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Research Article

Ficus thonningii Stem Bark Extracts Prevent High Fructose Diet Induced Increased Plasma Triglyceride Concentration, Hepatic Steatosis and Inflammation in Growing Sprague-Dawley Rats

Eliton Chivandi[1*], Yvonne Mhosva[1], Trevor Nyakudya[2], Pilani Nkomozepi[3]

[1]Department of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[2]Department of Physiology, Faculty of Health Sciences, University of Pretoria, Arcadia, South Africa
[3]Department of Human Anatomy and Physiology, Faculty of Health Sciences, University of Johannesburg, Johannesburg, South Africa

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ABSTRACT

Background: *Ficus thonningii* extracts exhibit hypoglycaemic, hypolipidaemic and antioxidant activities. We investigated the potential of methanolic *F. thonningii* stem-bark extracts (MEFT) to protect growing Sprague-Dawley (SD) against high-fructose diet-induced Metabolic Derangements (MD) in a model mimicking children fed obesogenic diets.

Methods: Eighty (40 males; 40 females) 21-days old SD rat pups were randomly allocated to and administered, for 8 weeks, five treatment regimens: 1-standard rat chow (SC)+water (PW), 2-SC+20% (w/v) fructose solution (FS), 3-SC+FS+fenofibrate at 100 mg/kg bwt/day, 4-SC+FS+low dose MEFT (LD; 50 mg/kg bwt/day) and 5-SC+FS+high dose MEFT (HD; 500 mg/kg bwt/day). Body weight, glucose load tolerance, fasting blood glucose and triglyceride, plasma insulin concentration, sensitivity to insulin, liver mass and fat content, steatosis and inflammation were determined.

Results: Fructose had no effect on the rats' growth, glucose and insulin concentration, glucose tolerance and insulin sensitivity (P>0.05) but increased triglycerides in females; induced hepatic microsteatosis and inflammation in both sexes but macrosteatosis in females (P<0.05). In females, MEFT prevented fructose-induced plasma triglyceride increase. Low dose MEFT increased liver lipid content in females (P<0.05). The MEFT protected the rats against hepatic steatosis and inflammation but fenofibrate protected against hepatic microsteatosis.

Conclusion: MEFT can be used as prophylaxis against dietary fructose-induced elements of MD but caution must be taken as low dose MEFT increases hepatic lipid accretion in females predisposing to fatty liver disease.

Keywords: *F. thonningii*, Ethnomedicine, Obesity, Lipid profile, Liver disease

***Correspondence:** Eliton Chivandi, Department of Physiology, University of the Witwatersrand, Johannesburg, South Africa, E-mail: Eliton.Chivandi@wits.ac.za

INTRODUCTION

Obesity is a global public health concern with 13% of the global human adult population and 340 million children obese (World Health Organisation, 2018). In sub-Saharan Africa (SSA) 10.6% of the children are obese (Muthuri SK, *et al.*, 2014) and in South Africa 13% of them are obese (Pienaar AE, 2015). Epigenetics contribute to obesity but lack and inadequate exercise and intake of obesogenic diets increases the development of obesity (Sekgala MD, *et al.*, 2018). The risk of developing dyslipidaemia (Zhang YI, *et al.*, 2014), insulin resistance, non-alcoholic fatty liver diseases (Jensen VS, *et al.*, 2018), metabolic syndrome (Matsuzawa Y, 2014) and type II diabetes (Jensen, *et al.*, 2018) is increased in obese individuals.

Metformin is used to manage type II diabetes mellitus and fenofibrate is used to manage dyslipidaemia associated with metabolic syndrome (Kraja A, *et al.*, 2010; Li XM, *et al.*, 2011). These conventional pharmacological agents are monotherapeutic, relatively expensive, inaccessible to the majority global population and elicit side effects (Rodgers RJ, *et al.*, 2012) hence the dire need for less costly, more accessible and less toxic alternatives. Majority of the global population makes use of plant-derived ethnomedicines (Amiot MJ, *et al.*, 2016). Eighty percent of the SSA population (World Health Organisation, 2018) and 27 million South Africans depend on plant-derived ethnomedicines for health care (Oyebode O, *et al.*, 2016). Research on efficacy and safety of these alternative medicines is critical to increasing access to global primary health care. *Ficus thonningii* is an ethnomedicine used to treat a number of conditions (Teklehaymanot T and Giday M, 2007). Its parts and extracts contain tannins, saponins and flavonoids (Egharevba HO, *et al.*, 2015) with antiobesity, antioxidant and antidiabetic activities (Fitrilia T, *et al.*, 2015) making it a potential prophylactic agent against diet-induced metabolic derangements (MDs). We evaluated the prophylactic potential of crude methanolic *F. thonningii* stembark extracts to protect against dietary fructose-induced MDs in growing Sprague-Dawley rats mimicking children fed obesogenic diets.

MATERIALS AND METHODS

Plant collection, identification and extract preparation

Fresh *F. thonningii* stem bark was collected at a farm (GPS: Longitude 20° 13' 47" and latitude 28° 45' 9") in Bulawayo, Zimbabwe. The stem barks and samples of the tree's small branches were transported overnight to the University of the Witwatersrand, South Africa where John Burrows, a nature conservationist, identified and authenticated the plant.

Cut strips of *F. thonningii* stem barks were dried in an oven at 40°C for 24 hours and then milled into a fine powder. The stem bark extract was prepared as described by Musabayane CT, *et al.*, 2007. . (Musabayane CT *et al.*, 2007). Briefly, 25 g of the powder were macerated in 100 mL of 80% methanol (Merck Chemicals, Johannesburg South Africa) for 24 hours with continuous stirring. Immediately thereafter the mixture was filtered using a filter paper (Whattman®, No 1, size 185 mm, pore size 7-11 µm). The filtrate was rotor-evaporator concentrated at 60°C and then dried in an oven at 40°C for 12 hours. The dried extract was stored at 4°C in sealed glass bottles until use.

Study site and ethical clearance

The study, approved by the Animal Ethical Screening Committee of Wits University (AESC number: 2016/05/24/C), was conducted within Wits Research Animal Facility and School of Physiology of Wits University. Handling and procedures on the rats were as per international guidelines on animal use in research.

Rat management

Eighty 21-day olds SD rat pups used were given a 2-day habituation period to familiarize with handling and the experimental environment. Each rat was individually housed in an acrylic cage with a feeding trough and a drinker. Bedding from clean wood shavings was changed twice weekly. Room temperature was maintained at 24 ± 2°C. A light/dark cycle conjuncture maintained: maintained: Lights on from 0700 to 1900 hours. A standard rat chow (Epol RCL Food, Centurion, South Africa) and drinking fluid: Tap water and 20% (w/v) fructose solution, depending on treatment, were availed *ad libitum*.

Study design

Eighty 21-days old SD rat pups (40 males; 40 females) were randomly allocated to and administered, for 8 weeks, five treatment regimens: 1-standard rat chow (SRC)+water (W), 2-SRC+20% (w/v) fructose solution (FS), 3-SRC+FS+fenofibrate at 100 mg/kg bwt/day (FEN), 4-SRC+FS+low dose MEFT (LD; 50 mg/kg bwt/day) and 5-SRC+FS+high dose MEFT (HD; 500 mg/kg bwt/day). Gelatine cubes, vehicles for the administration of *F. thonningii* extract and fenofibrate, were prepared as described by Kamerman (Kamerman PR, *et al.*, 2004). In order to maintain a constant fenofibrate and *F. thonningii* extract dose and assess growth, the rats were weighed twice per week.

Oral glucose tolerance test

Following 54 days on treatments (post-natal day 77), the rats were subjected to an Oral Glucose Tolerance Test (OGTT) following an overnight fast but with *ad libitum* access to drinking water. A Contour-plus glucometer was used to determine the fasting blood glucose concentration with blood from a pin-prick of each rat's tail vein (Loxham SJG, *et al.*, 2007). Immediately thereafter, each rat was gavaged with 2 g/kg body weight of sterile 50% (w/v) D-glucose (Sigma, Johannesburg, South Africa) solution. Post-gavage blood glucose concentrations were measured at 15, 30, 60 and 120 minutes.

Terminal procedures and measurements

After a 48-hour recovery from the OGTT on their respective treatments, the rats were again fasted for 12-hours. Fasting blood glucose and triglyceride concentrations were measured using a calibrated Contour Plus® glucometer and an Accutrend GCT meter. Each rat was then euthanised by intraperitoneal injection of 200 mg/kg bwt sodium-pentobarbitone (Euthanaze, Centaur labs, Johannesburg, South Africa). Each rat's blood, collected into heparinised blood collection tubes *via* cardiac puncture was then centrifuged for 10 min at 5000 × g. Plasma was decanted into microtubes and stored at -20°C pending assay for plasma insulin concentration. Livers were dissected out, each weighed, and a sample preserved in 10% phosphate-buffered formalin and the remainder was frozen-stored at -20°C for liver lipid content determination.

Insulin determination and estimation of insulin resistance

An ELISA (ElabScience Biotechnology, Texas, USA) kit with monoclonal insulin antibodies specific for rat insulin, was used to determine plasma insulin concentration. Absorbencies were read 450 nm off a plate reader (Multiskan Ascent, Lab System Model354, Helsinki, Finland). Insulin concentrations were determined from the constructed standard curve. Fasting blood glucose and plasma insulin data were used to compute fasting whole-body insulin sensitivity and the β-cell function using the homeostasis model assessment of insulin resistance as follows: HOMA-IR=fasting plasma glucose (mg/dL) × fasting plasma insulin (μU/mL)]/405 (Matthews DR, *et al.*, 1985).

Liver lipid content and histology

Liver lipid content was determined as described by the Association of Analytical Chemists (Horwitz W and Latimer GW, 2005) 20 using a Tecator Soxtec apparatus. Assays were done in triplicate. The formalin-preserved liver samples were processed in an automatic tissue processor (Microm STP 120 Thermoscientific, Massachusetts, USA), embedded in paraffin wax, rotary microtome-sectioned (RM 2125 RT, Leica Biosystems, Germany) at 3 μm, mounted on glass slides and then haematoxylin and eosin stained. A Leica ICC50 HD video-camera linked to a Leica DM 500 microscope (Leica, Wetzlar, Germany) captured photomicrographs of stained sections that were analysed using ImageJ software. Stained liver sections were scored semi-quantitatively for macro-/micro-steatosis and inflammation according to Liang (Liang W, *et al.*, 2014). Hepatocellular vesicular micro-/macro-steatosis were analyzed based on the total area of the liver parenchyma affected per camera field (× 20) and scored according to the criteria: Grade 0=<5%; grade 1=5-33%, grade 2=33-66% and grade 3=>66%. The number of inflammatory cell aggregates in the liver parenchyma were counted per camera field (× 100) and scored as follows: Grade 0=none or no foci per camera field; grade 1=0.5 to 1.0 foci per camera field, camera field; grade 2=1-2 foci per camera field; grade 3 ≥ 2 foci per camera field (×100).

Statistical analysis

Parametric data is presented as mean ± SD and non-parametric data as median and interquartile ranges. GraphPad Prism 6.0 (Graph Pad Software, San Diego, California, USA) was used to analyse data. A repeated measures ANOVA was used to analyse OGTT data. Other parametric data was analyzed using a one-way ANOVA and mean comparisons done *via* Bonferroni *post hoc* test. Kruskal-Wallis test was used to analyse scores for macro-/micro-steatosis and inflammation. Medians of non-parametric data were compared using the Dunns *post hoc* test. Significance was set at P<0.05.

RESULTS

Growth performance and tolerance of an oral glucose load

Figures 1A and 1B show the induction and terminal body masses of the male and female rats, respectively. The induction body weights of the male rats and female rats *(Figures 1a and 1b)*, respectively, were similar. Treatment regimens had no effect (P>0.05) on the rats' terminal body masses but the rats grew significantly during the trial (P<0.05).

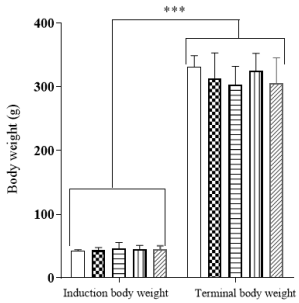

Figure 1a: The induction and terminal body weights of male rats
***** P<0.0001. Mean induction body weights and mean terminal body weights of rats were not significantly different (P>0.05). SC+PW=standard rat chow+plain drinking water+plain gelatine cube; SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body weight/day); SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *Ficus thonningii* extract (50 mg/kg body weight/day. SC+FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *Ficus thonningii* extract (500 mg/kg body weight/day. Data presented as mean ± SD; n=7-8. Note: (▭) SC+PW; (▭) SC+FS; (▭) SC+FS+FEN; (▭) SC+FS+LD; (▭) SC+FS+HD**

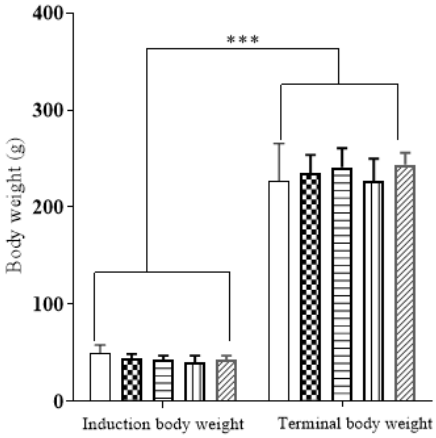

Figure 1b: The induction and terminal body weights of the female rats
***** P<0.0001. The mean induction body weights and mean terminal body weights of female rats were not significantly different (P> 0.05). SC+PW=standard rat chow+plain drinking water+plain gelatine cube; SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body weight/day); SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *Ficus thonningii* extract (50 mg/kg body weight/per day); SC+ FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *Ficus thonningii* extract (500 mg/kg body weight) per day. Data presented as mean ± SD; n=7-8. Note: (▭) SC+PW; (▩) SC+FS; (▭) SC+FS+FEN; (▭) SC+FS+LD; (▨) SC+FS+HD**

Figures 2a and 2b show the area under the curve for male and female rats, respectively, calculated from the OGGT data. Treatment regimens had no effect (P>0.05) on tolerance to an oral glucose load.

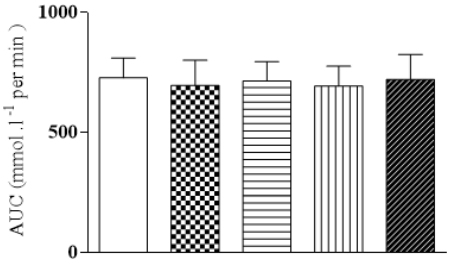

Figure 2a: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on the area under curve of oral glucose tolerance of male rats fed a high fructose diet

No significant difference in the total area under the curve (AUC) of Oral Glucose Tolerance Test (OGTT) for male rats across treatment regimens (P>0.05). SC+PW=standard rat chow+plain drinking water+plain gelatine cube; SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body weight/day); SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *Ficus thonningii* extract (50 mg/kg body weight/day). SC+ FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *Ficus thonningii* extract (500 mg/kg body weight/day). Data presented as mean ± SD; n=7-8 Note: (▭) SC+PW; (▩) SC+FS; (▭) SC+FS+FEN; (▭) SC+FS+HD; (▨) SC+FS+LD

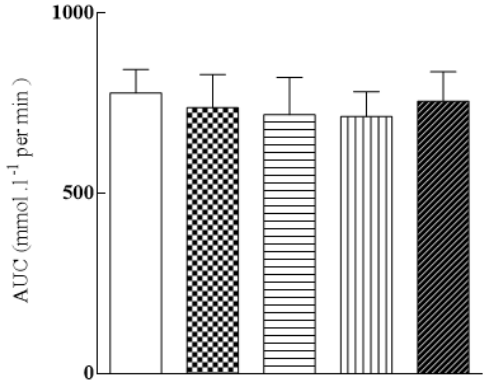

Figure 2b: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on total area under curve of oral glucose tolerance of female rats fed a high fructose diet

***No significant difference in the total area under the curve of oral glucose tolerance test for female rats across treatment regimens (P>0.05). SC+PW=standard rat chow+plain drinking water+plain gelatine cube; SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body weight/day); SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *Ficus thonningii* extract (50 mg/kg body weight/day). SC+FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *Ficus thonningii* extract (500 mg/kg body weight/day. Data presented as mean ± SD; n=7-8. Note: (▭) SC+PW; (▩) SC+FS; (▭) SC+FS+FEN; (▭) SC+FS+LD; (▨) SC+FS+HD**

Circulating metabolites concentration and insulin sensitivity

Effects of the methanolic *F. thonningii* stem bark extracts on blood glucose and triglyceride and plasma insulin concentrations and HOMA-IR of the male and female rats are shown in *tables 1a and 1b*, respectively. The triglyceride, glucose and insulin concentrations and HOMA-IR index of male rats across treatment regimens was similar (P>0.05). In females blood glucose and plasma insulin concentration and HOMA-IR index of the rats were similar (P>0.05) across treatments but females administered a high fructose diet alone had higher (P<0.05) plasma triglyceride concentration than rats fed the control diet.

Liver lipid content and liver macro-/micro-morphometry

Figures 3a and 3b show the effects of crude methanolic *F. thonningii* stem-bark extracts (MEFT) on the hepatic lipid content of male and females rats, respectively, fed a high-fructose diet. Hepatic lipid content of male rats was similar across treatment regimens. Female rats fed a high fructose diet and the low dose of the crude MEFT had significantly more hepatic lipid compared to that of rats administered other treatments (P<0.001). *(Figures 2a and 2b)* show the effects of crude MEFT on the liver weights, hepatic steatosis and hepatic inflammation of male and female rats, respectively, fed a high-fructose diet. Male rats fed the high fructose diet had higher hepatic micro-steatosis and inflammation scores compared to control (P<0.05). Relative to body mass, fenofibrate caused heavier livers in both male and female rats but elicited hepatic inflammation only in males (P<0.05). The low and high dose MEFT prevented the fructose-induced hepatic micro-steatosis and inflammation. Fenofibrate prevented microsteatosis in males *(Figure 4a and Table 2a)*. Dietary fructose induced macro- and micro-steatosis and hepatic inflammation (P<0.05) in female rats which were prevented by both MEFT doses but not fenofibrate *(Figure 4b and Table 2b)*.

Table 1a: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on blood triglyceride, glucose and insulin concentration and HOMA-IR index of male rats fed a high-fructose diet

Parameter	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance
Triglyceride (mmol/L)	1.38 ± 0.61[a]	1.9 ± 0.93[a]	1.51 ± 0.55[a]	1.39 ± 0.36[a]	1.39 ± 0.24[a]	ns
Glucose (mmol/L)	4.54 ± 0.77[a]	4.26 ± 0.64[a]	4.49 ± 0.54[a]	3.93 ± 0.41[a]	4.26 ± 0.57[a]	ns
Insulin (µg/L)	38.68 ± 29.2[a]	37.02 ± 20.9[a]	31.42 ± 18.99[a]	29.02 ± 21.7[a]	29.59 ± 13.68[a]	ns
HOMA-IR	7.73 ± 5.60[a]	6.82 ± 3.69[a]	5.99 ± 3.57[a]	5.21 ± 4.13[a]	5.40 ± 2.61[a]	ns

Note: Where ns: not significant; P>0.05
Rats' mean triglyceride, glucose and insulin concentrations and HOMA-IR indices were similar across treatment regimens. SC+PW=standard rat chow+plain drinking water+plain gelatine cube; SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube

Table 1b: Effects of crude methanolic *Ficus thonningii* stem-bark extracts on blood triglyceride, glucose and insulin concentration and HOMA-IR index of female rats fed a high-fructose diet

Parameter	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance
Triglyceride (mmol/L)	1.48 ± 027[a]	2.04 ± 0.33[b]	1.60 ± 0.25[ab]	1.60 ± 0.35[ab]	1.43 ± 0.34[ac]	*
Glucose (mmol/L)	4.26 ± 0.64[a]	4.24 ± 0.48[a]	4.49 ± 0.54[a]	3.94 ± 0.46[a]	4.34 ± 0.72[a]	ns
Insulin (µg/L)	27.85 ± 13.47[a]	28.01 ± 19.27[a]	24.67 ± 18.65[a]	22.71 ± 15.77[a]	37.57 ± 20.6[a]	ns
HOMA-IR	5.07 ± 3.34[a]	5.09 ± 3.32[a]	4.85 ± 3.51[a]	4.52 ± 3.25[a]	7.09 ± 3.35[a]	ns

Note: Where ns: not significant; P>0.05; *P<0.05; [abc]: Within row means with different superscripts are significantly different (P<0.05)
Rats fed a high fructose diet alone had significantly higher blood triglyceride concentration compared to control rats (P<0.05). Rats fed a high fructose diet with a high dose of F.thonningii as an intervention had significantly lower blood triglyceride concentration P(<0.05) compared to that of rats fed a high fructose diet alone. SC+PW=standard rat chow+plain drinking water+plain gelatine cube; SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body mass/day); SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *Ficus thonningii* extract (50 mg/kg body mass/day). SC+FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *Ficus thonningii* extract (500 mg/kg body mass/day). Data presented as mean ± SD; n=7-8

Table 2a: Effect of crude methanolic *Ficus thonningii* stem-bark extract on the macro- and micro-morphometry of the liver from male rats fed a high-fructose diet

Parameters	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance
Liver (g)	11.10 ± 1.20[a]	10.50 ± 0.98[a]	13.30 ± 3.09[a]	12.00 ± 3.06[a]	12.00 ± 3.06[a]	ns
Liver (% bwt)	3.35 ± 0.34a[b]	2.56 ± 1.49[a]	4.44 ± 1.23[b]	3.50 ± 0.51[ab]	3.47 ± 0.33[ab]	**
Liver (rTL g/mm)	27.50 ± 3.16[a]	25.50 ± 2.37[a]	34.10 ± 7.90[a]	30.2 ± 7.43[a]	27 ± 4.84[a]	ns
Macrosteatosis	0(0;0)[a]	0(0;0)[a]	0(0;0)[a]	0(0;0)[a]	0(0;0)[a]	ns
Microsteatosis	0(1;0)[a]	1(2;1)[b]	0.5(1;0)[a]	0.5(1;0)[a]	0.5(1;0)[a]	*
Inflammation	0(1;0)[a]	1(1;0)[b]	1(1;0)[b]	0(1:0)[a]	0(1;0)[a]	*

Note: ns: not significant, P>0.05; *P ≤ 0.05; **P<0.01; [ab]: Within row means with different superscripts are significantly different at P<0.05).
Rats fed a high fructose diet with fenofibrate had significantly heavier livers compared to the rats fed the control diet (P<0.01). Rats fed a high fructose diet alone had a significantly higher (P<0.05) micro-steatosis score compared to the rats in other treatment regimens. Rats fed a high fructose diet and those fed a high fructose diet with fenofibrate as an intervention had significantly higher (P<0.05) hepatic inflammation scores than their counterparts administered other treatment regimens. SC+PW=standard rat chow+plain drinking water+plain gelatine cube; SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body weight/day); SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *Ficus thonningii* extract (50 mg/kg body weight/day). SC+FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *Ficus thonningii* extract (500 mg/kg body weight/day). Data presented as mean ± SD, median and IQR; n=7-8

Table 2b: Effect of crude methanolic *Ficus thonningii* stem-bark extract on the macro-and micro-morphometry of the liver from female rats fed a high-fructose diet

Parameters	SC+PW	SC+FS	SC+FS+FEN	SC+FS+LD	SC+FS+HD	Significance
Liver (g)	7.35 ± 2.12[a]	8.07 ± 2.03[a]	9.73 ± 1.74[a]	8.02 ± 1.39[a]	8.54 ± 1.58[a]	ns
Liver (% bwt)	3.19 ± 0.39[a]	3.4 ± 0.60[a]	4.02 ± 0.49[b]	3.52 ± 0.39[ab]	3.50 ± 0.59[ab]	*
Liver (rTL g/mm)	18.90 ± 5.80[a]	21.50 ± 5.36[a]	26.00 ± 3.85[a]	21.60 ± 4.22[a]	24.00 ± 3.94[a]	ns
Macrosteatosis	0(0;0)[a]	2.5(3.0; 2.0)[b]	0(0;0)[a]	0(0;0)[a]	0(0;0)[a]	***
Microsteatosis	0(1;0)[a]	2(3;1)[b]	1(2;1)[a]	0.5(1;0)[a]	0(0;0)[a]	*
Inflammation	0(0;0)[a]	1(1;0)[b]	0(1;0)[ab]	0(0:0)[a]	0(0;0)[a]	**

Note: Ins: not significant; P>0.05; *P<0.05. **P<0.01; [ab]: Within row means with different superscripts are significantly different at P<0.05.
Rats fed a high fructose diet with fenofibrate had significantly heavier livers) compared to the rats fed the control diet (P<0.01). Rats fed a high fructose diet had significantly higher steatosis scores compared to rats fed a control diet or a high fructose diet with fenofibrate or *F. thonningii* extracts (P<0.05). SC+PW=standard rat chow+plain drinking water+plain gelatine cube; SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body weight/day); SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *F. thonningii* extract (50 mg/kg body weight/day). SC+FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *F. thonningii* extract (500 mg/kg body weight/day). Data presented as mean ± SD, median and IQR; n=7-8

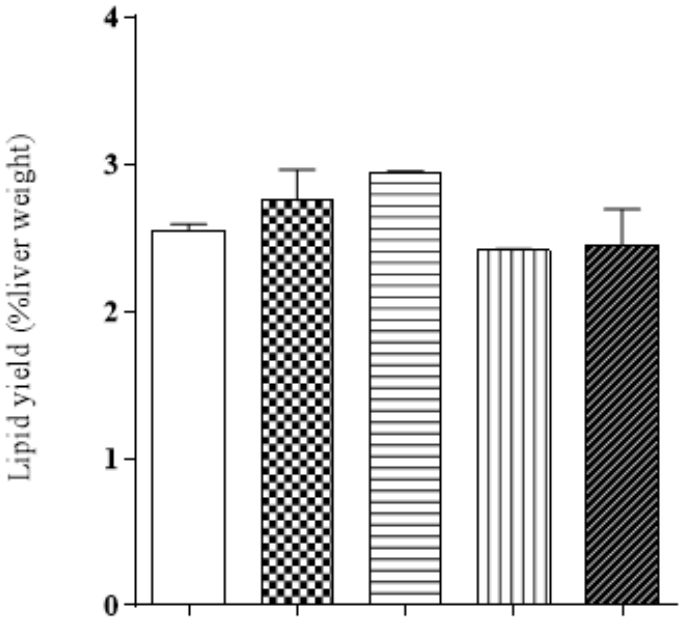

Figure 3a: Effect of crude methanolic *F. thonningii* stem bark extracts on liver lipid content of male rats fed a high fructose diet Rats' liver lipid content similar (across treatment regimens P>0.05). SC+PW=standard rat chow+plain drinking water+plain gelatine cube; SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body weight/day); SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *F. thonningii* extract (50 mg/kg bodyweight/day). SC+ FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *F. thonningii* extract (500 mg/kg body weight/day). Data presented as mean ± SD; n=7-8. Note: (▭) SC+PW; (▩) SC+FS; (▤) SC+FS+FEN; (▨) SC+FS+HD; (▥) SC+FS+LD

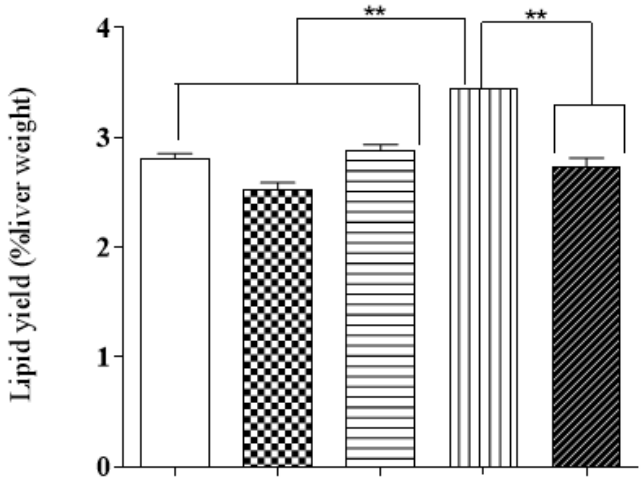

Figure 3b: Effect of crude methanolic *F. thonningii* stem bark extracts on liver lipid content of male rats fed a high fructose diet Rats' liver lipid content similar (across treatment regimens P>0.05). SC+PW=standard rat chow+plain drinking water+plain gelatine cube; SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body weight/day); SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *F. thonningii* extract (50 mg/kg bodyweight/day). SC+ FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *F. thonningii* extract (500 mg/kg body weight/day). Data presented as mean ± SD; n=7-8. Note: (▭) SC+PW; (▩) SC+FS; (▤) SC+FS+FEN; (▨) SC+FS+HD; (▥) SC+FS+LD

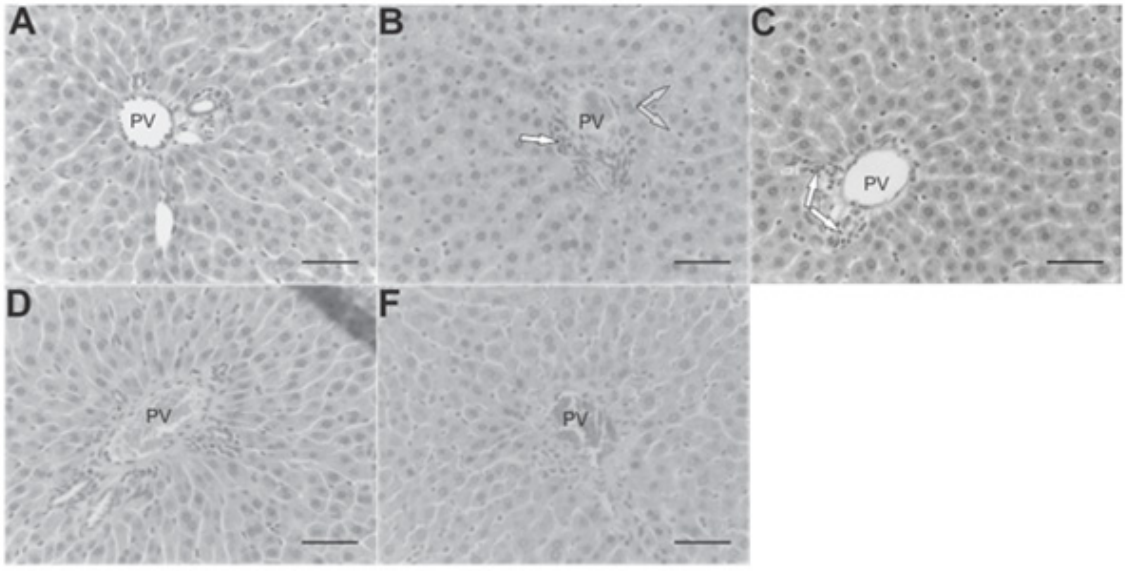

Figure 4a: Representative photomicrographs showing histopathological features of the haematoxylin and eosin (HE, × 40) stained liver sections of male rats from each treatment group.
A: SC+PW=standard rat chow+plain drinking water+plain gelatine cube (n=7); B: SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; C: SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body weight/day); D: SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *F. thonningii* extract (50 mg/kg body weight/day; E: SC+ FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *F. thonningii* extract (500 mg/kg body weight/day). PV=portal vein. White, grey and black arrows indicate inflammatory cell aggregates, micro and macro steatosis respectively; Scale bar=50 μm

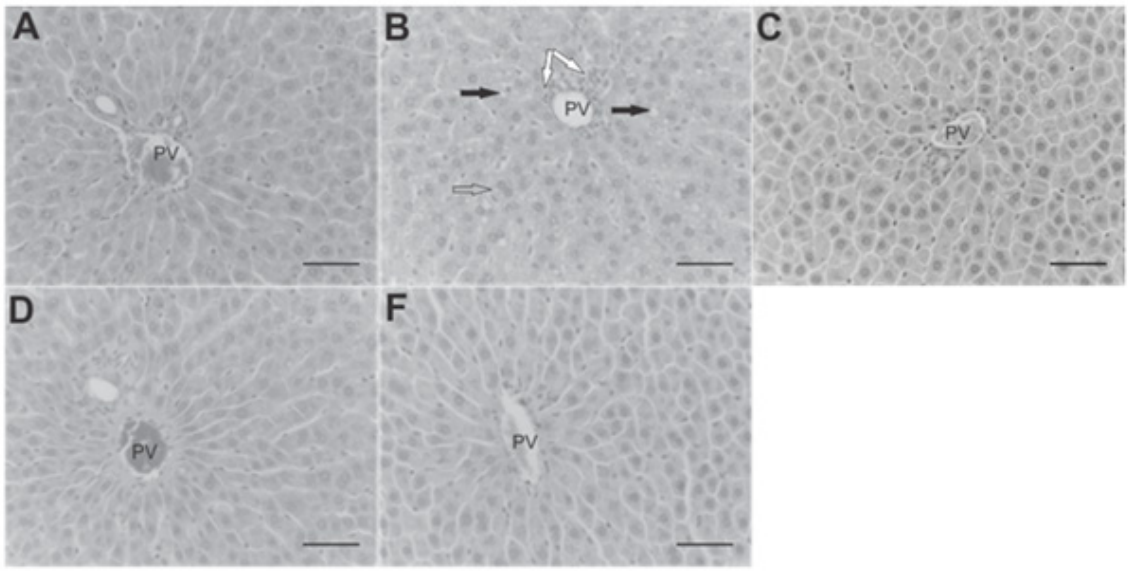

Figure 4b: Representative photomicrographs showing histopathological features of the haematoxylin and eosin (HE, × 40) stained liver sections of female rats from each treatment group.
A: SC+PW=standard rat chow+plain drinking water+plain gelatine cube (n=7); B: SC+FS=standard rat chow+20% fructose (FS) in drinking water (w/v)+plain gelatine cube; C: SC+FS+FEN=standard rat chow+20% FS in drinking water (w/v)+fenofibrate (100 mg/kg body weight/day); D: SC+FS+LD=standard rat chow+20% FS in drinking water (w/v)+low dose *F. thonningii* extract (50 mg/kg body weight/day) SC+ FS+HD=standard rat chow+20% FS in drinking water (w/v)+high dose *F. thonningii* extract (500 mg/kg body weight/day). PV=portal vein. White, grey and black arrows indicate inflammatory cell aggregates, micro and macro steatosis, respectively. Scale bar=50 μm

DISCUSSION

Dietary fructose alone and or with fenofibrate or crude MEFT as interventions had no effect on rats' growth performance suggesting that these interventions did not compromise growth. Our findings disagree with Pektaş (Pektaş MB, *et al.*, 2015; Toop and Gentili, 2016) who observed increases in body weight of fructose-fed rats. We contend that the difference in our findings was due to differences in the age of the rats. We used weanling growing rats that are known to channel "extra" fructose calories to support growth and development unlike adult rats that accrete "excess" calories as adipose tissue. The lack of effect of dietary fructose on body weight we report is in tandem with Grau (Grau MM, *et al.*, 2018) observations of similarities in terminal body weight of adolescent SD rats fed 60% fructose solution as a drinking fluid. Badiora (Badiora AS, *et al.*, 2016) showed that orally administered *F. thonningii* stem-bark extracts increased the body weight of rats. In our study the crude MEFT neither compromised nor promoted rat growth thus they can used without the risk of compromising animal and or child growth.

Dietary fructose-induced hyperglycaemia, deranged lipid profile and insulin resistance, is well documented (Bellamkonda R, *et al.*, 2018). Our findings show that the high-fructose diet alone one and or with fenofibrate or crude MEFT as interventions did not alter the rats' glucose and insulin concentrations and HOMA-IR indices but the chronic consumption of dietary fructose increased female rats' blood triglyceride concentration. Thus we infer that the consumption of a high fructose diet for 8 weeks induced hypertriglyceridaemia in female rats only but did not elicit hyperglycaemia and insulin resistance in growing male and female rats. Crude MEFT and fenofibrate did not elicit dysregulation of blood glucose and insulin concentration. Grau MM, *et al.* contend that fructose consumption stimulates *de novo* hepatic lipogenesis (DNL) which increases blood triglycerides in rodents. We report, in female rats, significant increase in plasma triglycerides with chronic fructose consumption compared to control counterparts and contend that increased *de novo* hepatic lipogenesis generates triglycerides that are exported to the systemic circulation hence the increase in plasma triglycerides. We showed similarities in the plasma triglyceride concentration of rats administered the control and that of counterparts fed the high-fructose diet with fenofibrate and or crude MEFT. This demonstrates that both orally administered of low and high dose MEFT and fenofibrate prevented dietary-fructose mediated plasma triglyceride concentration increase in female rats. Crude MEFT can be used as prophylaxis against fructose-induced hypertriglyceridaemia in growing female rats and possibly girl-children.

Mapfumo, *et al.* (Mapfumo M, *et al.*, 2020; Lê KA, *et al.*, 2009) reported that fructose-rich diets caused hepatic lipid accretion in growing rats' livers but we show that dietary fructose did not impact the rats' liver lipid across treatment regimens suggesting that dietary fructose *per se* and or with either orally administered high dose MEFT or fenofibrate did not alter liver lipid storage of the rats. We also show that female rats fed a high-fructose diet with the low dose MEFT had the highest liver lipid content. This suggests that the low dose MEFT may stimulate excessive hepatic lipid accretion and thereby predispose female rats to higher risk of developing fatty liver diseases. Therefore, despite its prophylactic potential against elements of dietary fructose induced MD, use of low dose MEFT must to be with caution in growing females.

In growing rats we show that chronic dietary fructose intake elicited hepatic inflammation in both rat sexes, micro-and macro-steatosis in females and micro-steatosis in males. In male rats crude MEFT and fenofibrate prevented the dietary fructose induced hepatic micro-steatosis but hepatic inflammation was only prevented by the MEFT. The low and high dose MEFT and fenofibrate mitigated the dietary fructose-induced hepatic steatosis and inflammation in female rats. The crude MEFT appear more efficacious in protecting against dietary fructose induced steatosis and inflammation compared to fenofibrate which did not attenuate dietary fructose induced hepatic inflammation in growing male rats. We speculate that the mutli-therapeutic effects of phytochemicals in crude MEFT make them better prophylactic agents compared to the monotherapeutic fenofibrate.

CONCLUSION

Fructose elicited hypertriglyceridaemia in a sexually dimorphic manner and caused hepatic inflammation and steatosis in both rat sexes. Crude low and high dose MEFT prevented dietary fructose induced hypertriglyceridaemia, hepatic inflammation and steatosis hence they can be used as prophylaxis against elements of diet-induced MD in growing SD rats and maybe in children. Caution must be taken as low dose MEFT can predispose females to increased risk of developing fatty liver disease.

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