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ABSTRACT:
Hyperandrogenism has been implicated in patients with polycystic ovary syndrome, which is the most common endocrine-metabolic disorder among women of reproductive age. Hyperandrogenism has been linked with renal disorders, particularly chronic kidney disease. Therefore, the present study investigated the effect of letrozole-induced hyperandrogenism on renal function in female Wistar rats. Eight-week-old female Wistar rats were allotted into two groups (n=6 per group) which include: Control, and letrozole-treated (LET). The control group received vehicle (*p.o.*) and the LET-treated group received letrozole (1 mg/kg; *p.o.*). The administration was carried out once daily for 21 days. The results showed that the hyperandrogenic rats had increased kidney weight, metabolic profile (fasting insulin and homeostatic model of assessment of insulin resistance), renal free fatty acids, renal inflammatory biomarkers (tumor necrosis factor and interleukin-6), renal malondialdehyde, γ-glutamyl transferase, lactate production, lactate dehydrogenase, plasma creatinine and urea, and plasma and renal uric acid with a subsequent decrease in renal glutathione peroxidase. Moreover, the study revealed an increased plasma testosterone level when compared with control animals. The present study therefore indicates that letrozole-induced hyperandrogenism resulted in elevated levels of testosterone and insulin resistance which causes renal dysfunction, further accompanied by renal lipid peroxidation and inflammation.

Keywords: Hyperandrogenism; Kidney; Letrozole.; Polycystic ovarian syndrome
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INTRODUCTION:

The kidneys are important organs in the body that regulate water and electrolyte balance as well as blood pressure. Alterations of renal function, which is commonly linked to cardiometabolic and endocrine disorders, including polycystic ovarian syndrome (PCOS) have been associated with cardiovascular disorders (CVD), which remains the huge cause of death globally [1]. PCOS is an endocrine / metabolic disorder occurring in about 6–21% of reproductive-aged women [2] and is often characterized by polycystic ovaries, hyperandrogenism and anovulation / oligomenorrhea [3]. PCOS usually progresses to a number of metabolic-related pathologies, such as chronic kidney disease (CKD), which is characterized by inflammation, oxidative stress as well as endothelial dysfunction [4]. Insulin resistance (IR) is an early metabolic event in CKD [5], and this may result in end-stage renal failure with deteriorated IR. Although, the indisputable link between PCOS (hyperandrogenism)-engendered IR and CKD is unclear, several studies have linked the underlying pathogenesis to excessive lipid peroxidation and imbalance between reactive oxygen species and antioxidant enzymes [6]. Nevertheless, further investigation of its pathophysiological mechanism would provide a therapeutic target for the management of metabolic/endocrine-associated kidney disease.

The pathophysiological involvement of IR in renal disease is multifactorial in nature which is possibly secondary to perturbations that are prominent in renal diseases, including oxidative stress, chronic inflammation, metabolic acidosis and adipokine derangement. IR plays a major role in the progression of renal disease, which deteriorates renal hemodynamics by activation of the sympathetic nervous system [7]. Impaired insulin signaling has been reported as a pathogenic factor in polycystic kidney disease [8], by possibly increasing uremic toxin such as endogenous nitric oxide synthase inhibitor. This aggravates glucose dyshomeostasis in various pathological conditions [9].

Hyperandrogenism is an important criterion in diagnosing PCOS. In patients with PCOS, the rate of hyperandrogenism could be as high as 60%-80%. Androgen hyperactivation leads to ovulation disorder, menstrual disorder and acne, suggesting that hyperandrogenism is not only a clinical characteristic of PCOS, but also an important risk factor. The current anti-androgen therapies in clinical settings have not achieved satisfactory effects, which lies in the complicated mechanisms of androgen production and its wide-ranging effects [10]. The menstrual cycle abnormality is also one of the critical characteristics in patients with PCOS. Accompanied by a prolonged menstrual cycle, anovulation becomes more frequent, leading to amenorrhoea, endometrial hyperproliferation

and even carcinogenesis. Polycystic ovaries are another vital feature in women with PCOS; defined as the presentation of at least 12 antral follicles (AFC) with a diameter from 2 to 9 mm in the whole ovary and/or an ovarian volume over 10mL [11]. Excessive AFC could lead to the secretion of large amounts of oestrogen, which inhibits the secretion of follicle-stimulating hormone (FSH) via negative feedback of the gonadal axis and leads to anovulation. Therefore, the length of the menstrual cycle and the number of AFC in ovaries are the two main indicators to estimate the disease severity of PCOS. The serum level of testosterone is positively correlated with the length of the menstrual cycle and the numbers of AFC [12], suggesting that hyperandrogenism is a promoter of PCOS. In addition, excess androgen can damage granulosa cells (GCs) and change the microenvironment of follicles, resulting in follicular atresia in PCOS [13].

Chronic kidney disease (CKD) is manifested as a decrease in glomerular filtration rate (GFR; GFR < 60 mL/min) over 3 months and proteinuria. Due to the high correlation between CKD and metabolic disorders, there may be a close correlation between PCOS and kidney diseases. Previous studies revealed the presence of pre-microalbuminuria and an increase in cystatin C (a biomarker for renal function) in PCOS women [14,15]. Considering that there are abundant androgen receptors in renal cells, such as mesangial cells and proximal tubular cells, excessive androgen could be a causal risk factor for kidney diseases. Reports show that androgen/AR imposes the susceptibility to severe infections in the upper urinary tract and a high rate of urinary citrate and sodium excretion in women [16,17]. Furthermore, a significantly positive correlation between serum testosterone and renal tubular cell injury has been implicated in patients with PCOS [18], and the follicular fluid collected from patients with PCOS could induce fibrotic lesions in cultured renal proximal tubular cells [18].

However, the specific mechanism is not clear. Testosterone was also reported to induce the apoptosis in renal tubular epithelial cells, as well as necrosis via activation of hypoxia-inducible factor 1α/Bcl-2 interacting protein 3 (HIF-1α/BNIP3) pathway [19]. Another study has shown that androgen/AR and Fgf10/Fgfr2 signalling participate in renal fibrosis [20]. In addition, evidence shows that decreased androgens protect against renal injury by reducing T-cell infiltration and enhancing anti-inflammatory cytokine production [21]. Studies also show that prenatal testosterone induces proteinuria in adulthood [22], which may explain the results of pre-microalbuminuria in patients with PCOS. Although previous studies have implicated the possible molecular mechanisms of hyperandrogenism in PCOS and its related complications, necessity is laid on research to uncover its dynamic nature.

METHODOLOGY:

Experimental Animals and Grouping:

Eight-week-old female Wistar rats were used in this study. The rats were given unlimited access to standard rat chow and tap water. The study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

After 2 weeks of acclimatization, the animals were randomly distributed into two groups (n=6 per group); the Control and letrozole-treated (LET) groups.

Rats were maintained in a colony under standard environmental conditions of temperature (22-26^{0}C), relative humidity (50-60%), and a 12-hour dark/light cycle. Rats were treated with letrozole (1.0 mg/kg) for 21 days as previously described [23,24,25,26].

Treatment:

Vehicle was given by oral gavage to the control group while LET group received 1.0 mg/kg of letrozole obtained from Sigma-Aldrich, in St Louis, MI by oral gavage. The treatments were done for 21 days.

Metabolic Indices:

The oral glucose tolerance test was performed 48 hours before the sacrifice of the rats:

After a 12-hour overnight fast, basal blood glucose was determined, and the rat were loaded with glucose (2.0 g/kg; oral gavage). Then blood was obtained sequentially at 30, 60, 90 and 120 minutes and the area under the curve (AUC) of glucose were monitored with a hand-held glucometer manufactured by ONETOUCH Life Scan, Inc., Milpitas, CA, USA. Insulin resistance was determined using the homeostatic model assessment of IR (HOMA-IR = fasting glucose (mmol/l)* fasting insulin (µU/l)/ 22.5) as described in the previous studies [26,27].

Collection of Samples:

Blood was collected by cardiac puncture into a heparinized tube after anesthetizing the animals with sodium pentobarbital (50 mg/kg, *ip*) and the blood was centrifuged at 704 *g* for 5 min at room temperature. Plasma was stored frozen until it was needed for biochemical assay.

Preparation of kidney homogenate:

After weighing the kidney, 100 mg section of the tissue was 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 was required for biochemical assays.

Biochemical analysis:

Plasma endocrine profile:

Plasma insulin and testosterone concentrations were determined with Rat ELISA kits obtained from Calbiotech Inc. in Cordell Ct., El Cajon, CA 92020, USA.

Plasma and kidney lipid profile:

Standard colorimetric methods were used to determine free fatty acids from the plasma and tissue homogenate by using assay kits obtained from Fortress Diagnostics Ltd. in Antrim, UK.

Plasma and kidney lipid peroxidation and antioxidant markers:

Malondialdehyde (MDA) is a marker of lipid peroxidation while glutathione peroxidase (GPx) assesses antioxidant capacity [28]. Malondialdehyde was determined from the plasma and tissue homogenate by standard non-enzymatic spectrophotometric method using assay kits from Randox Laboratory Ltd. in Co. Antrim, UK. Glutathione peroxidase was determined from the plasma and tissue homogenate by standard enzymatic spectrophotometric method using assay kits from Oxford Biomedical Research Inc. in Oxford, USA.

Inflammatory biomarkers:

The levels of tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were determined in the plasma and tissue homogenate by quantitative standard sandwich ELISA technique using a monoclonal antibody specific for these parameters with rat kits obtained from Elabscience Biotechnology Inc. in Wuhan, Hubei, P.R.C., China.

Uric acid, Creatinine and Urea:

The level of plasma creatinine was determined by colorimetric method using assay kits from Randox Laboratory Ltd. in Co. Antrim, UK. Whereas plasma urea and plasma and renal uric acid concentrations were determined t by standard spectrophotometric methods, using kits from Oxford Biomedical Research Inc. in Oxford, UK.

Plasma and renal lactate, lactate dehydrogenase (LDH) and γ-glutamyl transferase (GGT):

Lactate concentration and LDH activity were determined from the plasma and tissue homogenate by standardized non-enzymatic and enzymatic colorimetric method respectively using assay kits obtained from Randox Laboratory Ltd. in Co. Antrim, UK. Whereas plasma and renal GGT activities were assayed by standardized enzymatic colorimetric method using assay kits obtained from Fortress Diagnostics Ltd. in Antrim, UK.

Immunohistochemical assessment of kidney:

Immunohistochemical evaluation of renal tissue was performed using an inflammasome antibody (NLRP3) obtained from Elabscience Biotechnology Inc. in Wuhan, Hubei, P.R.C., China, in adherence to the procedures described in the previous study [28].

Data analysis and statistics:

All data were expressed as means ± S.D. Statistical group analysis was performed with

Graphpad Prism software version 9. Student T-test was used to compare the mean values of variables between the groups. Statistically significant differences were accepted at $p<0.05$.

Ethical approval:

The study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Ethical Review Committee of Afe Babalola university.

Consent to participate is not applicable.

RESULTS:

Effect of LET-induced hyperandrogenism on kidney weight in animal model: There was a significant increase in the kidney weight of letrozole-induced hyperandrogenic animals compared to the control animals (Figure 1).

Effect of LET-induced hyperandrogenism on metabolic profile in animal model: Metabolic profiles such as fasting insulin and HOMA-IR significantly increased in experimental hyperandrogenic animals compared with the control (Figure 2).

Effect of LET-induced hyperandrogenism on plasma and renal free fatty acids in animal model: There was a significant increase in the renal but not plasma FFA in experimental hyperandrogenic animals compared with the control animals (Figure 3).

Figure 1: Effect of letrozole induced hyperandrogenism on kidney weight in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*$p<0.05$ vs. CTL; #$p<0.05$ vs LET). Control (CTL); Letrozole (LET).

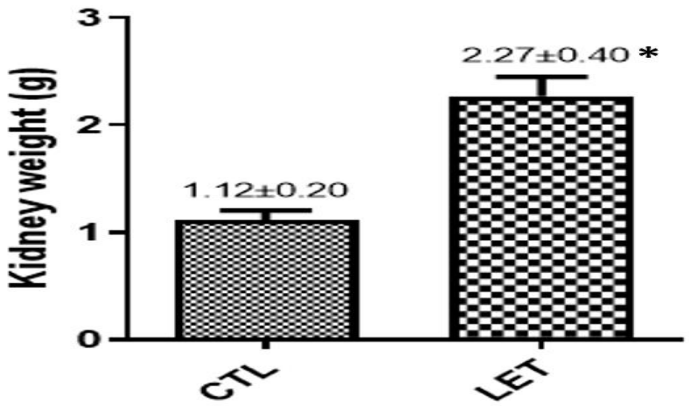


Figure 2: Effect of letrozole induced hyperandrogenism on metabolic profile: fasting blood glucose (a) fasting insulin (b) and HOMA-IR (c) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*p<0.05 vs. CTL; #p<0.05 vs LET). Control (CTL); Letrozole (LET); Homeostatic model of assessment of insulin resistance (HOMA-IR).

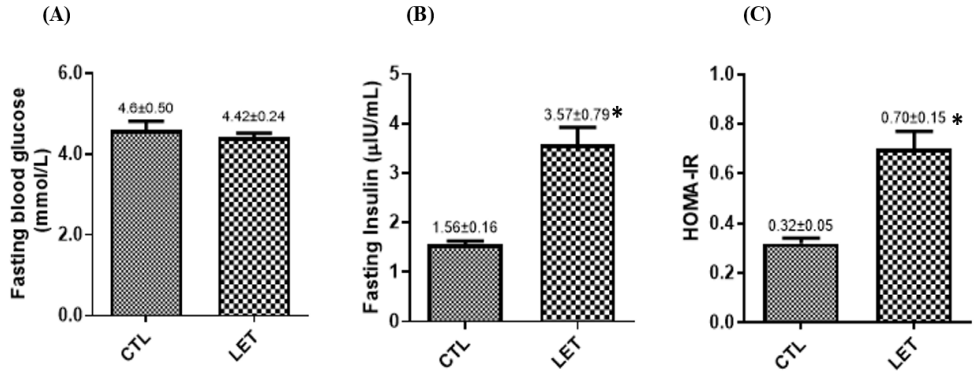
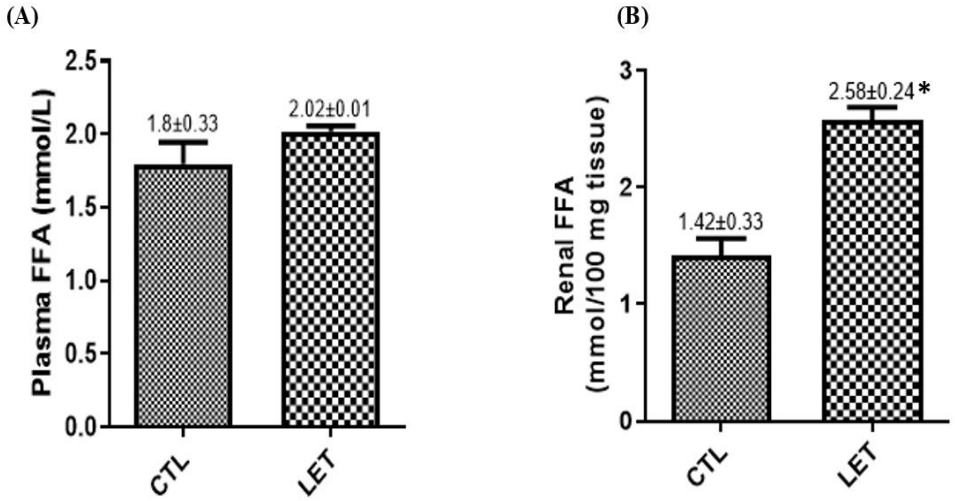


Figure 3: Effect of letrozole induced hyperandrogenism on lipid profile: plasma FFA (a) and renal FFA (b) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*$p<0.05$ vs. CTL; #$p<0.05$ vs LET). Control (CTL); Letrozole (LET); Free fatty acid (FFA).



Effect of LET-induced hyperandrogenism on renal inflammatory biomarkers in animal model: Inflammatory biomarkers such as TNF-α and IL-6 increased significantly in the renal tissue of experimental hyperandrogenic animals compared with the control (Figure 4).

Effect of LET-induced hyperandrogenism on renal malondialdehyde, g-glutamyl transferase

and glutathione peroxidase in animal model: In experimental hyperandrogenic animals, there was a significant increase in renal MDA and GGT while GPx decreased significantly in the kidney compared to control animals (Figure 5).

Effect of LET-induced hyperandrogenism on renal lactate and lactate dehydrogenase in animal model: There was a significant increase in lactate and LDH in experimental hyperandrogenic animals when compared with the control (Figure 6).

Effect of LET-induced hyperandrogenism on plasma and renal electrolytes in animal model: There was a significant increase in the plasma and renal uric acid, plasma creatinine and urea in experimental hyperandrogenic animals compared with the control animals (Figure 7).

Effect of LET-induced hyperandrogenism on plasma testosterone in animal model: There was a significant increase in the plasma testosterone levels in experimental hyperandrogenic animals compared with the control animals (Figure 8).

Figure 4: Effect of letrozole induced hyperandrogenism on renal TNF-α (a) and renal IL-6 (b) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*$p<0.05$ vs. CTL; #$p<0.05$ vs LET). Control (CTL); Letrozole (LET); Tumor necrotic factor-α (TNF-α) Interleukin-6 (IL-6).

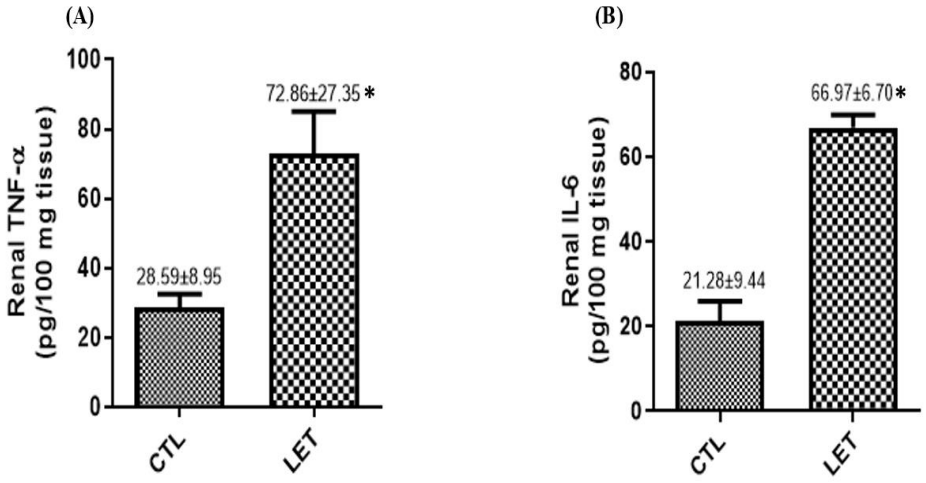


Figure 5: Effect of letrozole induced hyperandrogenism on lipid peroxidation: renal MDA (a) renal GPx (b) and renal GGT (c) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (**p<0.05* vs. CTL; *#p<0.05* vs LET). Control (CTL); Letrozole (LET); Malondialdehyde (MDA); Glutathione peroxidase (GPx); Gamma Glutamyl transferase (GGT).

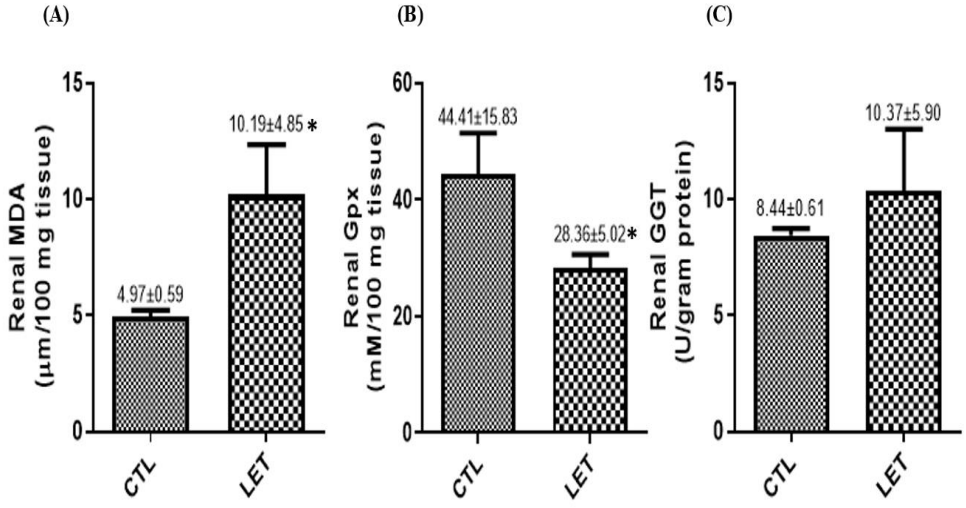


Figure 6: Effect of letrozole induced hyperandrogenism on lipid peroxidation: renal lactate (a) and renal LDH (b) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (**p<0.05* vs. CTL; *#p<0.05* vs LET). Control (CTL); Letrozole (LET); Lactate Dehydrogenase (LDH).

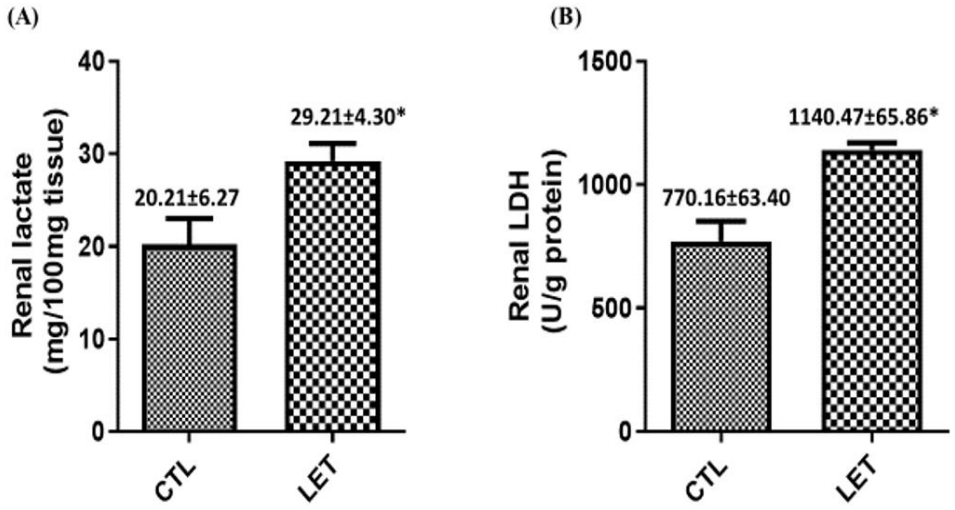


Figure 7: Effect of letrozole induced hyperandrogenism on renal electrolyte: plasma uric acid (a) renal uric acid (b) plasma creatinine (c) and plasma urea (d) in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*$p<0.05$ vs. CTL; #$p<0.05$ vs LET). Control (CTL); Letrozole (LET).

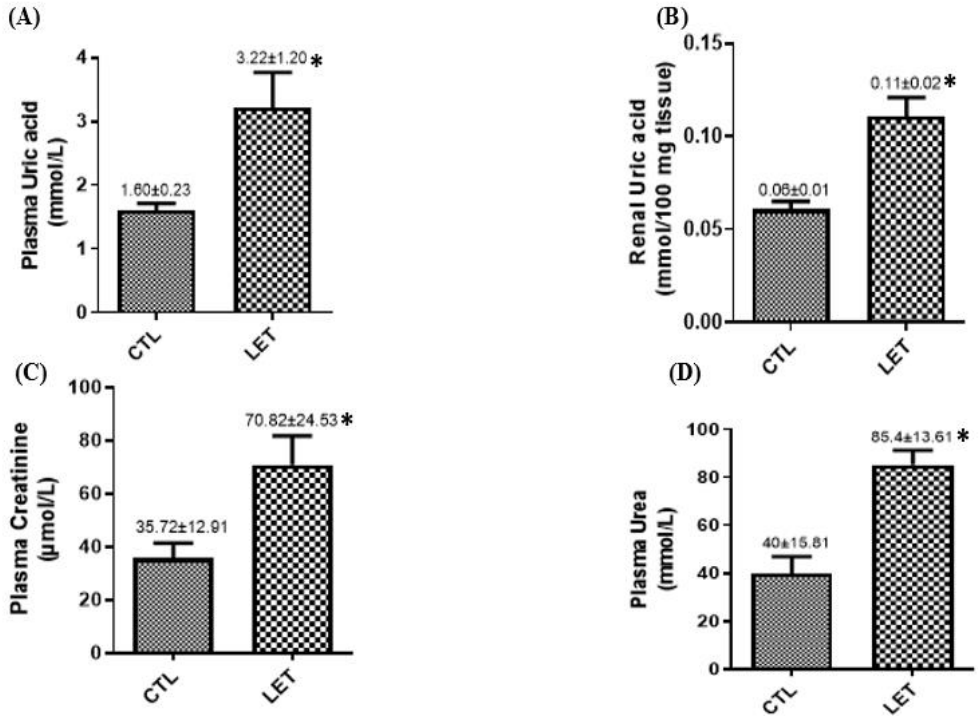


Figure 8: Effect of letrozole induced hyperandrogenism on plasma testosterone level in LET-induced PCOS. Data are expressed as means ± S.D., n=6. (*$p<0.05$ vs. CTL; #$p<0.05$ vs LET). Control (CTL); Letrozole (LET).

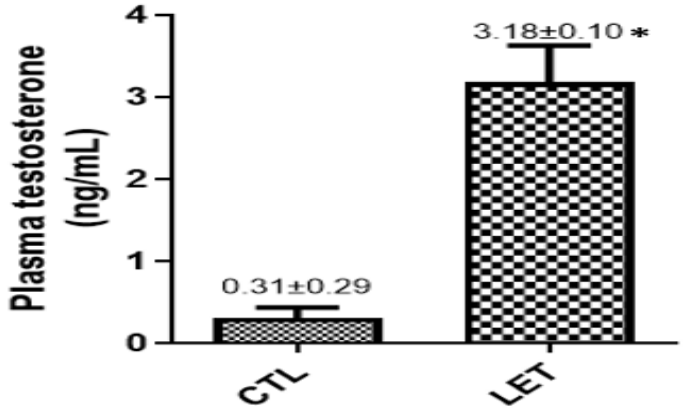
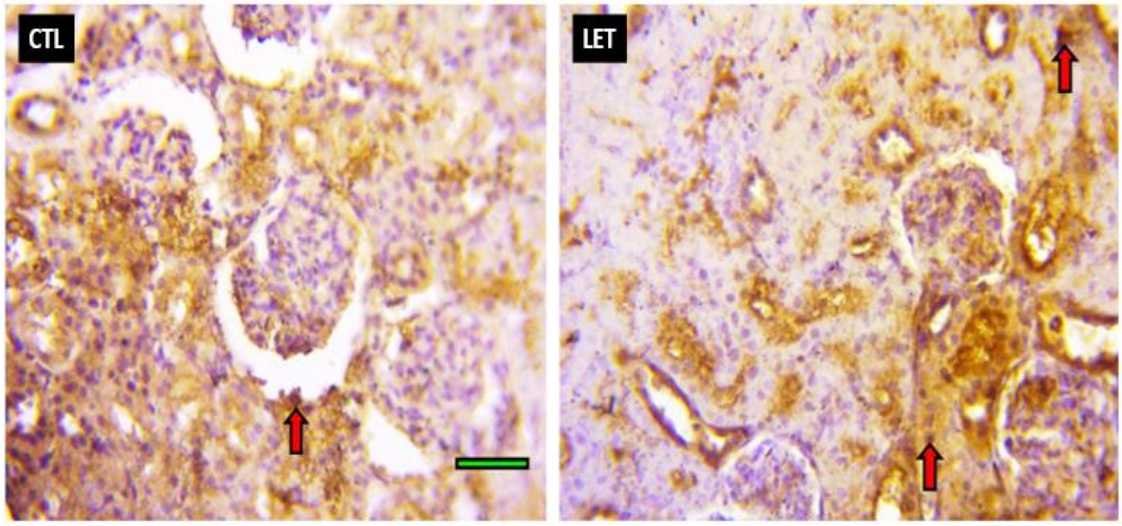


Figure 9: Immunohistochemical staining for inflammasome (NLRP3) antibody in the renal section. Control groups showed no expression of inflammasome (a) and letrozole-induced hyperandrogenic animal showed severe expression of inflammasome (b). Scale bar: 51 µm (red arrow indicate a positive response to inflammasome antibody).



Immunohistochemical staining for inflammasome (NLRP3) antibody in the renal section:

Immunohistochemical staining for inflammasome (NLRP3) antibody in the renal section. Control groups showed no expression of inflammasome. However, letrozole-induced hyperandrogenic animal showed severe expression of inflammasome (Figure 9).

DISCUSSION:

The novel finding in this present study is that hyperactivity of androgen hormones which is a well-known characteristic of PCOS can affect renal functions. PCOS has a close association with the progression of metabolic abnormalities such as obesity, diabetes and hypertension, which are the major causes of kidney disease [29,30]. These suggest PCOS may have an intimate association with kidney injury. Few studies have investigated the underlying mechanism of PCOS-associated kidney injury. In this study, we have established a link in PCOS with kidney injury through experimental research.

To test the hypothesis that PCOS is associated with kidney injury, we evaluated kidney function in two group of female Wistar rats, PCOS induced with letrozole and control. The present study provides different metabolic risks, and IR is a hallmark of the classic hyperactivation of androgens through increased free fatty acid. Obesity is strongly associated with PCOS [31]. Although, obesity is not a diagnostic criterion for

PCOS, both obese and non-obese PCOS patients have more visceral adipose tissue (VAT) than that of women without PCOS, and VAT has been positively correlated with total androgen levels suggesting obesity plays a crucial role in PCOS [32]. Compared with normal-weight PCOS patients, overweight PCOS patients exhibit significantly higher serum-free testosterone and free androgen indices [33]. All evidence indicates that androgens are closely related to obesity in PCOS patients. However, current data on the association between hyperandrogenism and obesity are limited and controversial. Abdominal obesity is a condition of relative hyperandrogenism. Androgens have been shown to induce abdominal adipose accumulation [34] and may cause adipose tissue dysfunction, including increased lipid accumulation and insulin resistance [35,36]. Lipogenic enzymes and antilipolytic genes are overexpressed in the omental adipose tissue of women with PCOS compared with that in non-hyperandrogenic women, meaning that androgens may play an important role in adipose lipid accumulation [36]. Androgens have also been implicated in insulin-mediated glucose dysmetabolism in adipose tissue. Previous study observed insulin resistance in subcutaneous adipose tissue in PCOS women, due to elevated levels of Testosterone (T), which inhibits insulin-mediated glucose uptake via impairment of phosphorylation of protein kinase C zeta in obese women [35]. Obesity, particularly abdominal obesity, along with its accomplice insulin resistance, also aggravates hyperandrogenism [37]. Obesity mainly manifests as increased levels of free fatty acids (FFAs), cholesterol, triglycerides and various apolipoprotein abnormalities [38]. Increased FFAs decrease insulin sensitivity and reduce glucose uptake in intramyocellular lipids [39]. FFAs can also activate serine/threonine kinases and ultimately decrease the tyrosine phosphorylation of IRS-1. Those reactions can promote insulin resistance [40]. Abdominal obesity and insulin resistance cooperatively stimulate excess androgen synthesis in the ovaries as well as the adrenal glands [41], with subsequent increases in abdominal obesity and inflammation, thus creating a pathological cycle. In addition, adipocytes further produce leptin and adiponectin via paracrine and autocrine glands, in order to regulate androgen levels in circulation. Serum leptin is increased in some PCOS patients, and high leptin concentrations inhibit the expression of aromatase mRNA in GCs [39], thus preventing the conversion of androgens to oestrogens, leading to increased serum androgens levels and ultimately promoting follicular atresia. Adiponectin is secreted by adipose tissue and is one of the most important adipose factors. Adiponectin has been observed to improve insulin sensitivity, which in turn reduces FFA intake as well as gluconeogenesis. Shorakae and team reported high adiponectin levels, which were negatively correlated with the free-androgen index and

fasting insulin. These were observed to be lower in women with PCOS than in non-PCOS women [42] Thus, concluding the diverse impact of obesity on androgen levels via various pathways.

Most studies have focused on the lipid nephrotoxicity hypothesis based on Moorhead's work [43]. Scholars who buttress this hypothesis believe that inflammation could be attributed to hyperlipidemia. However, to date, the cellular and molecular mechanism associating hyperandrogenism with renal disease is limited. Lipid accumulation triggers renal dysfunction through excessive ROS production. One of the main sites for renal lipid accumulation is the renal proximal tubule cells. High levels of albumin-bound long-chain saturated fatty acids are known to promote the progression of renal tubular damage and interstitial fibrosis; excess of Ox-HDL induced pro-inflammatory pathways, including TNF-α and IL-6, and increased the production of ROS [44,45]. PCOS causing increased FFA leads to chronic inflammation and develop severe renal degeneration and glomerular damage, indicating that the aggravation of obesity may itself exacerbate existing kidney damage, perhaps due to the overexpression of TNFα, IL-6, and monocyte chemotactic protein-1 (MCP-1) during inflammation, which results in the thickening of the glomerular basement membrane, extracellular matrix, glomerulosclerosis [46]. In particular, lipid accumulation can initiate ER stress to enhance TNFα or IL-6 in HMC and HK2 cells, resulting in the increased production of ROS and direct toxic effects on the kidney [46]. Elevated levels of ROS markers will aggravate endothelial dysfunction and vascular disease in CKD and cause an increase in uremic toxins uric acid and creatinine [47]. As a result of oxidative stress levels being increased, it is naturally bound for lipid peroxidation level (MDA and GSH) to increase and lipid antioxidant level will decrease [48]. As a result of insulin resistance causing hyperglycaemia, lipid peroxidation increases. However, due to increase in insulin resistance, naturally lactate and lactate dehydrogenase are bound to be elevated which was pointed out in the result [49].

CONCLUSION:

The present study therefore indicates that letrozole-induced hyperandrogenism resulting in elevated levels of testosterone and insulin resistance causes renal dysfunction, which is accompanied by renal lipid peroxidation and inflammation.

Conflict of interests: The authors declare no conflict of interest

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REFERENCES:

1. Osibogun O, Ogunmoroti O, Michos ED. Polycystic ovary syndrome and cardiometabolic risk: Opportunities for cardiovascular disease prevention. Trends in cardiovascular medicine. 2020 Oct 1;30(7):399-404.
2. Baldani DP, Skrgatic L, Ougouag R. Polycystic ovary syndrome: important underrecognised cardiometabolic risk factor in reproductive-age women. International journal of endocrinology. 2015 Jun 1;2015.
3. Barbosa G, de Sá LB, Rocha DR, Arbex AK. Polycystic ovary syndrome (PCOS) and fertility. Open J of Endocrine and Metabolic Diseases.2016Jan12;6(1):58-65.
4. Askari H, Rajani SF, Poorebrahim M, Haghi-Aminjan H, Raeis-Abdollahi E, Abdollahi M. A glance at the therapeutic potential of irisin against diseases involving inflammation, oxidative stress, and apoptosis: an introductory review. Pharmacological research. 2018 Mar 1;129:44-55.
5. Spoto B, Pisano A, Zoccali C. Insulin resistance in chronic kidney disease: a systematic review. American Journal of Physiology-Renal Physiology. 2016 Dec 1;311(6):F1087-108.
6. Chen Q, Wang Q, Zhu J, Xiao Q, Zhang L. Reactive oxygen species: key regulators in vascular health and diseases. British journal of pharmacology. 2018 Apr;175(8):1279-92.
7. Fu S, Ping P, Wang F, Luo L. Synthesis, secretion, function, metabolism and application of natriuretic peptides in heart failure. Journal of biological engineering. 2018 Dec;12:1-21.
8. De Cosmo S, Menzaghi C, Prudente S, Trischitta V. Role of insulin resistance in kidney dysfunction: insights into the mechanism and epidemiological evidence. Nephrology Dialysis Transplantation. 2013 Jan 1;28(1):29-36.
9. Nigam SK, Bush KT. Uraemic syndrome of chronic kidney disease: altered remote sensing and signalling. Nature Reviews Nephrology. 2019 May;15(5):301-16.
10. Baldani DP, Skrgatic L, Ougouag R, Kasum M. The cardiometabolic effect of current management of polycystic ovary syndrome: strategies of prevention and treatment. Gynecological Endocrinology. 2018 Feb 1;34(2):87-91.
11. Abinaya S, Siva D, Sabitha R, Achiraman S. An overview of hyperandrogenism in PCOS and the prospective underlying factors. Res. J.Life Sci.Bioinform. Pharmac. Chem. Sci. 2019 Jan;1(5):179-86.
12. Paixão L, Ramos RB, Lavarda A, Morsh DM, Spritzer PM. Animal models of hyperandrogenism and ovarian morphology changes as features of polycystic ovary syndrome: a systematic review. Reproductive Biology and Endocrinology. 2017 Dec;15(1):1-1.
13. Zeng X, Xie YJ, Liu YT, Long SL, Mo ZC. Polycystic ovarian syndrome: correlation between hyperandrogenism, insulin resistance and obesity. Clinica chimica acta. 2020 Mar 1;502:214-21.
14. Ziaee A, Oveisi S, Ghorbani A, Hashemipour S, Mirenayat M. Association between metabolic syndrome and premicroalbuminuria among Iranian women with polycystic ovary syndrome: a case control study: met syn. and premicroalbuminuria in PCOS. Global journal of health science. 2013 Jan;5(1):187.
15. Gozashti MH, Gholamhosseinian A, Musavi F, Mashrouteh M. Relationship between serum cystatin C and polycystic ovary syndrome. Iranian Journal of Reproductive Medicine. 2013 Jan;11(1):71.
16. Olson PD, McLellan LK, Hreha TN, Liu A, Briden KE, Hruska KA, Hunstad DA. Androgen exposure potentiates formation of intratubular communities and renal abscesses by Escherichia coli. Kidney international. 2018 Sep 1;94(3):502-13.

17. Fedrigon D, Alazem K, Sivalingam S, Monga M, Calle J. Nephrolithiasis and polycystic ovary syndrome: a case-control study evaluating testosterone and urinary stone metabolic panels. Advances in Urology. 2019 Oct 17;2019.

18. Song Y, Ye W, Ye H, Xie T, Shen W, Zhou L. Serum testosterone acts as a prognostic indicator in polycystic ovary syndrome-associated kidney injury. Physiological Reports. 2019 Aug;7(16):e14219.

19. Peng Y, Fang Z, Liu M, Wang Z, Li L, Ming S, Lu C, Dong H, Zhang W, Wang Q, Shen R. Testosterone induces renal tubular epithelial cell death through the HIF-1α/BNIP3 pathway. Journal of Translational Medicine. 2019 Dec;17:1-3.

20. Sun WL, Zhu YP, Ni XS, Jing DD, Yao YT, Ding W, Liu ZH, Ding GX, Jiang JT. Potential involvement of Fgf10/Fgfr2 and androgen receptor (AR) in renal fibrosis in adult male rat offspring subjected to prenatal exposure to di-n-butyl phthalate (DBP). Toxicology letters. 2018 Jan 5;282:37-42.

21. Patil CN, Wallace K, LaMarca BD, Moulana M, Lopez-Ruiz A, Soljancic A, Juncos LA, Grande JP, Reckelhoff JF. Low-dose testosterone protects against renal ischemia-reperfusion injury by increasing renal IL-10-to-TNF-α ratio and attenuating T-cell infiltration. American Journal of Physiology-Renal Physiology. 2016 Aug 1;311(2):F395-403.

22. Bábíčková J, Borbélyová V, Tóthová LU, Kubišová K, Janega P, Hodosy J, Celec P. The renal effects of prenatal testosterone in rats. The Journal of Urology. 2015 May 1;193(5):1700-8.

23. Balen AH. Ovulation induction in the management of anovulatory polycystic ovary syndrome. Molecular and cellular endocrinology. 2013 Jul 5;373(1-2):77-82.

24. Kafali H, Iriadam M, Ozardalı I, Demir N. Letrozole-induced polycystic ovaries in the rat: a new model for cystic ovarian disease. Archives of medical research. 2004 Mar 1;35(2):103-8.

25. Adeyanju OA, Falodun TO, Michael OS, Soetan OA, Oyewole AL, Agbana RD. Spironolactone reversed hepato-ovarian triglyceride accumulation caused by letrozole-induced polycystic ovarian syndrome: tissue uric acid—a familiar foe. Naunyn-Schmiedeberg's Archives of Pharmacology. 2020 Jun;393:1055-66.

26. Olaniyi KS, Oniyide AA, Adeyanju OA, Ojulari LS, Omoaghe AO, Olaiya OE. Low dose spironolactone-mediated androgen-adiponectin modulation alleviates endocrine-metabolic disturbances in letrozole-induced PCOS. Toxicology and Applied Pharmacology. 2021 Jan 15;411:115381.

27. Hsing AW, Gao YT, Chua Jr S, Deng J, Stanczyk FZ. Insulin resistance and prostate cancer risk. Journal of the National Cancer Institute. 2003 Jan 1;95(1):67-71.

28. Olaniyi KS, Areloegbe SE. Acetate: A therapeutic candidate against renal disorder in a rat model of polycystic ovarian syndrome. The Journal of Steroid Biochemistry and Molecular Biology. 2023 Jan 1;225:106179.

29. Cooney LG, Lee I, Sammel MD, Dokras A. High prevalence of moderate and severe depressive and anxiety symptoms in polycystic ovary syndrome: a systematic review and meta-analysis. Human reproduction. 2017 May 1;32(5):1075-91.

30. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Human reproduction. 2018 Sep 1;33(9):1602-18.

31. Ma X, Hayes E, Prizant H, Srivastava RK, Hammes SR, Sen A. Leptin-induced CART (cocaine-and amphetamine-regulated transcript) is a novel intraovarian mediator of obesity-related infertility in females. Endocrinology.2016 Mar 1;157(3):1248-57.

32. Jena D, Choudhury AK, Mangaraj S, Singh M, Mohanty BK, Baliarsinha AK. Study of visceral and subcutaneous abdominal fat

thickness and its correlation with cardiometabolic risk factors and hormonal parameters in polycystic ovary syndrome. Indian journal of endocrinology and metabolism. 2018 May;22(3):321.

33. Lazúrová I, Lazúrová Z, Figurová J, Ujházi S, Dravecká I, Mašlanková J, Mareková M. Relationship between steroid hormones and metabolic profile in women with polycystic ovary syndrome. Physiological research. 2019 Jun 1;68(3).

34. Milutinović DV, Nikolić M, Veličković N, Djordjevic A, Bursać B, Nestorov J, Teofilović A, Antić IB, Macut JB, Zidane AS, Matić G. Enhanced inflammation without impairment of insulin signaling in the visceral adipose tissue of 5α-dihydrotestosterone-induced animal model of polycystic ovary syndrome. Experimental and Clinical Endocrinology & Diabetes. 2017 Sep;125(08):522-9.

35. Corbould A. Chronic testosterone treatment induces selective insulin resistance in subcutaneous adipocytes of women. Journal of Endocrinology. 2007 Mar 1;192(3):585-94.

36. Cortón M, Botella-Carretero JI, Benguria A, Villuendas G, Zaballos A, San Millán JL, Escobar-Morreale HF, Peral B. Differential gene expression profile in omental adipose tissue in women with polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism. 2007 Jan 1;92(1):328-37.

37. Sirotkin AV, Fabian D, Babeľová J, Vlčková R, Alwasel S, Harrath AH. Body fat affects mouse reproduction, ovarian hormone release, and response to follicular stimulating hormone. Reproductive Biology. 2018 Mar 1;18(1):5-11.

38. Torre-Villalvazo I, Bunt AE, Alemán G, Marquez-Mota CC, Diaz-Villaseñor A, Noriega LG, Estrada I, Figueroa-Juárez E, Tovar-Palacio C, Rodriguez-López LA, López-Romero P. Adiponectin synthesis and secretion by subcutaneous adipose tissue is impaired during obesity by endoplasmic reticulum stress. Journal of cellular biochemistry. 2018 Jul;119(7):5970-84.

39. Chow LS, Mashek DG, Wang Q, Shepherd SO, Goodpaster BH, Dubé JJ. Effect of acute physiological free fatty acid elevation in the context of hyperinsulinemia on fiber type-specific IMCL accumulation. Journal of Applied Physiology. 2017 Jul 1;123(1):71-8.

40. Pereira S, Park E, Moore J, Faubert B, Breen DM, Oprescu AI, Nahle A, Kwan D, Giacca A, Tsiani E. Resveratrol prevents insulin resistance caused by short-term elevation of free fatty acids in vivo. Applied Physiology, Nutrition, and Metabolism. 2015;40(11):1129-36.

41. Delitala AP, Capobianco G, Delitala G, Cherchi PL, Dessole S. Polycystic ovary syndrome, adipose tissue and metabolic syndrome. Archives of gynecology and obstetrics. 2017 Sep;296:405-19.

42. Shorakae S, Abell SK, Hiam DS, Lambert EA, Eikelis N, Jona E, Sari CI, Stepto NK, Lambert GW, de Courten B, Teede HJ. High-molecular-weight adiponectin is inversely associated with sympathetic activity in polycystic ovary syndrome. Fertility and Sterility. 2018 Mar 1;109(3):532-9.

43. Moorhead JF, El-Nahas M, Chan MK, Varghese Z. Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. The Lancet. 1982 Dec 11;320(8311):1309-11.

44. Kennedy DJ, Chen Y, Huang W, Viterna J, Liu J, Westfall K, Tian J, Bartlett DJ, Tang WW, Xie Z, Shapiro JI. CD36 and Na/K-ATPase-α1 form a proinflammatory signaling loop in kidney. Hypertension. 2013 Jan;61(1):216-24.

45. Gao X, Wu J, Qian Y, Fu L, Wu G, Xu C, Mei C. Oxidized high-density lipoprotein impairs the function of human renal proximal tubule epithelial cells through CD36. International Journal of Molecular Medicine. 2014 Aug 1;34(2):564-72.

46. Yang P, Xiao Y, Luo X, Zhao Y, Zhao L, Wang Y, Wu T, Wei L, Chen Y.

Inflammatory stress promotes the development of obesity-related chronic kidney disease via CD36 in mice. Journal of lipid research. 2017 Jul 1;58(7):1417-27.

47. Modaresi A, Nafar M, Sahraei Z. Oxidative stress in chronic kidney disease. Iranian journal of kidney diseases.2015 May 1;9(3).

48. Abdelkhalek NK, Ghazy EW, Abdel-Daim MM. Pharmacodynamic interaction of Spirulina platensis and deltamethrin in freshwater fish Nile tilapia, Oreochromis niloticus: impact on lipid peroxidation and oxidative stress. Environmental Science and Pollution Research. 2015 Feb;22:3023-31.

49. Adeva-Andany M, López-Ojén M, Funcasta-Calderón R, Ameneiros-Rodríguez E, Donapetry-García C, Vila-Altesor M, Rodríguez-Seijas J. Comprehensive review on lactate metabolism in human health. Mitochondrion. 2014 Jul 1;17:76-100.