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RESEARCH ARTICLE

Ficus asperifolia Miq-enriched biscuit diet protects against L-NAME induced hyperlipidemia and hypertension in rats

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Abstract

Dietary sources of functional foods and nutraceutical have shown strong potentials in the management of hypertension and its complications. Sandpaper leaves, *Ficus asperifolia* Miq (FA), particularly found in Africa has a rich folkloric history in the management of diabetes and hypertension. This study produced biscuits supplemented with blends of FA at 2.5% and 5% fed to N_w (G)-nitro-L-arginine-methyl-ester (L-NAME, 40 mg/kg/day) induced-hypertensive rats for 14 days followed by the assessment of blood pressure, lipid profile, and atherogenic index in hypertensive rats. The phenolic constituents of FA blends were analyzed using high-performance liquid chromatography diode-anode (HPLC-DAD). Thereafter, the mean arterial blood pressure (MABP) and systolic blood pressure (SBP) was measured using the tail-cuff method after which the heart and lungs of rats were collected, weighed, and the antioxidant status and lipid cholesterol profile were assessed. We realized that recorded phenolic constituents in extracts of FA was at a high level and FA enriched biscuit-diet caused a significant decrease in SBP and MABP in L-NAME-induced hypertensive rats, body weight, atherogenic index and cholesterol profile in treated rats. However, FA enriched biscuit resulted in increased activities of catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) antioxidant enzymes in the heart and lungs of hypertensive rats. This study revealed that FA enriched biscuit-diet does not only have antihypertensive and antioxidant potential in L-NAME-induced hypertensive rats but also plays a protective role in the management of hyperlipidemia.

KEYWORDS
anti-hyperlipidemia, antihypertensive, *Ficus asperifolia* Miq (FA), HPLC-DAD, N(G)-nitro-L-arginine-methyl-ester (L-NAME)

1 | INTRODUCTION

Dyslipidemia is the occurrence of abnormal lipid content in the blood while hyperlipidemia results from elevated lipids such as cholesterol, triglycerides, LDL-cholesterol, and depleted HDL-cholesterol in the blood (Sa'adah et al., 2017). The occurrence of hyperlipidemia contributes to the emergence of coronary heart diseases (hypertension, atherosclerosis, ischemic shock), stroke, and in severe cases death

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(Kumar et al., 2014). Interestingly, Das et al. (2016) illustrates the interlink between total cholesterol and cardiovascular diseases (CVD) development wherein the level of angiotensin II produced can be negatively affected by lipid metabolism. This is by reducing adiponectin produced by the adipose tissue that is necessary for reducing inflammation and promotion of coronary heart disease (Poornima et al., 2007). Hence, combination therapeutic approach that considers both hyperlipidemia and hypertension is a reliable means of depleting the incidence of cardiovascular complications in metabolic syndrome (Aluko et al., 2018; Nilay et al., 2011).

The burden of hypertension and related complications in sub-Saharan Africa is ever increasing and individual's reliance on folkloric plants as a therapeutic option is rampant nowadays among African indigens. A symptomatic feature of hypertension or elevated blood pressure is deficit nitric oxide (NO) level. Nitric oxide is an anti-atherogenic agent reported to inhibit low-density lipoprotein oxidation, act as a vascular smooth muscle proliferator, and an anti-hypertrophic agent active in the adhesion of monocytes to endothelium (Alonso & Ramsoki, 2003; Anacak & Catravas, 2006). Inhibition of nitric oxide synthase (NOS) with L-arginine analog-like Nω-Nitro-L-arginine-methyl-ester (L-NAME), the level if NO reduces hence, resulting in arterial hypertension. Arterial hypertension and NOS inhibition in experimental rats can lead to dyslipidemia (Aluko et al., 2018). Dyslipidemia occurs in about 90-95% of hypertensive patients (Nyadjeu et al., 2013).

Apart from the use of medication as the first line of action in managing dyslipidemia, other effective options include but not limited to improving diet options and lifestyle. The availability of fresh fruits, vegetables, and medicinal plants with abundant antioxidants and phytochemical compounds contributes to the management of coronary heart diseases and hyperlipidemia (Nakajima et al., 2014). There are obvious limitations faced with alternative medicinal treatments particularly that include limited scientific knowledge on their mechanism of action however, the urgent demand for available natural products as phytomedicine is of preference since they are readily available and cheap (Chen et al., 2019). The leaves of *Ficus asperifolia Miq* (FA) have reported beneficial therapeutic roles in numerous diseases across countries in sub-Saharan Africa. Accordingly, Ojo et al. (2014) claimed it possesses alkaloids, saponin, phenol, tannin, cardiac glycoside, steroid, cardenolides, and phlorotannins. Soforowa (1996) identifies FA as an African drug used as a laxative and in treating parasitic worm. A brief experiment by Ojo and Akintayo (2014) revealed the role of FA in the treatment of kidney disease, as an antibacterial agent tenable in assisting against oxidative organ damage, and a claim by Ojo et al. 2016 is that it is a detoxifier.

However, so far there is a paucity of informative report on the anti-hyperlipidemic effect of FA, traditionally used in the dual treatment of hypertension and weight loss therapy in sub-Saharan Africa. Therefore, this study investigates the effects of FA- enriched biscuit diets on hypertensive rats' serum lipid profile, blood pressure, oxidative related injuries in L-NAME-induced hypertensive rats and FA phenolic content.

2 | MATERIALS AND METHODS

2.1 | Sample collection

The leaves of the Ficus plant (FA, *Ficus spp.*) commonly called "sandpaper leaves" or "Epin" by the Yorubas of western Nigeria were obtained from farming areas in Akure, Nigeria. The authentication of the plants was carried out at the Centre for Research and Development (CERAD) Federal University of Technology, Akure, Nigeria, and registered with voucher registration number (0186).

2.2 | Chemicals

Thiobarbituric acids (TBA), L-NAME, L-arginine, 5, 5′- dithiols-bis-2-nitrobenzoic acid (DNTB), coomassie blue G, sulphanilamide, acetylthiocholine iodide, thiobarbituric acid, adenosine, and Ellman's reagent were purchased from Sigma–Aldrich Chemical Co. with purity 99%, while the measure of total cholesterol, LDL-cholesterol, VLDL-cholesterol, and HDL-cholesterol level was done using kit reagents from DiaSys®, Germany with water purity $\leq 1\,\mu$S.

2.3 | Animal source

Thirty mature male rats weighing between 230 and 250 g were grouped into five groups (n = 6). They were incurred from Animal house, Department of Biochemistry, Federal University of Technology, Akure, Nigeria, and weighed daily. According to the National and Institutional Guidelines for Animal Protection and Welfare, handling of experimental animals was strictly followed and approved by the departmental ethical committee with ethical number- FUTA/ ETH/ 20/ 28 at room temperature (25°C) with free access to a diet formulated and exposed to 12 h light/dark cycle while housed in aerated plastic cages.

2.4 | Diet formulation

The leaves of FA were washed, dried, and then blended in powdered form, thereafter, they were mixed with other ingredients to make biscuit dough then placed in a Binatone Mini Electric Oven Model No-Mo-4500 made in China batch code NE 1301031, till biscuits turned golden brown. The composition of inclusive diet of *Ficus asperifolia* made into biscuits is represented in Table 1. The biscuit intake of each rat was monitored daily throughout the experiment.

2.5 | Experimental protocol

Hypertensive state was induced in rats by orally administering nitric oxide synthase (NOS) inhibitor,LL-NAME (40 mg/kg/day) for 14 days as described by Nyadjeu et al. (2013).

TABLE 1 Composition ingredients of Ficus asperifolia formulated biscuit distributed across the experimental groups. Source: Gbenga-Fabusiwa et al. (2018)

Biscuit Constituents	Flour	Cocoa butter	Aspartame	Water	Egg Albumin	Skimmed milk	FA	Total
Group A	85	2	0.5	4	4	5	–	100 g
Group B	85	2	0.5	4	4	5	–	100 g
Group C	85	2	0.5	4	4	5	–	100 g
Group D	82.5	2	0.5	4	4	5	2.5	100 g
Group E	80	2	0.5	4	4	5	5	100 g

Group A: Normotensive rats fed with basal composite biscuits.

Group B: Hypertensive rats induced with L-NAME orally.

Group C: Hypertensive rats induced with L-NAME orally and treated with (10 mg/kg/day) Atenolol orally.

Group D: Hypertensive rats induced with L-NAME orally and fed with 2.5% *Ficus asperifolia* enriched composite biscuits.

Group E: Hypertensive rats induced with L-NAME orally and fed with 5% *Ficus asperifolia* enriched composite biscuits.

2.6 | Determination of hemodynamic parameter

Measurement was carried out using non-invasive tail-cuff plethysmography under stable signal in awake male rats while considering 30 min stabilization interval before each recording using Kent Scientific; RTBP1001 Rat Tail Blood Pressure System for rats, Litchfield, USA. The result of systolic blood pressure (SBP) and mean arterial blood pressure (MABP) was complied at the end of the experiment. According to Meaney et al. (2000), the MABP was calculated with the following formula: **MABP = DBP + 0.412 (SBP − DBP)**.

2.7 | Biochemical assays

After treatment, animals were anesthetized, dissected, the heart and lungs were collected, washed in saline, and weighed. Blood was taken from the inferior vena cava of the heart into ethylene-diamine tetra-acetic acid-containing tubes without protease inhibitors and centrifuged at 3000 × *g* for 15 min using an MSC bench centrifuge. Finally, the homogenization process was done using a Teflon-glass homogenizer and centrifuged for 10 min at 5000 × *g*. The antioxidant parameters were measured using biochemical assay according to Belle et al. (2004) that includes catalase activity (CAT) using Sinha (1972) method. Superoxide dismutase (SOD) and glutathione-S-transferase (GST) activity were measured according to the method of Alia et al. (2003) and Mannervik and Guthenberg (1981), respectively.

2.8 | Collection of blood serum and lipid parameter assessment

The blood serum was separated from blood cells by centrifuging at 3000 rpm for 10 min, collected in microtube (Pratiwi & Aulanni'am dan, 2013), and kept at −20°C for lipid assay. The low-density lipoprotein (LDL) content was calculated from the other lipid parameters according to the method of Friedewald et al. (1972) as follows:

$$\text{LDL} = \text{TC} - (\text{TG}/5) - \text{HDL}$$

following Norbert (1995) formula of assessing serum very low-density lipoprotein (VLDL) as follows:

$$\text{VLDL} = \frac{\text{Triglycerides}}{5} - \text{Total Cholesterol Concentration}$$

whereas the atherogenic index was calculated using the formula of Yokozawa et al. (2006) where Atherogenic Index is as follows:

$$\text{Atherogenic Index} = \frac{\text{Total Cholesterol} - \text{High Density Lipoprotein (HDL)}}{\text{High Density Lipoprotein (HDL)}}$$

2.9 | Characterization of phenolic compounds with HPLC-DAD

The blended powdered extract of FA was analyzed using reverse-phase chromatography on a C_{18} column at a concentration of 15 mg/mL following the method of Amaral et al. (2013) with slight modifications of the reverse phase chromatographic analyses were carried out using standard flavonoids and phenolic acids (Sigma-Aldrich Chemie GmbH, Steinheim, North Rhine-Westphalia, Germany, 99% purity). The C_{18} column (4.6 mm × 150 mm) was packed with 5 μm diameter particles while water served as the mobile phase containing solvent systems of A: contained 3% acetic acid and B: methanol (80:20 v/v) with composition gradient of 20%, 40%, 60%, 80%, and 100% derived at 10, 15, 20, 25, and 30 min, respectively, following the method of Barbosa Filho et al. (2014) and detected at a wavelength of 280 nm. The flow rate was 0.6 ml/min and the injection volume was at 50 μl. Identification of phenolic compounds was performed on aqueous extracts of FA and the peaks of the chromatography were confirmed comparing their retention time with reference standards with DAD spectra (200–500 nm). The calculations of limit of detection (LOD) and limit of quantification (LOQ) were calculated using 3.3 and 10 δ/S (δ is equivalent to standard deviation and S to slope of calibration curve).

TABLE 2 Effects of FA- supplemented biscuit diet on L-NAME induced hypertensive rats' hemodynamic parameters (where * $p < 0.05$)

Hemodynamic Parameters	Treatments				
	Control	L-NAME (40 mg/kg p.o)	Atenonol	L-NAME + 2.5% FA	L-NAME +5% FA
SBP (mmHg)	129.67± 21.21	183.25± 8.84*	140.50± 9.19*	127± 0.47*	116.50± 3.54*
MABP (mmHg)	118.44± 17.18*	168.28± 8.45*	122.23± 2.20*	116.73± 3.92*	105.32± 1.19*

Abbreviation: FA, *Ficus asperifolia*; SBP, systolic blood pressure; MABP, mean arterial blood pressure.

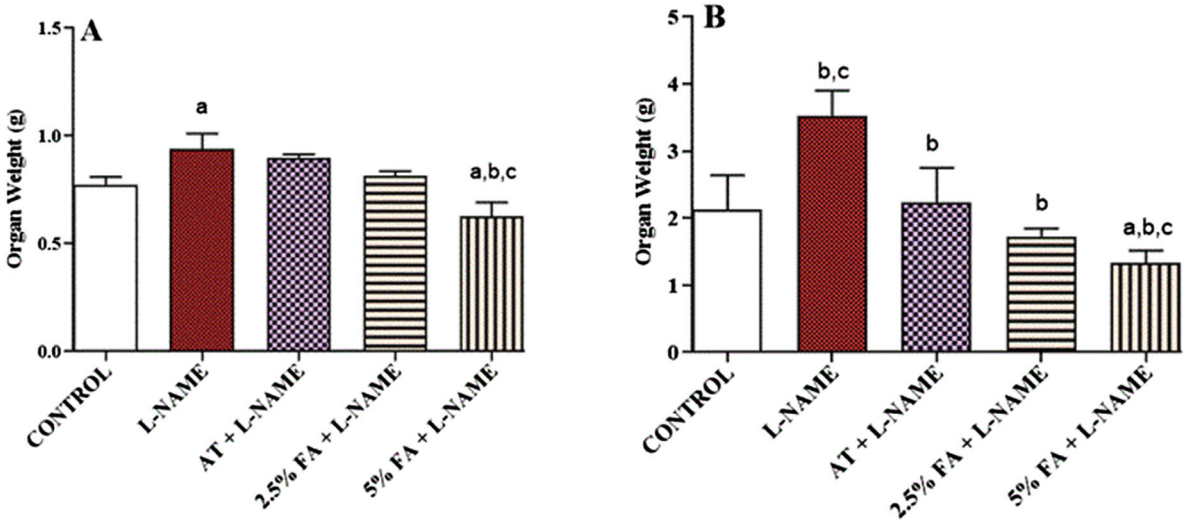


FIGURE 1 The effect of FA-formulated biscuit (2.5% and 5%) on organ weight of the A) Heart and B) Lungs of L-NAME induced hypertensive rats. Values represent the mean ± standard error mean, SEM (n = 6) rats for each group. [a] $p < 0.05$ significantly different when compared with control, [b] $p < 0.05$ significantly different when compared with L-NAME induced rats, [c] $p < 0.05$ significantly different when compared with L-NAME + AT treated rats. FA- *Ficus asperifolia Miq*, L-NAME - N_w (G)-nitro-L-arginine-methyl-ester, AT- Atenolol

2.10 | Determination of total protein content

The protein content in the heart samples was determined according to the standard method of Bradford (1976) using bovine serum albumin (BSA) as standard.

2.11 | Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). GraphPad Prism version 5.00 for windows (GraphPad Prism Software, Inc.) was used to analyze and construct graphs using a one-way analysis of variance followed by the Bonferroni post hoc multiple range test.

3 | RESULT

3.1 | Effects of *Ficus asperifolia* enriched biscuit on the systolic blood pressure (SBP) and mean arterial blood pressure (MABP) in L-NAME-induced hypertensive rats

Experimentation using tail-cuff plethysmograph revealed the blood pressure of experimental rats administrated with L-NAME with significantly ($p < 0.05$) elevated SBP and MABP after 14 days treatment when compared with control rat (normotensive) groups, whereas significantly decreased SBP and MABP were observed in hypertensive rats treated with FA enriched biscuits as shown in Table 2 and hypertensive rats group treated with the commercially available drug, Atenonol, when compared with control and hypertensive rat as shown in Table 2 to near normal.

3.2 | Effects of *Ficus asperifolia*-enriched biscuit diets on the relative organ weight and organo-somatic weight index in L-NAME induced hypertensive rats

Figures 1 and 2 illustrate the effect of FA-enriched biscuits on organ weight(s) and organo-somatic weight index in hypertensive rats induced with L-NAME. There was a significant increase in the absolute organ weight and organo-somatic index in the heart and lungs of L-NAME treated with Atenolol when compared to the control, although the organo-somatic index was not significantly different when treated with 5% FA formulated biscuit diets in the heart. Overall, the treatment of rats with FA enriched biscuits at 2.5% and 5%, respectively, decreased the absolute and organo-somatic index in both the heart and lungs.

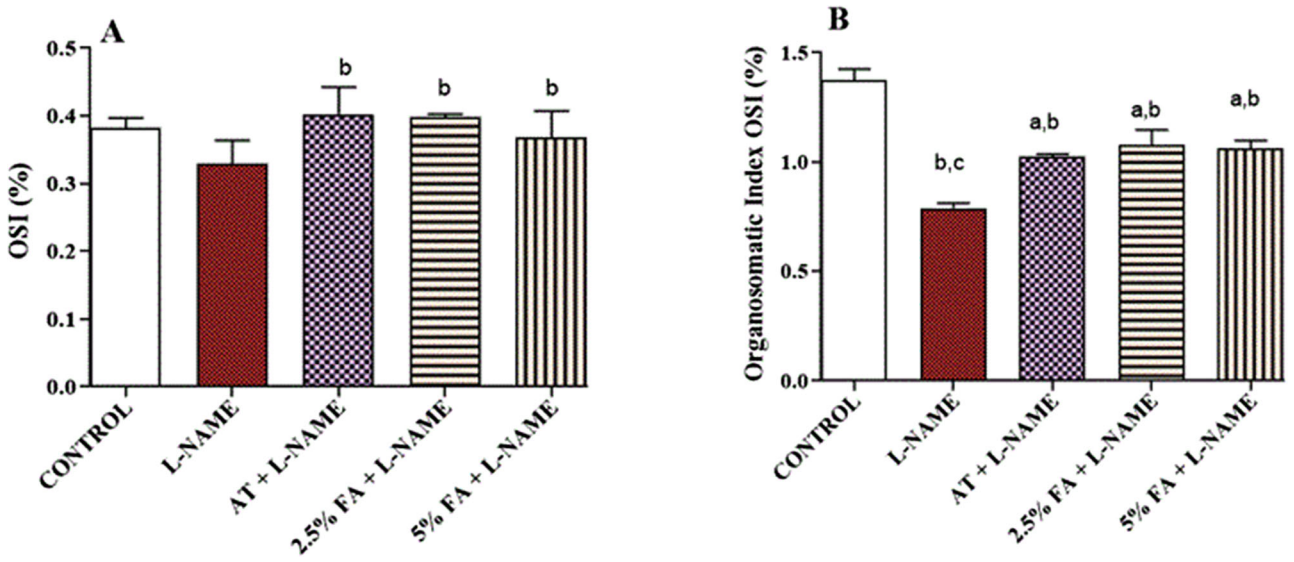


FIGURE 2 The effect of FA-formulated biscuit (2.5% and 5%) on organosomatic index of the A) Heart and B) Lungs of L-NAME induced hypertensive rats. Values represent the mean ± standard error mean, SEM (n = 6) rats for each group. [a] $p < 0.05$ significantly different when compared with control, [b] $p < 0.05$ significantly different when compared with L-NAME induced rats, [c] $p < 0.05$ significantly different when compared with L-NAME + AT treated rats. FA- *Ficus asperifolia Miq*, L-NAME - N_w (G)-nitro-L-arginine-methyl-ester, AT- Atenolol, OSI-organosomatic index

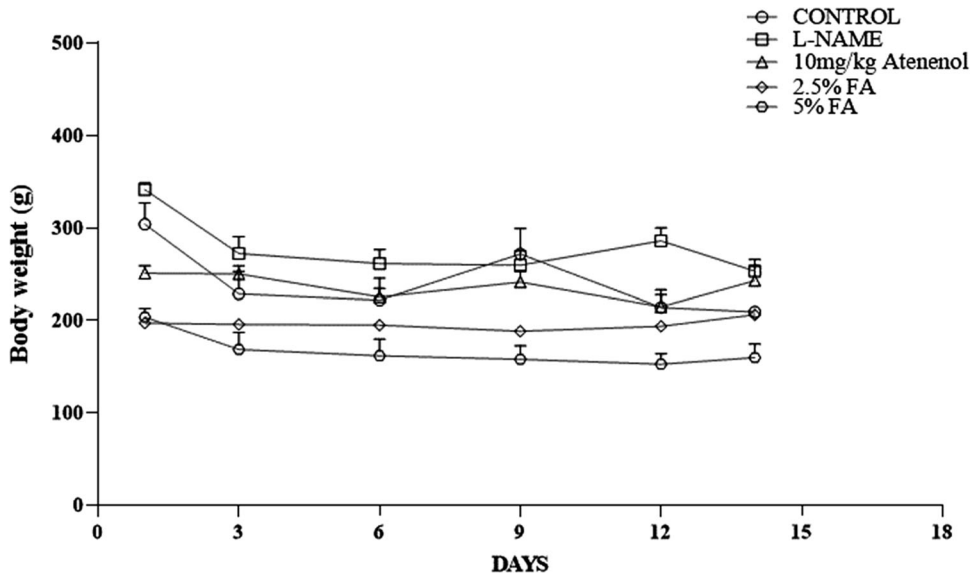


FIGURE 3 The effect of change in body weight of administered FA-formulated biscuit (2.5% and 5%) on hypertensive rats induced with L-NAME. Values represent the mean ± standard error mean, SEM (n = 6) rats for each group, FA- *Ficus* asperifolia

3.3 | Effects of *Ficus asperifolia*-enriched biscuit diets on changes in body weight in L-NAME induced hypertensive rats

The change in mean body weight of the treated rat groups and control rats is represented in Figure 3. The initial and progressive weight change of all the groups was significantly different when compared to the control group. Additionally, the change in body weight recorded in hypertensive rat groups and L-NAME-induced rats treated with FA-enriched biscuits was significantly different when compared to the control ($p < 0.05$) with the exception to Atenolol treated rats that were insignificant when compared with L-NAME-induced group. Interestingly, rats treated with FA-enriched biscuits had the lowest decline in weight after treatment when compared with L-NAME hypertensive rats.

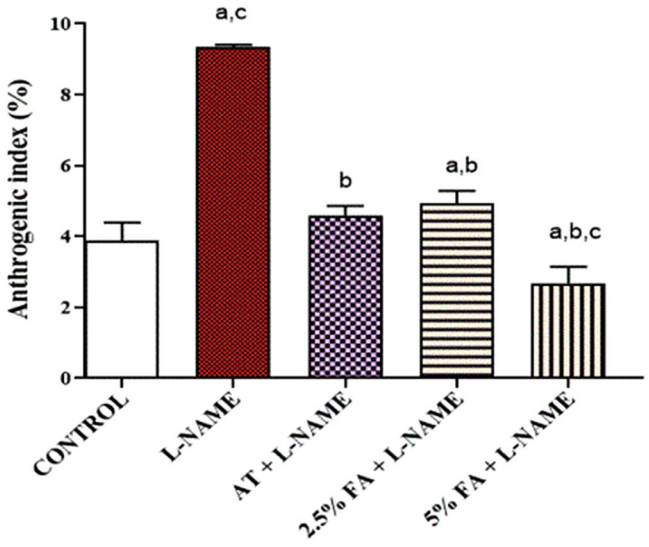


FIGURE 4 The effect of FA-formulated biscuit (2.5% and 5%) on AI of the A) Heart and B) Lungs of L-NAME induced hypertensive rats. Values represent the mean ± standard error mean, SEM (n = 6) rats for each group. [a] $p < 0.05$ significantly different when compared with control, [b] $p < 0.05$ significantly different when compared with L-NAME induced rats, [c] $p < 0.05$ significantly different when compared with L-NAME + AT treated rats. FA- *Ficus asperifolia Miq*, L-NAME - N_w (G)-nitro-L-arginine-methyl-ester, AT- Atenolol, AI- Atherogenic Index

3.4 | Effects of *Ficus asperifolia*-enriched biscuit diets on the atherogenic index (AI) in L-NAME induced hypertensive rats

Figure 4 revealed the atherogenic index across the treatment groups. There was a significant elevation in the AI in L-NAME- INDUCED hypertensive rats group when compared with control and treated group with Atenolol as represented. Hypertensive rats treated with Atenolol and FA enriched biscuit diets showed a significant decrease in measured AI after 14 days treatment.

3.5 | Effects of *Ficus asperifolia*-enriched biscuit diets on lipid profile, LDL), VLDL-C, and HDL level in L-NAME induced hypertensive rats

The level of LDL, HDL, and VLDL-cholesterol in experimental rats is shown in Figure 5. There was no significant increase in both LDL and VLDL in L-NAME-treated rats at the end of the experiment when compared to the control group. However, there was a significant decrease in LDL and VLDL-cholesterol levels in the hypertensive rats group treated with FA supplemented diets and Atenonol when compared with the L-NAME-induced rat groups. Importantly, the HDL in FA-treatment groups and Atenonol-treated rats was significantly high when compared with the control and L-NAME-induced rats.

3.6 | Effects of *Ficus asperifolia*-enriched biscuit diets on SOD, GST, and CAT activity in L-NAME-induced hypertensive rats

Furthermore, we evaluated the effect of FA on the activity of SOD, GST, and CAT in the heart and lungs of hypertensive rats and the results are presented in Figures 6–8. The activities of SOD, GST, and CAT in hypertensive rats were significantly reduced when compared to normotensive rats. Treatment with Atenolol and FA enriched biscuits (2.5% and 5%) resulted in significantly ($p < 0.05$) in hypertensive rats increased the measured activity of SOD, GST, and CAT in the heart and lungs respectively when compared with L-NAME induced rat group.

3.7 | Phenolic composition of *Ficus asperifolia* extract

The HPLC fingerprinting of *Ficus asperifolia* revealed the presence of high concentration amounts of the following compounds namely, salicylic acid, vanillic acid, syringic acid, naringenin, kaempferol, luteolin, ellagic acid, quercetin, chlorogenic acid, and myricetin (Table 3 and Figure 9).

4 | DISCUSSION

Phenolic and flavonoid compounds of plant extracts' have reported protective effects and reduced mortality rates in coronary heart diseases by acting as potential antioxidant agents against LDL oxidation which results in atherogenic disease , coronary heart disease (Yokozawa et al., 2006), and lipase inhibition by depleting lipase enzyme activity (Martins et al., 2010; Yang et al., 2008). The excess availability of LDL-cholesterol provides lipid to the tissues system causing notable detrimental condition one of which includes atherosclerosis (an inherent condition of hyperlipidemia) and oxidative damage. There have been reported cases of patients with hypertension suffering from the abnormalities namely dyslipidemia and hyperlipidemia (Nyadjeu et al., 2013).

The mechanistic role of L-arginine analog-like compound, L-NAME in experimental animals is to inhibit NOS since it is a competitor of the substrate, arginine at the enzyme' active site thus, declining NO produced and resulting in the generation of arterial blood pressure. Interestingly, there is an interlink between elevated lipid profile and hypertension when the activity of nitric oxide synthase is blocked (Aluko et al., 2018; Biwer et al., 2016). In this study, we established the occurrence of elevated threshold in the systolic blood pressure and mean arterial blood pressure in rats induced with 40 mg/kg body weight in experimental rats seen in Table 2.

Observations from hypertensive patients commonly show cardiac ventricles and arterioles structure with enlarged left atrium due to hypertrophy of the left ventricle (LV) and affiliated diastolic dysfunction (Marek et al., 2017). Vaziri et al. (1995) experiments justify the

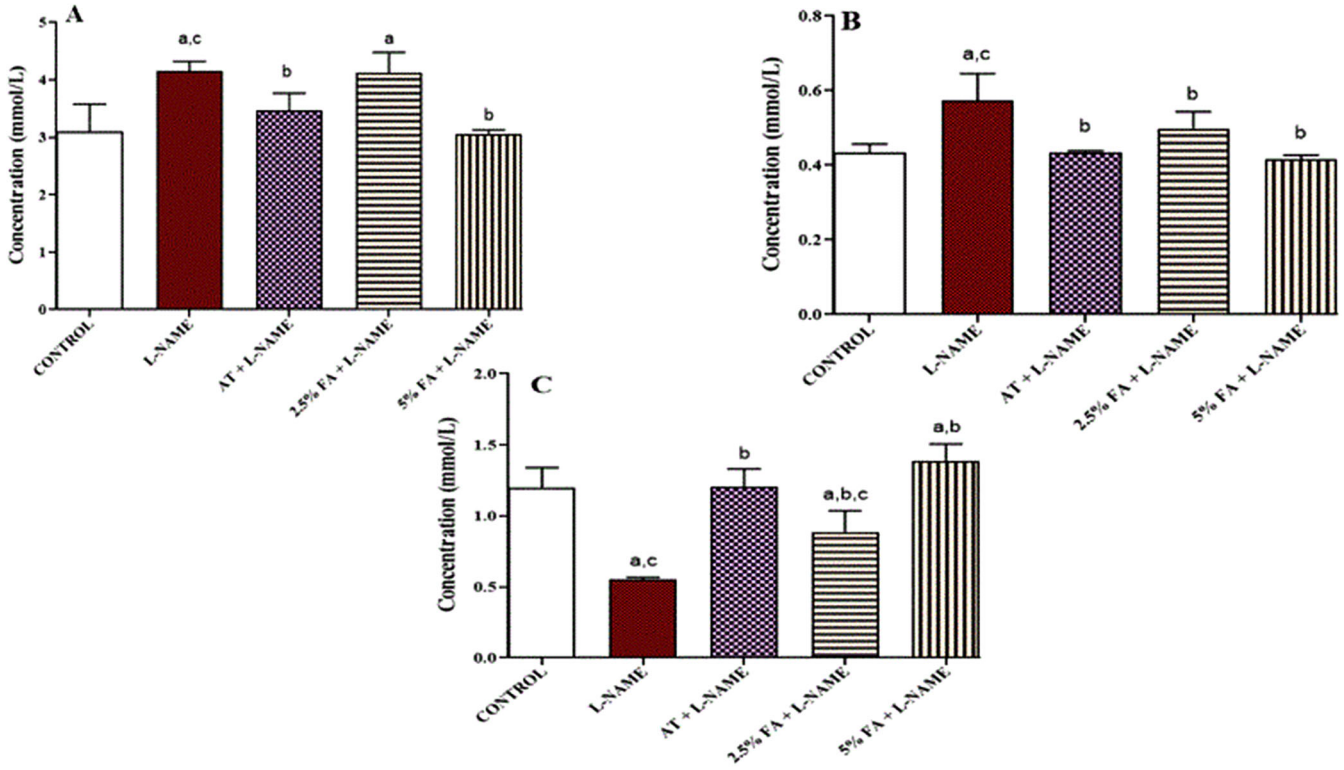


FIGURE 5 The effect of FA-formulated biscuit diet (2.5% and 5%) the A) LDL- cholesterol B) VLDL-cholesterol and C) HDL levels in the serum of L-NAME induced hypertensive rats. Values represent the mean ± standard error mean, SEM (n = 6) rats for each group. [a] $p < 0.05$ significantly different when compared with control, [b] $p < 0.05$ significantly different when compared with L-NAME induced rats, [c] $p < 0.05$ significantly different when compared with L-NAME + AT treated rats. FA- *Ficus asperifolia Miq*, L-NAME - N_w (G)-nitro-L-arginine-methyl-ester, AT- Atenolol, LDL- low-density-lipoprotein, VLDL- very-low-density-lipoprotein, HDL- high density lipoprotein

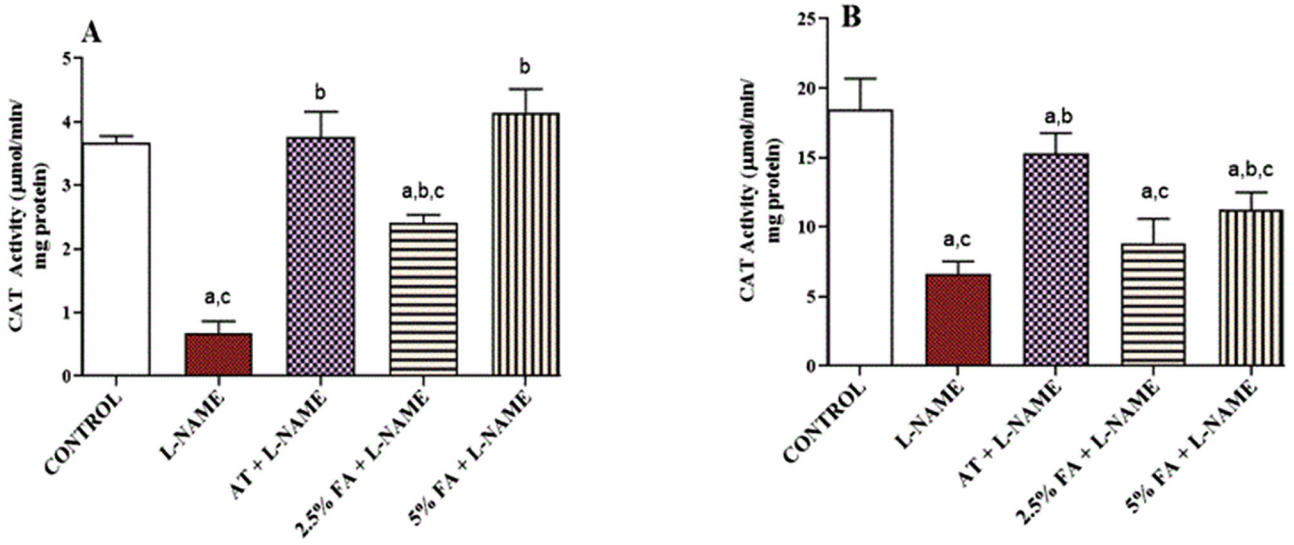


FIGURE 6 The effect of FA-formulated biscuit diet (2.5% and 5%) on CAT activity in the A) Heart and B) Lungs of L-NAME induced hypertensive rats. Values represent the mean ± standard error mean, SEM (n = 6) rats for each group. [a] $p < 0.05$ significantly different when compared with control, [b] $p < 0.05$ significantly different when compared with L-NAME induced rats, [c] $p < 0.05$ significantly different when compared with L-NAME + AT treated rats. FA- *Ficus asperifolia Miq*, L-NAME - N_w (G)-nitro-L-arginine-methyl-ester, AT- Atenolol, CAT- catalase

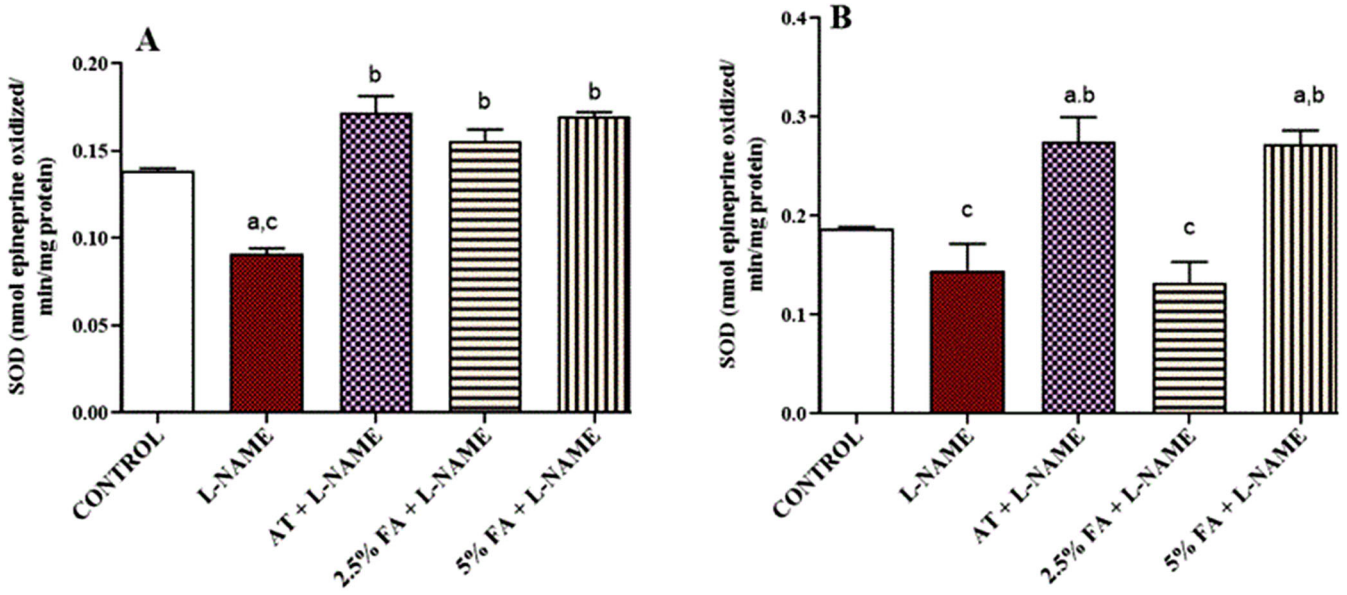


FIGURE 7 The effect of FA-formulated biscuit diet (2.5% and 5%) on SOD activity in the A) Heart and B) lungs of hypertensive rats induced with L-NAME. Values represent the mean ± standard error mean, SEM (n = 6) rats for each group. [a] $p < 0.05$ significantly different when compared with control, [b] $p < 0.05$ significantly different when compared with L-NAME induced rats, [c] $p < 0.05$ significantly different when compared with L-NAME + AT treated rats. FA- *Ficus asperifolia Miq*, L-NAME - N_w (G)-nitro-L-arginine-methyl-ester, AT- Atenolol, SOD- superoxide dismutase

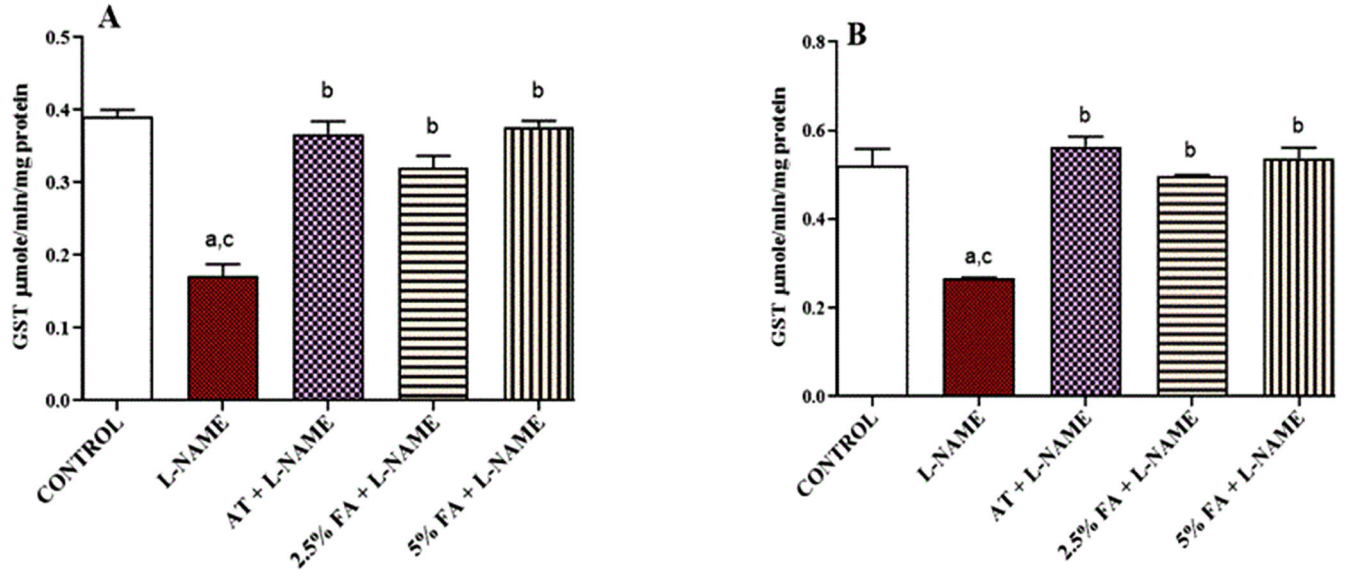


FIGURE 8 The effect of FA-formulated biscuit diet (2.5% and 5%) on GST activity in the A) Heart and B) lungs of hypertensive rats induced with L-NAME. Values represent the mean ± standard error mean, SEM (n = 6) rats for each group. [a] $p < 0.05$ significantly different when compared with control, [b] $p < 0.05$ significantly different when compared with L-NAME induced rats, [c] $p < 0.05$ significantly different when compared with L-NAME + AT treated rats. FA- *Ficus asperifolia Miq*, L-NAME - N_w (G)-nitro-L-arginine-methyl-ester, AT- Atenolol, GST- Glutathione- S-Transferase

occurrence of increased cardiac and aorta organ weight when treated with L-NAME as observed in this study and in L-NAME induced rats which showed increased organ weight of both the heart and lungs (Figure 1). Decreased organo-somatic index (OSI) is based on swelling, atrophy, or hypertrophy of organs (Adedara et al., 2014), and in most instances, this is as a result of contributing effect of reactions due to intoxication on organ system (Li et al., 2017). Hypertrophy was observed in the organs of L-NAME induced rats in this study (Figure 2). The reduced OSI index of the heart and lungs in hypertensive rats implies that atrophy occurred in these organs from damages in the cardiac muscle owing to elevated diastolic blood pressure as compared to normotensive and treated rats.

TABLE 3 shows the composition of FA extracts, retention time, limit of detection (LOD) and limit of quantification (LOQ)

Compounds	Amount (mg/100 g)	Retention Time (Min)	LOD (μg/ml)	LOQ (μg/ml)
Salicyclic acid	10.75 ± 1.18^{c}	9.34	0.0031	0.01
Vanillic acid	5.35 ± 0.06^{b}	11.78	0.0057	0.017
Syringic acid	317.36 ± 3.28^{d}	16.04	0.0085	0.026
Naringenin	2.64 ± 0.01^{a}	16.54	0.0048	0.015
Kaempferol	16.77 ± 0.02^{c}	19.52	0.0051	0.015
Luteolin	6.19 ± 0.01^{b}	20.13	0.0026	0.008
Ellagic acid	2.41 ± 0.00^{a}	22.51	0.0049	0.015
Quercetin	10.30 ± 0.02^{c}	22.97	0.0049	0.015
Myricetin	1.23 ± 0.77^{a}	24.73	0.0025	0.008
Chlorogenic acid	1.82 ± 0.01^{a}	25.01	0.0020	0.010

Abbreviation: FA,***Ficus asperifolia* Miq**.Note: The results are expressed as mean ± SEM of three determinations. Averages calculated at $p < 0.05$. Values with different alphabet superscripts along the column are significantly ($p < 0.05$) different.

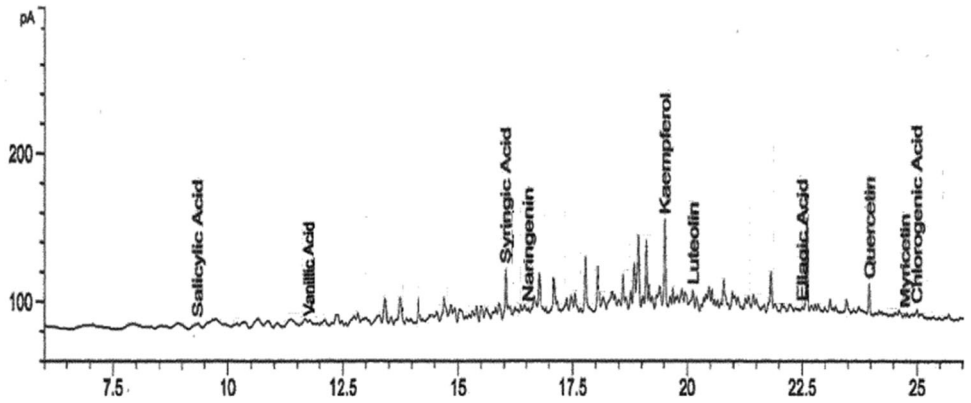


FIGURE 9 Representation of phenolic profile of FA chromatogram at 280 nm in order of hierarchy of amount (mg/100 g). FA- *Ficus asperifolia Miq*

The measure of nutritional status in individuals over a given time is calculated from body mass index (BMI, an 'anthropometric index' marker). When the range of atherogenic index is above 1.6, it depletes atherosclerosis (Yokozawa et al., 2006). In addition to this, the decline in body weight of treated rats corresponds to better nutritional status in hypertensive rats (Figure 3) as compared to increased body weight in rats in hypertensive state. In instances whereby there is an elevated HDL level, it represents an inverse indicator to the state of atherogenic index (AI) and can be used to predict the chances of developing atherosclerosis which in the case of high HDL, AI will be less. Therefore, it is important to acknowledge the beneficial role of HDL in mopping-up excess cholesterol moving from the tissue system to the liver. Our result revealed the HDL level to be a reciprocate of atherogenic index. Hence, the marked decrease in AI in a concentration-dependent manner in the treated rats reveals the anti-hyperlipidemic role of FA-biscuit diet in hypertensive rats as represented in Figure 4.

Hyperlipidemia results from elevated blood serum lipid levels namely, LDL-cholesterol and VLDL-cholesterol which are keen reflections of the abundance of total cholesterol and triglycerides in the extra-hepatic tissues transferable to body tissues (Adekunle et al., 2013). The toxic effect of L-NAME in rats caused an elevation in LDL-cholesterol owing to deficit in nitric oxide (NO) level in a hypertensive state as depleted in Figure 5. Similar findings by Murugesan and Raja (2012) reported increased cholesterol, LDL, VLDL, and decreased HDL concentration in experimental rats. Additionally, the non-significant increase in VLDL level in L-NAME induced rats in our study corresponds vividly with the marginal concentration of VLDL also reported by Aluko et al. (2018).

During oxidative damage, one of the primary antioxidant enzymes involved in promoting defense in body cells is superoxide dismutase (SOD) while catalase (CAT) evacuates hydrogen peroxide (H_2O_2) generated as a result of the reaction by SOD enzyme and/ or from reactions with an environmental agent. Chronic heart failure patients are clinically seen to have deteriorated endothelial-bound SOD activity due to increased vascular oxidative stress as a result of the endothelial dysfunction (Landmesser et al. 2002). The activities of CAT and SOD in the

heart and lungs were significantly reduced in LL-NAME hypertensive rats which could be as a result of the excessive burden from free radicals or reactive oxygen species (ROS) generated (Figures 6 and 7). The enzyme, glutathione-S-transferase (GST) is pivotal in the maintenance of oxidative balance in the presence of its substrate (GSH) in cellular systems. Hence, a case in point is that the decline in GST activity in L-NAME -induced hypertensive rats is due to reduced GSH substrate bioavailability and/or elevated free radical generation (Figure 8). Thus, our study revealed the role of FA- supplemented biscuit diet as an antioxidant responsive in scavenging free radical generated from oxidative damage caused by L-NAME.

The role of *Ficus asperifolia* in regulating the hyperlipidemia in hypertensive rats was ascertained by assessing its phytochemicals constituent using HPLC-DAD. Amongst the numerous compounds screened, ten (10) active polyphenolic compounds were found which are salicylic acid, vanillic acid, syringic acid, naringenin, kaempferol, luteolin, ellagic acid, quercetin, chlorogenic acid and myricetin. However, the most prominent polyphenolic compounds in the extract which have also been found from reports to be functional in regulating systemic lipid profile and antioxidant level are namely, syringic acid, kaempferol, quercetin, vanillic acid, and naringenin (Table 3). Syringic acid (SA) administered at 50 mg/kg/bw to rats exerts anti-hypertensive and antihyperlipidemic activity by reducing blood pressure and lipid peroxides while increasing nitric oxide availability and antioxidant level in blood samples of rats (Kumar et al., 2012). We reported SA as the most abundant phenolic compound of which experimentation using pure compound of SA by Jalili et al. (2006) showed its effectiveness in ameliorating organ damage in hypertensive rats (Figure 9). However, experimental work by Wang et al. (2020) done on kaempferol at 200 mg/kg in diet illustrates its beneficial role in modulating body and organ weight gain, serum cholesterol triglyceride levels by identifying its anti-obesity effects in high-fat diet (HFD) fed mice. Dietary quercetin at 0.04% and 0.08% improves hypertriglyceridemic conditions and antioxidant status (Jeong et al., 2012). In addition, Kumar et al. (2014) portrayed vanillic acid to have anti-hyperlipidemic effects in nitric oxide deficient hypertensive rats by enhancing the regulation of lipids and lipoproteins and suggested it as a useful candidate in hypertension therapy. Finally, naringenin assists in the reduction of VLDL overproduced during dyslipidemia in Western-fed Ldlr $^{-}$/$^{-}$ mice and obesity associated with accelerated atherogenesis, therefore, preventing atherosclerosis (Mulvihill et al., 2010). Overall, it can be said that the theory of Moure et al. (2001) is observed in this study wherein an interrelationship exists between plants' extracts with high phenolic content leading to improved antioxidant activity in the systematic organs of hypertensive rats. Therefore, this implies that a structure-function relationship is applicable to the activity of FA in both hypertensive and hyperlipidemic conditions.

5 | CONCLUSION

Taking insights from this study, the functional mechanism of action of *Ficus asperifolia* formulated biscuits involves the reduction of elevated blood pressure, body weight and hyperlipidemia while improving antioxidant activities in rats exposed to L-NAME. Additionally, reports on FA phenolic compounds constituents attests to its folkloric use as a therapeutic agent in the management of hypertension and hyperlipidemia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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None declared

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