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Original Article

Letrozole ameliorates fructose-induced hyperlipidaemia and uric acid accumulation in male Wistar rats

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ABSTRACT

Introduction: High fructose consumption is commonly associated with increased risk of cardiovascular disease (CVD). However, cardiovascular effects of aromatase inhibitors remain unresolved, although they are effective in the treatment of breast cancer. Thus, this study investigated the effect of letrozole on CVD indicators in Wistar rats exposed to high fructose intake.

Methods: Twenty male rats were randomly placed in four groups (n=5/group): control (distilled water), fructose (10% fructose in drinking water), letrozole (1mg/kg) and fructose+ letrozole. After 21-day exposure, fasting blood glucose was taken and the rats were sacrificed, while blood and heart were collected and prepared for biochemical analyses.

Results: Our data showed that 10% fructose induced hyperglycaemia and lipid peroxidation. It reduced serum high-density lipoprotein cholesterol, elevated serum total cholesterol (TC), triglycerides and free fatty acid but did not alter serum low-density lipoprotein cholesterol significantly, when compared with the control. Furthermore, high fructose-intake increased serum or cardiac adenosine deaminase (ADA), xanthine oxidase and uric acid. Our findings revealed that letrozole, when taken with 10% fructose, attenuated all the observed fructose-induced alterations. However, when administered alone, letrozole elevated serum TC as well as cardiac malondialdehyde and ADA.

Conclusion: This study showed that high fructose-intake promoted the risk of CVDs in rats, while administration of letrozole attenuated fructose effects. Hence, letrozole may serve as a potential adjuvant therapy for attenuating CVD risk. However, further pre-clinical and clinical findings are necessary to thoroughly investigate the cardiometabolic effects of letrozole.

Keywords:
CVD
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Cardiovascular diseases (CVDs) remain the commonest forms of non-communicable diseases and the leading causes of global death, claiming about 17.8 million lives, especially in middle-income countries (Roth et al., 2018). Several factors such as cardiometabolic, behavioral, environmental and social risk factors have been established as the main causes of CVD worldwide (Roth et al., 2018). While the etiology of CVDs is multifactorial, high intake of added sugar has been associated with the risk of CVDs mortality (Yang et al*.,* 2014).

Fructose is a monosaccharide that is commonly used as an additive in products like soft drinks and biscuits. It is metabolized mainly by fructokinase or ketohexokinase (Khitan and Kim, 2013). Since fructokinase lacks negative feedback mechanism, continued fructose metabolism depletes intracellular phosphate, activates adenosine monophosphate deaminase and generates uric acid formation (Tappy and Lê, 2010). Meanwhile, high level of serum uric acid is an independent predictor of coronary artery disease which promotes CVD morbidity and mortality in general adult population (Bickel et al., 2002; Peng et al., 2015). More importantly, fructose can serve as a substrate for *de novo* lipogenesis and lipid production (Malik and Hu, 2015). Hence, studies have associated high fructose intake with dyslipidaemia- a hallmark feature of CVD (Rutledge and Adeli, 2007; Olaniyi and Olatunji, 2019).

Currently, the non-steroidal aromatase inhibitors (AIs) including letrozole, are considered as the preferred adjuvant treatment options for post-menopausal women with oestrogen receptor-positive breast cancer (Early Breast Cancer Trialists' Collaborative Group, 2015). Functionally, AIs inhibit aromatase activity and suppress the peripheral oestrogen production- the major source of oestrogen synthesis in post-menopausal women (Geisler et al*.,* 2002). Oestrogen is physiologically important as it prevents CVD risk before menopause, while its post-menopausal loss increases both incidence and severity of the disease (Hasan et al*.,* 2021). Nonetheless, the exact effect of AIs on cardiovascular system remains controversial (Blondeaux et al*.,* 2016; Abdel-Qadir et al*.,* 2016; Kamaraju et al*.,* 2019; Khosrow-Khavar *et al.,* 2020). Similarly, while some studies have associated the use of AIs with hypercholesterolemia (Bundred, 2005; Buzdar et al*.,* 2006), others found no effect on serum cholesterol level (Wasan et al*.,* 2005; Hozumi et al*.,* 2011). Hence, more studies are needed to elucidate the cardiovascular effect of AIs. In this study, we investigated the effects of letrozole in fructose-exposed Wistar rats. Although letrozole is commonly prescribed for breast cancer treatment, male rats were used in this study to investigate cardiovascular and possible therapeutic effects of letrozole on fructose-induced cardiometabolic disorder, independent of sex. Future studies will focus on its role in female rats with or without endogenous oestradiol.

Materials and methods

Experimental animals

Twenty male Wister rats with an average weight of 106.04g were obtained from the animal holdings of Ladoke Akintola University of Technology Ogbomosho, Oyo state, Nigeria. The rats were transferred to the animal house, Zoology Department, University of Ilorin, Ilorin, Nigeria. They were fed with standard rat chow and were given clean tap water, *ad libitum*. After 2 weeks of acclimatization, the rats were randomly grouped into four (n=5/group). They were kept and maintained under standard conditions of temperature, relative humidity and 12h day/night cycle. Handling of the rats were in conformity to the regulation of the University of Ilorin Ethical Committee (UERC/ASN/2016/513) and in accordance with the National Institutes of Health guidelines on the care and use of laboratory animal.

Treatment

Control group received distilled water daily via oral route. Fructose group received 10% fructose (Sigma, USA; w/v) in drinking water (Olaniyi and Olatunji, 2019). Letrozole group was administered orally with letrozole (Sun Pharma, India, 1mg/kg body weight) (Xu et al., 2020), while fructose + letrozole group received a combination of 10% fructose (w/v) in drinking water and 1mg/kg letrozole. Food consumption and water intake rates were monitored daily, while body weight was measured weekly. The treatments continued for 3 weeks.

Fasting blood glucose and sample preparation

At the end of the experiment, fasting blood glucose (FBG) was measured using Accu-Chek® Active (Roche Diagnostics GmbH, Mannheim, Germany). Thereafter, the animals were sacrificed by cervical dislocation and blood was collected through cardiac puncture into plain

bottle. The blood was then left to settle at room temperature and later centrifuged at 3000 rpm for 15min to obtain serum. Serum was stored frozen, prior to use, for biochemical assays. The heart was quickly removed, cleared of attached connective tissues and homogenized as previously reported for biochemical analyses (Abdulkareem et al*.,* 2019).

Biochemical assays

Fortress Diagnostics Limited, Antrim, United Kingdom assay kits were used for all the biochemical parameters, except otherwise stated. Procedures for carrying out the assays followed strictly instructions provided by the kit's manufacturer and Readings were taken (throughout) by Molecular Devices Spectramax 250 Microplate Reader, Sunnyvale, USA. The actual values of each parameter were generated through interpolation from the standard curves. Brief protocols for each of the assays are given below:

Malondialdehyde (MDA)

Estimation of MDA was based on thiobarbituric acid (TBA) assay. Briefly, 0.5ml of serum samples was added to 2.5ml of 20% tricholoroacetic acid in a 10ml test tube and the mixture was shaken vigorously. Then, 1ml of 0.6% TBA was added to the mixture, shaken (to form MDA-TBA adduct), incubated at 95°C for 1h and then cooled on ice bath for 15min. The 200µl of MDA-TBA adduct was then pipetted into a 96-well microplate in duplicate and the absorbance was measured at a wavelength of 532nm.

High density lipoprotein cholesterol (HDL-c)

Samples were appropriately mixed with Reagent 1, containing lipoprotein antibody and incubated for 5min at 37^{o}C. Thereafter, Reagent 2 (which contains cholesterol oxidase and esterase) was added and incubated for 5min at 37^{o}C to produce a blue colour which was measured at 600nm.

Low density lipoprotein cholesterol (HDL-c)

Samples were mixed with Reagent 1, containing enzyme solution and incubated for 5 min at 37^{o}C. Decomplexing agent was then added and incubated for 5min at 37^{o}C to form a blue colour complex which was measured at 600nm.

Total cholesterol (TC) and triglycerides (TG)

The 200µl of either cholesterol or triglycerides reagent was added to 2µl samples and mixed well. The solution was left to incubate for 15min at 37^{o}C and absorbance was measured at 550nm.

Free fatty acid (FFA)

For the estimation of FFA, 10µl of the sample was pipetted into the wells of 96-well micro titer plate, followed by 2µl of Acyl-CoA Synthetase Reagent. The mixture was protected from light, gently mixed and incubated at 37°C for 30min. Thereafter, 50µl reaction mix (prepared according to the kit's manufacturer specification) was added, incubated for another 30min at 37°C and the reading was taking at 570nm wavelength.

Adenosine deaminase/xanthine oxidase (XO)/uric acid pathway

Nonenzymatic colorimetric assay kit (Oxford Biomedical Research Inc., Oxford, USA) was used for the estimation of serum and cardiac uric acid. The 5µl of samples was pipetted into the wells of 96-well plate in duplicate. Thereafter, 200µl working reagent was added and mixed together by tapping lightly. The mixture was then incubated for 30min at room temperature and absorbance was measured at 520nm. Activity of adenosine deaminase (ADA) was estimated in serum and heart by standard enzymatic colorimetric method. The 50µl of samples was dispensed into sample well of 96-well plate in duplicate. The 50µl of reaction mix was added to each well. The plate was pre-incubated at 37°C for 5min and reading was taking at 293nm. Estimation of XO activity was performed following the same procedures for ADA except that the plate was read immediately at 570nm at T1 to read A1 before being left at 25°C (protected from light) for 20min and measured again at T2 to read A2. The final signal was then taken as: $\Delta A = A2 - A1$

Statistical analysis

All the data were analyzed and presented as mean ± SEM. Statistical analysis was performed using GraphPad Prism software version 8.0 (GraphPad Software, USA) and One-way analysis of variance (ANOVA), followed by Tukey post hoc test, was used to compare the mean values among the groups. The significant difference was determined at 95% confidence level and $P<0.05$, $P<0.01$ and $P<0.001$were considered statisti-

TABLE 1: Effect of fructose (10% w/v), letrozole (1mg/kg body weight) and fructose (10% w/v)+ letrozole (1mg/kg body weight) on average daily food consumption rate per group.

GROUP	food consumption rate (g/day)			
	WEEK ZERO	WEEK ONE	WEEK TWO	WEEK THREE
CONTROL	112.4 ± 8.567	161.14 ± 15.04	139.8 ± 13.98	133.2 ± 9.430
FRUCTOSE	99.47 ± 14.22	125.4 ± 5.986	92.39 ± 9.932	110.9 ± 11.52
LETROZOLE	124.3 ± 23.78	155.0 ± 18.31	83.02 ± 12.06	119.7 ± 9.459
FRUC + LETR	93.45 ± 14.99	103.1 ± 8.416	87.13 ± 17.04	95.53 ± 22.04

Letrozole or fructose did not alter food consumption rate. Data were expressed as mean±SEM (n=3). Data were analyzed by one-way ANOVA, while Tukey post-hoc test was used to compare the mean values among the groups in corresponding week. Fruc + Letr: fructose + letrozole group.

TABLE 2: Effect of fructose (10% w/v), letrozole (1mg/kg body weight) and fructose (10% w/v)+ letrozole (1mg/kg body weight) on average daily water intake per group.

GROUP	Rate of water intake (ml/day)			
	WEEK ZERO	WEEK ONE	WEEK TWO	WEEK THREE
CONTROL	155.3 ± 18.75	183.1 ± 19.74	257.6 ± 30.41	253.4 ± 25.83
FRUCTOSE	177.0 ± 18.45	165.6 ± 9.761	197.9 ± 1.53*	269.4 ± 9.319
LETROZOLE	144.8 ± 6.60	158.4 ± 16.29	216.1 ± 24.77	218.7 ± 18.53*
FRUC + LETR	142.8 ± 11.89	147.3 ± 10.29	175.6 ± 4.16*	188.6 ± 15.66*

Fructose intake did not alter rate of water intake at the end of exposure, when compared with control in corresponding week. In contrast, administration of letrozole reduced water intake by 2nd and 3rd weeks of exposure, when compared with control in corresponding week. Data were expressed as mean±SEM (n = 3). Data were analyzed by one-way ANOVA, while Tukey post-hoc test was used to compare the mean values among the groups in corresponding week. Fruc + Letr: fructose + letrozole group.

cally significant.

Results

Effects of letrozole with or without high fructose intake on body weight gain, food intake and water intake

Table 1 shows that both fructose and letrozole did not significantly affect ($P>0.05$) food consumption rate, when compared with the control in corresponding week. Similarly, fructose intake did not alter rate of water intake at the end of exposure, when compared with the control in corresponding week (Table 2). In contrast, administration of letrozole reduced ($P<0.05$) water intake by 2nd and 3rd weeks of exposure, when compared with the control in corresponding week. Our results also revealed that high fructose intake did not alter ($P>0.05$) percentage body weight gain in Wister rats when compared with the control (Fig. 1). However, treatment with letrozole with or without fructose intake, significantly lowered ($P<0.05$) percentage body weight gain when compared with the control and fructose groups.

Letrozole attenuates fructose-induced hyperglycaemia and lipid peroxidation in Wistar rats

Hyperglycaemia induces generation of oxidative stress and promotes lipid profile derangement, thus, increasing the risk of cardiovascular diseases. As shown in Figure 2a, our data revealed elevated ($P<0.01$) FBG in fructose group, while letrozole treatment attenuated ($P<0.001$) fructose-induced hyperglycaemia. Similarly, high fructose intake significantly increased both serum and cardiac MDA, whereas letrozole alone decreased ($P>0.05$) serum MDA but elevated ($P<0.05$) cardiac MDA level (Fig. 2b), when compared with the control group. In contrast, letrozole treatment in fructose-administered group significantly lowered ($P<0.05$) serum MDA level and relatively reduced ($P>0.05$) cardiac MDA level.

Letrozole lowers serum TC and TG, and elevates HDL-c in rats taking high fructose

As presented in Figure 3, both fructose and letrozole

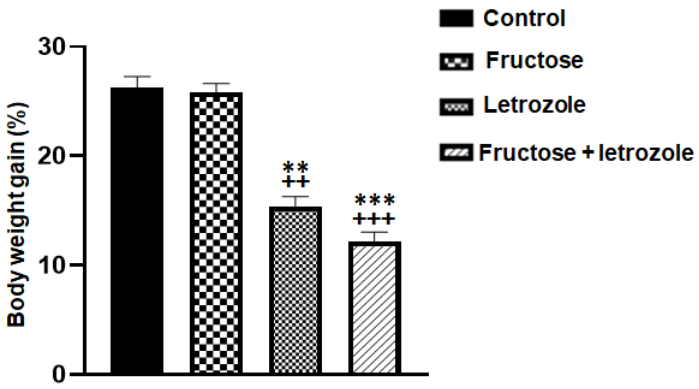

FIGURE 1. Effect of Fructose (10% w/v), Letrozole, and Fructose + letrozole on the body weight of rats. Letrozole treatment with (Fructose + letrozole) or without fructose reduced body weight gain. Data were expressed as mean ± SEM (n = 3). Data were analyzed by One-way ANOVA followed by the Bonferroni post hoc test. ($^{**}P < 0.01$ vs control; $^{***}P < 0.001$ vs control; $^{++}P < 0.001$ vs fructose; $^{+++}P < 0.001$ vs fructose).

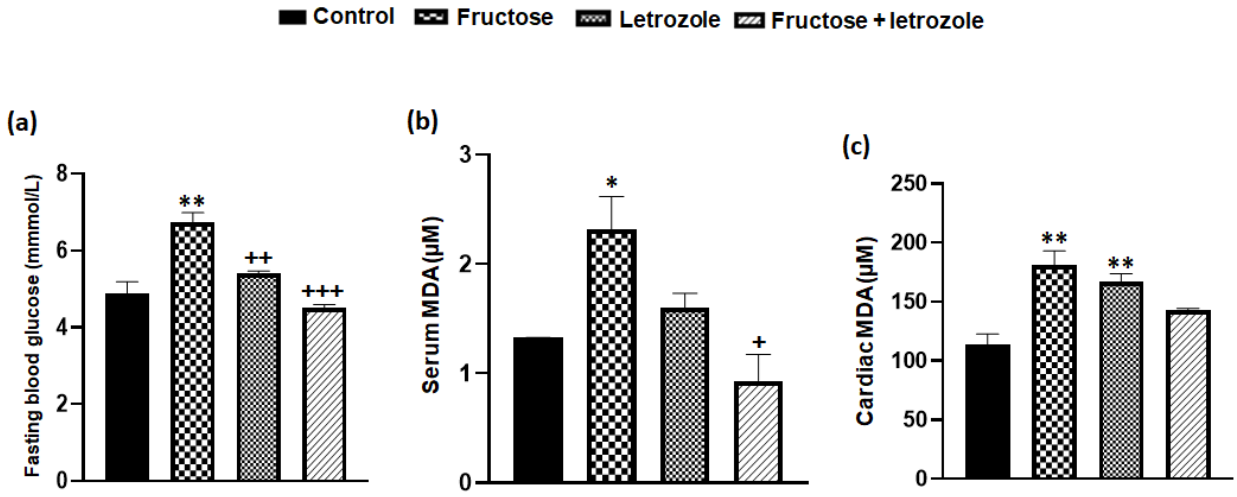

FIGURE 2. Effect of Fructose (10% w/v), Letrozole, and Fructose + letrozole on fasting blood glucose (a), serum malondialdehyde (MDA) (b), and cardiac MDA (c) in Wistar rats. High fructose intake but not letrozole elevated fasting blood glucose and serum MDA, whereas both fructose and letrozole alone increased cardiac MDA. However, treatment with letrozole attenuated fructose-induced hyperglycaemia and lipid peroxidation. Data were expressed as mean ± SEM (n = 3). Data were analyzed by one-way ANOVA followed by the Tukey post hoc test. ($^{*}P< 0.05$ vs control; $^{**}P< 0.01$ vs control; $^{+}P < 0.05$ vs fructose; $^{++}P< 0.01$ vs fructose; $^{+++}P< 0.001$ vs fructose).

alone elevated serum TC ($P<0.001$ and $P<0.01$, respectively), while fructose but not letrozole reduced serum HDL-c ($P<0.05$), elevated serum TG ($P<0.01$) and FFA ($P<0.001$). However, letrozole treatment in fructose-exposed rats alleviated serum TC and TG ($P<0.05$ and $P<0.01$, respectively) but did not significantly lower ($P>0.05$) serum FFA, when compared with rats exposed to fructose alone. The LDL-c was not significantly altered by either fructose or letrozole.

Effects of letrozole with or without high fructose intake on ADA/XO/uric acid pathway

ADA and XO catalyze uric acid formation, thus, promoting risk of CVDs. Increases in serum and cardiac ADA ($P<0.05$ and $P<0.001$, respectively), XO ($P<0.01$, respectively) and uric acid ($P<0.001$ and $P<0.01$, respectively) were observed in fructose-exposed rats (Figs. 4a-f), whereas letrozole alone increased ($P<0.01$) cardiac ADA but reduced serum and cardiac XO ($P<0.01$ and $P<0.05$, respectively), uric acid ($P<0.001$ and $P<0.01$, respectively) and serum ADA ($P<0.01$) as compared with the control group. Administration of letrozole in fructose-exposed rats significantly decreased serum and cardiac ADA ($P<0.01$), XO ($P<0.01$) and uric acid ($P<0.05$ and $P<0.01$, respectively), when compared with the fructose group.

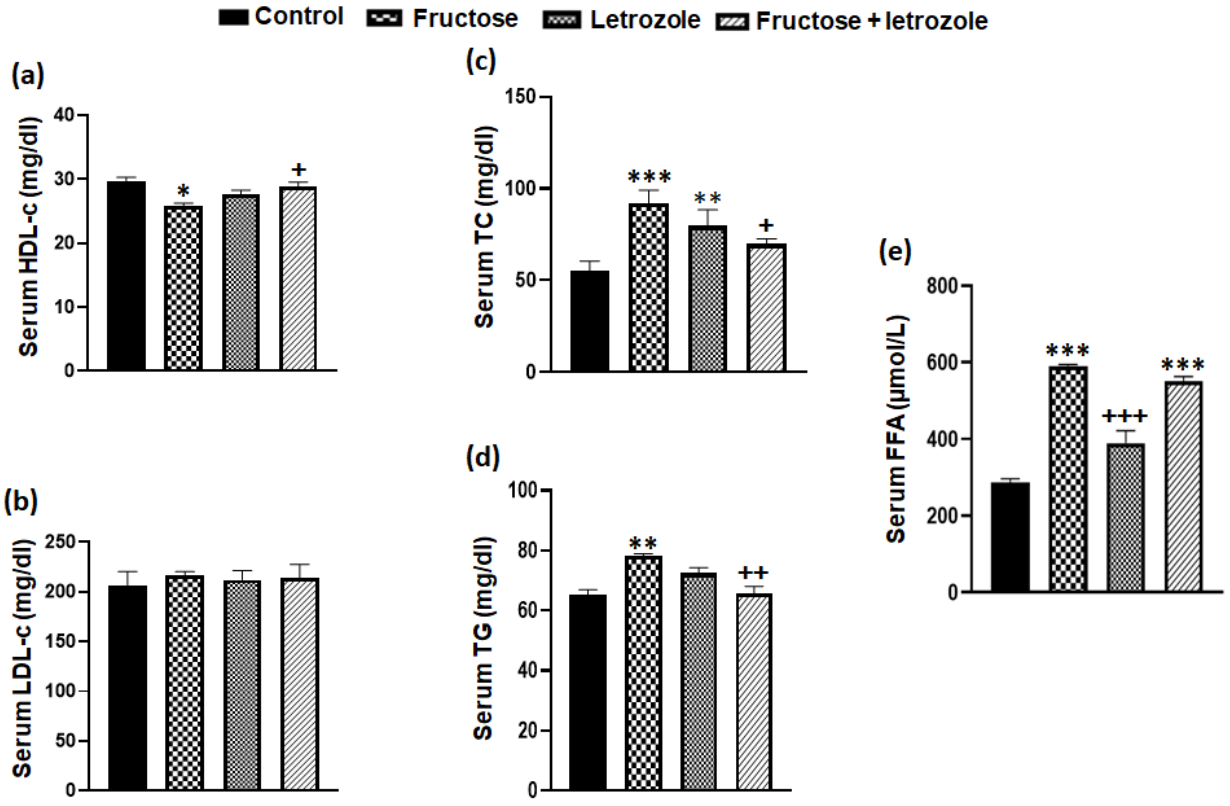

FIGURE 3. Effect of Fructose (10% w/v), Letrozole, and Fructose + letrozole on serum high-density lipoprotein cholesterol (HDL-c) (a), serum low-density lipoprotein cholesterol (LDL-c) (b), serum total cholesterol (TC) (c), serum triglycerides (TG) (d) and serum free fatty acid (FFA) (e) in Wistar rats. Fructose and letrozole individually elevated serum TC, while only fructose elevated serum TG and FFA and lowered HDL-c. However, letrozole administration attenuated fructose-induced elevation of serum TC and TG but did not significantly reduce fructose-induced FFA when compared with the control. LDL-c did not change significantly following fructose or letrozole administration. Data were expressed as mean ± SEM (n = 3). Data were analyzed by one-way ANOVA followed by the Tukey post hoc test. ($^{*}P < 0.05$ vs control; $^{**}P < 0.01$ vs control; $^{***}P < 0.001$ vs control; $^{+}P < 0.05$ vs fructose; $^{++}P < 0.01$ vs fructose; $^{+++}P < 0.01$ vs fructose).

Discussion

This current study investigated the role of letrozole on fructose-induced cardiometabolic disorder in male Wistar rats. We demonstrated that food and water intakes were not affected by high fructose intake but letrozole decreased water intake at the end of the experiment. Similarly, high fructose intake did not interfere with body weight gain, while letrozole lowered percentage gain in body weight. Our findings also revealed elevated levels of FBG, serum and cardiac lipid peroxidation (as evidenced by the rising level of MDA), serum TC, TG, FFA and ADA/XO/uric acid pathway and reduced serum HDL-c in rats exposed to high fructose intake. In contrast, letrozole alone did not affect FBG, serum MDA and TG. Similarly, letrozole alone did not alter serum and cardiac XO, uric acid and serum ADA but increased serum TC as well as cardiac MDA and ADA. Furthermore, our results proved the efficacy of letrozole in attenuating fructose-induced lipid peroxidation, hyperglycemia, dyslipidaemia and uric acid accumulation.

Effect of high fructose intake on food appetite is controversial (Tappy and Lê, 2010). It has been suggested that unlike glucose, fructose reduces the circulating levels of insulin and leptin, decreases postprandial ghrelin concentration and therefore, increases caloric intake, body weight gain and obesity (Teff et al., 2004; Dornas et al., 2015). However, in this study, we did not observe any significant change in food consumption rate due to fructose intake. Our result is in consistence with previous studies from our group (Olaniyi and Olatunji, 2019) and another group (Stanhope et al., 2009). Lack of change in food consumption rate after 3-week of fructose exposure may be due to improved circulating levels of leptin and ghrelin. It has been reported that chronic exposure to high fructose for 1-4 week in healthy individuals increases leptin concentration, suggesting possible long-term suppressing effect of high fructose on food intake (Lê et al., 2006). Similarly, our result that

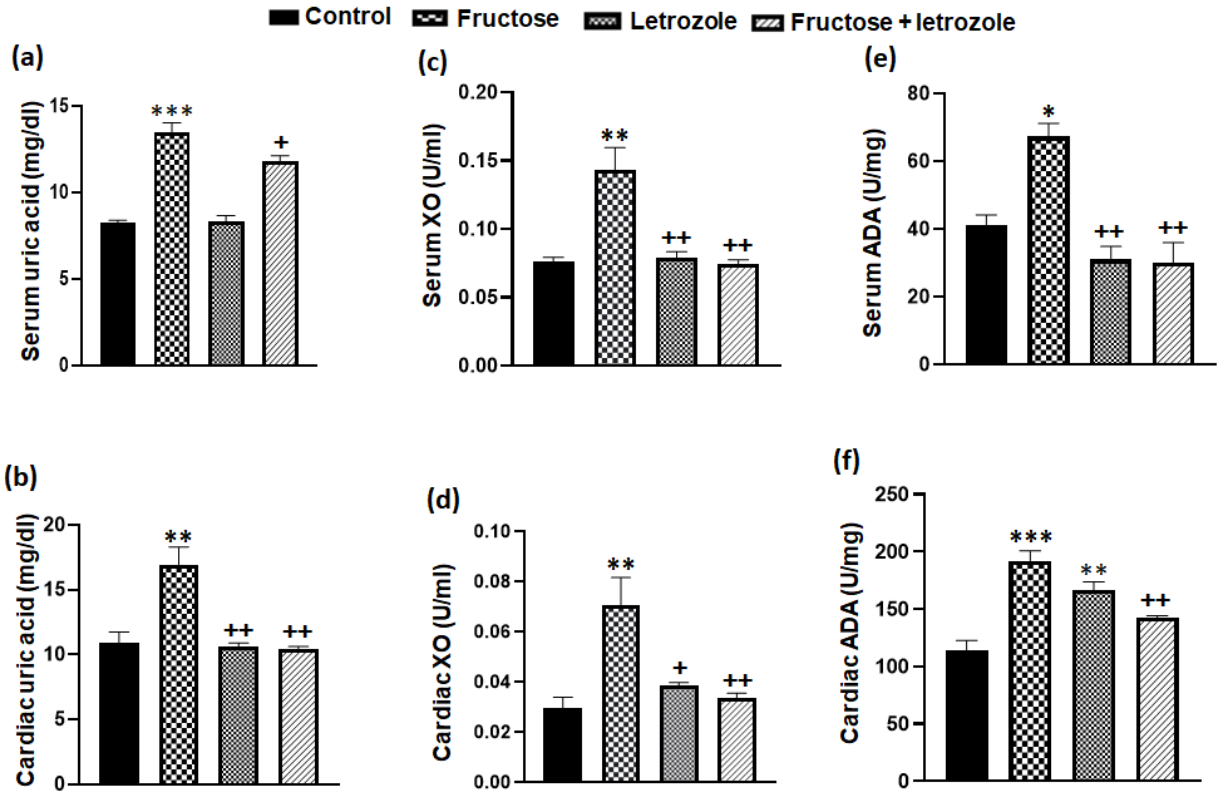

FIGURE 4. Effect of Fructose (10% w/v), Letrozole, and Fructose + letrozole on serum uric acid (UA) (a), cardiac UA (b), serum xanthine oxidase (XO) (c), cardiac XO (d), serum adenosine deaminase (ADA) (e), and cardiac ADA (f) in male Wistar rats. Fructose but not letrozole elevated serum and cardiac uric acid and XO as well as serum ADA, whereas both fructose and letrozole increased cardiac ADA. Administration of letrozole in fructose-taking rats attenuated serum and cardiac UA, XO, and ADA. Data were expressed as mean ± SEM (n = 3). Data were analyzed by one-way ANOVA followed by the Tukey post hoc test. ($^{*}P < 0.05$ vs control; $^{**}P < 0.01$ vs control; $^{***}P < 0.001$ vs control; $^{+}P < 0.05$ vs fructose; $^{++}P < 0.01$ vs fructose; $^{+++}P < 0.001$ vs fructose).

showed no effect of fructose intake on percentage body weight gain agrees with our previous finding (Olaniyi and Olatunji, 2019) as well as finding of others (Ramos et al., 2017), but disagrees with other study (Yoo et al., 2017). This finding may imply that leptin and ghrelin concentrations remain intact in fructose group of the current study. There is evidence that high fructose-induced body weight gain in human is particular to hypercaloric trials (Sievenpiper et al., 2011), therefore, the difference in our finding and that of others may be due to different percentage of fructose used. For instance, we used 10% fructose in drinking water, while Yoo et al. (2017) used 30% fructose diet.

Increased in FBG observed in fructose group of our study agrees with previous studies (Olaniyi and Olatunji, 2019; Lê et al., 2006) and suggests excessive fructose intake as a possible predisposing factor to metabolic syndrome. However, in contrast to study of Skarra et al. (2017) who had earlier reported increased level of FBG in pubertal letrozole-exposed female mice (Skarra et al., 2017), our data showed no significant elevation in FBG level in rats that received letrozole alone. The discrepancy may be due to difference in sex of the animals used and/or duration of exposure. It is well established that hyperglycaemia activates several pathological pathways, including lipid peroxidation, to generate oxidative stress (Furukawa et al., 2017). Interestingly, oxidative stress has been implicated as a major cause of fructose-induced cardiac hypertrophy (Park et al., 2018a). In this study, we demonstrated increased levels of serum and cardiac MDA, following 3-week fructose exposure. This may mean that, high fructose intake leads to hyperglycaemia which consequently results in lipid peroxidation that is capable of generating organs damage, including the heart, and increasing the risk of CVD. Although the mechanism of fructose-induced hyperglycaemia was not investigated in this study, we have previously reported that high fructose intake causes in-

sulin resistance and degenerates β-cell function (Olaniyi and Olatunji, 2019). Our result that revealed increase in cardiac MDA level by letrozole alone, without increase in fasting blood glucose level, may suggest potentiality of letrozole to cause cardiac oxidative stress independent of hyperglycaemia. Meanwhile, administration of letrozole in fructose-exposed rats attenuated fructose-induced hyperglycaemia and oxidative stress, indicating the efficacy of letrozole in maintaining glucose homeostasis and preventing hyperglycaemia-induced oxidative damage during high fructose intake.

Dyslipidaemia is an established risk factor for the development of atherosclerosis and coronary heart disease. It is characterized by elevation of serum TC, LDL-c or TG and reduced serum HDL-c concentration (Hedayatnia et al., 2020). Current estimates put the global incidence of elevated TC alone at about 28.5 million people adult population (aged 20 years or older) with prevalence of 11.9% (Benjamin et al., 2018). This current study demonstrated that fructose increased TC, suggesting that excessive fructose intake promotes the incidence of hypercholesterolaemia. Furthermore, high TG and low HDL-C correlate with metabolic syndrome and are associated with increased risk of atherosclerotic cardiovascular disease (ASCVD) in general population, independent of LDL-c (Vega et al., 2014). Hence, our findings that revealed increased serum TG and decreased serum HDL-c following fructose administration further establish excessive fructose intake as a possible risk factor for ASCVD. Administration of letrozole in fructose-taking rats decreased serum levels of TC and TG, and increased serum HDL-c, which is suggestive of therapeutic efficiency of letrozole in alleviating CVD risk following fructose consumption. However, letrozole alone significantly elevated serum total cholesterol but had no effect on serum triglycerides. This observation concurs with the previous report of Boutas et al. (2015) in ovariectomized rats and it may be an indication that positive influence of estrogen on cholesterol level was compromized when letrozole was administered alone.

Furthermore, this study showed that elevated level of serum TG in fructose group was accompanied by increase in serum FFA, signifying de novo synthesis of TG from FFA. Elevation in serum FFA is an indication of lipotoxicity, thus, the observed increase in serum FFA may imply that fructose increases metabolic risk. High level of plasma FFA has been previously associated with impaired insulin secretion and increased endogenous glucose production (Delarue and Magnan, 2007). It may therefore be inferred that the observed fructose-induced hyperglycaemia in this current study is, in part, due to an increased FFA synthesis. However, other mechanism cannot be ruled out since letrozole which attenuated fructose-induced hyperglycaemia, could not significantly lower serum FFA in fructose-taking rats. Our finding that revealed no significant change in LDL-c in fructose-administered rats suggests that fructose promotes dyslipidaemia of obesity, which is characterized with increased TG and FFA, decreased HDL-c and normal or slightly increased LDL-c (Klop et al., 2013).

Our data which demonstrated elevated serum and cardiac levels of ADA, XO and uric acid in fructose-exposed rats agree with previous studies (Olaniyi and Olatunji, 2019; Le et al*.,* 2020). ADA and XO play critical roles in uric acid synthesis as an end product of purines and endogenous purine metabolism, thus, controlling serum uric acid concentration (Le et al*.,* 2020). Unlike an acute increase in uric acid that may be beneficial as an antioxidant, chronic hyperuricaemia has been associated with several precursors of CVDs, including metabolic syndrome and coronary artery disease (Bickel et al*.,* 2002; Jin et al*.,* 2012). Thus, excessive fructose consumption may increase incidence of cardiometabolic syndrome via uric acid production, as revealed in the current study. Since inhibition of ADA and XO activities decreases uric acid synthesis (Zhao et al*.,* 2012; Park et al*.,* 2018b), decrease in ADA and XO activities observed in fructose+ letrozole group may imply that, letrozole treatment reduces uric acid synthesis by suppressing fructose-induced elevation of ADA and XO activities in both serum and heart. Suppressing effect of letrozole on ADA and XO activities in fructose-taking rats may further prove the potency of letrozole to mitigate fructose-induced cardiometabolic risk.

Conclusion

This study clearly demonstrated that fructose induced hyperglycaemia, lipid peroxidation, dyslipidaemia, FFA accumulation and elevated uric acid synthesis in male Wistar rats, while administration of letrozole attenuated the fructose effects. The implication of these findings is that, letrozole treatment could be a promising pharmacological agent that can attenuate fructose-induced cardiometabolic syndrome, thus, preventing the risk of

CVDs. Although letrozole alleviated fructose-induced lipid peroxidation and hypercholesterolaemia in this study, we also reported increased cardiac MDA and serum TC in rats administered with letrozole only. Hence, further animal and clinical findings are recommended to thoroughly investigate the cardiometabolic effects of letrozole.

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Conflicts of interest

The authors report no conflict of interest.

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