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# Acetate causes renoprotection like androgen and mineralocorticoid receptors blockade in testosterone-exposed pregnant rats

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## Abstract

The kidney plays a critical role in human health and deviation from its normal function can lead to severe morbidity and mortality. Exposure to excess testosterone in women has been linked to several disorders, including kidney disorder and acting undoubtedly through androgen receptor (AR), whereas the involvement of mineralocorticoid receptor (MR) is unclear. Likewise, the renal effect of sodium acetate (SAc) during late gestational exposure to testosterone is not well known. We hypothesized that SAc or MR blockade would protect the kidney of testosterone-exposed pregnant rats against glutathione and adenosine depletion. Twenty-five pregnant Wistar rats were treated (sc) with olive oil, testosterone propionate (0.5 mg/kg) singly or in combination with SAc (200 mg/kg; *p.o.*), androgen receptor (AR) blocker, flutamide (Flu; 7.5 mg/kg; *p.o.*) or (MR) blocker, eplerenone (Eple; 0.5 mg/kg) between gestational days 14 and 19. Glutathione, adenosine and nitric oxide were decreased while uric acid (UA), xanthine oxidase (XO), malondialdehyde (MDA), lactate dehydrogenase activity and free fatty acids were increased in the kidneys of gestational rats exposed to testosterone. Also, plasma urea and creatinine were elevated. SAc and Eple reversed tested testosterone-induced effects in gestational rats. The exposure to testosterone impairs renal antioxidant defense via AR and MR during late gestation in pregnant rats. The study also provides evidence that sodium acetate protects the kidneys of gestational testosterone-exposed rats against defective antioxidant defense in like manner as MR or AR antagonist.

**Keywords** Maternal testosterone · Mineralocorticoid receptor · Androgen receptor · Renal anti-oxidant · SCFA

## Introduction

Maternal testosterone exposure and the associated low birth weight due to fetal growth restriction and adverse adult health consequences have recently received growing attention of scientific studies. Higher testosterone levels are reported in several obstetric complications such as preeclampsia, leading to fetal growth restriction and preterm birth [1, 2]. In addition, it has been demonstrated that excess gestational androgen exposure resulted in kidney disorders with consequent renal failure [3, 4].

Intact kidneys are essential for healthy living as they play pivotal roles in fluid and electrolyte balance, regulation of blood pressure, excretion and loss of these functions can contribute to increased morbidity and mortality, especially in pregnant women [5, 6]. Nevertheless, the kidney is one among the organs significantly impacted by pregnancy-derived alterations [7], which include mineralocorticoid receptor (MR) activation and the impact here is shown to be worsened by elevated testosterone [8]. In addition, mineralocorticoid receptor (MR) has been documented as the genomic effector of the activities of mineralocorticoids, including aldosterone and this serves as the basis of using MR blockade to cause diuresis and subsequently reduce blood pressure. MR blockade has also been shown to have

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other beneficial effects such as amelioration of inflammation and oxidative stress.

The nucleoside, adenosine, has vasodilatory, anti-inflammatory and antioxidant properties. Negative regulation of renin-angiotensin system by adenosine in addition to its anti-inflammatory and antioxidant properties protects the kidney from damage [9–11]. Adenosine deaminase (ADA), which clears and reduces adenosine irreversibly catalyzes conversion of adenosine to inosine (a substrate for uric acid production). It is a ubiquitous polymorphic enzyme and its activity in the kidney is highly predictable. By reducing adenosine, the activity of ADA has been correlated with inflammatory response [11]. The last two steps of purine metabolism which produces uric acid is catalyzed by xanthine oxidase (XO) activity [12]. Findings from relevant studies have led to the perception that enhanced XO activity underlies the pro-oxidant property of uric acid and its adverse effects [13]. Increase in uric acid production is involved in the pathogenesis of kidney disorders with consequent cardiovascular disease [14].

Overproduction of ROS or inadequate antioxidant defense leads to oxidative stress (OS) in tissues and organs leading to maladies like nephropathy which especially associated with diabetic patients [15]. Cells are protected from ROS by an endogenous antioxidant barrier including enzymatic or nonenzymatic components like reduced glutathione (GSH). GSH converts hydrogen peroxides and lipid peroxides to $H_2O$ and lipid alcohols respectively [16]. It also plays a role in preventing the oxidation of proteins sulfhydryl group. Glucose-6-phosphate dehydrogenase (G6PD) the enzyme that catalyzes the rate limiting step in the pentose phosphate pathway (PPP) maintains GSH pool [16].

Mineralocorticoid receptor mediates genomic activities of mineralocorticoids such as aldosterone and this serves as the basis of using MR blockade to cause diuresis in order to reduce blood pressure. MR blockade has also been shown to have other beneficial effects such as amelioration of inflammation and oxidative stress. The involvement of mineralocorticoid receptor activation in various ailments is not new. A previous study from our laboratory has shown that MR activation is involved in cardiac derangements caused by late gestational testosterone exposure in rats [17].

Short chain fatty acids (SCFAs) are end products of fermentation of complex carbohydrates by colonic microbiota especially anaerobic bacteria [18]. Acetate represents about 65% of the SCFA composition produced in the colon avoiding hepatic clearance. It reaches micromolar concentrations in the circulation [19]. Reports revealed that SCFAs ameliorate kidney disease [20]. Reports also showed that acetate attenuated intracellular ROS induced by lipopolysaccharide with an increase in glutathione [21].

We therefor tested the hypothesis that both AR and MR activation are involved in renal defective antioxidant defense in testosterone-exposed pregnant rats and that sodium acetate (SAc) like AR or MR antagonists would protect the rats against renal glutathione and adenosine depletion.

## Materials and methods

### Animals and treatment

The investigation was conducted in adherence to the National Institutes of Health Guide for theCare and Use of Laboratory Animals and the study protocol was approved by the University Ethical Review Committee (UERC), University of Ilorin, Ilorin, and every effort was made to minimize both the number of animals used and their suffering. Animals were also treated in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press). Pregnant Wistar rats with an average age of twelve (12) weeks weighing between 150 and 180 g were used for this study. Rats were given access to food and water ad libitum. The animals were randomly assigned to 5 groups ($n = 5$/group). Rats were maintained under standard environmental conditions of temperature (22–26 °C), relative humidity (50–60%), and 12-h dark/light cycle. The animals were treated either with vehicle (olive oil; *sc* and distilled water; *po*) or testosterone propionate (0.5 mg/kg; sc) singly (Tes) or in combination with sodium acetate (Ace; 200 mg/kg; *po*), flutamide (Flu), an AR blocker (7.5 mg/kg; *po)* or eplerenone (Epl; 1.0 mg/kg; *po*) from day 14 to 19 of gestation.

### Sample preparation

#### Blood sample

At the termination of the experiment, animals were anesthetized with sodium pentobarbital (50 mg/kg, *ip*) after an overnight 12-hourfast. Blood was collected by cardiac puncture into heparinized bottles and centrifuged at 3000 rpm for 15 min. Plasma was obtained and stored frozen until needed for biochemical assay.

#### Tissue sample

The kidneys of the animals were excised, blotted and weighed without connective tissues before being homogenized in ice-cold phosphate buffer saline (PBS) solution. The homogenate was centrifuged at 10000 rpm for 10 min and the supernatant was decanted and stored frozen until needed for biochemical assay.



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## Biochemical assay

### Evaluation of redox biomarkers

Glucose-6-phosphate dehydrogenase (G6PD) activity was determined, using an assay kit from Fortress diagnostics (Antrim, UK), by the Kinetic enzyme method [22]. Glutathione peroxidase (GPx) was determined using an established method as described by Paglia and Valentine [23]. Reduced and oxidized glutathione (GSH and GSSG) were measured by standardized enzymatic colorimetric assay using a previously described standard method [24]. The protein levels were estimated spectrophotometrically by the Bradford method [25].

### Evaluation of tissue injury markers

Lactate dehydrogenase (LDH) activity was estimated using an assay kit from Fortress diagnostics (Antrim, UK). NAD was reduced to NADH by LDH to produce a color by interacting with a specific probe. The estimation of LDH was done at OD 450 nm (T1) on a microplate reader in a kinetic mode, every 2–3 min, for at least 30–60 min at room temperature protected from light. Also, lactate was estimated using an assay kit from Fortress diagnostics (Antrim, UK). LDH oxidized lactate to generate a product that interacts with a probe to produce a color at optical density (OD) of 450 nm. The levels of thiobarbituric acid reactive substances (TBARS), product of malonyldialdehyde (MDA), were assayed using kit obtained from Oxford Biomedical Research Ltd. (Rochester Hills, MI, USA) by measuring the pink color chromophore formed by the reaction of 2-thiobarbituric acid with MDA [26].

### Estimation of uric acid production

Uric acid was measured by assay kit obtained from Fortress Diagnostics (Co Antrim UK). After the preparation of the enzyme reagent, 20 µl of the standard was added to each sample and 800 µl of the buffer was added, mixed and incubated for 1 min and initial absorbance read. 200 µl of the enzyme reagent was then added to each well containing the sample and the absorbance read after exactly 5 min. Hepatic adenosine was estimated following a multi-step enzymatic approach resulting in the generation of an intermediate that reacts with the adenosine probe to form a fluorescent product. The fluorescence was then measured immediately on a microplate reader at Ex/Em = 535/587 nm. Adenosine deaminase (ADA) activity was measured by a standardized enzymatic colorimetric method [27]. Xanthine oxidase was measured by a standardized enzymatic colorimetric method where XO oxidized xanthine to $H_2O_2$ which reacts stoichiometrically with OxiRed Probe to generate color ($\lambda$ = 570 nm).

### Liver injury enzyme markers

Gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured by a standardized enzymatic colorimetric method using assay kit obtained from Fortress Diagnostics Limited, Antrim, UK.

## Statistical analysis

All data were expressed as means ± SEM. Statistical group analysis was performed using One-way analysis of variance (ANOVA) and Bonferroni's Post Hoc analysis was used to identify the significance of pair wise comparison of mean values among the groups. Statistically significant differences were accepted at $p < 0.05$.

## Results

### Effects of acetate, flutamide and eplerenone on renal lipid in testosterone-exposed pregnant rats

Gestational testosterone exposure led to elevated renal tissue free fatty acid level, which was attenuated by androgen receptor blocker (flutamide), mineralocorticoid receptor blocker (eplerenone) as well as sodium acetate (Fig. 1a). However, renal triglyceride and total cholesterol were not affected by testosterone exposure with or without AR blockade, MR receptor blockade, and acetate (Fig. 1b, c).

### Effects of sodium acetate, flutamide and eplerenone on renal uric acid production, lactate production, nitric oxide and malondialdehyde in testosterone-exposed pregnant rats

Gestational testosterone exposure led to elevated uric acid production with concomitant increase in xanthine oxidase but not adenosine deaminase (Fig. 2b–d). Also, malondialdehyde level was elevated in gestational testosterone exposure (Fig. 2e). Although testosterone did not affect adenosine deaminase activity, adenosine was reduced (Fig. 1a). Renal nitric oxide and lactate levels were reduced despite elevated lactate dehydrogenase in gestational testosterone exposure (Fig. 2f–h). Treatment with flutamide, eplerenone and acetate during gestational testosterone exposure restored adenosine, lactate and nitric oxide levels and attenuated uric acid production and malondialdehyde level (Fig. 2).



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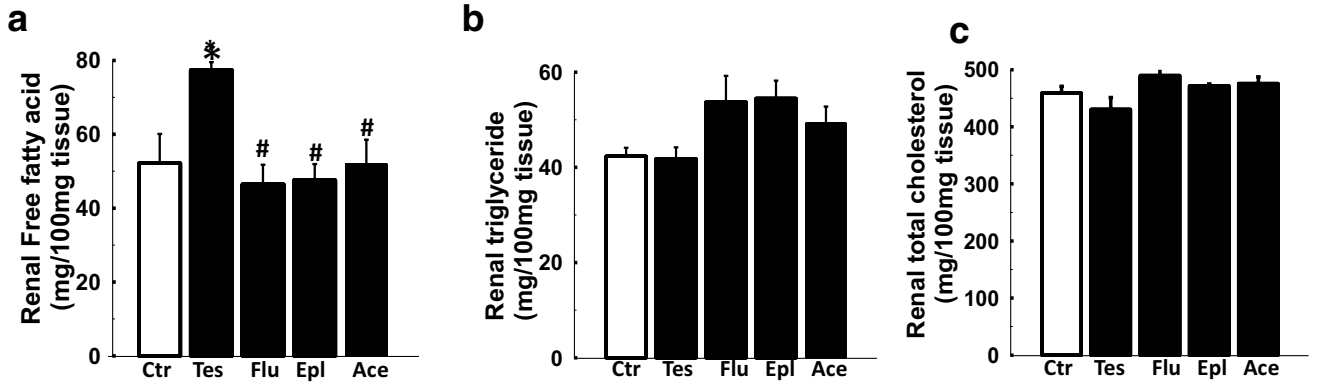


**Fig. 1** Effects of sodium acetate, flutamide and eplerenone on renal free fatty acid (**a**), triglyceride (**b**) and total cholesterol (**c**) in testosterone-exposed pregnant rats. Testosterone exposure caused an increase in renal free fatty acid that was attenuated following treatment with flutamide, eplerenone or sodium acetate. However, testosterone singly or in combination with flutamide, eplerenone or sodium acetate did not affect renal triglyceride or total cholesterol levels. Values are expressed as mean ± SEM of 6 rats per group; *p<0.05 vs Ctr, #p<0.05 vs Tes group. Data were analyzed by one-way ANOVA and Bonferroni's Post hoc analysis; *Ctr* control, *Tes* testosterone, *Flu* flutamide, *Epl* eplerenone, *Ace* sodium acetate

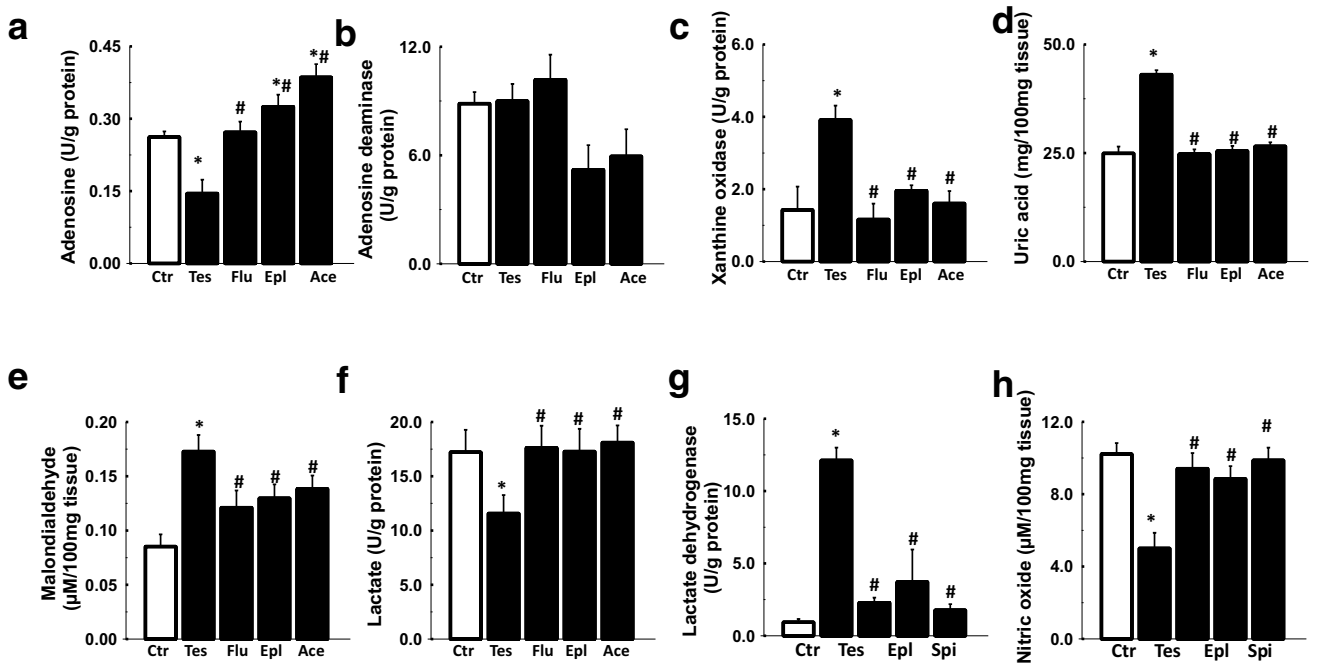


**Fig. 2** Effects of sodium acetate, flutamide and eplerenone on renal adenosine (**a**), adenosine deaminase (**b**), xanthine oxidase (**c**), uric acid (**d**), malondialdehyde (**e**), lactate (**f**), lactate dehydrogenase (**g**) and nitric oxide (**h**) in testosterone-exposed pregnant rats. Testosterone exposure caused a decrease in adenosine, lactate and nitric oxide that was replenished following treatment with flutamide, eplerenone or sodium acetate. On the other hand, testosterone exposure caused an increase in xanthine oxidase, uric acid, malondialdehyde and lactate dehydrogenase which were reversed by flutamide, eplerenone or sodium acetate treatment. Adenosine deaminase was not affected by testosterone either singly or in combination with flutamide, eplerenone or sodium acetate. Values are expressed as mean ± SEM of 6 rats per group; *p<0.05 vs Ctr, #p<0.05 vs Tes group. Data were analyzed by one-way ANOVA and Bonferroni's Post hoc analysis; *Ctr* control, *Tes* testosterone, *Flu* flutamide, *Epl* eplerenone, *Ace* sodium acetate



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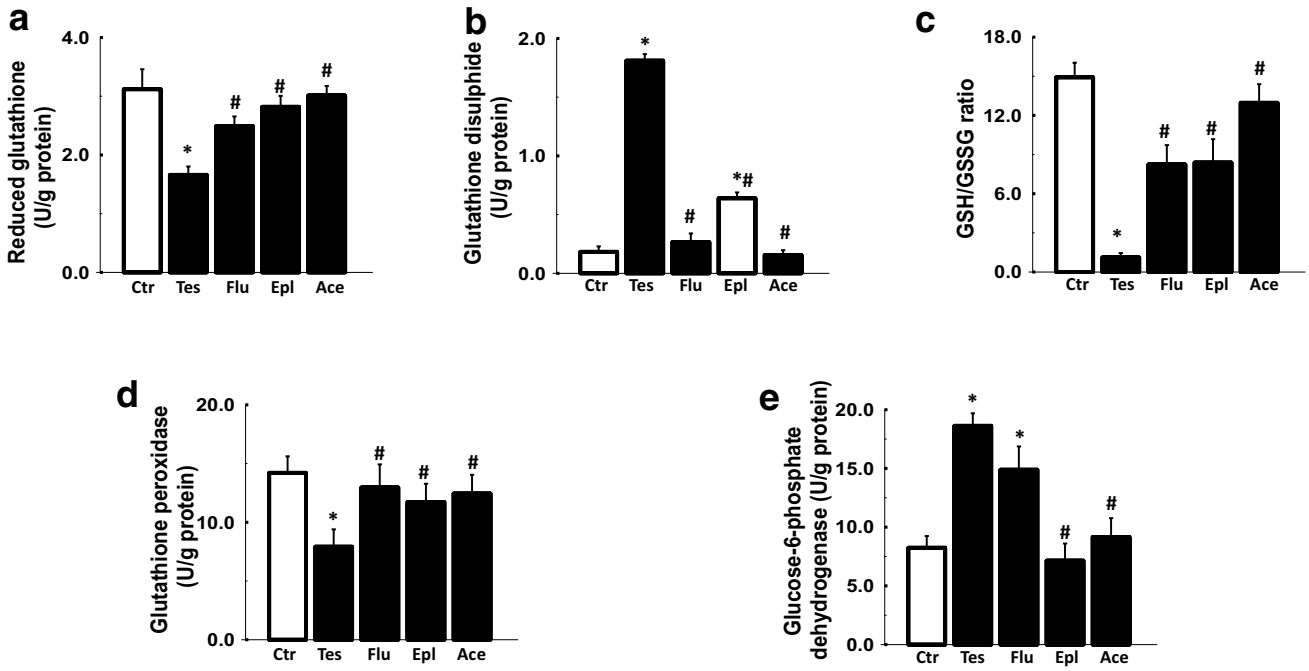


**Fig. 3** Effects of sodium acetate, flutamide and eplerenone on renal reduced glutathione (GSH, **a**), glutathione disulphide (GSSG, **b**), GSH/GSSG ratio (**c**), glutathione peroxidase (**d**) and glucose-6-phosphate dehydrogenase (**e**) in testosterone-exposed pregnant rats. Testosterone exposure caused a decrease in GSH, GSH/GSSG ratio and glutathione peroxidase that was replenished following treatment with either flutamide, eplerenone or sodium acetate. On the other hand, testosterone exposure caused an increase in GSSG which was reversed by flutamide, eplerenone or sodium acetate treatment. Testosterone also caused an increase in glucose-6-phosphate dehydrogenase that was reversed by either eplerenone or sodium acetate but was not affected by flutamide treatment. Values are expressed as mean ± SEM of 6 rats per group; *$p<0.05$ vs Ctr, $^{\#}p<0.05$ vs Tes group. Data were analyzed by one-way ANOVA and Bonferroni's Post hoc analysis; *Ctr* control, *Tes* testosterone, *Flu* flutamide, *Epl* eplerenone, *Ace* sodium acetate

### Effects of sodium acetate, flutamide and eplerenone on renal glutathione anti-oxidandant defense in testosterone-exposed pregnant rats

Gestational testosterone exposure caused reduction in reduced GSH and reduced GSH/GSSG ratio, glutathione peroxidase and increase in GSSG, and glucose-6-phosphate dehydrogenase (Fig. 3a–e). Treatment with AR (flutamide), MR (eplerenoene) and acetate restored GSH, GSSG/GSH and glutathione peroxidase, whereas GSSG was attenuated. However, only eplerenone and sodium acetate attenuated glucose-6-phosphate dehydrogenase (Fig. 3).

### Effects of sodium acetate, flutamide and eplerenone on renal enzyme markers of tissue injury in testosterone-exposed pregnant rats

Gestational testosterone exposure led to increased GGT, ALT, AST, and ALP levels. All the enzymes were attenuated by flutamide, eplerenone and acetate treatment during testosterone exposure (Fig. 4).

### Effects of sodium acetate, flutamide and eplerenone on plasma creatinine and urea in testosterone-exposed pregnant rats

Gestational exposure to testosterone led to over 15-fold increase in plasma creatinine and over 25-fold increase in plasma urea levels. Blockade of AR with flutamide, MR blockade with eplerenone and acetate attenuated the testosterone-induced elevated creatinine and urea levels (Table 1) (Fig. 5).

## Discussion

The current study demonstrates that exposing pregnant rats to excess testosterone between gestational days 14 to 19 led to depletion of renal glutathione and adenosine which were accompanied by increased uric acid (UA), xanthine oxidase (XO), malondialdehyde (MDA), lactate dehydrogenase, free fatty acids and decreased nitric oxide (NO). The study also shows that blockade of the MR, AR or SCFA, acetate (SAc), comparably protects the kidney against testosterone-induced renal dysfunction. However, whether SAc can modulate MR



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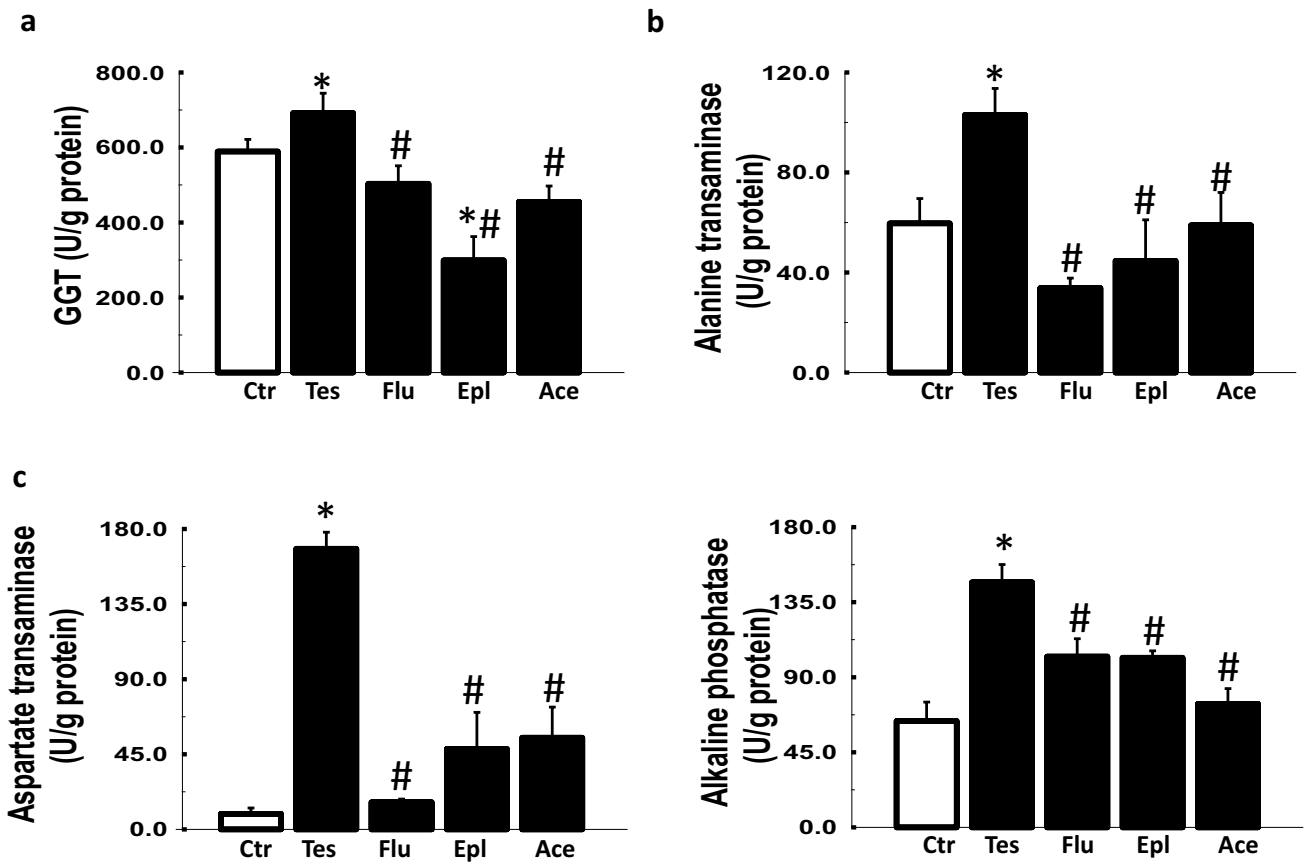


**Fig. 4** Effects of sodium acetate, flutamide and eplerenone on renal gamma glutamyl transferase (GGT, **a**), alanine transaminase (**b**), aspartate transaminase (**c**) and alkaline phosphatase in testosterone-exposed pregnant rats. Testosterone exposure caused an increase in gamma glutamyl transferase, alanine transaminase, aspartate transaminase and alkaline phosphatase which was reversed by either flutamide, eplerenone or sodium acetate treatment. Values are expressed as mean ± SEM of 6 rats per group; *p<0.05 vs Ctr, #p<0.05 vs Tes group. Data were analyzed by one-way ANOVA and Bonferroni's Post hoc analysis; *Ctr* control, *Tes* testosterone, *Flu* flutamide, *Epl* eplerenone, *Ace* sodium acetate

**Table 1** Effects of sodium acetate, flutamide and eplerenone on plasma urea and creatinine levels in testosterone-exposed pregnant rats

| | Ctr | Tes | Flu | Epl | Ace |
|---|---|---|---|---|---|
| Creatinine (mg/dl) | 68.3 ± 2.5 | 1651.2 ± 71.6* | 54.8 ± 4.6# | 119.1 ± 5.4# | 91.6 ± 6.7# |
| Urea (mg/dl) | 0.3 ± 0.04 | 5.0 ± 0.12* | 0.4 ± 0.04# | 0.3 ± 0.01# | 0.5 ± 0.04# |

Values are expressed as mean ± SEM of 6 rats per group; Data were analyzed by one-way ANOVA and Bonferroni's Post hoc analysis

*Ctr* control, *Tes* testosterone, *Flu* flutamide, *Epl* eplerenone, *Ace* sodium acetate

*$p < 0.05$ vs Ctr, #$p < 0.05$ vs Tes group

or AR needs further studies. The findings of the present study show that SAc might trigger signals that counteract deleterious renal effects of AR or MR activation due to late gestational testosterone exposure. The protective effects of SAc were similar to the protection offered by both AR and MR blockade.

Clinical and experimental studies have suggested that elevated androgen level during pregnancy is associated with various deleterious conditions [28–31]. During pregnancy, the kidney is significantly impacted [7] exposing it to deleterious intra mural events. This is usually worsened in pregnancies characterized by elevated testosterone [8]. Kidney disorders create a serious health problem [5]. Scientists presently concede that SCFAs are histone deacetylase (HDAC) inhibitors and participates in epigenetic modifications [32] and accumulating evidence support the beneficial and protective role of SCFAs [33]. Acetate, a 2-carbon fatty acid, is the most abundant SCFA in the circulation [34]. A dietary



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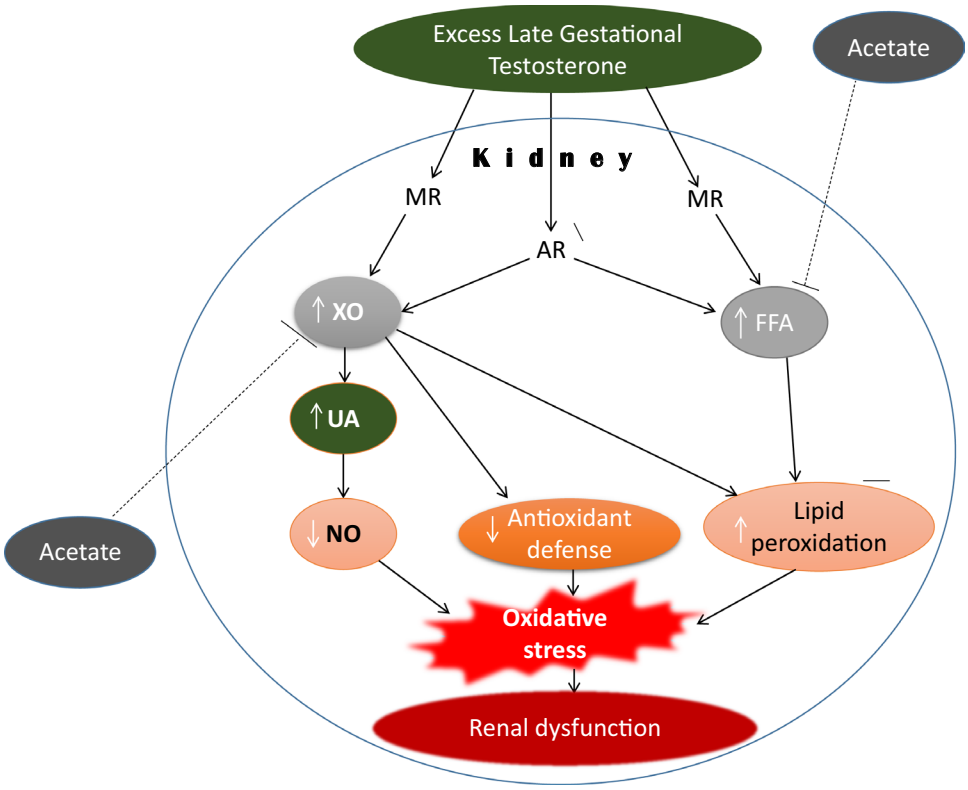


**Fig. 5** Schematic diagram depicting the possible pathway through which late gestational testosterone exposure causes renal dysfunction through AR and MR activation and the protective effect of SAc. *AR* Androgen receptor, *MR* Mineralocorticoid receptor, *XO* Xanthine oxidase, *FFA* Free fatty acid, *NO* Nitric oxide, *SAc* Sodium acetate, *UA* uric acid

elevation of this molecule may plausibly represent a safe curative measure for various diseases especially those with a trace of metabolic derangement. In addition, MR activation has been connected to several metabolic complications [35, 36] and recently to testosterone-induced dysmetabolism in rats in our laboratory [37]. As a novel finding in this study, SAc treatment mimics the therapeutic outcome of MR blockade and AR blockade in the kidneys of testosterone-exposed pregnant rats showing that it might be effective in conferring protection on the kidney during MR and/or AR activation.

In females, supra-physiological levels of androgens cause oxidative stress-mediated tissue damage like cardiomyopathy [31]. Likewise, androgens increase mitochondrial ROS and lead to apoptosis in vascular smooth muscle cell (VSMC) [38]. The findings of this study also corroborate the existent data showing the link between supra-physiological androgens in females, especially during pregnancy, and imbalanced renal redox status. In the current study, testosterone-exposed rats at late gestation, had increased renal lipid peroxidation indicated by MDA level, depressed glutathione-dependent antioxidant capacity and markers of tissue injury. Lipid peroxides the product of lipid peroxidation are oxidative species that assist to induce cellular damage, inflammatory responses and apoptosis during stress. Oxidative stress is established when the rate of free radical production exceeds the rate of elimination by endogenous antioxidant systems such as the enzymes glutathione peroxidase (GPx) as well as low molecular weight reductants like GSH [39]. In this study, renal G6PD, which fuels the glutathione antioxidant system, [40] was reduced alongside GSH and GPx in testosterone-exposed rats, with corresponding increase in enzyme markers of tissue injury and MDA. The finding in this study that SAc, MR and/or AR blockade replenishes the renal G6PD and glutathione antioxidant defenses as well as decrease the MDA levels is also in agreement with the previous study in diabetic hypertensive rats, although that study used the MR blocker spironolactone [41]. Also, the result of the present study that exposure of pregnant rats to elevated testosterone level led to increased FFA is consistent with earlier studies [42], and increased tissue FFA level has been demonstrated to cause lipotoxicity in the experimental model of nephrotic syndrome [43], suggesting that elevated gestational testosterone-induced oxidative stress is associated with kidney lipotoxicity. Tissue free fatty acid can exert deleterious effects through interaction with toll-like receptor type 4 leading to ROS production and inflammation. Insulin resistance can also occur due to serine phosphorylation of insulin receptor substrate by fatty acid intermediates. The testosterone-induced defective glutathione-dependent antioxidant defense and increased FFA in the kidneys were attenuated by SAc, AR and MR blockade, comparably. These results promote the link between AR and MR activation in androgen-induced renal lipotoxicity. Hence, the findings imply that acetate could confer renoprotective effect via AR- and/or MR-dependent mechanism.



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Inflammatory response usually succeeds chronic oxidative stress as a result of dysregulated activation of immune cells. ADA plays a pivotal role in immune-mediated inflammatory response [11]. Although testosterone administration did not alter the kidney ADA activity, however, the kidney adenosine level was reduced with consequent increase in XO and uric acid when compared to the control group. ADA clears adenosine, an anti-inflammatory molecule while signaling production of inflammatory cytokines from macrophages [44]. As described above, the testosterone-induced depletion of adenosine without corresponding increase in the activity of its negative regulator, ADA, may suggest that testosterone causes decrease in ATP production due probably to mitochondrial dysfunction leading to reduced availability of purines. However, adenosine depletion is detrimental in chronic injury due to activation of macrophages which will promote excessive tissue repair and fibrotic responses [45]. SAc and MR blocker prevented the kidney adenosine depletion, suggesting that SAc can be of a therapeutic benefit to prevent inflammatory process in the kidney by augmenting adenosine. The purine degradation pathway is associated with generation of oxidative species, which is essentially due to the activity of XO [46]. Several metabolic disorders are characterized by elevated XO activity with corresponding surge in uric acid concentration. Similarly, inhibition of XO activity has been noted to be therapeutic in metabolic-related disorders, including kidney disorders [47]. XO and UA were elevated in the testosterone-exposed rats independent of ADA activity, although with corresponding adenosine depletion. The enhanced activity of XO increases kidney UA production as validated in this study. Increased UA production can inhibit production of nitric oxide by activating arginase activity, the negative regulator of L-arginine reducing the substrate of nitric oxide synthase. Reduced kidney NO can compromise blood flow to the kidney and lead to apoptotic and consequent fibrotic response. SAc and MR blocker reversed the deleterious alterations of XO activity, UA and NO levels observed in the testosterone-exposed pregnant rat.

The beneficial effect of MR blockade has been reported in previous studies [37,38]. In addition, AR blockade has known beneficial effect especially in androgen-induced abnormality [48]. The present study reports an attenuating effect of AR blocker, flutamide and MR blocker, eplerenone on testosterone-induced renal depleted adenosine and glutathione, although the effect of MR blockade seems to be more prominent. This outcome shows that AR and MR activations are involved in the kidney adenosine and glutathione depletion induced by gestational testosterone. A novel finding in this study is that Sac reversed the testosterone-induced kidney dysfunction, which is characterized by elevated lipid peroxidation and defective glutathione, adenosine and nitric oxide contents comparably with MR and AR blockade. Although evidence exists that SCFAs interact with membrane bound G protein coupled receptor family which is also believed to be activated by androgens [42, 49], an interaction of SCFA with AR is not yet established and the mechanisms involved in SCFA prevention of androgen-induced renal disorders is unclear. The relationship between SCFAs and MR could be linked to the role of SCFAs as HDAC inhibitors. This is because HDAC inhibition has been reported to cause acetylation of MR thereby reducing its transcription and subsequently preventing the deleterious effects associated with MR activation [50]. A previous study by Marques et al. [51] also reported that acetate protects against renal injury caused by MR activator DOCA. The study further reported that the protective effect of acetate on the kidney could be due to the upregulation of protective pathways in the kidney such as the respiratory electron chain while downregulating pathways associated with lipid metabolism and endoplasmic reticulum stress [51]. Similarly, Gray et al. explored the possibility of adjusting maternal diet to harness the ability of the intestinal microbiome to generate SCFAs, including SAc, which could then exert its protective effects [52]. However, future study would focus on the direct effect of SAc on both AR and MR.

## Conclusion

The study indicates that late gestational testosterone exposure induces kidney dysfunction by depletion of kidney glutathione, adenosine and nitric oxide contents via AR and MR activation. The study also demonstrates that the AR and MR blockade-like renoprotective effect of acetate in testosterone-exposed pregnant rats, presumably, is mediated by enhanced glutathione-dependent antioxidant capacity.

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**Author contributions** OLA and UTO conceived and designed the research. BOO conducted experiments. OLA contributed new reagents and analytical kits. OLA, AOA, UTO, AED, IPO, OKS and BOO analyzed and interpreted data. AOA, UTO, AED OKS and IPO drafted the manuscript. OLA, AOA, UTO, AED OKS, IPO, BOO and OAO read and approved the final manuscript.

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## Compliance with ethical standards

**Conflict of interest** LAO received eplerenone (PF-02845980) used in this study from Pfizer Inc. (NY, USA) via the Compound Transfer Program (CTP). TOU, OAA, EDA, OOB, IPO, KSO and AOO do not have any conflicts of interest to declare.



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**Consent for publication** All authors gave consent for this manuscript to be published.

**Ethical approval** This study protocol was approved by the University Ethical Review Committee (UERC), University of Ilorin, Ilorin.

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