

Low-dose spironolactone combats dyslipidemia and hepatic inflammation by modulating PCSK9 in rat model of polycystic ovarian syndrome

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ARTICLE INFO

Editor: Lawrence Lash

Keywords:

Low dose spironolactone
Dyslipidemia
Liver
PCSK9
PCOS

ABSTRACT

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder among women and it is associated with overt metabolic derangement. Circulating lipids are regulated by proprotein convertase subtilisin/kexin type 9 (PCSK9) which blocks low density lipoprotein (LDL) receptors especially in the liver. The liver is highly vulnerable in dyslipidemia as lipid accumulation leads to progression of non-alcoholic fatty liver disease (NAFLD). An array of scientific endeavours hold that low-dose spironolactone (LDS) is beneficial as intervention for PCOS traits, but this claim is yet to be fully elucidated. The aim of this study was to investigate the effect of LDS on dyslipidemia and hepatic inflammation in rats with letrozole (LET)-induced PCOS and to assess the possible involvement of PCSK9 in these effects. Eighteen female Wistar rats were randomly assigned into 3 groups. The control group received vehicle (distilled water; *p.o.*), LET-treated group received letrozole (1 mg/kg; *p.o.*), LET+LDS-treated group received LET plus LDS (0.25 mg/kg, *p.o.*) for 21 days. Exposure to LET increased body and hepatic weights, plasma and hepatic total cholesterol (TC), TC/HDL, LDL, interleukin-6, MDA, PCSK9, ovarian degenerated follicles and hepatic NLRP3 intensity, reduced GSH and normal ovarian follicles. Interestingly, LDS averted dyslipidemia, NLRP3-dependent hepatic inflammation and ovarian PCOS traits. It is evident herein that LDS ameliorates PCOS traits and combats dyslipidemia and hepatic inflammation in PCOS by a PCSK9-dependent mechanism.

1. Introduction

Polycystic ovarian syndrome (PCOS), a neuroendocrine disorder in epidemic proportions is the most common neuroendocrine disorder among women worldwide and it is associated with overt metabolic derangement. The metabolic disruptions that characterize PCOS engender overt glucose dysregulation and dyslipidemia (Akbarzadeh et al., 2019) and is strongly linked to characteristic hyperandrogenism in PCOS. This is associated with oxidative stress and inflammation in tissues, leading to insulin resistance and lipid dysmetabolism (Mancini et al., 2021). Lipid dysmetabolism is a known malign process that

introduces toxicity to vital organs such as the liver leading to macrovascular and microvascular complications, morbidity and plausibly mortality. A lot of molecular factors ranging from transporters to enzymes regulate lipid metabolism and a good number of them have become of immense pharmaceutical importance being a target for pharmacological intervention.

Circulating lipids, especially low-density lipoproteins (LDL) are regulated by proprotein convertase subtilisin/kexin type 9 (PCSK9) (Stoekenbroek et al., 2015). Elevated PCSK9 degrades the LDL receptor (LDLR) mostly on hepatocyte membrane, leading to reduced absorption of LDL into the liver and pooling of it in circulation (Stoekenbroek et al.,

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<https://doi.org/10.1016/j.taap.2023.116604>

Received 22 February 2023; Received in revised form 30 May 2023; Accepted 13 June 2023

Available online 14 June 2023

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2015). The consequent dyslipidemia affects microcirculations and vital organs such as liver, which is the peripheral metabolic hub leading to gross peripheral dysmetabolism of important macromolecules. Intramural hepatic dysmetabolism involving especially lipids derange redox status and lead to chronic low-grade inflammation and eventual non-alcoholic fatty liver disease (NAFLD). Increased prevalence of NAFLD has been reported in patients with PCOS (Asfari et al., 2020; Won et al., 2021; Chen et al., 2022). It is therefore an interesting fact that derangement of endocrine functions in PCOS may require non-conventional interventions as the complications involved transcends the direct effects of the cause. Ultimately, the perturbation in PCOS have been traced to largely hyperandrogenism (Goodarzi et al., 2011) whereas, the cause of the androgen-producing cysts in the ovary is quite yet to be elucidated (Ashraf et al., 2019). Although, abnormal high expression of PCSK9 in the blood and ovary has been suggested in the pathogenesis of PCOS by affecting lipid metabolism and ovarian function (Wang et al., 2019). However, further investigation of the involvement of PCSK9 in hepatic dysmetabolism and attendant disorder in PCOS would possibly provide a therapeutic target and insight for the management of endocrinometabolic-driven hepatic derangement associated with PCOS.

Low-dose spironolactone (LDS), 0.25 mg/kg, has been shown by scientific studies to be beneficial in PCOS traits (Adeyanju et al., 2019; Olaniyi et al., 2021). Higher doses of spironolactone were found to be associated with adverse effects especially in metabolic derangements involving estrogen deficiency or androgen excess (de Resende et al., 2006). The mechanisms by which LDS combats PCOS traits and its complications are still inconclusive. It is therefore expedient to study this thoroughly so as to consider how the drug can be directly repositioned, from being just a diuretic for treatment of volume/salt-related hypertension to a pharmacologic candidate for treatment of PCOS and its complications by either dose manipulation or fortification with other molecules. The present study focused on assessment of the therapeutic benefits of LDS on lipid derangement and hepatic inflammation caused by letrozole (LET)-induced PCOS traits in association with the PCSK9 pathway.

2. Materials and methods

2.1. Experimental animals and grouping

Female Wistar rats that are eight-week-old were procured from the animal house of the College of Health Sciences, Afe Babalola University, Ado-Ekiti. These rats were allowed unlimited access to standard rat chow and tap water and the study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The approval of protocol (ABUADERC/17A/2021) was obtained from the Institutional Ethical Review Board of Afe Babalola University (Ado-Ekiti, Nigeria). All the animals used for the study were on the same estrous stage, which was determined via vaginal smear for each rat. After 2 weeks of acclimatization, the animals were randomly assigned into three groups ($n = 6$ per group) namely: Control, PCOS (LET-treated) and LET+LDS-treated groups and treatments given by oral gavage. Rats were maintained in a colony under standard environmental conditions of temperature (22–26 °C), relative humidity (50–60%), and 12-h dark/light cycle.

2.2. Treatment

The control group received vehicle (distilled water; *p.o.*), LET-treated group received letrozole (1 mg/kg; *p.o.* Sigma-Aldrich, St Louis, MI) and LET+LDS-treated group received LET plus LDS (0.25 mg/kg, *p.o.* Pfizer Limited, Kent, UK). The treatment was done via oral gavage once daily for 21 days (Balen et al., 1993; Kafali et al., 2004; Adeyanju et al., 2019; Olaniyi et al., 2021). Initial and final body weights were determined, and the body weight gain was estimated.

2.3. Collection of samples

At the end of the treatment, the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). Blood was collected by cardiac puncture into heparinized tube and was centrifuged at 704g for 5 min at room temperature. Plasma was stored frozen until it was needed for biochemical assay.

2.4. Preparation of liver homogenate

After weighing the liver, 100 mg section of the tissue was carefully removed and homogenized with a glass homogenizer in phosphate buffer solution, centrifuged at 8000g for 10 min at 4 °C and the supernatant was collected and stored frozen until it is required for biochemical assays.

2.5. Determination of plasma and liver lipid profile

Standard colorimetric methods using assay kits obtained from Fortress Diagnostics Ltd. (Antrim, UK) were used to determine total cholesterol (TC), high-density lipoprotein-cholesterol (HDLc), and low-density lipoprotein cholesterol (LDLc) from the plasma and liver. Besides, plasma and liver TC/HDLc was determined as previously reported (Miralles et al., 2015; Zhu et al., 2018).

2.6. Determination of plasma and liver lipid peroxidation and glutathione concentration

Malondialdehyde (MDA), a marker of lipid peroxidation was determined from the plasma and liver homogenate by standard non-enzymatic spectrophotometric method using assay kits from Randox Laboratory Ltd. (Co. Antrim, UK), while glutathione (GSH) was determined using a non-enzymatic spectrophotometric method with assay kits obtained from Oxford Biomedical Research Inc. (Oxford, USA). Glutathione concentration was determined by spectrophotometric method based on the oxidation of GSH in the sample by the sulfhydryl reagent 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5'-thio-2-nitrobenzoic acid (TNB), measured at 412 nm.

2.7. Determination of interleukin-6 (IL-6)

Plasma and liver concentration of interleukin-6 was determined by the quantitative standard sandwich ELISA technique using monoclonal antibody specific for these parameters with rat kits obtained from Elabscience Biotechnology Inc. (Wuhan, Hubei, P.R.C., China).

2.8. Determination of lactate dehydrogenase (LDH) and γ -glutamyl transferase (GGT) activities

The LDH and GGT activities were determined from the liver homogenate by standardized enzymatic colorimetric method using assay kits obtained from Randox Laboratory Ltd. (Co. Antrim, UK), and following the manufacturers' procedures.

2.9. Determination of testosterone and sex hormone binding globulin (SHBG) concentration

The plasma concentration of testosterone and SHBG were determined using rat ELISA kits obtained from Elabscience Biotechnology Inc. (Wuhan, Hubei, P.R.C., China) and following the manufacturers' procedures.

2.10. Estimation of plasma and liver level of proprotein convertase subtilisin kexin type 9

The levels of plasma and liver PCSK9 were determined by rat ELISA

kits obtained from Elabscience Biotechnology Inc. (Wuhan, Hubei, P.R. C., China) with cat number E-EL-R2487. This kit recognized Rat PCSK9 in samples and no significant cross-reactivity or interference between Rat PCSK9 and analogues was observed. This method used the Sandwich-ELISA principle.

2.11. Histological assessment of ovaries

For hematoxylin and eosin (H & E) stains, a section of the ovary was fixed in 10% formol saline overnight and thereafter dehydrated, embedded in paraffin and sectioned at 5- μ m thickness. The slides were prepared and examined using OPTO-Edu industrial camera light microscope and a computer (Nikon, Japan).

2.12. Stereological assessment of ovarian follicles

For the stereological assessment of ovarian follicles was determined as previously reported (Malamed et al., 1992; Olaniyi et al., 2021).

2.13. Immunohistochemical assessment of liver

Immunohistochemistry of hepatic tissue was performed to detect the expression of inflammasome antigens using the heat method of antigen retrieval. Processed hepatic tissues were sectioned and baked in an oven at 50 °C (30 min). Slides were treated in xylene and dehydrated in ascending grades of alcohol for 10 mins each. The slides were rinsed and placed in antigen retrieval solution for 40 min till it cooled down to room temperature. Slides were then rinsed in distilled water and PBS for 5 min each. On each slide, tissue area was encircled using PAP pen and then incubated in H₂O₂ for 30 mins at room temperature to block endogenous peroxidase followed by protein block solution for 10 min at room temperature. Thereafter, slides were incubated in NLRP3 polyclonal antibody from Elabscience Biotechnology Inc. (Wuhan, Hubei, P. R.C., China) at room temperature for 180 min. The slides were rinsed and incubated in polyperoxidase-antimouse/rabbit IgG at room temperature (Elabscience Biotechnology Inc. Wuhan, Hubei, P.R.C., China) for 20 min and treated with 3, 3'-diaminobenzidine (DAB) working solution. Slides were counterstained in 1% aqueous hematoxylin solution for 20 min and then dehydrated in ascending order of ethanol for 10 min each. They were later cleared in xylene for 10 min and mounted using DPX mountant and examined using OPTO-Edu industrial camera light microscope and a computer (Nikon, Japan).

2.14. Data analysis and statistics

All data were expressed as means $\pm$ SD. and statistical group analysis was performed with Graphpad Prim software version 9. One-way ANOVA was used to compare the mean values of variables among the groups and Bonferroni's test was used for *post hoc* analysis. Statistically significant differences were accepted at $p < 0.05$.

3. Result

3.1. Impact of low-dose spironolactone on ovarian morphology, testosterone and sex hormone binding globulin concentration in LET-induced PCOS

Histological analysis of ovaries revealed ovarian tissues with degenerated follicles and disrupted granulosa cells, antrum and oocyte in animals with PCOS compared with normal antrum, granulosa cells and oocyte in animals without PCOS. However, the ovarian tissues of animals with PCOS plus LDS treatment showed normal antrum, granulosa cells and oocyte. In addition, normal follicles were significantly lower while degenerated follicles were significantly higher in PCOS animals compared to the control. Nevertheless, normal follicles significantly increased while degenerated follicles were significantly lower in

LET+LDS group compared with LET group. Levels of testosterone were higher while SHBG levels were lower in PCOS animals than control group. Testosterone levels were decreased while SHBG levels were increased in LET+LSD group compared with LET group (Fig. 1).

3.2. Impact of low-dose spironolactone on body and liver weight in LET-induced PCOS

The body weight and liver weight were significantly higher in PCOS group than control group. Administration of LDS significantly reduced the body weight and liver weight in PCOS group compared with untreated PCOS group (Fig. 2).

3.3. Impact of low-dose spironolactone on plasma and liver total cholesterol, total cholesterol/high-density lipoprotein cholesterol and low-density lipoprotein cholesterol in LET-induced PCOS

The plasma and liver TC were significantly higher in PCOS group than control group. However, administration of LDS significantly decreased the liver but not plasma TC in PCOS group compared with untreated PCOS group. The plasma and liver TC/HDLc were significantly higher in PCOS group than in control group, and administration of LDS significantly decreased the plasma and liver TC/HDLc in PCOS group compared with untreated PCOS group. Similarly, the plasma and liver LDLc were significantly higher in PCOS group than in control group. Nonetheless, administration of LDS significantly reduced the plasma and liver LDLc in PCOS group compared with untreated PCOS group (Fig. 3).

3.4. Impact of low-dose spironolactone on plasma and liver interleukin 6 in LET-induced PCOS

The plasma and liver IL-6 were significantly higher in PCOS group than control group. However, administration of LDS significantly lowered the plasma but not liver IL-6 in PCOS group compared with untreated PCOS group (Fig. 4).

3.5. Impact of low-dose spironolactone on plasma and liver reduced glutathione and lipid peroxidation in LET-induced PCOS

The plasma and liver GSH were significantly lower in PCOS group than control group, and administration of LDS significantly increased the plasma and liver GSH in PCOS group compared with untreated PCOS group. Likewise, the plasma and liver MDA were significantly higher in PCOS group than control group. Administration of LDS significantly decreased the liver and plasma MDA in PCOS group compared with untreated PCOS group (Fig. 5).

3.6. Impact of low-dose spironolactone on liver γ -glutamyl transferase and lactate dehydrogenase activities in LET-induced PCOS

The liver GGT and LDH activities were significantly higher in PCOS group than control group. Administration of LDS significantly reduced the liver GGT and LDH activities in PCOS group compared with untreated PCOS group (Fig. 6).

3.7. Impact of low-dose spironolactone on plasma and liver proprotein convertase subtilisin kexin 9 in LET-induced PCOS

The plasma and liver PCSK9 levels were significantly higher in PCOS group than in control group. Administration of LDS significantly lowered the plasma and liver PCSK9 in PCOS group compared with untreated PCOS group (Fig. 7).

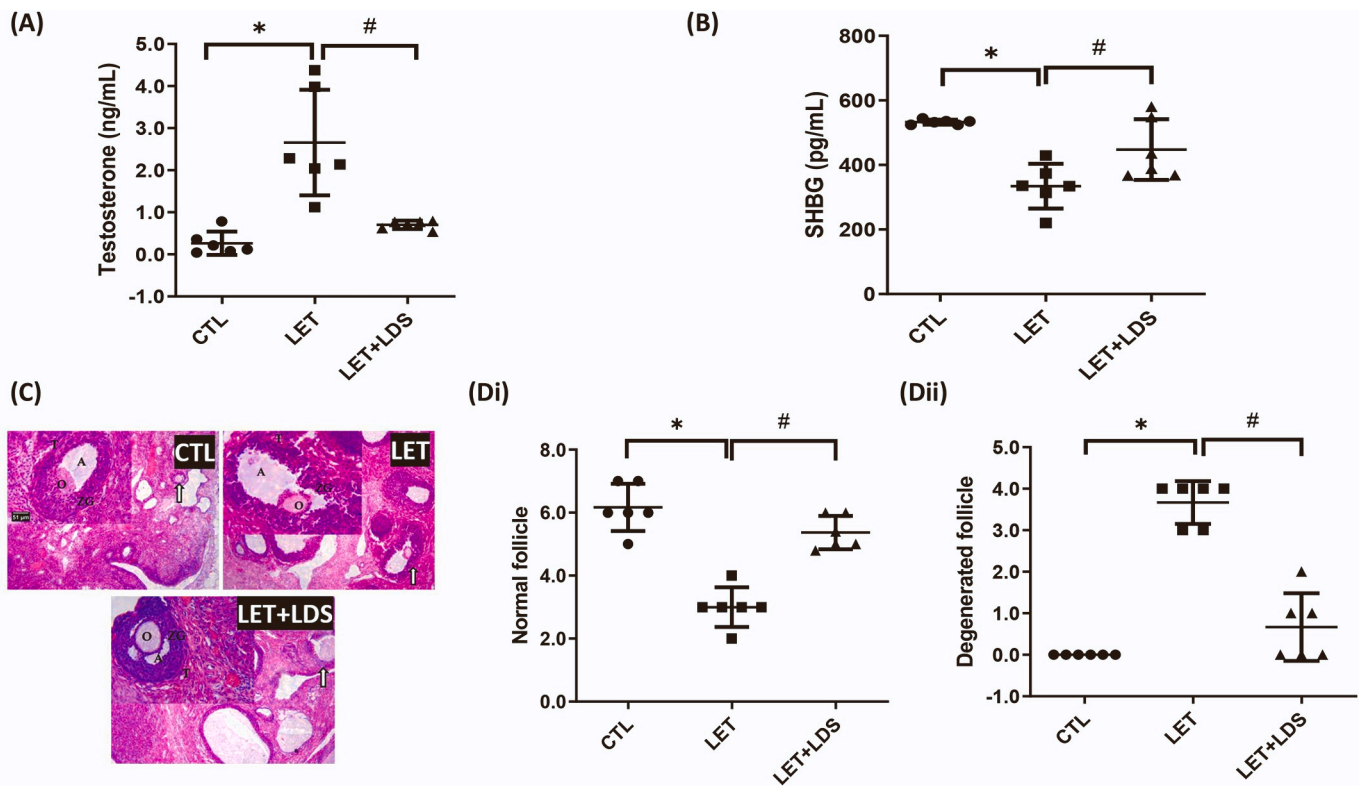


Fig. 1. Effects of low-dose spironolactone on testosterone (a), sex hormone binding globulin (b), ovarian morphology (c) and follicular count (d) in LET-induced PCOS rat model. Data are expressed as SD, n = 6. (*p < 0.05 vs. CTL, #p < 0.05 vs. LET). Control (CTL); Letrozole (LET); Low-dose spironolactone (LDS); Sex hormone binding globulin (SHBG).

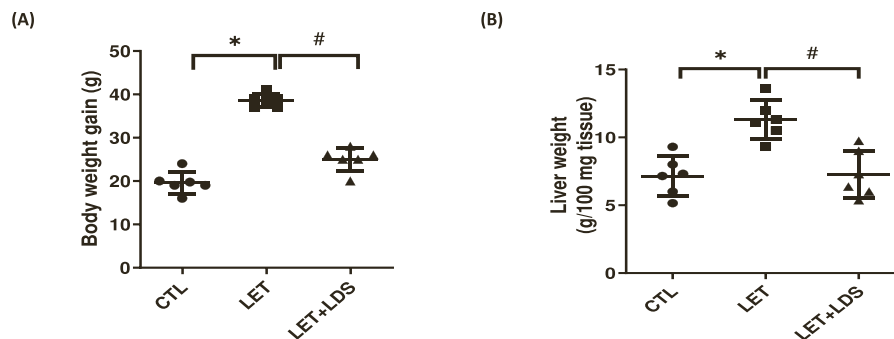


Fig. 2. Effects of low-dose spironolactone on body weight gain (a) and liver weight (b) in LET-induced PCOS rat model. Data are expressed as SD, n = 6. (*p < 0.05 vs. CTL, #p < 0.05 vs. LET). Control (CTL); Letrozole (LET); Low-dose spironolactone (LDS).

3.8. Impact of low dose spironolactone on immunohistochemistry of liver in LET-induced PCOS

Photomicrographs showed inflammatory responses by Inflammasome (NLRP3) antibody in the hepatic tissue of animals with PCOS compared with control animals with no expression of inflammasome. However, inflammasome expression significantly decreased in LET+LDS group compared with control group (Fig. 8).

4. Discussion

The present study showed that induction of PCOS traits by LET was associated with body and hepatic weight gain, increased ovarian follicular degeneration, caused dyslipidemia involving LDLc dysmetabolism and led to NLRP3-associated hepatic redox imbalance and inflammatory response. This study shows also that LET increased both

circulating and hepatic PCSK9 showing that PCSK9 elevation is a factor involved in the dyslipidemia associated with LET-induced PCOS traits. However, LDS normalized body and hepatic weights, ameliorated follicular degeneration, dyslipidemia and hepatic NLRP3-dependent inflammatory response. These effects were accompanied by a reduction in circulating and hepatic PCSK9 showing that improvement of LDL receptor functions by decreasing PCSK9 is a pathway involved in the prevention of dyslipidemia and hepatic inflammation in PCOS traits by LDS (Fig. 9).

The present study showed that a significant increase in body weight gain is associated with LET-induced PCOS traits. This body weight phenotype shows that there are possibilities of fat accumulation and central deregulation of energy metabolism following treatment of female rats with LET. Although the aim of this study was not to assess central or hypothalamic functions in PCOS dysmetabolism, it is worthy of note that increased body weight is a manifestation of metabolic

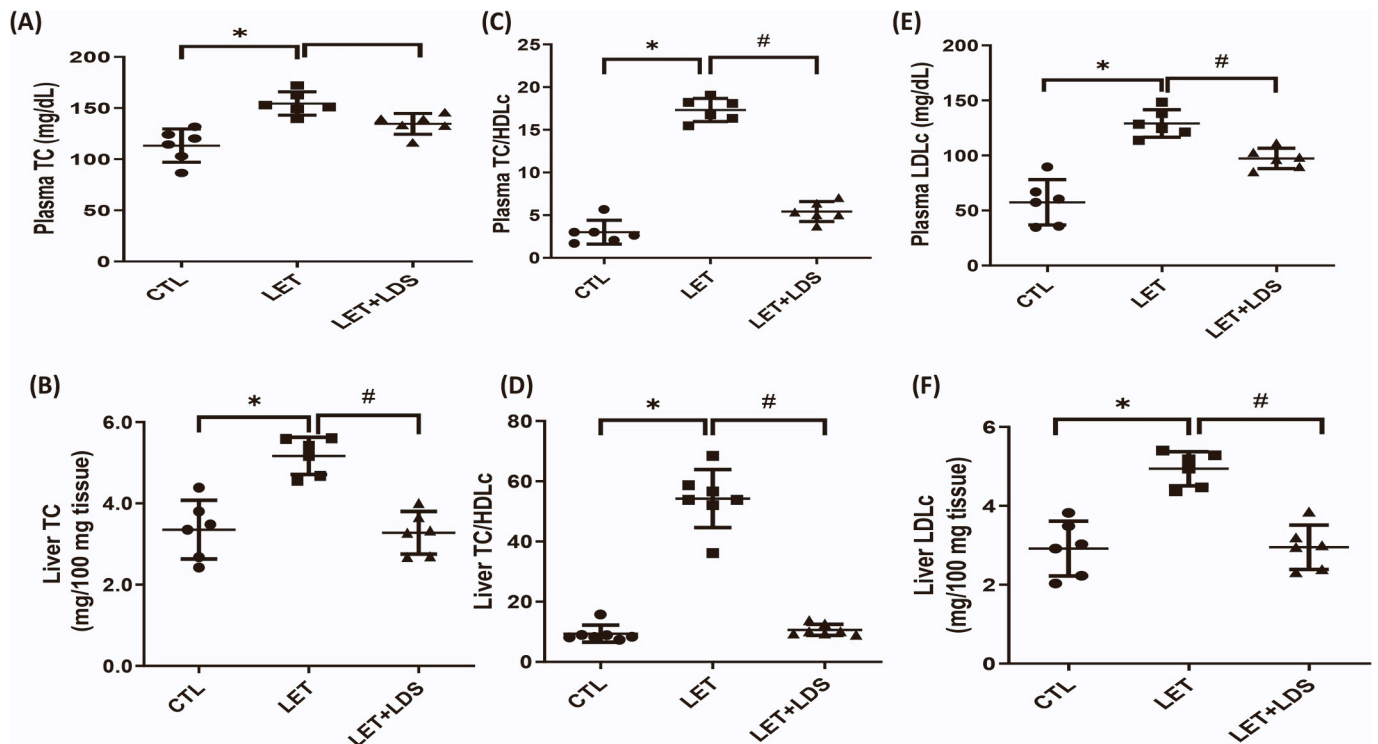


Fig. 3. Effects of low-dose spironolactone on plasma and liver total cholesterol (a and b), TC/HDLc (c and d) and low-density lipoprotein cholesterol (e and f) in LET-induced PCOS rat model. Data are expressed as SD, $n = 6$. (* $p < 0.05$ vs. CTL, # $p < 0.05$ vs. LET). Control (CTL); Letrozole (LET); Low-dose spironolactone (LDS); Total cholesterol (TC); Low-density lipoprotein cholesterol (LDLc); High-density lipoprotein cholesterol (HDLc).

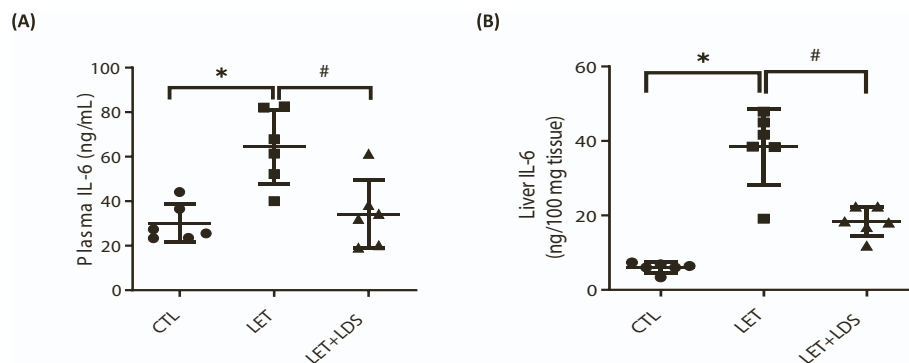


Fig. 4. Effects of low-dose spironolactone on plasma interleukin-6 (a) and liver interleukin-6 (b) in LET-induced PCOS rat model. Data are expressed as SD, $n = 6$. (* $p < 0.05$ vs. CTL, # $p < 0.05$ vs. LET). Control (CTL); Letrozole (LET); Low-dose spironolactone (LDS); Interleukin-6 (IL-6).

derangement and is one of the phenotypes with high prognostic value in metabolic syndrome and PCOS (Mancini et al., 2021). Along with body weight, this study assessed liver weight and found that the same treatment (LET, 1 mg/kg) that increased body weight gain also increased hepatic weight significantly. The factors that may contribute to increased hepatic weight in mature animals include hepatic fat accumulation and inflammation which are not unlikely in obesity and obesity-related diseases. Excess body weight gain remains handy and useful in clinical practice and data here shows that the odds of increased hepatic weight is high when body weight increases. In addition to elevated body and hepatic weights, LET exposure led to increased follicular degeneration in the ovaries. The mechanism of LET action is inhibition of aromatase which converts testosterone to estrogen causing hyperandrogenism and hypoestrogenism. Estrogen is important for follicular development which is why LET exposure caused elevated degenerated follicles and reduced normal follicles as shown in histological quantitative analysis of ovarian follicles.

This study also showed that following LET exposure to female rats, plasma and liver cholesterol portrayed dyslipidemia and hepatic fat accumulation favouring increased circulating LDL and hepatic total cholesterol. Elevated circulating LDL has been associated with cardiometabolic and cardiovascular diseases owing to high risk of atherosclerosis, tissue hypoxia and inflammatory responses (Leutner et al., 2016). In the present study, elevated circulating and hepatic PCSK9 accompanied plasma and hepatic LDL status showing that PCSK9 as its biological function describes would have reduced the bioavailability of LDL receptors on the liver and other tissues leading to reduced internalization of LDL and pooling in the circulation. This shows that LET-induced PCOS traits engenders dyslipidemia via PCSK9-associated mechanism. Lipid derangement is chiefly implicated in tissue damage via lipotoxicity (Trauner et al., 2010). Nevertheless, damage to the liver is pivotal to metabolic complications because of its role as the peripheral metabolic hub. In the present study, accompanying circulating and hepatic lipid dysmetabolism are plasma and hepatic decline in GSH,

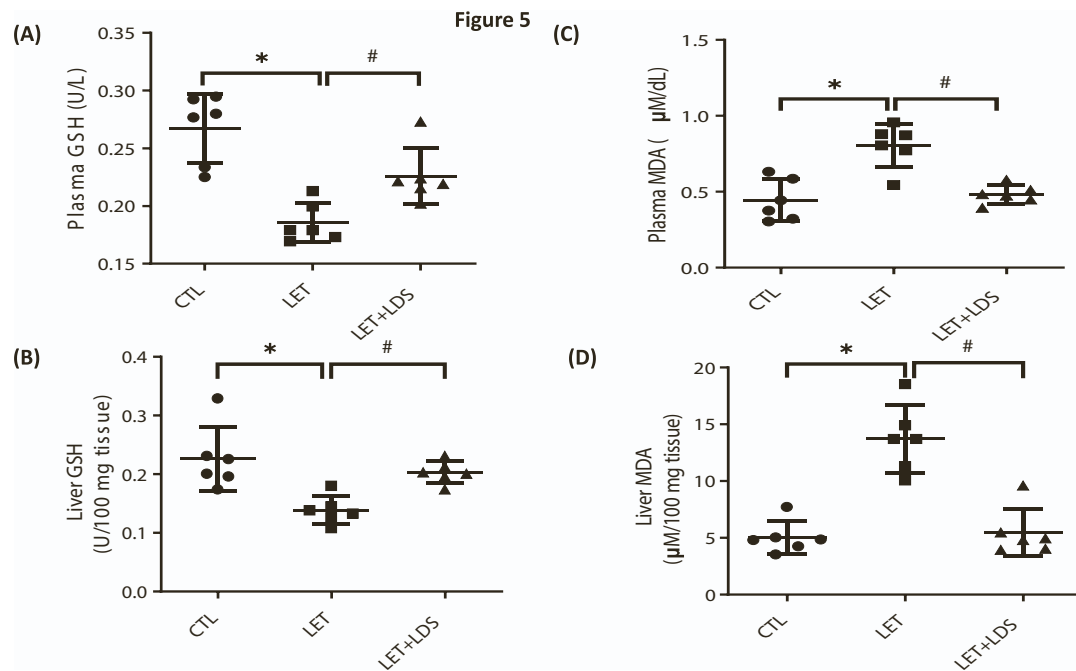


Fig. 5. Effects of low-dose spironolactone on plasma and liver reduced glutathione (a and b) and lipid peroxidation (c and d) in LET-induced PCOS rat model. Data are expressed as SD, $n = 6$. (* $p < 0.05$ vs. CTL, # $p < 0.05$ vs. LET). Control (CTL); Letrozole (LET); Low-dose spironolactone (LDS); Reduced glutathione (GSH); Malondialdehyde (MDA).

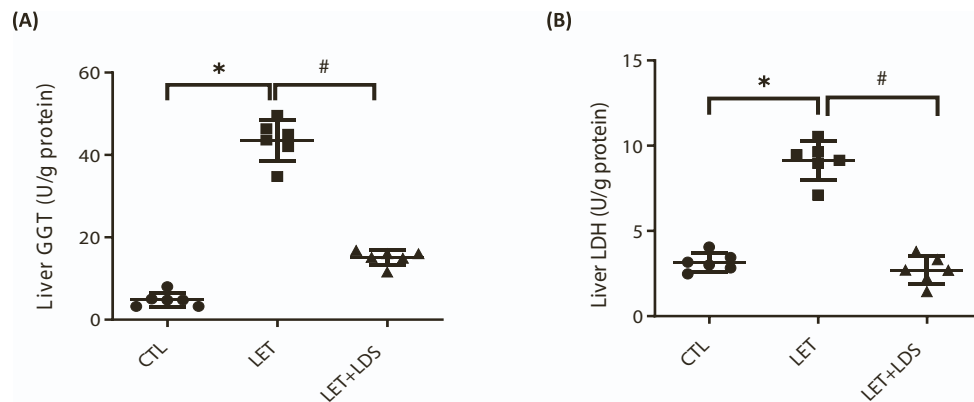


Fig. 6. Effects of low-dose spironolactone on liver γ -glutamyl transferase activity (a) and lactate dehydrogenase activity (b) in LET-induced PCOS rat model. Data are expressed as SD, $n = 6$. (* $p < 0.05$ vs. CTL, # $p < 0.05$ vs. LET). Control (CTL); Letrozole (LET); Low-dose spironolactone (LDS); γ -glutamyl transferase (GGT); Lactate dehydrogenase (LDH).

elevated malondialdehyde, IL-6, hepatic NLRP3 staining intensity, GGT and LDH in LET-exposed animals. This milieu shows dysregulation of redox and inflammatory responses following LET exposure and that the pathway for hepatic inflammation in this study is associated with NLRP3 inflammasome which stimulates inflammatory response in association with caspase in a cascade of reactions. As markers of tissue injury, hepatic GGT and LDH were elevated following LET-exposure.

The administration of LDS to LET-treated animals averted dyslipidemia. This benefit of LDS was accompanied by amelioration of PCSK9, showing that inhibition of this enzyme may be beneficial in the treatment of dyslipidemia or lipid dysregulation and that spironolactone at low doses might possess some PCSK9 inhibitory properties. Nevertheless, the present study did not investigate whether higher doses of spironolactone might elicit the same effect on PCSK9. LDS also prevented NLRP3 inflammasome-related hepatic inflammation by significantly ameliorating NLRP3 inflammasome and IL-6. In addition, LDS demonstrated antioxidant property by augmenting plasma and hepatic GSH and reducing lipid peroxidation in PCOS animals. This was supported by

the fact that GGT and LDH were also ameliorated by LDS in LET-exposed animals as markers of oxidant or inflammation-induced injury. Interestingly, PCOS phenotypes such as body weight gain, hyperandrogenism and ovarian follicular dysregulation were resolved by LDS in LET-exposed animals. Although, studies have shown that spironolactone has anti-androgenic properties (Rathnayake and Sinclair, 2010; Burinkul et al., 2021). This may be the reason LDS might be regarded as a suitable pharmacologic candidate in arresting conditions associated with hypoestrogenism and hyperandrogenism. Therefore, the present results suggest for the first time that LDS ameliorates PCOS-associated dyslipidemia and hepatic inflammation, a beneficial effect that is accompanied by suppression of circulating/hepatic PCSK9. However, the current study is not without a caveat in such that the cause effect relationship between the PCSK9 and other biochemical alterations in PCOS rat model were not evaluated. Nevertheless, the present data provide justification for future molecular study and perhaps clinical relevance for prevention and management of PCOS-associated hepatic disorder.

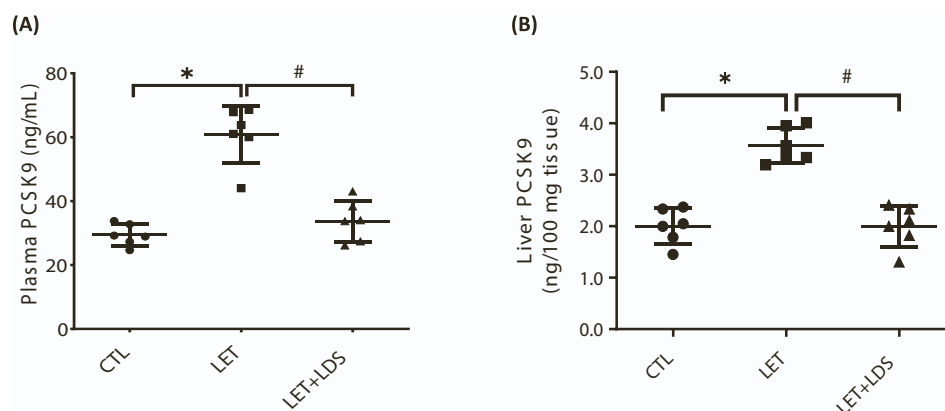


Fig. 7. Effects of low-dose spironolactone on plasma proprotein convertase subtilisin kexin 9 (a) and liver proprotein convertase subtilisin kexin 9 (b) in LET-induced PCOS rat model. Data are expressed as SD, n = 6. (* $p < 0.05$ vs. CTL, # $p < 0.05$ vs. LET). Control (CTL); Letrozole (LET); Low-dose spironolactone (LDS); Proprotein convertase subtilisin kexin 9 (PCSK9).

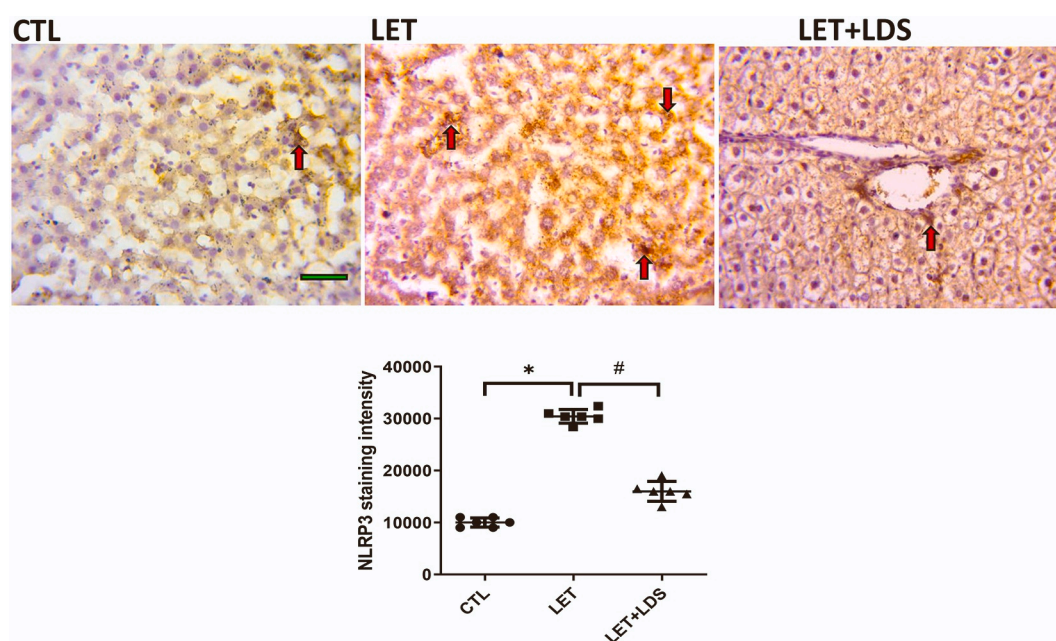


Fig. 8. Effects of low-dose spironolactone on immunohistochemistry of liver in LET-induced PCOS rat model using inflammasome antibody. Data are expressed as SD, n = 6. (* $p < 0.05$ vs. CTL, # $p < 0.05$ vs. LET). Control (CTL); Letrozole (LET); Low-dose spironolactone (SPL); Nod-like receptor protein 3 (NLRP3).

5. Conclusion

Experimentally induced PCOS causes dyslipidemia, hepatic lipid accumulation and inflammation, which are the pathogenic features of non-alcoholic fatty liver disease and this hepatic disorder was averted by low-dose spironolactone. The study in addition reported that the beneficial effect of low-dose spironolactone in PCOS-associated hepatic lipid disturbance/inflammation is accompanied by suppressed level of PCSK9. This suggests that low-dose spironolactone possesses PCSK9 inhibitory property and might be suitable for combating dyslipidemia-related conditions and hepatic disorders with minimal side effects.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

Kehinde S, Olaniyi: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing-original draft, Writing-review and editing.

Stephanie E. Areloegbe: Data curation, Methodology, Formal analysis, Project administration, Resources, Writing-review and editing.

Emmanuel D. Areola: Methodology, Writing-original draft, Writing-review and editing.

Isaiah W. Sabinari: Methodology, Visualization, Writing-original draft, Writing-review and editing.

Adedamola A. Fafure: Formal analysis, Methodology, Visualization, Writing-review and editing.

Richard D. Agbana: Visualization, Resources, Writing-review and editing.

Chukwubueze L. Atuma: Visualization, Resources, Writing-review and editing.

Mohd Z. ul haq Shah: Methodology, Resources, Software, Validation,

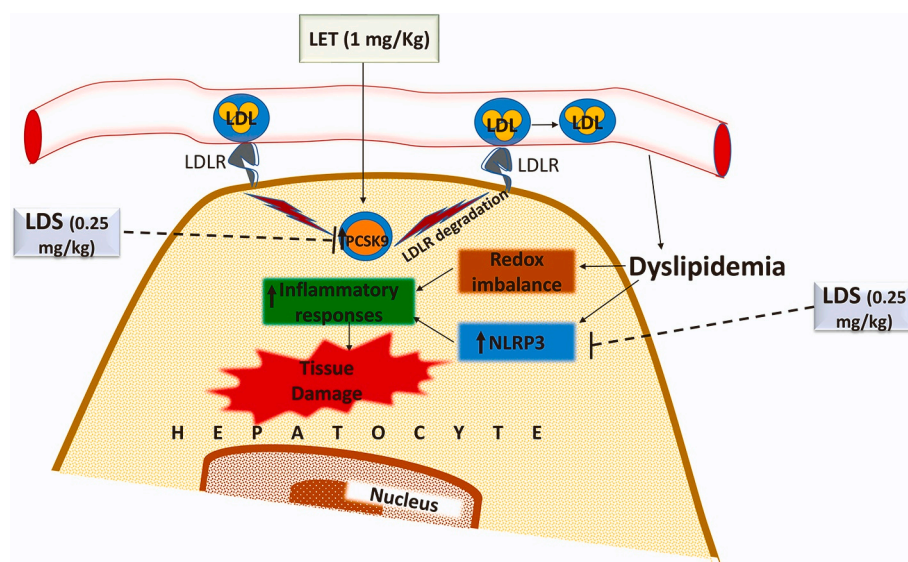


Fig. 9. Schematic diagram showing the involvement of PCSK9 and NLRP3 in PCOS associated hepatic pathology and therapeutic impact of LDS. Low-dose spironolactone (LDS); Letrozole (LET); Low-density Lipoprotein cholesterol (LDLc); Proprotein convertase subtilisin kexin 9 (PCSK9); Nod-like receptor protein 3 (NLRP3).

Visualization, Writing-review and editing.

Lawrence A. Olatunji: Supervision, Resources, Validation, Writing-review and editing.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

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