Novel antihypertensive action of rutin is mediated via inhibition of angiotensin converting enzyme/mineralocorticoid receptor/angiotensin 2 type 1 receptor (ATR1) signaling pathways in uninephrectomized hypertensive rats

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Abstract

Hypertension is the most common cardiovascular disease that affects approximately 26% of adult population, worldwide. Rutin is one of the important flavonoids that is consumed in the daily diet, and found in many food items, vegetables, and beverages. Uninephrectomy (UNX) of the left kidney was performed, followed by induction of hypertension. The rats were randomly divided into four groups of 10 rats: group 1—Sham-operated rats; group 2—UNX rats, group 3—UNX-L-NAME (40 mg/kg) plus rutin (100 mg/kg bwt), and groups 4—UNX-L-NAME plus lisinopril (10 mg/kg bwt), orally for 3 weeks. Results revealed significant heightening of arterial pressure and oxidative stress indices, while hypertensive rats treated with rutin had lower expressions of angiotensin converting enzyme (ACE) and mineralocorticoid receptor in uninephrectomized rats. Together, rutin as a novel antihypertensive flavonoid could provide an unimaginable benefits for the management of hypertension through inhibition of angiotensin converting enzyme and mineralocorticoid receptor.

Practical applications

Hypertension has been reported to be the most common cardiovascular disease, affecting approximately 26% of the adult population worldwide with predicted prevalence to increase by 60% by 2025. Recent advances in phytomedicine have shown flavonoids to be very helpful in the treatment of many diseases. Flavonoids have been used in the treatment and management of cardiovascular diseases, obesity and hypertension. The study revealed that rutin, a known flavonoid inhibited angiotensin converting enzyme (ACE), angiotensin 2 type 1 receptor (ATR1), and mineralocorticoid receptor (MCR), comparable to the classic ACE inhibitor, Lisinopril, indicating the novel antihypertensive property of rutin. Therefore, flavonoids such as rutin found in fruits and vegetables could, therefore, serve as an antihypertensive drug regimen. Combining all, functional foods rich in flavonoids could be used as potential therapeutic candidates for managing uninephrectomized hypertensive patients.

KEYWORDS: hypertension, mechanism of action, phytochemical, rutin, uninephrectomy

1 INTRODUCTION

Hypertension has been reported to be the most common cardiovascular disease, affecting approximately 26% of the adult population worldwide with predicted prevalence to increase by 60% by 2025 (Dusing, 2010). The kidneys function to regulate body fluid volume and electrolyte balance, thereby maintaining blood pressure. This homeostasis can be altered by removal of one kidney. The development of proteinuria, hypertension with concomitant gradual decrease in renal function in the donor after surgical resection of a kidney has been positively correlated with hyperfiltration. Therefore, potential therapeutic strategies are necessary to mitigate hyperfiltration-mediated injury to the remaining kidney after uninephrectomy. In uninephrectomized animals, hyperfiltration has been reported to subject podocytes to increased tensile stress and fluid shear stress (Srivastava et al., 2017).

This further reduces the response of podocytes to mechanical stress upon elevation of intraglomerular capillary pressure (Martineau et al., 2004).

The production of vasoactive hormones, such as prostaglandin E2 (PGE2), and increased gene and protein expressions of cyclooxygenase (COX)-2 has been documented to contribute significantly to podocyte morphology, and compromise glomerular permeability (Srivastava et al., 2014). Hence, glomerular hyperfiltration is one of the major risk factors for progression of chronic kidney disease (CKD) as earlier described (Helal et al., 2012). A previous study showed that higher glomerular filtration rate can predict low risk of developing chronic kidney disease in living kidney donors (Tsai et al., 2013).

The kidneys are known to play a pivotal role in blood pressure control, and reduced nephron number is a risk factor for arterial hypertension (Zohdi et al., 2012). Therefore, uninephrectomized subjects are at greater risk of becoming hypertensive as reported elsewhere (Zohdi et al., 2012). Angiotensin II, because of its potent vasoconstrictor activity, increases disease progression in renal/cardiovascular injury (Costantino et al., 2019; Zhu et al., 2019). Angiotensin II has been associated with the induction of oxidative stress in smooth muscle cells that is germane to renal injury (Agbo et al., 2019; Bekpinar et al., 2019). Oxidative stress that is precipitated in smooth muscle cells has been found to arise from the stimulation of membrane-bound NADH/NADPH oxidase with accompanied generation of superoxide anion radical (Guntani et al., 2011; Veerappan & Malarvili, 2019). The involvement of angiotensin II-induced oxidative stress has been documented to alter vascular responses that contribute to systemic hypertension and vascular injury (Lu et al., 2020). The renin-angiotensin-aldosterone system modulates volume, sodium and potassium homeostasis (Macchiavello et al., 2019). Therefore, enhanced endothelial cell-specific mineralocorticoid receptor activates endothelial dysfunction, renal artery fibrosis/stiffening, and impaired NOS (NO synthase) with resultant tubulointerstitial fibrosis (Aroor et al., 2019). Primary aldosteronism is the most common cause of secondary hypertension (Satoh et al., 2019). Hence, patients suffering from primary aldosteronism have an increased propensity to develop cardiovascular disease conditions and target organ damage (Stavropoulos et al., 2018). Together, mineralocorticoid receptor antagonists have opened a novel therapeutic window for the management of patients with secondary/ resistant hypertension (Faulkner & Belin de Chantemèle, 2019; Imprialos et al., 2018; Sato, 2019).

The rennin-angiotensin-aldosterone system (RAAS) has been well documented and reported for the regulation of blood pressure, fluid volume, and sodium balance (Zhang et al., 2019). Therefore, over activity of RAAS has been reported to promote both systemic and regional glomerular capillary hypertension leading to renal damage, vasoconstriction, endothelial dysfunction, thrombosis, inflammation, and fibrosis via proinflammatory pathways (Gromotowicz-Poplawska et al., 2016; Zhang et al., 2019). Angiotensin-converting enzyme (ACE) inhibitors prevent the formation of more physiologically active angiotensin II from its precursor angiotensin I, and are, therefore, effective for the management of hypertension due to excessive generalized vasoconstriction in the cardiovascular system (Bhandari & Chadburn, 2019; Podzolkov & Tarzimanova, 2018). Blockers of the renin-angiotensin-aldosterone system (RAAS), including renin inhibitors, angiotensin (Ang)-converting enzyme (ACE) inhibitors, Ang II type 1 receptor antagonists, and mineralocorticoid receptor

antagonists, are the hallmarks in the treatment of hypertension (Te Riet et al., 2015; Zhang et al., 2017).

Recent advances in phytomedicine have shown flavonoids to be very helpful in the treatment of many diseases. Rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside) is one of the important flavonoids that is consumed in the daily diet and found in many food items, vegetables, and beverages (Hosseinzadeh & Nassiri-Asl, 2014). It is specifically found in citrus fruits, mulberry, cranberries, and buckwheat (Singh et al., 2018). Flavonoids are phenolic compounds existing as secondary metabolites in fruits and vegetables as well as fungi, and are classified as flavonols, flavones, flavanones, isoflavones, flavanols, and anthocyanidins (Budzynska et al., 2019). Flavonoids are major dietary constituents of plant-based food found ubiquitously in the plant kingdom where they are usually present in substantial amounts (Budzynska et al., 2019). Rutin has been reported to have a wide range of biological and pharmacological properties against inflammatory bowel disease, diabetes mellitus, Alzheimer's, hypertension, and cancer (Habtemariam, 2016; Habtemariam & Belai, 2018, Olaleye et al., 2014) Alzheimer's disease (Habtemariam, 2016), diabetes mellitus (Habtemariam & Lentini, 2015), and hypertension (Olaleye et al., 2014).

This study seeks to unravel the link between flavonoids application, specifically rutin, for the treatment of uninephrectomized hypertensive rats and the mechanism of action involved. Therefore, fruits and vegetables that are rich in rutin might offer natural remedies for hypertensive patients following uninephrectomy. We also hypothesized that a rutin-flavonoid cocktail might provide uninephrectomized hypertensive patients with new therapeutic drug regimen.

2 MATERIALS AND METHODS

2.1 Chemicals

All chemicals used in this study were of analytical grade, and they include rutin, xylenol orange (XO), hydrochloric acid, sulfuric acid, sorbitol, dichloronitrobenzene, thiobarbituric acid, trichloro acetic acid, potassium hydroxide, reduced glutathione, sodium hydroxide, O-dianisidine, hydrogen peroxide, guanidine hydrochloride, potassium chloride, ethanol, dinitrophenylhydrazine, ethyl acetate, acetic acid, normal goat serum, biotinylated antibody, horse radish Peroxidase, anti-angiotensin 2 type 1 receptor, anti-mineralocorticoid receptor, anti-angiotensin converting enzyme, and anti-caspase 3 antibodies.

2.2 Method of uninephrectomy

Following the induction of anesthesia in rats with ketamine (21.2 mg/kg) and xylazine (4.2 mg/kg), the left paralumbar was shaved, aseptically prepared, and an incision of about 3 centimeters was made. The left kidney was exteriorized, and the renal vessel ligated with catgut suture material. The proximal aspect of the ligature was removed. Thereafter, closure of the muscle (with catgut suture) and skin (with nylon suture) was done. The rats were allowed to heal with for 14 days post-surgery. Water, feed, and exposure to alternate cycle of 12 hr of darkness and 12 hr light were provided.

2.3 Animal ethics statement

This study was approved (UI-ACUREC.18.0133) by the Committee of the University of Ibadan for concerned with use of animals for research.

2.4 Hypertension induction and experimental protocol

The N-omega-nitro-L-arginine methyl ester (L-NAME, 40 mg/kg) was administered orally to uninephrectomized rats for the induction of hypertension. Animal grouping was as follows:

Group 1 (10 rats): Sham-operated.

Group 2 (10 rats): Uninephrectomized (UNX).

Group 3 (10 rats): UNX+ L-NAME+ rutin.

Group 4: UNX-L-NAME + lisinopril.

Rutin was administered at 100 mg/kg bwt, whereas lisinopril was administered at 10 mg/kg bwt.

2.5 Blood pressure measurements

The non-invasive method for the measurement of blood pressure parameters in conscious rats was adopted for this study using a computerized plethysmograph (Kent Scientific, USA).

2.6 Serum and Tissue preparation

Whole blood was collected from the venous sinus of the eye by inserting an aseptic capillary tube into the medial canthus of the eye. The blood was allowed to clot, centrifuged and a Pasteur pipette was used to obtain the clear serum that was refrigerated and subsequently used for biochemical analyses. The kidneys and testes were homogenized in 50 mM Tris–HCl buffer and cold centrifugation (4°C, 10,000 *g* for 15 min) was done to obtain post mitochondrial fractions (PMFs) that were subsequently used for biochemical analyses.

2.7 Biochemical analysis for oxidative stress and antioxidant markers

In this study, the markers of oxidative stress evaluated included hydrogen peroxide, malondialdehyde, protein carbonyl, advanced oxidation protein product (AOPP), and myeloperoxidase using standard methods. The analysis of hydrogen peroxide was based on the principle that hydrogen peroxide selectively causes the oxidation of ferrous to ferric ion in dilute acid, with the addition of xylenol orange leading to the formation of blue-purple complex (Wolff, 1994). The thiobarbituric acid method was use to assay for malondialdehyde, an index of lipid peroxidation, as described by Varshney and Kale (1990). The spectrophotometric method for carbonyl assay which estimates oxidative damage to proteins was used to measure protein carbonyl contents in the renal and testicular tissues (Reznick & Packer (1994). The vitamin C contents were measured by precipitating the post mitochondria fractions in trichloroacetic acid and using dinitrophenyl hydrazine and

thiourea as color reagents (Jacques-Silva et al. (2001). The advanced oxidation protein products (AOPP) which are the uremic toxins produced during oxidative stress were assayed based on the method of Kayali et al. (2006) that involves treatment of the post mitochondrial fractions with phosphate buffer, reading the absorbance of the mixture at 340 nm wavelength. The method described by Akaike et al. (1990) was used for the assay of xanthine oxidase, whereas myeloperoxidase was assayed by suspending 0.1 ml of the post mitochondrial fraction with 2.9 ml of 50 mM phosphate buffer containing O-dianisidine and hydrogen peroxide and read at 450 nm wavelength (Beutler et al., 1963).

The biuret method (Gornal et al., 1949) was used for the measurement of protein concentration at 540 nm wavelength with BioTek ELx800 plate reader. The antioxidant markers were assayed using standard methods are superoxide dismutase (Misra & Fridovich, 1972; Oyagbemi et al., 2015), reduced glutathione (Jollow et al., 1974), glutathione peroxidase (Beutler et al., 1963), Glutathione S-transferase (Habig et al., 1974). The protein thiol (PSH) and non-protein thiol (NPSH) contents were determined using Ellman's reagent (DTNB) 5′-5′–dithiobis-(2-dinitrobenzoic acid) as previously described and read at 412 nm wavelength (Ellman, 1959). Serum nitric oxide which is an endogenous vasodilator was measured as described by Olaleye et al. (2007). Serum myeloperoxidase (MPO) activity was determined according to the method described by Xia and Zweier (1997).

2.8 Quantitative and Dipstick Urinalysis for markers of kidney function

Dipstick Urinalysis was employed for determination of metabolites in the urine samples. Urine was collected from rats in metabolic cages 24 hr prior to termination of experiment. The strips had reagent pads for semiquantitative assessment of ascorbic acid, bilirubin, urobilinogen, ketones, glucose, proteins, neutrophils, leukocytes, pH, and specific gravity using standard kits (ACON Laboratories, USA). Also, blood urea nitrogen and creatinine were assayed using standard kits according to manufacturer's instructions (Randox Laboratories).

2.9 Testosterone assay

Serum testosterone was measured using standard kits (Wuhan USCN) based on the competitive inhibition enzyme immunoassay technique using a microplate precoated with a monoclonal antibody specific to testosterone. A 96-well plate pre-coated with anti-testosterone antibodies ELISA kit was used. The serum samples and the testosterone-HRP conjugate were subsequently added to the wells. The testosterone in the serum bound with the added testosterone-HRP. The intensity of signal was inversely proportional to the amount of Testosterone in the sample and the intensity was measured at 450 nm.

2.10 Sperm abnormalities and characteristics

The sperm counts, motility, and livability were determined by the method of Wells and Awa based on the established protocol as described below. Percentage sperm motility was estimated after dilution of the semen on a pre-warmed clean glass slide with a drop of warm 2.9% sodium citrate and examined under a light microscope at a magnification of x 40 (Wells & Awa, 1970; Wheeler & Andrew, 1943). The spermatozoa livability % was determined by preparing a smear of semen, and then stained with 1% Eosin and 5% Nigrosin

in 2.9% sodium citrate dehydrate solution as previously described by (Oyeyemi & Babalola, 2006). The spermatozoa were counted with the aid of the Improved Neubauer hemocytometer (Deep 1/10 mm, LABART, Germany) chamber as demonstrated by Pant and Srivastava (2003).

2.11 Histopathology

Small pieces of kidney and testes were fixed in 10% neutral buffered formalin, passed through graded concentrations of alcohol and xylene, embedded in paraffin wax before sections of 5–6 mm in thickness were made on the microtome, and thereafter stained with Hematoxylin and Eosin (H & E) for histopathological examination according to the methods described by Drury and Wallington (1976). Thereafter, photomicrographs were taken with a light microscope at different magnifications.

2.12 Immunohistochemical analysis

The immunohistochemical expressions of angiotensin 2 type 1 receptor (ATR1), angiotensin converting enzyme (ACE), mineralocorticoid receptor (MCR), caspase 3 were evaluated in kidney and testes, respectively, using paraffin embedded tissues that were deparaffinized in xylene and rehydrated with graded alcohol. Antigen retriever, peroxidase quenching, blocking non-specific binding, and overnight incubation with anti-ATR1, anti-ACE, anti-caspase 3, and anti-MCR primary antibodies were carried out as previously described (Oyagbemi et al., 2019). The immune-positive reactions were enhanced with 3, 3′-Diaminobenzidine (DAB; AMRESCO LLC. OHio, USA) as the substrate. The immunoreactive positive expressions of ATR1, MCR, ACE, and caspase 3 anti-rabbit as intensive regions were viewed with light microscope (Leica LAS-EZ®, Germany) powered with Leica software application suite version 3.4 equipped with a digital camera. Immunoreactivity was also quantified using Image J software.

2.13 Data processing

Analysis of variance (ANOVA) was used to compare means across groups and Dunnett's post test was also carried out. The test of significance between two groups was estimated by Student's *t* test. All values are expressed as mean ± *SD*. Values of probability less that .05 were taken to be statistically significant.

3 RESULTS

3.1 Markers of kidney damage/inflammation

Our results indicate significant $p < .05$ increase in serum blood urea nitrogen (BUN), creatinine, advanced oxidative protein products (AOPPs), and myeloperoxidase activity together with significant $p < .05$ reduction in nitric oxide (NO) bioavailability in uninephrectomized hypertensive rats compared to the sham control (Table 1). Furthermore, the uninephrectomized hypertensive rats treated with rutin or lisinopril had significant $p < .05$ reduction in BUN, creatinine, advanced oxidative protein products (AOPPs), and myeloperoxidase along with significant improvement in the NO bioavailability when

compared to the uninephrectomized hypertensive rats (Table 1). Also, the anti-inflammatory property of either rutin or lisinopril demonstrated significant $p < .05$ reduction in the activity of MPO relative to the sham control and uninephrectomized hypertensive untreated rats (Table 1).

TABLE 1. Serum markers of renal damager and inflammation

Experimental groups	Sham	Uninephrectomized (UNX)	UNX + Rutin (100 mg/kg)	UNX + Lisinopril (10 mg/kg)
Blood urea nitrogen (U/L)	1.39 ± 0.53	1.84 ± 0.46	0.96 ± 0.34[b]	0.86 ± 0.18[b]
Creatinine (U/L)	2.00 ± 0.37	2.87 ± 0.25[a]	2.62 ± 0.16[a,b]	2.79 ± 0.08[a]
Nitric oxide (µmole/mg protein)	0.70 ± 0.09	0.50 ± 0.05[a]	0.58 ± 0.05[a]	0.65 ± 0.03[b]
AOPPs (nmole/mg protein)	32.76 ± 1.71	36.27 ± 6.54	29.68 ± 4.37	31.66 ± 5.57
Myeloperoxidase (µmole/min/mg protein)	4.96 ± 0.63	13.12 ± 3.41[a]	6.15 ± 1.50[b]	7.02 ± 0.69[b]

Note: Values are presented as mean ± standard deviation (*n* = 10). Superscript([a]) indicate significant difference when Groups B (Sham), C (Uninephrectomized; UNX) and D (UNX + Lisinopril (10 mg/kg) compared with Group A at $p < .05$, Superscript ([b]) indicate significant difference when Groups C and D compared with Group B in each row. Group A (Sham; Control), Group B Uninephrectomized (UNX) Group C (UNX + Rutin (100 mg/kg), Group D (UNX + Lisinopril (10 mg/kg).

Abbreviations: AOPP, advanced oxidation protein products (nmole/mg protein); MPO, myeloperoxidase (µmole/min/mg protein); NO, nitric oxide (µmole/mg protein).

3.2 Renal antioxidant defence status and markers of oxidative stress

The renal antioxidant defence status revealed that the uninephrectomized hypertensive rats had statistically significant $p < .05$ reductions in reduced glutathione (GSH), non-protein thiol (NPSH), and vitamin C content though not significant compared with the sham control (Table 2). However, the protein thiol (PSH) content of uninephrectomized hypertensive rats increased significantly when compared to the sham and the UNX+ L-NAME+ rutin and UNX+ L-NAME+ lisinopril, respectively (Table 2). Interestingly, the UNX+ L-NAME+ lisinopril group had significant improvement in renal GSH, PSH, NPSH, and vitamin C contents (Table 2).

TABLE 2. Renal non-enzymic antioxidant status in uninephrectomized hypertensive rats

Experimental groups	Sham	Uninephrectomized (UNX)	UNX + Rutin (100 mg/kg)	UNX + Lisinopril (10 mg/kg)
Reduced glutathione	107.32 ± 11.19	77.34 ± 6.34[a]	105.50 ± 8.42[b]	100.41 ± 8.72[b]
Non-protein thiol (NPSH)	45.10 ± 5.44	36.62 ± 2.90[a]	45.21 ± 1.93	40.17 ± 1.05[b]
Protein thiol (PSH)	76.34 ± 8.11	87.04 ± 3.17[a]	78.47 ± 8.91	55.45 ± 4.90[a,b]
Vitamin C	1.86 ± 0.08	1.88 ± 0.03	1.96 ± 0.12	1.91 ± 0.04

Note: Values are presented as mean ± standard deviation (*n* = 10). Superscript([a]) indicate significant difference when Groups B (Sham), C (Uninephrectomized; UNX) and D (UNX + Lisinopril (10 mg/kg) compared with Group A at $p < .05$, Superscript ([b]) indicate significant difference when Groups C and D compared with Group B in each row. Group A (Sham; Control), Group B Uninephrectomized (UNX) Group C (UNX + Rutin (100 mg/kg), Group D (UNX + Lisinopril (10 mg/kg).

Abbreviations: GSH, reduced glutathione (micromole/g tissue); NPSH, non- protein thiol (nmole/mg protein); PSH, protein thiol (nmole/mg protein); VITC, vitamin C (nmole/mg protein).

The renal protein carbonyl (PCO), malondialdehyde (MDA), and H_2O_2 generated in uninephrectomized hypertensive rats increased significantly compared to the Sham control (Table 3). In contrast, the UNX+ L-NAME+ rutin and UNX+ L-NAME+ lisinopril had statistically significant reduction of PCO, MDA and H_2O_2 generated (Table 3). In another experiment, the renal superoxide dismutase (SOD), and GST in uninephrectomized hypertensive rats increased significantly relative to the Sham. Similarly, the renal SOD and GST activity of the UNX+ L-NAME+ rutin and UNX+ L-NAME+ lisinopril increased significantly (Table 4).

TABLE 3. Renal markers of oxidative stress in uninephrectomized hypertensive rats

Experimental groups	Sham	Uninephrectomized (UNX)	UNX + Rutin (100 mg/kg)	UNX + Lisinopril (10 mg/kg)
Protein carbonyl (PCO)	66.23 ± 6.72	115.69 ± 8.97^{a}	63.34 ± 5.21^{b}	46.62 ± 1.15^{a,b}
Malondialdehyde (MDA)	1.80 ± 0.70	9.60 ± 0.04^{a}	1.40 ± 0.26^{b}	1.86 ± 0.64^{b}
H_2O_2 generated	61.40 ± 2.55	66.86 ± 3.22^{a}	61.86 ± 4.39^{a}	52.39 ± 5.25^{a,b}

Note: Values are presented as mean ± standard deviation (*n* = 10). Superscript(^{a}) indicate significant difference when Groups B (Sham), C (Uninephrectomized; UNX) and D (UNX + Lisinopril (10 mg/kg) compared with Group A at $p < .05$, Superscript (^{b}) indicate significant difference when Groups C and D compared with Group B in each row. Group A (Sham; Control), Group B Uninephrectomized (UNX) Group C (UNX + Rutin (100 mg/kg), Group D (UNX + Lisinopril (10 mg/kg).

Abbreviations: H_2O_2, hydrogen peroxide (µmol/mg protein); MDA, malondialdehyde (micromole MDA formed/mg protein); PCO, protein carbonyl (µmol/mg protein).

TABLE 4. Renal enzymic antioxidant defence system in uninephrectomized hypertensive rats

Experimental groups	Sham	Uninephrectomized (UNX)	UNX + Rutin (100 mg/kg)	UNX + Lisinopril (10 mg/kg)
Superoxide dismutase (SOD)	44.37 ± 8.05	52.09 ± 8.65 ^{a}	86.88 ± 3.62^{a,b}	56.77 ± 5.99^{a}
Glutathione peroxidase (GPx)	207.05 ± 8.98	209.01 ± 4.63	208.61 ± 4.20	209.92 ± 5.67^{a}
Glutathione S-transferase (GST)	16.21 ± 2.24	24.92 ± 4.32^{a}	29.16 ± 5.02^{a}	24.28 ± 4.08^{a}

Note: Values are presented as mean ± standard deviation (*n* = 10). Superscript (^{a}) indicate significant difference when Groups B (Sham), C (Uninephrectomized; UNX) and D (UNX + Lisinopril (10 mg/kg) compared with Group A at $p < .05$, Superscript (^{b}) indicate significant difference when Groups C and D compared with Group B in each row. Group A (Sham; Control), Group B Uninephrectomized (UNX) Group C (UNX + Rutin (100 mg/kg), Group D (UNX + Lisinopril (10 mg/kg).

Abbreviations: GPx, glutathione peroxidase (µmole/min/mg protein); GST, glutathione S-transferase (mmole1-chloro-2,4-dinitrobenzene-GSH/min/mg protein); SOD, superoxide dismutase (units'/mg protein).

3.3 Testicular antioxidant defence status and markers of oxidative stress

Testicular non-enzymic antioxidant of GSH, vitamin C and protein thiol contents significantly reduced in UNX+ L-NAME relative to the sham-operated (Table 5). Also, there was improvement in testicular GSH, PSH, and vitamin C contents in UNX+ L-NAME+ rutin and UNX+ L-NAME+ lisinopril compared to the sham-operated and uninephrectomized hypertensive rat. Similar to renal tissues, testicular PCO, and H_2O_2 generated increased significantly in reference to sham-operated group (Table 6). However, treatment of

uninephrectomized hypertensive rats treated with rutin or lisinopril diminished testicular PCO content and H_2O_2 generated (Table 6). In another experiment, reductions in testicular SOD of uninephrectomized hypertensive rats and uninephrectomized hypertensive rats with either rutin or lisinopril compared to the Sham control (Table 7). The testicular GST of uninephrectomized hypertensive rats and uninephrectomized hypertensive rats with rutin reduced significantly relative the Sham while uninephrectomized hypertensive rats with lisinopril increased significantly compared to uninephrectomized hypertensive rats (Table 7).

TABLE 5. Testicular non-enzymic antioxidant status in uninephrectomized hypertensive rats

Experimental groups	Sham	Uninephrectomized (UNX)	UNX + Rutin (100 mg/kg)	UNX + Lisinopril (10 mg/kg)
Reduced glutathione	100.34 ± 0.55	105.33 ± 3.98[a]	104.18 ± 1.35[a]	104.80 ± 2.11[a]
Non-protein thiol (NPSH)	36.26 ± 3.51	34.39 ± 2.18	34.43 ± 4.18	36.06 ± 4.46
Protein thiol (PSH)	51.5 ± 0.82	38.77 ± 0.77[a]	36.72 ± 0.63[a,b]	36.21 ± 0.70[a,b]
Vitamin C	4.67 ± 0.61	1.74 ± 0.25[a]	1.81 ± 0.15[a]	1.81 ± 0.09[a]

Note: Values are presented as mean ± standard deviation (*n* = 10). Superscript ([a]) indicate significant difference when Groups B (Sham), C (Uninephrectomized; UNX) and D (UNX + Lisinopril (10 mg/kg) compared with Group A at $p < .05$, Superscript ([b]) indicate significant difference when Groups C and D compared with Group B in each row. Group A (Sham; Control), Group B Uninephrectomized (UNX) Group C (UNX + Rutin (100 mg/kg), Group D (UNX + Lisinopril (10 mg/kg).

Abbreviations: GSH, reduced glutathione (micromole/g tissue); NPSH, non- protein thiol (nmole/mg protein); PSH, protein thiol (nmole/mg protein); VITC, vitamin C (nmole/mg protein).

TABLE 6. Testicular markers of oxidative stress in uninephrectomized hypertensive rats

Experimental groups	Sham	Uninephrectomized (UNX)	UNX + Rutin (100 mg/kg)	UNX + Lisinopril (10 mg/kg)
Protein carbonyl (PCO)	90.47 ± 2.52	118.91 ± 10.40[a]	80.86 ± 9.40[b]	93.00 ± 3.90[b]
Malondialdehyde (MDA)	2.30 ± 0.24	2.60 ± 0.36[a]	2.10 ± 0.40[b]	2.00 ± 0.48[b]
H_2O_2 generated	38.18 ± 0.64	40.73 ± 1.87[a]	37.00 ± 3.30[b]	37.64 ± 1.30[b]

Note: Values are presented as mean ± standard deviation (*n* = 10). Superscript ([a]) indicate significant difference when Groups B (Sham), C (Uninephrectomized; UNX) and D (UNX + Lisinopril (10 mg/kg) compared with Group A at $p < .05$, Superscript ([b]) indicate significant difference when Groups C and D compared with Group B in each row. Group A (Sham; Control), Group B Uninephrectomized (UNX) Group C (UNX + Rutin (100 mg/kg), Group D (UNX + Lisinopril (10 mg/kg).

Abbreviations: H_2O_2, hydrogen peroxide (μmol/mg protein); MDA, malondialdehyde (micromole MDA formed/mg protein); PCO, protein carbonyl (μmol/mg protein).

TABLE 7. Testicular enzymic antioxidant defence system in uninephrectomized hypertensive rats

Experimental groups	Sham	Uninephrectomized (UNX)	UNX + Rutin (100 mg/kg)	UNX + Lisinopril (10 mg/kg)
Superoxide dismutase (SOD)	105.44 ± 8.50	45.15 ± 4.43[a]	46.40 ± 5.54[a]	103.78 ± 10.35[b]
Glutathione peroxidase (GPx)	211.01 ± 2.58	207.08 ± 3.45[a]	210.55 ± 2.73	210.74 ± 2.58
Glutathione S-transferase (GST)	24.14 ± 4.03	16.63 ± 1.78[a]	18.23 ± 3.77[a]	26.86 ± 5.47[b]

Note: Values are presented as mean ± standard deviation (n = 10). Superscript([a]) indicate significant difference when Groups B (Sham), C (Uninephrectomized; UNX) and D (UNX + Lisinopril (10 mg/kg) compared with Group A at $p < .05$, Superscript ([b]) indicate significant difference when Groups C and D compared with Group B in each row. Group A (Sham; Control), Group B Uninephrectomized (UNX) Group C (UNX + Rutin (100 mg/kg), Group D (UNX + Lisinopril (10 mg/kg).

Abbreviations: GPx, glutathione peroxidase (µmole/min/mg protein); GST, glutathione S-transferase (mmole1-chloro-2,4-dinitrobenzene-GSH/min/mg protein); SOD, superoxide dismutase (units'/mg protein).

3.4 Body, kidney, and testes weights

A significant decrease in body weight of uninephrectomized hypertensive rats and other treatment groups when compared the sham control was recorded (Figure 1), but, the weight of kidney increased in UNX and UNX+ L-NAME+ lisinopril group in reference to the sham (Figure S1).

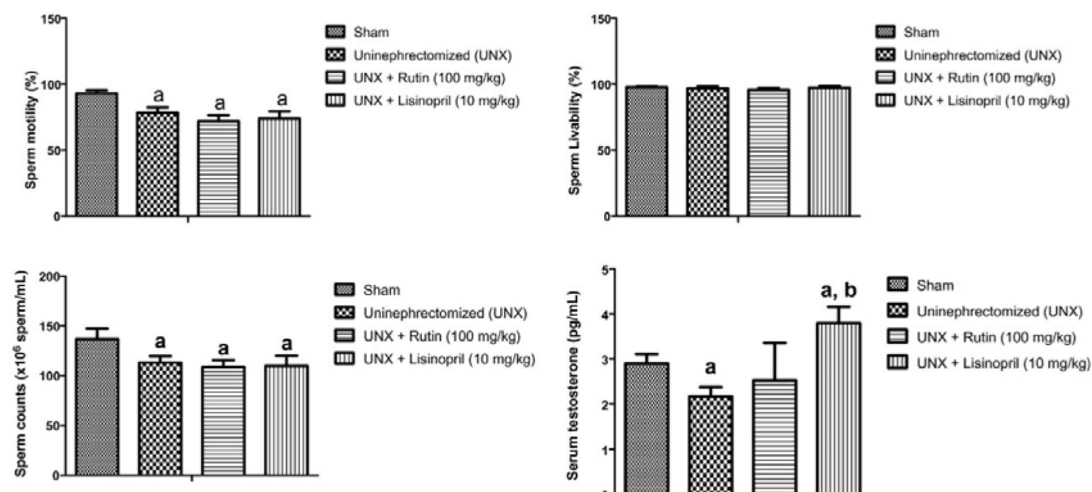

FIGURE 1. Sperm characteristics of uninephrectomized hypertensive rats

3.5 Hemodynamic parameters

In Figure S2, there was no significant difference in hemodynamic parameters of pre-uninephrectomized normotensive rats across all groups. However, 3 weeks post hypertensive state, uninephrectomized hypertensive rats showed significant increases in systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP), whereas the converse was true for UNX+ L-NAME+ lisinopril and UNX+ L-NAME+ lisinopril groups (Figure S3).

3.6 Spermiogram and serum testosterone level

The motility and counts of spermatozoa decreased in UNX, UNX+ L-NAME+ rutin, and UNX+ L-NAME+ lisinopril (Figure 1). Sperm livability was not significantly affected across all experimental groups. Furthermore, the serum testosterone level decreased in UNX group compared with the UNX+ L-NAME+ lisinopril (Figure 1).

3.7 Urinalysis

From Table S1, the urinalysis revealed high presence of bilirubin, urobilinogen, ketones, proteins, blood, neutrophils, leukocytes, and pH of uninephrectomized hypertensive rats relative to the sham control and other treatment groups. However, the UNX+ L-NAME+ rutin and UNX+ L-NAME+ lisinopril groups had no bilirubin, blood, glucose but with few leukocytes, neutrophils and small presence of proteins in their urine samples.

3.8 Histology

Renal histology showed moderate hyperplasia of the mesangium in the sham control, moderate glomerular and tubular epithelial atrophy with ectatic lumen containing cellular casts in the uninephrectomized hypertensive rats. Attenuation of tubular epithelial cells and patchy tubular epithelial coagulation necrosis were also observed in the uninephrectomized hypertensive rats treated with either rutin or lisinopril, respectively (Figure S4).

Testicular histology showed seminiferous tubules with germ cells at different developmental stages of development with no observable lesions in the sham control. The seminiferous tubules also showed germ cells at different stages of development in uninephrectomized hypertensive rats. There were a few pyknotic cells and scanty germinal epithelium in the seminiferous tubules of uninephrectomized hypertensive rats treated with rutin, while seminiferous tubules showed germ cells at different stages of development with no observable lesions in uninephrectomized hypertensive rats treated with lisinopril (Figure S5).

3.8.1 Angiotensin converting enzyme (ACE)

Figures 2 and S6a show that expressions of ACE increased in uninephrectomized group, but decreased in UNX+ L-NAME+ rutin and UNX+ L-NAME+ lisinopril in comparison with the sham-operated rats. The reduction in the ACE expressions was more profound in the lisinopril treated rats (Figures 2 and S6a).

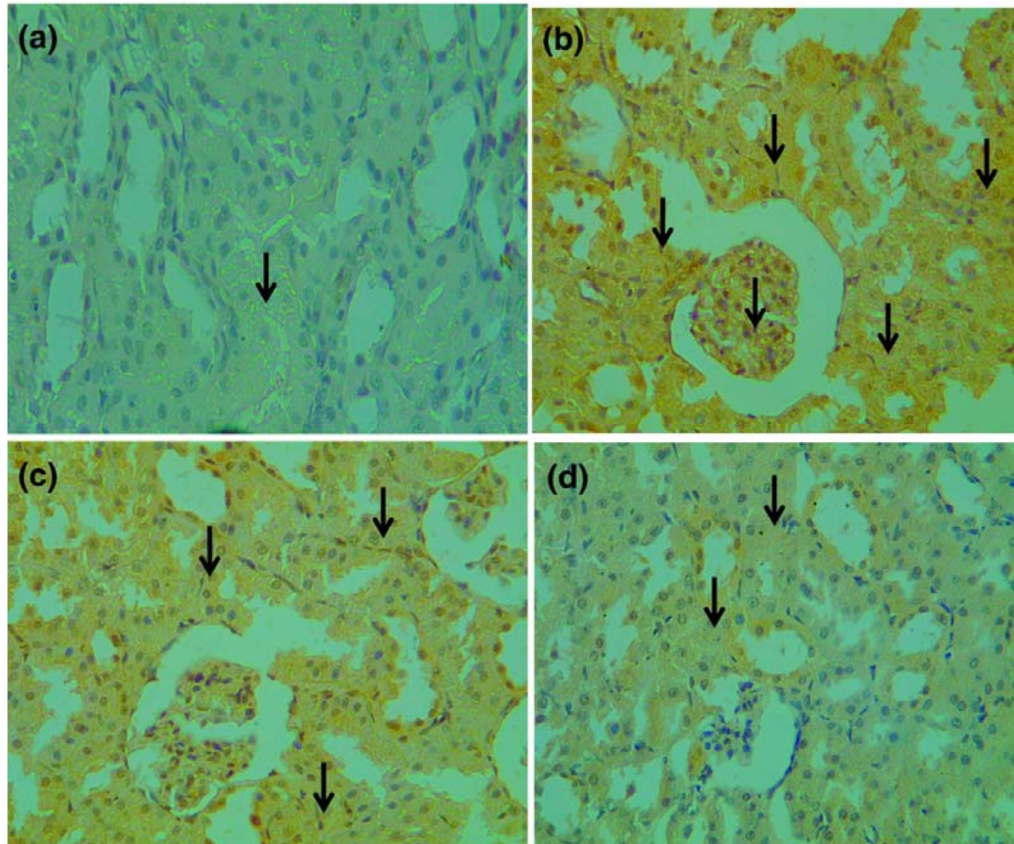

FIGURE 2. The immunohistochemistry of angiotensin converting enzyme (ACE) of uninephrectomized hypertensive rats

3.8.2 Mineralocorticoid receptor (MCR)

In another experiment, the expressions mineralocorticoid receptor (MCR) were significantly higher in UNX group in comparison with sham and UNX+ L-NAME+ lisinopril and UNX+ L-NAME+ rutin (Figures 3 and S6b). The expressions of MCR in uninephrectomized hypertensive rats treated with rutin were higher than that of sham and uninephrectomized hypertensive untreated rats. However, the uninephrectomized hypertensive rats treated with lisinopril gave the lowest expressions of MCR in comparison to sham and uninephrectomized hypertensive rats (Figures 3 and S6b).

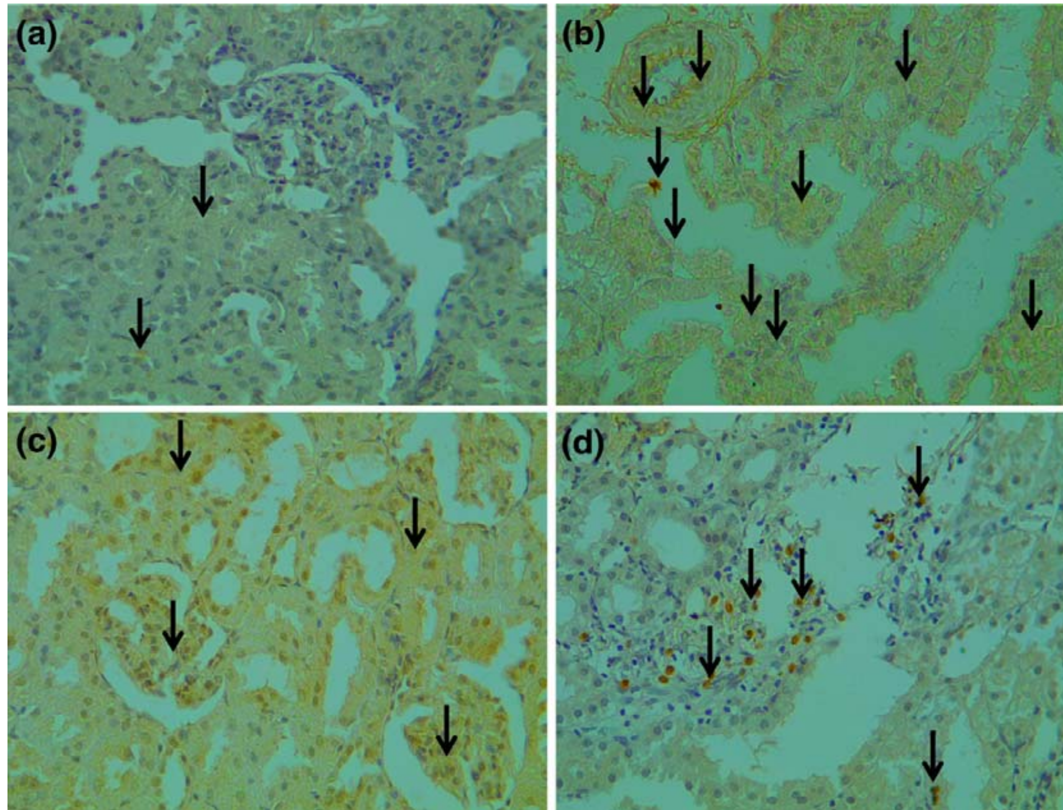

FIGURE 3. The immunohistochemistry of mineralocorticoid receptor (MCR) of uninephrectomized hypertensive rats

3.8.3 Angiotensin 2 type 1 receptor (ATR1)

The immunolocalization of ATR1 was found to be higher in UNX group and UNX+ L-NAME+ rutin, although, this was not statistically significant (Figures 4 and S6c). Furthermore, treatment of uninephrectomized hypertensive rats with lisinopril resulted in an appreciable reduction in the expressions of ATR1 relative to sham and uninephrectomized hypertensive rats (Figures 4 and S6c).

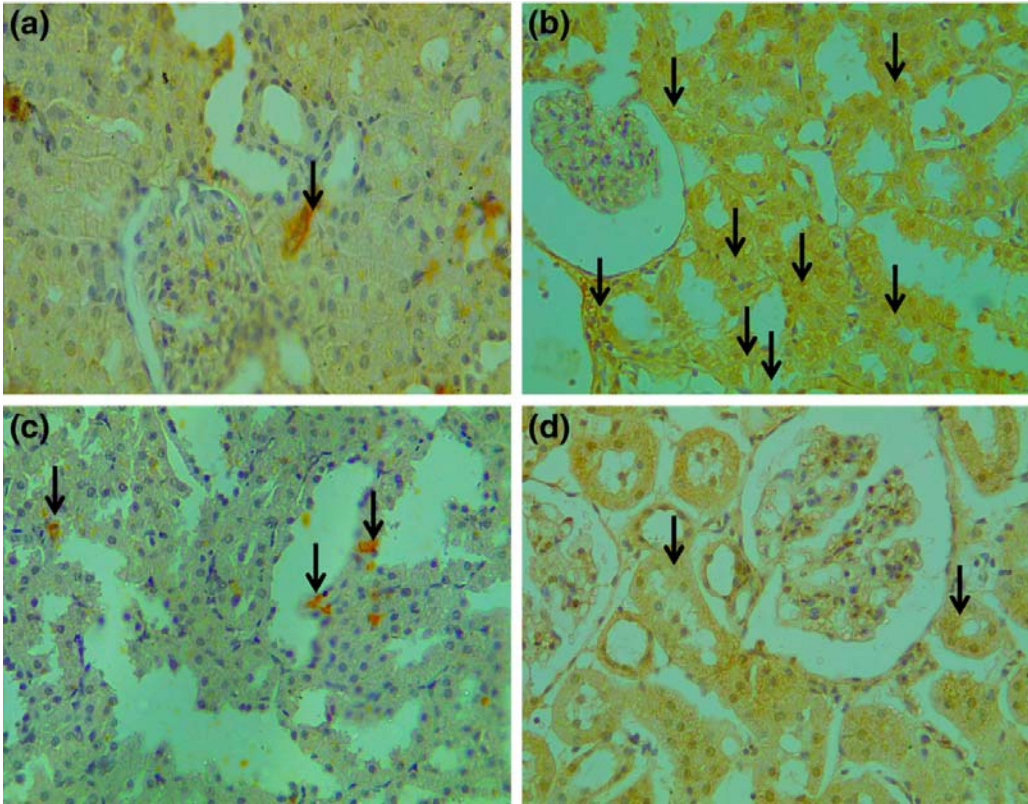

FIGURE 4. The immunohistochemistry of angiotensin 2 type 1 receptor (ATR1) of uninephrectomized hypertensive rats

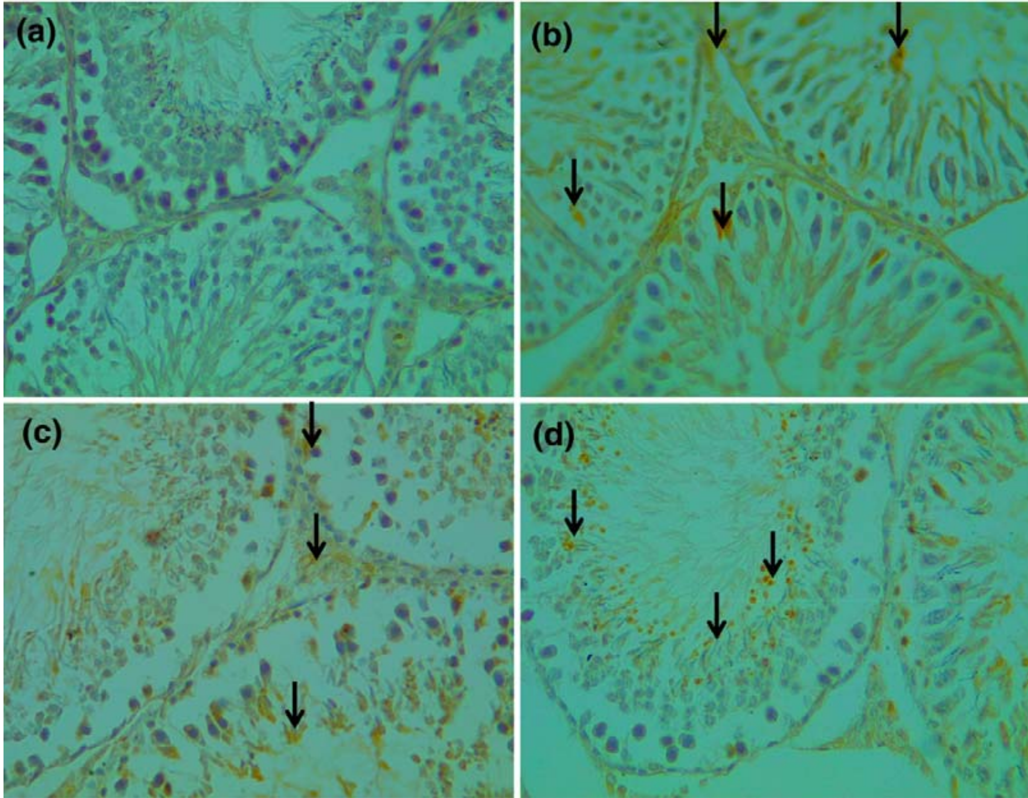

FIGURE 5. The immunohistochemistry of caspase 3 of uninephrectomized hypertensive rats

3.8.4 Testicular caspase 3

The testicular caspase 3 expressions were significantly higher in both uninephrectomized hypertensive rats and uninephrectomized hypertensive rats administered rutin compared to the sham (Figures 5 and S6d). Interestingly, uninephrectomized hypertensive rats treated with lisinopril significantly lowered the expressions of testicular caspase 3 when compared to uninephrectomized hypertensive rats and the sham (Figures 5 and S6d).

4 DISCUSSION

From the present study, a high presence of bilirubin, ketones, glucose, proteins, leukocytes, and neutrophils was found in the urine samples of UNX-hypertensive rats. This is indicative of loss of functional capacity of the nephrons and glomeruli that serve to filter blood, and toxic waste products. Proteinuria is a likely predictive risk factor for chronic renal damage as earlier described (Skowron et al., 2019). Other findings have reported the enhancement of proinflammatory cytokines in glomerulonephritis and glomerulonephropathy (Elblehi et al., 2019; Xu et al., 2019). Hence, the presence of leukocytes and neutrophils observed in the urine samples of UNX hypertensive rats was an indication of an on-going inflammatory reaction that might be associated with or precipitated by uninephrectomy. Rutin or lisinopril administration mitigated the aberration in the release of waste products, toxic metabolites, and nutrients that should be reabsorbed by the nephron into the urine. This might be indicative of the nephroprotective effect of rutin and lisinopril against UNX-induced renal damage.

The inflammatory response was corroborated with significant elevation of serum myeloperoxidase (MPO) activity of UNX group compared with the sham operated. Punuru et al. (2014) and Oztay et al. (2016) reported a significant increase in the renal activity of MPO accompanied with elevated levels of serum BUN and creatinine in unilateral renal ischemia reperfusion injury in rats. Exaggerated inflammation might also be responsible for the heightened presence of leukocytes in the urine of UNX hypertensive rats. This implies that renal damage could also be accompanied by inflammation. Interestingly, co-treatment with either rutin or lisinopril was able to reduce the activity of MPO, and it also normalized the serum levels of BUN and creatinine which are indicative of nephroprotective and inflammatory properties of rutin and lisinopril. Previous studies have reported the anti-inflammatory and nephroprotective effects of rutin and lisinopril (Kumar & Yin, 2018; Pfaff & Vallon, 2002). The UNX-induced inflammation might also exacerbate the abnormal elevation in serum BUN, creatinine and urinary bilirubin, urobilinogen, ketones, and proteins in UNX hypertensive rats. Therefore, hypertension coupled with UNX could precipitate renal hypertrophy and damage of the remaining kidney. UNX hypertensive patients could be encouraged to increase their intake of vegetables or phytochemicals rich in rutin alongside ACE inhibitors such as lisinopril.

The mineralocorticoid is an aldosterone that plays many causative roles in the pathogenesis of cardiovascular diseases (Shibata & Itoh, 2012). In the present study, the observed elevated systolic, diastolic, and mean arterial pressures which were consistent with higher ACE and MCR expressions in hypertensive subjects, since the formation of angiotensin II, a potent vasoconstrictor and stimulator of aldosterone release, requires the physiological

activity of ACE. Mineralocorticoid antagonists and ACE inhibitors are drugs of choice as antihypertensive agents (Gupta et al., 2019; Messerli et al., 2018; Muslih, 2012; Podzolkov & Tarzimanova, 2018). Aldosterone has been reported to activate MCR, thereby causing hypertension due to increased fluid retention associated with enhanced sodium ion reabsorption in the nephron (Frey et al., 2004). Therefore, a MCR antagonist will be effective in reducing and maintaining blood pressure parameters. Our results showed that rutin lowered the expressions of both ACE and MCR similar to the action of the standard ACE inhibitor, lisinopril. The implication of this is that rutin might be classified as both an ACE inhibitor and MCR antagonist. Rutin probably mediates its effect on hypertensive states through the inhibition of ACE and deactivation of MCR. Also, our result showed mild elevation of expressions of renal immunolocalization of angiotensin 2 type 1 receptor (ATR1) in UNX hypertensive rats when compared to the Sham group. We hypothesized that UNX combined with hypertension must have also contributed to the increased immunostaining of ATR1 and that the observable increase in the immunolocalization of renal ACE, MCR, and ATR1 underlines the modulatory role of the renin-angiotensin-aldosterone system in the pathogenesis of hypertension. Interestingly, ATR1 expressions were also reduced similar to that ACE and MCR UNX hypertensive untreated rats. From this study, lisinopril gave a better reduction in the expressions of ATR1. Hence, deactivation of renal ATR1 might be another classical pathway for the antihypertensive effect of lisinopril, indicating that the antihypertensive effect of rutin might not be through ATR1.

From our study, we noticed reduction in serum nitric oxide (NO), and this might be responsible for the observed elevation in mean arterial as well as the systolic and diastolic pressures of UNX hypertensive animals. Previous findings have shown that reduced serum NO bioavailability due to endothelial nitic oxide synthase (eNOS) inhibition is directly proportional to hypertension, endothelial dysfunction, and arterial stiffness (Guizoni et al., 2020; Oyagbemi et al., 2020). It could, therefore, be hypothesized based on this study that administration of rutin to UNX hypertensive rats was capable of restoring the functional integrity and capacity of eNOS following the combination of UNX and hypertension, and this was evident in the improved NO bioavailability in the UNX+ L-NAME+ rutin and UNX+ L-NAME+ lisinopril groups. More so, eNOS inhibition can also precipitate chronic kidney disease as previously described by Rajapakse et al. (2016).

The advanced oxidative protein products (AOPP) as an oxidative stress product have found clinical relevance in end-stage renal disease of hemodialyzed patients (Sangeetha et al., 2018). The elevated levels of serum AOPPs in UNX hypertensive rats could be extrapolated to on-going inflammation, oxidation, and probably initiation of renal fibrosis with attendant grave consequences of the cardiovascular system. The capacity of rutin to reduce the AOPP level was highly comparable to that of lisinopril. The inhibition of eNOS with production of peroxynitrite (ONOO-) which is a cytotoxic molecule could also aggravate the formation of AOPP, thereby fostering endothelial damage and ultimate cardiovascular system compromise (Khan et al., 2018). The production of ONOO- is from the reaction between the superoxide anion radical ($O_2\bullet^-$) and NO, with concomitant reduction in the NO bioavailability, thereby precipitating hypertension.

Our present study revealed a decrease in the renal and testicular GSH, as well as NPSH and vitamin C, while malondialdehyde (MDA), protein carbonyl, and H_2O_2 generation were

elevated in UNX group. The elevation of oxidative stress indicators such as MDA with concomitant reduction in the non-enzymic antioxidants confirm the earlier reports of oxidative mechanisms in hypertension (Sangeetha et al., 2018). The compensatory hypertrophy of the remaining kidney might also aggravate oxidative processes seen in this study, as exaggerated contents of MDA, protein carbonyl and H_2O_2 generation in both renal and testicular tissues alluding to the remote effect of UNX on the reproductive system. We also observed that both renal and testicular antioxidant defence system were compromised in UNX hypertensive rats. The antioxidant enzymes SOD, GPx, and GST were elevated specifically in the renal tissues of the UNX group, thus, suggesting an adaptive response that was accompanied with compensatory hypertrophy. In other words, the body was trying to rid itself of free radicals generated following UNX and hypertension.

Also, in this study, motility, livability and spermatozoa counts as well as testosterone level were reduced in addition to the observed increase in testicular apoptosis seen as heightened caspase 3 expressions in UNX hypertensive rats. Combination of UNX, hypertension and renal damage might be responsible for the observed complication of reproductive failure in UNX hypertensive rats. Sexual dysfunction, impaired spermatogenesis, lower fertility, decreased libido, and testosterone levels have been reported in uraemia and renal transplant recipients (Xu et al., 2009). The aberration observed in the sperm motility, livability, counts and serum testosterone was restored in UNX+ L-NAME+ rutin and UNX+ L-NAME+ lisinopril groups. The aforementioned higher expressions of testicular caspase 3 recorded in the UNX hypertensive rats were also downregulated in UNX hypertensive rats treated. This implies that the untreated UNX hypertensive state might progress to reproductive failure, infertility, and probably sterility. Our study, therefore, supported that of Akbari et al. (2003) who reported abnormality in the levels of testosterone, prolactin, and gonadotropins after renal transplantation.

5 CONCLUSION

This study, therefore, demonstrated the antihypertensive, renoprotective, and repro-protective of rutin in UNX hypertensive rats. It also highlighted antioxidant and anti-inflammatory activity of rutin in UNX hypertensive rats. The mechanism of antihypertensive action of rutin was hypothesized as renal angiotensin converting enzyme and the mineralocorticoid receptor inhibition. The antihypertensive beneficial effect of rutin was noticeable through augmentation of the serum nitric oxide bioavailability. Similarly, improvement in sperm counts, motility, livability and serum testosterone might also be associated with improvement in testicular antioxidant defence status and abrogation of testicular apoptosis. Taken together, fruits and vegetables rich in rutin could, therefore, serve as potential nutraceuticals for the management of hypertension and its complications in uninephrectomized hypertensive patients.

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CONFLICT OF INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHOR CONTRIBUTION

Ademola Adetokunbo Oyagbemi: Conceptualization; Data curation; Investigation; Methodology; Project administration; Supervision; Writing-original draft; Writing-review & editing. **Foluso Bolawaye Bolaji-Alabi:** Methodology; Writing-original draft; Writing-review & editing. **Temitayo Olabisi Ajibade:** Data curation; Formal analysis; Investigation; Methodology; Writing-original draft; Writing-review & editing. **Olumuyiwa Abiola Adejumobi:** Investigation; Methodology. **Olumide Samuel Ajani:** Data curation; Formal analysis; Investigation; Methodology; Resources. **Theophilus Aghogho Jarikre:** Formal analysis; Visualization. **Olufunke Eunice Ola-Davies:** Resources; Supervision. **Temidayo Olutayo Omobowale:** Investigation; Methodology; Project administration; Resources; Supervision; Writing-original draft; Writing-review & editing. **Kehinde Olugboyega Soetan:** Investigation; Methodology. **Abimbola Obemisola Aro:** Writing-original draft; Writing-review & editing. **Benjamin Obukowho Emikpe:** Visualization; Writing-review & editing. **Adebowale Benard Saba:** Supervision. **Adeolu Alex Adedapo:** Supervision. **Matthew Olugbenga Oyeyemi:** Data curation; Formal analysis; Methodology; Supervision. **Sanah Malomile Nkadimeng:** Writing-review & editing. **Prudence Ngalula Kayoka-Kabongo:** Writing-review & editing. **L.J. McGaw:** Supervision; Writing-review & editing. **Oluwafemi O. Oguntibeju:** Resources; Supervision; Writing-review & editing. **Momoh Audu Yakubu:** Resources; Supervision; Writing-review & editing.

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