



Low-dose spironolactone attenuates metabolic defects in the skeletal muscle of letrozole-induced hyperandrogenic female rats

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Abstract

Purpose Hyperandrogenism is characterized by high levels of testosterone, which has been reported to cause insulin resistance and other metabolic adversities. Spironolactone (SPL) is a mineralocorticoid receptor blocker with anti-inflammatory effects. However, it is unknown whether SPL would restore skeletal muscle defect against letrozole-induced hyperandrogenism. Therefore, the present study investigated the hypothesis that low-dose SPL would attenuate skeletal muscle defect in hyperandrogenic female rats.

Methods Eight-week-old female Wistar rats were divided into three groups, namely, control, letrozole (LET)-treated, and LET + SPL-treated groups. The control received vehicle, LET-treated group received 1 mg/kg of LET, and LET + SPL-treated group received a combination of LET and SPL (0.25 mg/kg). The administrations were done by oral gavage for 21 days uninterruptedly. Biochemical analyses were determined with appropriate assay methods.

Results The results of this experiment showed that letrozole increased plasma testosterone and leptin and caused insulin resistance and increased triglyceride, total cholesterol, free fatty acids, pro-inflammatory markers (TNF- α and NF- κ B), lipid peroxidation markers (MDA), and apoptosis in the skeletal muscle. Nevertheless, administration of low-dose SPL attenuated these alterations in skeletal muscles.

Conclusion The present study demonstrates that letrozole-induced hyperandrogenism causes metabolic-driven apoptosis in the skeletal muscles. Hence, low-dose SPL mitigates metabolic defect and apoptosis in the skeletal muscles of hyperandrogenic female rats.

Keywords Hyperandrogenism · Letrozole · Insulin resistance · Skeletal muscles · Spironolactone

Introduction

Polycystic ovary syndrome (PCOS) affects up to 20% of women of reproductive age, and it is clinically characterized by irregular menstrual cycles, hyperandrogenism, infertility, or subfertility and frequently by a characteristic ovarian morphology on ultrasonographic examination. There is no consensus as to the most appropriate diagnostic criteria

since it incorporates multiple phenotypes [8, 44]. The etiology of PCOS is complex and not completely understood. Nevertheless, a central pathophysiological feature of PCOS is insulin resistance (IR), which has been associated with increased risk of type 2 diabetes mellitus (T2D) [21, 75]. Many studies have documented that letrozole, an aromatase inhibitor experimentally induces an androgenized systemic and ovarian milieu that mimics clinical manifestations of PCOS, including hyperandrogenism, irregular menstrual cycle/anovulation, and polycystic ovaries [57, 84].

Insulin resistance occurs in 44–85% of women of PCOS [42] and appears to be independent of obesity in PCOS patients. The pathogenesis of IR in PCOS patients remains unknown. Skeletal muscles are responsible for approximately 80% of insulin-stimulated glucose uptake in the whole body. Previous studies demonstrated that PCOS patients have impaired insulin signaling in skeletal muscles.

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Women with polycystic ovary syndrome (PCOS) exhibit reduced skeletal muscle insulin-mediated glucose uptake. Altered muscle mass may affect IR and inflammation, thereby potentially aggravating reproductive status including ovulatory cyclicity and fertility potential. However, the relationship between PCOS and skeletal muscle mass is elusive giving conflicting reports on protective or detrimental influence of PCOS associated endocrine derangements (hyperandrogenism, IR) on muscles. The molecular mechanisms of this defect are distinctive. In insulin receptors isolated from cultured skin fibroblasts of ~ 50% of women with PCOS, there is constitutive serine phosphorylation that inhibits insulin-stimulated tyrosine phosphorylation [25]. Recent studies have confirmed that a serine kinase extrinsic to the IR inhibits its autophosphorylation in PCOS [25, 52]. The resulting IR is selective, affecting metabolic but not mitogenic pathways [12]. The mechanism for IR in the PCOS skin fibroblasts with normal IR serine phosphorylation has not been investigated but may involve serine phosphorylation of downstream signaling molecules such as insulin receptor substrate (IRS)-1. In skeletal muscles, the major site of insulin-mediated glucose uptake, IRS-1-associated phosphatidylinositol (PI) 3-kinase activity is decreased in women with PCOS *in vivo*, consistent with a defect in IR- or IRS-1-mediated signaling [24]. Insulin resistance clusters in PCOS families [51] and defects in insulin action persist in cultured skin fibroblasts of women with PCOS [12, 25], suggesting that there is genetic susceptibility to these abnormalities.

A range of treatment options are available for the management of PCOS, ranging from lifestyle modifications (diet and exercise interventions) and pharmacological treatments to more invasive strategies such as laparoscopic ovarian drilling and bariatric surgery [76]. Despite the major health and economic impacts of PCOS, there are no regulatory approved drugs for the treatment of PCOS [65], and the current management strategies are suboptimal as they rely on symptomatic treatment [79]. There is a global growing interest in the role of mineralocorticoid receptor (MR) in the pathogenesis of IR and other cardiometabolic disturbances [37]. The blockade of the MR in previous studies has shown improvement in such disturbances [3, 4, 38, 57]. Spironolactone (SPL) is a MR antagonist that has been in wide clinical use for several decades because of its therapeutic benefits [27]. SPL, besides being a diuretic, has been used in the treatment of hirsutism in ovulatory women [13, 73]. The use of SPL to treat PCOS has been used largely in combination with other drugs [31]. Recently, it was reported that the use of SPL is relatively safe in smaller doses [3, 4, 50]. We therefore hypothesized that spironolactone at a low dose [32] may protect against metabolic disturbances in the skeletal muscles of hyperandrogenic female Wistar rats.

Materials and methods

Experimental animals and groupings

The study was carried out and reported in accordance with the ARRIVE guidelines 2.0. Eight-week-old female Wistar rats were procured from the animal house of the College of Health Sciences, Afe Babalola University, Ado-Ekiti. These rats had unrestricted access to standard rat chow and tap water. This study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Institutional Ethical Review Board of Afe Babalola University (Ado-Ekiti, Nigeria), and every effort was made to minimize both the number of used animals and their suffering. All the animals chosen for the work were with at least three consecutive regular estrous cycles and 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, letrozole-treated (LET), and LET + SPL-treated groups. 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. Rats were treated with letrozole (1 mg/kg) for 21 days consecutively to induce experimental PCOS as previously described [3, 4, 45, 57].

Treatment

Vehicle (distilled water) was given by oral gavage to the control group, LET group received 1 mg/kg of letrozole (oral gavage, Sigma-Aldrich, St. Louis, MI), and LET + SPL group received spironolactone and letrozole. The treatments were done uninterruptedly for 21 days [3, 4, 9, 45]. To avoid disruption of the circadian rhythm, the administration of all treatments was carried out between 7:00 and 8:00 am.

Preparation of skeletal tissue homogenate

At the end of the treatment and after overnight fasting, 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 704 *g* for 5 min at room temperature. Plasma was stored frozen until it was needed for biochemical assay. After weighing the gastrocnemius skeletal muscles, 100-mg section of the tissues were carefully removed and homogenized with a glass homogenizer in phosphate buffer solution, centrifuged at 8000 *g* for 10 min at 4 °C, and the supernatant was collected and stored frozen until it is required for biochemical assays.

Metabolic indices

After overnight fasting, blood glucose levels were determined from the blood obtained through the tails of the animals using a hand-held glucometer (ONETOUCH-LifeScan, Inc., Milpitas, CA, USA). Insulin concentrations were determined with rat ELISA kits obtained from Calbiotech Inc. (Cordell Ct., El Cajon, CA 92,020, USA) by following the manufacturer's procedures. Homeostatic model of assessment of IR ($\text{HOMA-IR} = \text{Fasting glucose (mmol/l)} \times \text{fasting insulin (}\mu\text{U/l)} / 22.5$) was determined as previously described [39, 55].

Biochemical analysis

Lipid and hormonal assay

From the skeletal muscle homogenates, standard colorimetric methods using assay kits (BXC0271 and BXC0261) obtained from Fortress Diagnostics Ltd. (Antrim, UK) were used to determine the triglyceride (TG) and total cholesterol (TC), respectively, while free fatty acid (FFA) was determined using kit (E-BC-K014) obtained from Elabscience Biotechnology Inc. (Wuhan, Hubei, PRC, China). Plasma testosterone, leptin, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were determined with rat ELISA kits obtained from Calbiotech Inc. (Cordell Ct., El Cajon, CA 92,020, USA).

Malondialdehyde and glutathione

Malondialdehyde (MDA) was determined from the skeletal tissue by standard non-enzymatic spectrophotometric method using assay kits from Oxford Biomedical Research Inc. (Oxford, USA). This method involves the reaction of MDA in the sample with thiobarbituric acid (TBA) to generate MDA-TBA adduct (Uchiyama et al. 1978), which was quantified spectrophotometrically, whereas glutathione (GSH) was determined using a non-enzymatic spectrophotometric method with assay kits obtained from Randox Laboratory Ltd. (Co. Antrim, UK). Glutathione determination by spectrophotometric method was 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.

Pro-inflammatory biomarkers

The levels of $\text{TNF-}\alpha$ and $\text{NF-}\kappa\text{B}$ were determined in the skeletal muscle homogenates by quantitative standard

sandwich ELISA technique using a monoclonal antibody specific for these parameters with rat kits (cat number E-EL-R0019 and E-EL-R0674, respectively) obtained from Elabscience Biotechnology Inc. (Wuhan, Hubei, PRC, China). The assays were performed in accordance to the manufacturer's procedures.

Immunohistochemistry

Immunohistochemical evaluation was determined from the skeletal tissue using BAX antibody. BAX antibody is a marker of apoptosis. Skeletal muscle already processed and sectioned was baked in an oven at 50 °C for 30 min. Slides were treated in xylene for 10 min, dehydrated in ascending grades of alcohol for 10 min each, and then rinsed in distilled water. Slides were placed in antigen retrieval solution (hot citric acid solution) at pH 7.0 heated to 70 °C for 40 min until 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 and then incubated in H_2O_2 for 30 min at room temperature to block endogenous peroxidase followed by protein block solution for 10 min at room temperature. Slides were rinsed with PBS in between the incubation. Thereafter, slides were incubated in BAX antibody from Elabscience Biotechnology Inc. (Wuhan, Hubei, PRC, China; E-AB-13814; 1:25–1:100) at room temperature for 180 min. The slides were rinsed and incubated in polyperoxidase-anti-mouse/rabbit IgG at room temperature (Elabscience Biotechnology Inc., Wuhan, Hubei, PRC, China) for 20 min and treated with 3, 3'-diaminobenzidine (DAB) working solution. Slides were counterstained in 1% aqueous hematoxylin solution for 20 min. Slides were then dehydrated in ascending order of ethanol for 10 min each. They were later cleared in xylene for 10 min and mounted. Then, the slides were examined using OPTO-Edu industrial camera light microscope and a computer (Nikon, Japan). The staining intensity was quantified in the skeletal muscles by processing with an image-processing and analysis software Image-J (Version 1.52).

Data analysis and statistics

All data were expressed as means $\pm$ SD. Statistical group analysis was performed with GraphPad Prism software version 5. One-way ANOVA was used to compare the mean values of variables among the groups. Bonferroni's test was used for post-hoc analysis. Statistically significant differences were accepted at p less than 0.05.

Results

SPL treatment improved insulin resistance and endocrine profile in LET-induced hyperandrogenic rats

There was no significant change in fasting blood glucose of LET group compared with control. Nevertheless, there was a significant increase ($p < 0.05$) in insulin level (Table 1) and IR as well as testosterone and leptin levels, while a significant decrease in FSH/LH of LET group compared with control group. However, in LET + SPL group, a significant decrease was observed in IR, insulin, and leptin, while FSH/LH increased when compared with LET group (Fig. 1).

Table 1 Fasting blood glucose and insulin

Groups	CTRL	LET	LET+SPL
Blood glucose (mmol/L)	4.48 ± 0.33	4.50 ± 0.09	3.90 ± 0.50
Insulin (μIU/mL)	1.56 ± 0.07	3.74 ± 0.20*	1.98 ± 0.18 [#]

Values are expressed as mean ± SD of 6 rats per group; * $p < 0.05$ vs CTRL, [#] $p < 0.05$ vs LET group. CTRL (control), LET (letrozole (1 mg/kg; *po*)), SPL (spironolactone (0.25 mg/kg; *po*))

SPL treatment improved skeletal muscle lipids in LET-induced hyperandrogenic rat

There was a significant increase ($p < 0.05$) in skeletal muscle TG, TC, and FFA of letrozole-induced group compared with control group. There was a significant decrease ($p < 0.05$) in skeletal muscle TG, TC, and FFA in LET + SPL-treated group compared with LET group (Fig. 2).

SPL treatment improved lipid peroxidation and glutathione in LET-induced hyperandrogenic rat

There was a significant increase ($p < 0.05$) in skeletal muscle MDA and a significant decrease in skeletal muscle GSH of LET group compared with control group. There was a significant decrease ($p < 0.05$) in skeletal muscle MDA and a significant increase in skeletal muscle GSH in LET + SPL-treated group compared with LET group (Fig. 3).

SPL treatment decreased pro-inflammatory markers in LET-induced hyperandrogenic rat

There was a significant increase ($p < 0.05$) in skeletal muscle NF-κB and TNF-α of LET group compared with control group, while a significant decrease ($p < 0.05$) was observed in skeletal muscle NF-κB and TNF-α of LET + SPL group compared with LET group (Fig. 4).

Fig. 1 SPL decreased HOMA-IR (A), testosterone level (B), increased FSH/LH (C), and decreased leptin (D) in LET-induced hyperandrogenic female rats. Values are expressed as mean ± SD of 6 rats per group; * $p < 0.05$ vs CTRL, [#] $p < 0.05$ vs LET group. CTRL (control), LET (letrozole (1 mg/kg; *po*)), SPL (spironolactone (0.25 mg/kg; *po*))

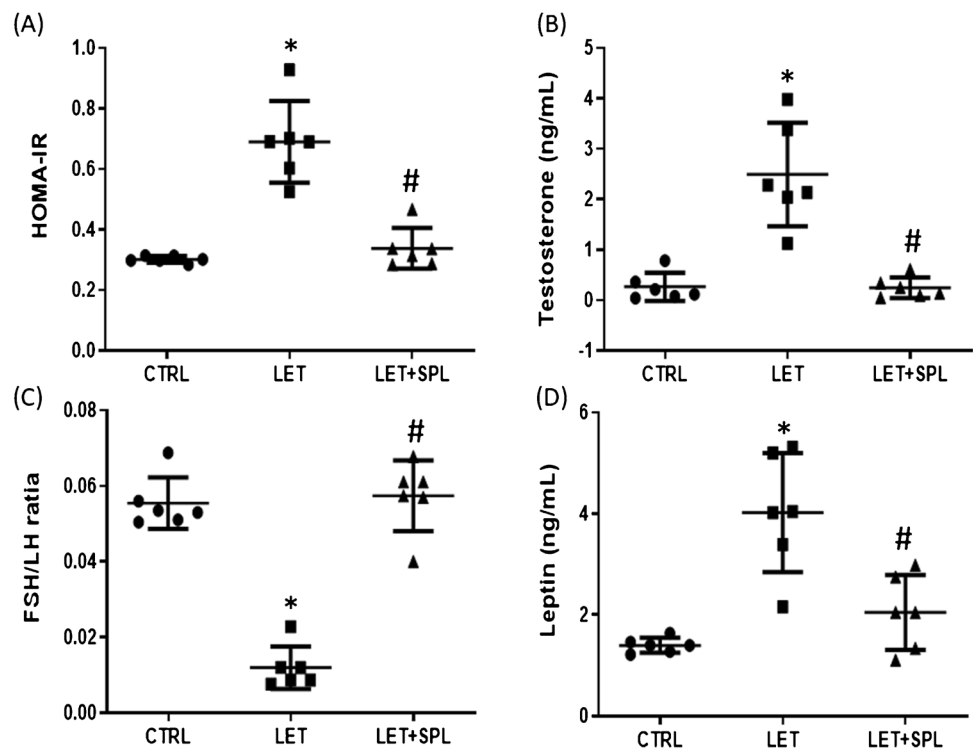


Fig. 2 SPL improved triglyceride (A), total cholesterol (B), and free fatty acid (C) in LET-induced hyperandrogenic rats. Values are expressed as mean $\pm$ SD of 6 rats per group; * p < 0.05 vs CTRL, # p < 0.05 vs LET group. CTRL (control), LET (letrozole (1 mg/kg; po)), SPL (spironolactone (0.25 mg/kg; po)), TG (triglycerides), TC (total cholesterol), FFA (free fatty acid)

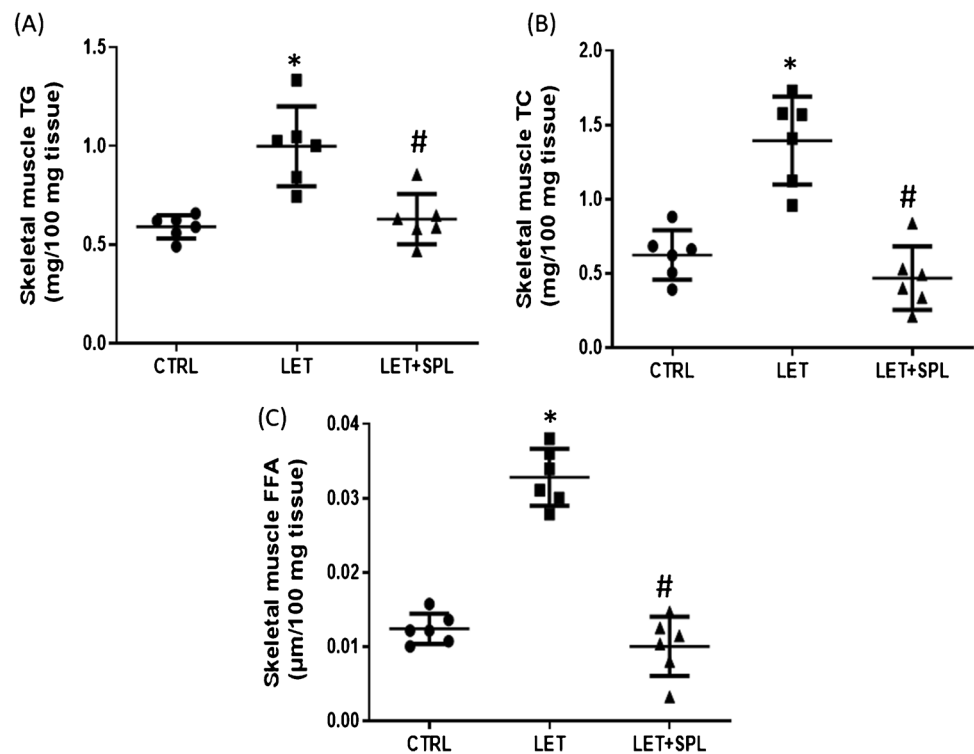


Fig. 3 SPL improved malondialdehyde (A) and glutathione (B) in LET-induced hyperandrogenic rats. Values are expressed as mean $\pm$ SD of 6 rats per group; * p < 0.05 vs CTRL, # p < 0.05 vs LET group. CTRL (control), LET (letrozole (1 mg/kg; po)), SPL (spironolactone; (0.25 mg/kg; po)), MDA (malondialdehyde), GSH (glutathione)

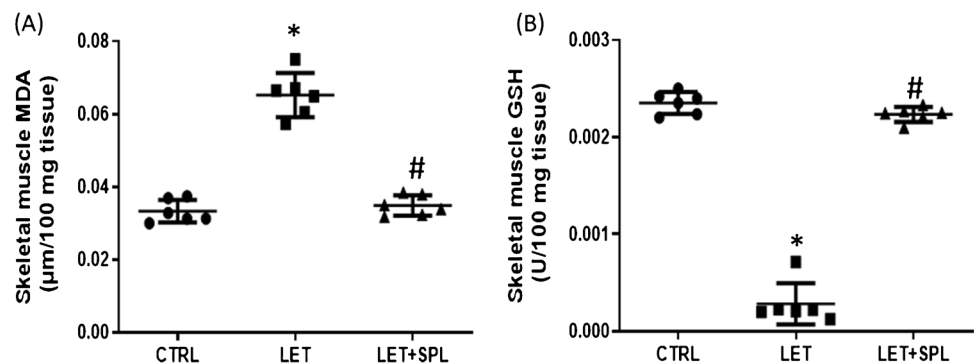
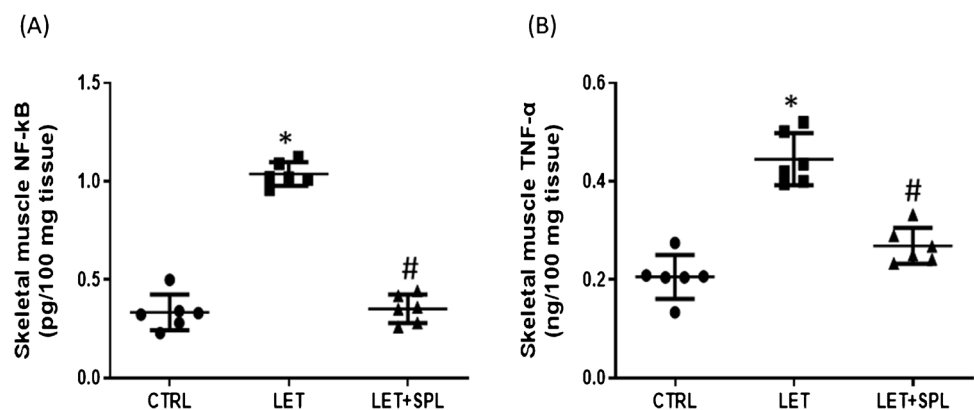


Fig. 4 SPL decreased NF- κ B (A) and TNF- α (B) in LET-induced hyperandrogenic rats. Values are expressed as mean $\pm$ SD of 6 rats per group; * p < 0.05 vs CTRL, # p < 0.05 vs LET group. CTRL (control), LET (letrozole (1 mg/kg; po)), SPL (spironolactone (0.25 mg/kg; po)), NF- κ B (nuclear factor-kappa B), TNF- α (tumor necrosis factor- α)



Immunohistochemical staining for BAX antibody in the skeletal muscle section

Immunohistochemical staining for BAX antibody in the skeletal muscle section of LET group showed severe expression of apoptosis with the BAX staining intensity significantly higher compared with control group. However, there was attenuation of apoptosis in the skeletal muscle of LET+SPL group with the BAX staining intensity showing significant reduction when compared with LET group (Fig. 5).

Discussion

The key finding of the present study is that SPL ameliorates metabolic perturbations and accompanied cellular apoptosis in the skeletal muscle of hyperandrogenic rats. The present study also demonstrated that LET treatment showed elevated circulating testosterone, accompanied by hyperinsulinemia and IR as well as metabolic disturbances. In addition,

the results revealed increment of lipid peroxidation, defective cellular antioxidant capacity, pro-inflammation/inflammation, and insulin resistance in LET group. Overall, these alterations were attenuated when animals were treated with SPL. Metabolic and related disorders such as insulin resistance, impaired glucose tolerance, T2D, and obesity among others have been well documented in women with PCOS [43, 88]. Similarly, in earlier studies, the animals with LET-induced PCOS showed impaired glucose tolerance, insulin resistance, and reduced insulin sensitivity with consequent hyperglycemia and compensatory hyperinsulinemia, which are crucial features of metabolic disorders [88].

Besides, hyperinsulinemia with or without hyperglycemia is a metabolic event that cause oxidative stress, thereby contributing to both structural and functional disorder of metabolic tissues, including the skeletal muscles. Similarly, reduced insulin sensitivity as indicated by increased HOMA-IR observed in animals with LET-induced PCOS might also affect the insulin-mediated functions of

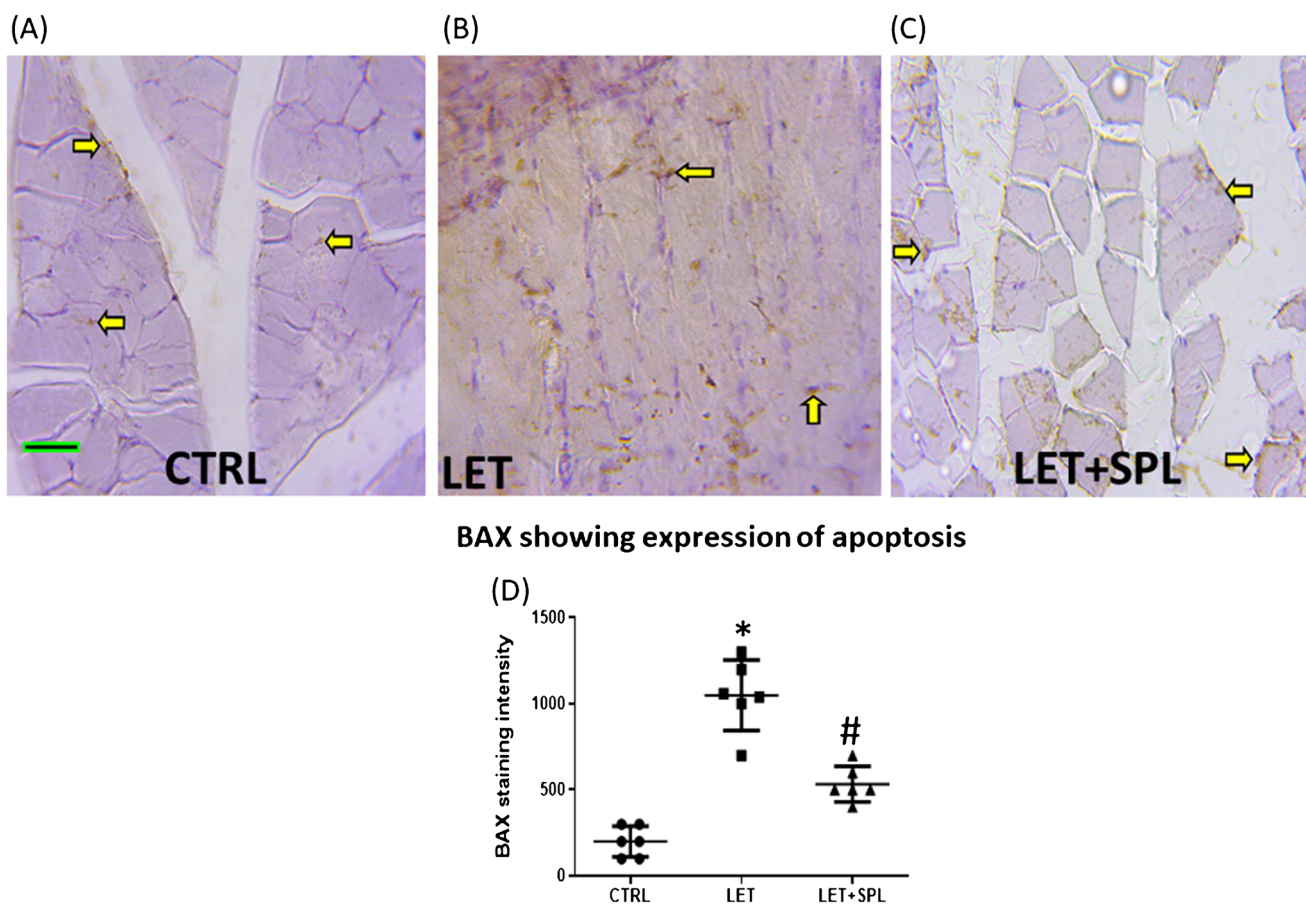


Fig. 5 Immunohistochemical staining for BAX antibody in the skeletal muscle section. Control groups showed no expression of BAX (A). LET group showed severe expression of apoptosis (B) and attenuation of apoptosis in LET+SPL (C). Scale bar: 51 μ m (yel-

low arrow indicates positive response to BAX antibody. Intensity of BAX expression (D) with values expressed as mean $\pm$ SD; * p < 0.05 vs CTRL, # p < 0.05 vs LET group (letrozole (1 mg/kg; *po*)), SPL (spironolactone (0.25 mg/kg; *po*))

metabolic tissues such as skeletal muscle, causing metabolic disturbances in skeletal tissues as a result of perturbed glucose-lipid regulation. Insulin resistance in skeletal muscle is manifested by decreased insulin-stimulated glucose uptake and results from impaired insulin signaling and multiple post-receptor intracellular defects including impaired glucose transport, glucose phosphorylation, and reduced glucose oxidation and glycogen synthesis. IR can affect skeletal muscles either from alterations in the insulin signal transduction pathway [62] or from alterations in either traffic or translocation of glucose transporter type 4 (GLUT4) to the plasma membrane [26]. Evidence from previous studies suggests that insulin signaling defects in muscle are associated with altered glucose homeostasis [68]. At physiological conditions, insulin activates glucose uptake by stimulating the canonical IRS-PI3K-Akt pathway and by phosphorylating and inactivating Akt substrate 160 (AS160), a protein that, when activated, prevents glucose transporter (GLUT) 4 translocation to the membrane. Thus, by inhibiting AS160, insulin promotes the GLUT4 translocation from inner vesicles, promoting fusion to the plasma membrane and consequently glucose uptake [69]. But IR-associated inflammation and increasing reactive oxygen species (ROS) primarily impair insulin signaling [48, 56], therefore leading to skeletal muscle defects.

Evidence exists that insulin resistance with consequent hyperinsulinemia together with free insulin growth factors stimulates ovarian tissues to produce androgen through inhibition of aromatase activity [30, 36]. The present result that showed elevated circulating testosterone in animals that received letrozole was consistent with the previous studies [3, 4, 30]. The increase in circulating testosterone might in part contribute to reduced insulin sensitivity with consequent reduction in peripheral glucose disposal in animals with LET-induced PCOS. It has been shown that LET induces PCOS by blocking the conversion of testosterone to estrogen [45]. The inhibition of the aromatase activity led to increase of ovarian androgens, which subsequently led to hyperandrogenism, the hallmark of PCOS [41, 85]. Decreased FSH/LH ratio was observed in this study. This is due to excess testosterone feedback to the pituitary gland, thereby resulting in excess LH and depressed FSH [2]. Therefore, LET in the present study might have inhibited aromatase activity, hence, conversion of testosterone to estrogen with the clinical manifestation of elevated circulating levels of testosterone increased levels of LH and decreased level of FSH [41, 85]. Synthesis and secretion of LH over FSH consequently decreased FSH/LH ratio in this study. This was in consonance with previous studies in women with PCOS [11, 33]. More so, in women with PCOS, elevated testosterone is typically driven by increased LH secretion [11, 33]. We found that treatment with SPL significantly decreased plasma testosterone levels in LET rats, indicating

that 21 days of continuous treatment with SPL at this very low-dose decreased testosterone levels induced by LET, hence positive effects on LH, FSH, and FSH/LH ratio, respectively. This is similar to a previous report [64]. It is worthwhile to note that SPL can bind to androgen receptor (AR) and partially inhibit adrenal steroidogenesis, which blocks 5 α -reductase and 17-hydroxysteroid dehydrogenase at the ovarian and adrenal level. This would stimulate aromatase and hence increase sex hormone-binding globulin [90]. It has also been reported that SPL could present intraovarian action similar to that in patients with hyperaldosteronism that shows a restoration of normal reactivity of glomerulosa to aldosterone after withdrawal of prolonged metabolite of SPL [7]. More so, elevated testosterone is typically driven by LH pulse frequency [33] which itself is likely a downstream of dysregulated steroidogenesis and sex steroid feedback [61]. These complex mechanisms of action of SPL are the reason behind normal testosterone values during SPL treatment in women with PCOS.

Inflammation is closely related to skeletal muscle insulin resistance, and increased inflammation promotes skeletal muscle insulin resistance [28]. Studies have shown that TNF- α , monocyte chemoattractant protein-1 (MCP-1), C-reactive protein (CRP), interleukins (ILs), and other inflammatory factors can promote skeletal muscle insulin resistance [29]. Inflammation primarily impairs insulin signaling by activating the IKK β /NF- κ B and JNK pathways, thereby promoting skeletal muscle insulin resistance [48]. Studies have shown that IL-1 can activate the skeletal muscle IKK β /NF- κ B pathway, reduce IRS-1 activity, and promote skeletal muscle insulin resistance [78]. Skeletal muscle insulin resistance improves after the IKK β /NF- κ B pathway is inhibited [83]. Moreover, IL-6 also promotes the expression of cytokine signaling inhibitor 1 (SOCS1) and SOCS3, thereby degrading IRS-1 [78] and promoting insulin resistance. The JNK pathway and TNF- α impair insulin signaling by inducing IRS-1 serine phosphorylation [48]. Therefore, inflammation impairs insulin signaling by activating inflammation-related factors as well as the IKK β /NF- κ B and JNK pathways, thereby increasing the risk of insulin resistance in skeletal muscle.

Oxidative stress can increase the risk of insulin resistance in skeletal muscle. Biomarkers of skeletal muscle oxidative damage such as malondialdehyde (MDA), protein carbonyl, 4-hydroxy-2-nonenal, hydroperoxide, protein oxidation products, 3-nitrotyrosine, advanced glycosylation products (AGEs), carbohydrate metabolites, and 8-hydroxy-2'-deoxyguanosine reduce skeletal muscle insulin sensitivity and increase the risk of insulin resistance [71, 89]. Studies have shown that after artificially increasing ROS production in myotubes, IRS-1 tyrosine phosphorylation, Akt activation, and GLUT4 translocation to the plasma membrane are impaired [20]. Studies of the underlying

mechanisms have shown that skeletal muscle oxidative stress can impair insulin signaling and induce insulin resistance [56]. Skeletal muscle oxidative stress activates several serine-threonine kinase pathways such as IKK β /NF- κ B and JNK, leading to IRS-1 degradation [14] and the inhibition of insulin signaling pathways. Moreover, oxidative stress can also inhibit the translocation of GLUT4 to the plasma membrane [53], further reducing the effect of insulin. In addition, oxidative stress can also induce insulin resistance by impairing mitochondrial function, revealing cellular apoptosis in the skeletal muscle of LET animals compared with control.

The beneficial effect of SPL treatment on the IR was associated with weight loss and reduced visceral fat in the LET-induced PCOS group [90] as a result of the SPL suppression of the elevated testosterone [32]. The underlying mechanism of action of SPL in this model suggests that the two major causes of PCOS, which are IR and elevated testosterone, closely interact [32]. Thus, if a drug has a protective effect on any of these two mechanisms, it has at least partial protective effect on the other mechanism. Furthermore, Adeyanju et al. previously reported that blockade of the MR with SPL can prevent IR-associated disorder [3, 4], hence, the effect could have been the blockade of MR. Insulin resistance was also observed in the LET group, but treatment with SPL had a protective effect on this as well. This was in agreement with a previous report [49]. Decreased IR concomitantly improves both metabolic and reproductive parameters of PCOS. The present results were also in consonance with earlier observation by Daneasa et al., who reported an improvement in PCOS-linked metabolic deregulation following SPL treatment (Daneasa et al., 2014). Lipid disturbances noticed in the skeletal muscles of LET group were also in line with previous reports [70, 87] and could be largely due to the elevated testosterone and visceral fat [60]. SPL treatment however attenuated these disturbances that might be a direct consequence of suppressed testosterone. Improved lipoprotein has been reported in PCOS state with blockade of MR [22]. As extension to earlier studies, including Daneasa et al. (2014), the inflammation (NF- κ B and TNF- α) and oxidative stress which were observed in the skeletal muscle of hyperandrogenic animals might be triggered by lipid accumulation in the skeletal muscles, and these possibly resulted in cellular apoptosis that is demonstrated with severe expression of BAX in LET-treated group. However, treatment with low dose of SPL attenuated inflammation/oxidative stress-driven apoptosis. Antioxidant enzymes provide cellular defense against ROS formation. Hence, prevention of oxidative stress through SPL treatment subsequently mitigated apoptosis in the skeletal muscle as characterized immunohistochemically by reduced BAX expression in LET + SPL group. Therefore,

the present results suggest that low dose of SPL blocks metabolic deregulation and oxidative stress-linked cellular apoptosis in the skeletal muscles of hyperandrogenic rats. Nevertheless, the present study was limited in such that immunohistochemical evaluation using Bcl2 antibody (anti-apoptotic marker) was not performed in the skeletal muscle, and the molecular mechanisms underlying the beneficial effects of SPL in the skeletal muscle of hyperandrogenic rats were not explored.

Conclusion

The present study demonstrates that administration of low-dose SPL mitigates letrozole-induced hyperandrogenism and associated metabolic deregulation as well as skeletal muscle apoptosis in female rats.

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Author contribution All the authors contributed to the manuscript.

Data availability The data supporting the present study will be made available from the corresponding author on request.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval and consent to participate The study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Independent Institutional Ethical Review Committee of Afe Babalola University (Ado-Ekiti, Nigeria). Consent to participate is not applicable.

Consent for participation All the authors participated and agreed to the submission.

Competing interest The authors declare no competing interests.

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