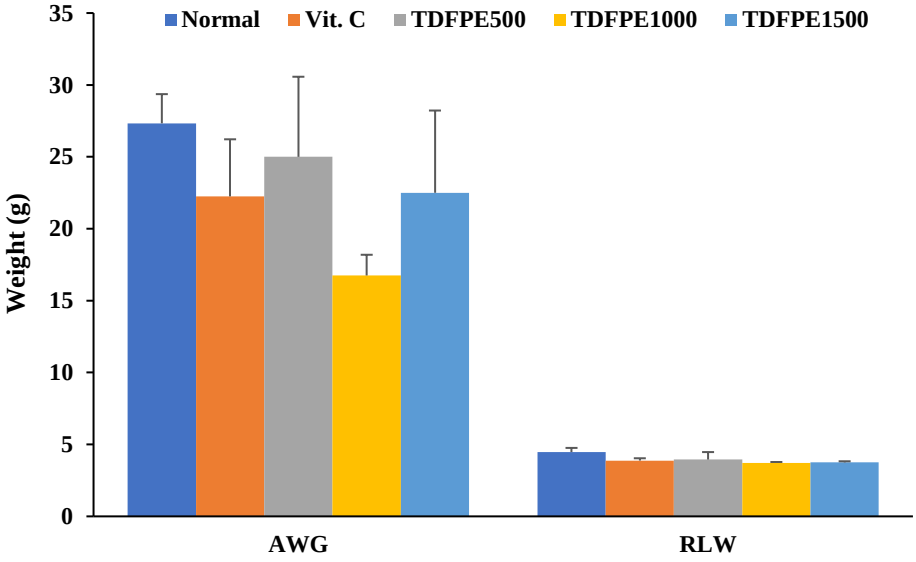

Figure 1: Effect of T. daniellii fruit pulp ethanolic extract on animal and relative liver weight.

Bars are mean ± SEM. AWG: Animal weight gain; RLW: Relative liver weight

Oxidative stress parameters

Oral administration of TDFPE graded dosage increased ($p<0.05$) liver SOD activity and MDA concentration while there was no alteration ($p>0.05$) in these parameters in the kidney of the rats when compared to normal and vitamin C treated rats. On the other hand, the GSH level was significantly higher in the liver and kidney of TDFPE-treated groups than in normal and vitamin C groups (Table 1).

Table 1: Effect of T. daniellii fruit pulp ethanolic extract on tissue antioxidant activity

Group	*SOD (U/mg protein)*		*GSH (µM/mg protein)*		*MDA (µM/mg protein)*	
	Liver	*Kidney*	*Liver*	*Kidney*	*Liver*	*Kidney*
Normal	7.76±0.43b	5.76±0.81a	3.70±0.34a	8.40±0.01a	2.73±0.54a	1.72±0.14a
Vit. C	3.94±0.76a	2.88±0.09a	2.52±1.18a	21.16±0.34 ab	2.03±1.00a	2.35±0.02a
TDFPE500	18.91±3.24c	5.43±1.55a	30.86±2.05 c	26.70±1.18 b	6.33±1.03b	2.41±0.12a
TDFPE1000	15.32±1.18c	4.88±0.88a	12.09±4.70 b	34.92±2.69 b	8.20±0.47b	3.16±0.22a
TDFPE1500	12.78±3.79c	2.74±0.66a	6.89±1.85b	23.85±0.34 b	6.33±1.03b	3.12±0.03a

Values are mean ± SEM. Different superscripts down a column = $p<0.05$. Same superscripts down a column = $p>0.05$.

Lipid profile parameters

There was no change (p>0.05) in plasma, hepatic and renal cholesterol levels of TDFPE-treated rats compared with the rats treated with vitamin C and distilled water (Table 2). A similar trend was observed (p>0.05) for liver and kidney triglyceride levels of normal, vitamin C and TDFPE groups. However, the plasma triglyceride concentration of TDFPE groups was significantly (p<0.05) higher than normal and vitamin C groups (Table 3). TDFPE treatment significantly (p<0.05) reduced plasma and hepatic HDL while increasing (p<0.05) LDL in the plasma and tissues compared to control groups (Tables 4 and 5). Nonetheless, TDFPE did not alter (p>0.05) renal HDL levels in comparison with the control groups.

Table 2: Effect of T. daniellii fruit pulp ethanolic extract on plasma and tissue cholesterol (mmol/L) level

Group	*Plasma*	*Liver*	*Kidney*
Normal	0.70±0.19a	0.51±0.11a	0.15±0.01a
Vit. C	0.59±0.01a	0.55±0.06a	0.20±0.05a
TDFPE500	0.62±0.04a	0.45±0.01a	0.12±0.03a
TDFPE1000	0.86±0.06a	0.69±0.11a	0.16±0.01a
TDFPE1500	0.76±0.06a	0.58±0.02a	0.13±0.02a

Values are mean ± SEM. Different superscripts down a column = p<0.05. Same superscripts down a column = p>0.05.

Table 3: Effect of T. daniellii fruit pulp ethanolic extract on plasma and tissue triglyceride (mmol/L)

Group	*Plasma*	*Liver*	*Kidney*
Normal	1.27±0.09a	1.32±0.09a	0.25±0.14a
Vit. C	1.24±0.25a	1.50±0.16a	0.45±0.12a
TDFPE500	1.84±0.04b	1.71±0.11a	0.35±0.14a
TDFPE1000	1.96±0.02b	1.56±0.13a	0.67±0.09a
TDFPE1500	1.18±0.07a	1.93±0.13a	0.47±0.18a

Values are mean ± SEM. Different superscripts down a column = p<0.05. Same superscripts down a column = p>0.05.

Table 4: Effect of T. daniellii fruit pulp ethanolic extract on plasma and tissue HDL (mmol/L) level

Group	*Plasma*	*Liver*	*Kidney*
Normal	1.12±0.12c	0.46±0.09b	0.05±0.02a
Vit. C	0.69±0.07b	0.24±0.06b	0.09±0.04a
TDFPE500	0.36±0.02a	0.11±0.03a	0.11±0.03a
TDFPE1000	0.44±0.01a	0.05±0.02a	0.08±0.03a
TDFPE1500	0.57±0.05a	0.09±0.04a	0.12±0.03a

Values are mean ± SEM. Different superscripts down a column = p<0.05. Same superscripts down a column = p>0.05.

Table 5: Effect of T. daniellii fruit pulp ethanolic extract on plasma and tissue LDL (mmol/L) level

Group	*Plasma*	*Liver*	*Kidney*
Normal	0.43±0.11a	0.22±0.08a	0.11±0.05a
Vit. C	0.50±0.02a	0.13±0.03a	0.08±0.03a
TDFPE500	0.82±0.18b	0.52±0.08b	0.46±0.15b
TDFPE1000	1.44±0.21b	0.85±0.12b	0.68±0.14b
TDFPE1500	1.57±0.15b	0.99±0.14b	0.62±0.10b

Values are mean ± SEM. Different superscripts down a column = p<0.05. Same superscripts down a column = p>0.05.

Liver function parameters

The activity of AST and ALT were significantly (p<0.05) increased in the plasma of rats administered TDFPE compared to the control and vitamin C-administered rats. Likewise, TDFPE treatment increased (p<0.05) total bilirubin concentration but was unable to alter (p>0.05) direct bilirubin concentration compared with vitamin C and distilled water treatment.

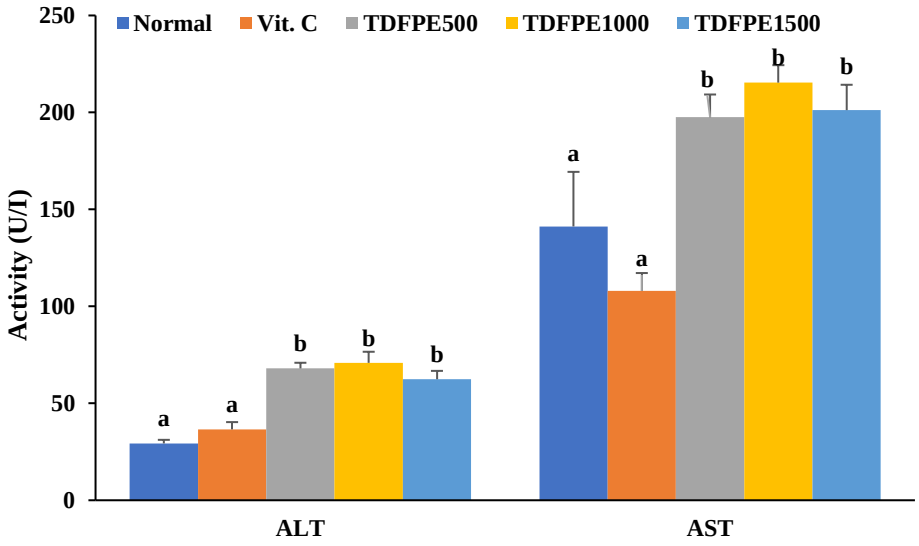

Figure 2: Effect of T. daniellii fruit pulp ethanolic extract on plasma ALT and AST activity. Bars are mean ± SEM of 6 biological replicates. Different superscripts for each assay = $p<0.05$. Same superscripts for each assay = $p>0.05$.

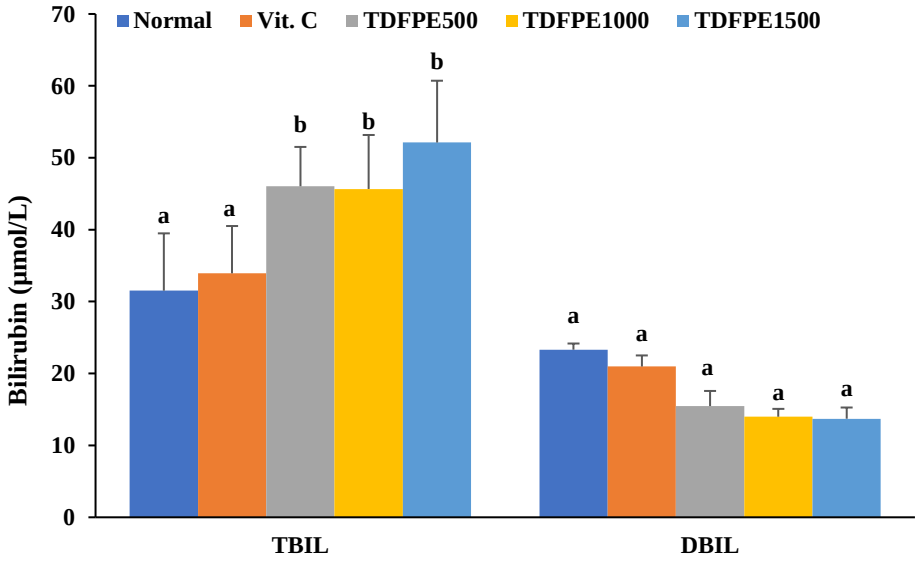

Figure 3: Effect of T. daniellii fruit pulp ethanolic extract on plasma direct and total bilirubin concentration. Bars are mean ± SEM of 6 biological replicates. Different superscripts for each assay = $p<0.05$. Same superscripts for each assay = $p>0.05$.

Histological examination of Liver

The hepatic histopathological sections of normal and vitamin C groups showed normal cellular architecture and distinct vacuole, while inflammatory cells in periportal regions, lysed blood in the vessels and distended sinusoid with fluid highlighting oedema was observed in the liver of TDFPE-treated animals (Figure 4).

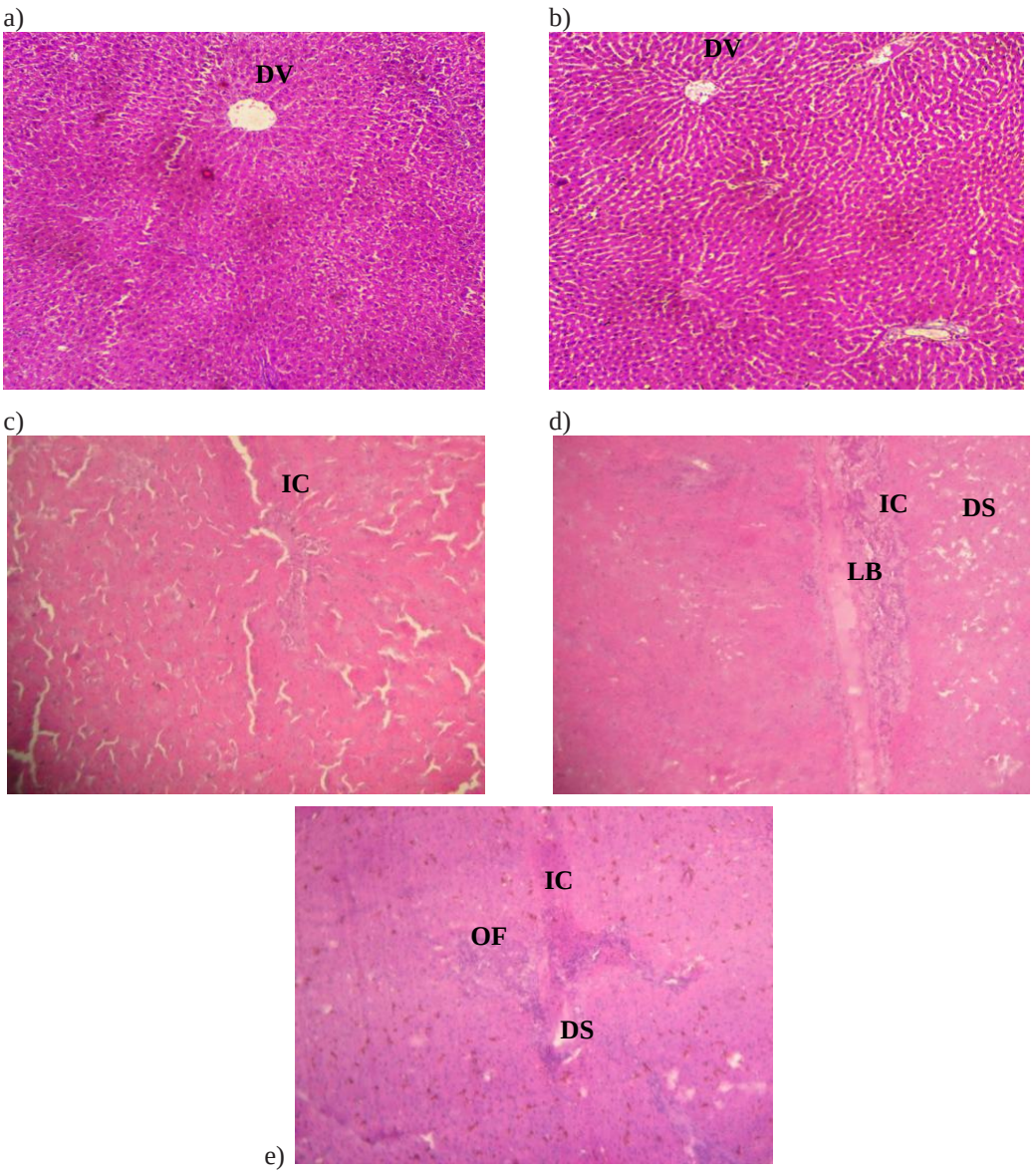

Figure 4: Liver histology of (a) normal, (b) vitamin C, (c) TDFPE500, (d) TDFPE1000 and (e) TDFPE1500 treatment groups (H&E ×400). DV: distinct vacuole; IC: inflammatory cells; LB: lysed blood in the vessels; DS: distended sinusoid; OF; fluid highlighting oedema

Discussion

Weight gain or loss is used to assess the holistic well-being of an organism. A reduction in body and organ weight indicates a decline in general health conditions.[21] The results of the current study indicated that treatment with T. daniellii fruit extract maintained the body and organ weight suggesting the animals were in good health despite the toxic effects of the extract.[22]

A study by Pérez-Vázquez, Ramírez[23] shows calcium and magnesium ions elicit a concerted activation effect on hepatic SOD activity. Hence their presence in T. daniellii fruit pulp might be responsible for the hepatic SOD activity increased in this study. The increase in GSH concentration may be attributed to the copious amount of flavonoids in the fruit pulp.[3] They are potent antioxidants and free radical scavengers via increased expression of γ-glutamylcysteine synthetase.[24] Interestingly, the increased SOD and GSH due to a chain of reaction by TDFPE administration in this study may indicate that rats treated with the extract experienced increased reactive oxygen species concentration. This observation was evidenced by the increase in hepatic and renal MDA concentration, which required activating the antioxidant system in this study. This finding is supported by studies on flavonoid-rich plants that reported toxicity in the liver and kidney.[25,26]

The liver is an essential tissue required for survival and detoxification roles in animals, with liver enzymes such as ALT and AST assessed as the hallmark of hepatic injury.[22,27] The rise in ALT, AST and BIL levels may be

a result of the oxidative stress on hepatic membranes, which are made up of lipid conjugates, particularly polyunsaturated fatty acids, which are highly susceptible to lipid peroxidation contributing to the increased permeability of these enzymes into the plasma.[28] The high level of bilirubin may result from obstructive jaundice due to dyslipidaemia-induced hepatic damage. This was further buttressed by the presence of inflammatory cells and distended sinusoids altering the liver histological profile of the rats treated with TDFPE. In addition, some hepatotoxicants might be responsible for the change in histological microarchitecture, which agrees with a previous study on bitter melon.[29]

Derangement of the plasma lipid profile is often associated with increased cardiovascular risk, poor overall health and dyslipidaemia.[30] An increase in triglyceride and LDL levels could be due to the reduced catabolism of triglyceride, which occurs as a result of a decreased stimulation of the lipolytic activity of plasma lipoprotein lipase.[31] The observed decrease in HDL might be due to the fruit extract's chain of oxidative stress reaction on liver lipid metabolism.[32] Hence, phytochemicals such as alkaloids and terpenoids in the extract may contribute to the resulting dyslipidaemia.[3] The inflammatory cells observed in the hepatic tissues may have been triggered by increased triglyceride, cholesterol and LDL concentrations.

Conclusion

It can be concluded that T. daniellii fruit pulp may exacerbate oxidative stress, dyslipidaemia and induce hepatic injury. Hence, caution should be taken on the quantity used to prepare ethnobotanical infusions and incorporated into animal feed. Further studies can be carried out to identify phytochemicals responsible for inducing these abnormal activities and their toxicokinetics.

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