BMC Research Notes

RESEARCH NOTE Open Access

Acetate attenuates hypothalamic pyroptosis in experimentally induced polycystic ovarian syndrome

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Abstract

This study hypothesized that SCFA, acetate impacts positively on hypothalamic pyroptosis and its related abnormalities in experimentally induced PCOS rat model, possibly through NrF2/HIF1-α modulation. Eight-week-old female Wister rats were divided into groups ($n=5$), namely control, PCOS, acetate and PCOS + acetate groups. Induction of PCOS was performed by administering 1 mg/kg body weight of letrozole for 21 days. After PCOS confirmation, the animals were treated with 200 mg/kg of acetate for 6 weeks. Rats with PCOS were characterized with insulin resistance, leptin resistance, increased plasma testosterone as well as degenerated ovarian follicles. There was also a significant increase in hypothalamic triglyceride level, triglyceride-glucose index, inflammatory biomarkers (SDF-1 and NF-kB) and caspase-6 as well as plasma LH and triglyceride. A decrease was observed in plasma adiponectin, GnRH, FSH, and hypothalamic GABA with severe inflammasome expression in PCOS rats. These were accompanied by decreased level of NrF2/HIF1-α, and the alterations were reversed when treated with acetate. Collectively, the present results suggest the therapeutic impact of acetate on hypothalamic pyroptosis and its related comorbidity in PCOS, a beneficial effect that is accompanied by modulation of NrF2/HIF1-α.

Keywords Acetate, GABA, Hypothalamus, Insulin resistance, Pyroptosis, PCOS

Introduction

Polycystic ovarian syndrome (PCOS) is an endocrine/metabolic disorder that affects about 10% of women and girls within reproductive years [1]. While the exact origin of PCOS remains unclear, a combination of genetic and environmental factors is believed to contribute to the development and progression of the disease [2]. Polycystic ovarian syndrome is notably associated with a complex of neuroendocrine changes that surface as hormonal imbalance, abnormal ovarian growths, anovulation, poor eating habits and weight gain [3]. Besides, PCOS has been clinically linked with the risk of mood disorders, obesity, diabetes mellitus, cardiovascular diseases (CVDs) and neuropsychiatric illnesses in the long run [4].

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The pathophysiology of PCOS is centered on a defective hypothalamo-pituitary-gonadal axis, in which neuroendocrine signals that regulate folliculogenesis are disrupted, leading to ovarian cysts formation and excessive androgen production [5]. Furthermore, increased LH secretion from intraovarian steroidogenesis anomalies and suppressed aromatase activity keep ovarian and circulating testosterone level higher in women with PCOS than in healthy women [3, 6, 7]. Polycystic ovarian syndrome-induced hyperandrogenemia alters body hair growth, hormonal regulation of nutrients signalling and fat distribution. Hence, metabolic disorders, particularly insulin resistance (IR) and dyslipidemia are intimately diagnosed with PCOS reproductive anomalies [8]. Insulin resistance mediates several adverse systemic conditions, including hyperinsulinemia, hyperlipidemia, protein glycation and atherogenesis, causing microvascular dysfunction, chronic inflammation and cellular death in vital tissues [9, 10]. However, sufficient understanding of the mechanisms responsible for progression of IR to these comorbid features in PCOS is still lacking.

Extensive research has demonstrated metabolic disorder-related organopathies in experimental PCOS [11, 12], by suppressing ovarian aromatase activity through letrozole (LET), this prevents the conversion of testosterone to estradiol, inducing thickening of theca interna cell layer, androgen secretion, anovulation and abdominal adiposity [11, 13]. Apart from its significant role in the neuromodulation of ovarian endocrine activities, the hypothalamus is critical in nutrient signalling and metabolism [14]. The blood-brain-barrier is formed by an endothelial microvasculature that may be negatively regulated through hypothalamic uptake and accumulation of atherogenic lipid species. We recently reported that hyperinsulinemia engenders metaflammation in the hypothalamus of PCOS animals [15]. Such deleterious implications could impair synaptic plasticity of hypothalamic neuronal circuits and cause nutrient/energy dysregulation.

The inflammasome complex is critical in the maintenance of immune response to internal and external pathogenic signals. Pyroptosis is a special cellular pro-inflammatory programming tasked to eliminate pathogens through NOD-like receptors (NLRs) activities [16, 17]. Moderate pyroptosis plays a protective role in the early stage of a harmful challenge, however, when cellular environment is overwhelmed with excessive pathogenic signals like excessive nutrient and macrophagic infiltration, the pyroptotic cascade could overreact by initiating vicious cellular damage. Morphologically, pyroptosis is distinguished from apoptosis with the presence of osmotic bulging of cell membrane, cytolysis and consequent release of cytosolic content into the extracellular space [18]. It has been proposed that the pathological mechanism underlying chronic inflammasome activation in IR involves the pyroptotic pathway [16, 17]. Hence, it would be important to clarify the role of pyroptotic cell damage in the hypothalamic metabolic dysregulation in PCOS.

Currently, there is no established cure for PCOS. However, various pharmacotherapy approaches to suppress related reproductive and metabolic symptoms have proven effective, but not without several concerns [19]. Short-chain fatty acids (SCFAs) are gaining attention as nutraceutical to aid recovery from PCOS-driven systemic neuroendocrine and metabolic problems [20]. They consist mainly of acetate, propionate and butyrate among others are synthesized in the gut microbiome [20, 21]. Gut microbiota dysbiosis, including short-chain fatty acids (SCFAs) deficiency play important role in the pathogenesis of several disorders, including inflammatory bowel diseases, colorectal cancer, and cardio-metabolic disorders [22, 23]. It has recently been of interest to fully elucidate the exact molecular mechanisms underlying the therapeutic potential of SCFAs in IR-driven chronic metabolic inflammation, seeing that supplementation of SCFAs is positively associated with weight loss, anti-inflammatory action, protein acetylation and apoptosis of cancer cells [23, 24]. Moreover, recent observations suggest that SCFAs may reverse cellular inflammatory damage in the hypothalamus of PCOS rats [15, 25]. This study hypothesized that SCFA, acetate would reverse hypothalamic pyroptosis-driven metaflammation, and further sought to probe the involvement of NrF2/HIF1-α in PCOS rat model.

Materials and methods

Experimental animals and grouping

Eight-week-old female Wistar rats, weighing between 130 and 150 g were used for the study. The animals were supplied from the central animal house of Afe Babalola University, Ado-Ekiti, Nigeria, and had unlimited access to standard rat chow and tap water. The animals were acclimatized for one week, and thereafter, through randomization, the rats were divided into four groups of $n=5$, control (CTL), sodium acetate (SAT), LET and LET + SAT groups, and maintained in standard environmental conditions of temperature (22-26^0 C), relative humidity (50–60%), and 12-hour dark/light cycle.

Induction and confirmation of PCOS

Polycystic ovarian syndrome (PCOS) was induced by administering 1 mg/kg of letrozole (oral gavage; Sigma-Aldrich, St Louis, MI) for 21 days and confirmed using Rotterdam criteria as previously documented [24–26].

Treatment

Distilled water (oral gavage) was given as a vehicle to the control and LET groups while SAT and LET + SAT groups received sodium acetate (200 mg/kg; Sigma-Aldrich, St Louis, MI) via oral gavage for six weeks [25, 27]. Initial and final body weights were determined and percentage body weight gain was estimated.

Sample collection

Following the completion of the treatments, the rats were subjected to overnight fasting, and sacrificed with anesthetic agent, 50 mg/kg body weight of sodium pentobarbital (*ip*), which was given as a chemical method of euthanasia as earlier documented [25, 27]. The blood sample was collected into heparinized tube via cardiac puncture and centrifuged at 704 g for 5 min at room temperature. Plasma was later stored at -80 °C prior to the period of biochemical assays. The brain of each rat was carefully isolated and weighed. After which, 100 mg section of tissue (hypothalamus) was carefully removed, minced and homogenized in 1 ml of 0.25 M sucrose / 0.2mM EDTA adjusted to pH 7.5 with Tris buffer and centrifuged at 4 °C for 12 min at 750 g. The supernatant was decanted and stored at -80 °C prior to the biochemical assays.

Biochemical analysis

The blood glucose levels were determined with a handheld glucometer (ONETOUCH-LifeScan, Inc., Milpitas, CA, USA). Plasma insulin, testosterone, LH, follicle stimulating hormone (FSH), leptin, adiponectin and gonadotropin releasing hormone (GnRH) were determined using Rat ELISA kit purchased from Calbiotech Inc. (Cordell Ct., El Cajon, CA 92020, USA). In addition, quantitative check of insulin sensitivity (QUICKI) was determined as a measure of insulin sensitivity [27]. The concentration of triglyceride (TG) was determined in the plasma and hypothalamic tissue using standard colorimetric methods with kits obtained from Fortress Diagnostics Ltd. (Antrim, UK). Thereafter, triglyceride-glucose index (TyG) was estimated as a surrogate marker of insulin resistance [28]. The levels of nuclear factor-kappaB (NF-kB), tumour necrosis factor-α (TNFα), stromal cell derived factor-1 (SDF-1), hypoxia inducible factor-1α and nuclear factor erythroid 2-related factor 2 (NrF2) were determined in the plasma and hypothalamus using rat ELISA kits from Elabscience Biotechnology Inc. (Wuhan, Hubei, P.R.C., China). Concentration of caspase-6 and Gamma-amino butyric acid (GABA) were determined in the hypothalamic tissue using rat ELISA kits obtained from ELK Biotechnology Co. Ltd. (1312 17th Street #692 Denver, CO 80202 USA).

Immunohistochemical assessment of hypothalamic tissue using inflammasome

Immunohistochemistry of hypothalamic tissue was performed to detect the expression of inflammasome antigens using NLRP3 polyclonal antibody from Elabscience Biotechnology Inc. (Wuhan, Hubei, P.R.C., China; E-AB-65952; 1:50 – 1:200), and the tissue was processed and evaluated as previously described [24, 25]. The expression of inflammasome in the neurons of the arcuate nucleus of the hypothalamus was quantified by processing with an image-processing and analysis software Image-J (Version 1.52).

Data analysis

The distribution of the data was confirmed using Shapiro-Wilk test and the data were normally distributed. Statistical group analysis was performed with GraphPad Prism software version 9, and data were expressed as means ± SD. Means were compared with one-way ANOVA and *Post hoc* analysis was done with Bonferroni's test. Statistically significant difference was considered at $p < 0.05$.

Results

Acetate attenuates excess body weight and improves metabolic indices in experimentally induced PCOS rat model

There was a significant increase ($p < 0.05$) in body weight gain and plasma insulin concentration, no change in plasma glucose and a decreased insulin sensitivity (QUICKI) in the LET-induced PCOS rat models compared to the CTL group. However, upon SAT administration, there was a significant decrease ($p < 0.05$) in body weight gain and plasma insulin concentration with an increased insulin sensitivity (QUICKI) in treated LET-induced PCOS animals (Table 1).

Table 1 Effects of acetate on body weight and metabolic indices in experimentally induced PCOS rat model

GROUPS	CTL	SAT	LET	LET + SAT
Initial body weight (g)	133.20 ± 4.50	135.80 ± 4.35	144.80 ± 2.17	133.80 ± 3.97
Body weight gain (%)	27.75 ± 0.93	26.15 ± 1.49	40.45 ± 0.07*	28.99 ± 1.25$^{\#}$
Fasting glucose (mmol/L)	3.40 ± 0.30	3.53 ± 0.32	3.87 ± 0.52	3.72 ± 0.38
Fasting insulin (μlU/mL)	1.45 ± 0.02	1.44 ± 0.11	3.70 ± 0.48*	1.85 ± 0.11$^{\#}$
QUICKI	1.44 ± 0.05	1.43 ± 0.03	0.87 ± 0.04*	1.19 ± 0.02$^{\#}$

Data are expressed as mean ± SD. $n = 5$. (*$p < 0.05$ vs. CTL; $^{\#}p < 0.05$ vs. LET)

Acetate normalizes endocrine profiles in experimentally induced PCOS rat model

There was a significant decrease ($p<0.05$) in the GnRH and FSH levels in LET-induced PCOS animals. Likewise, there was an increase in the LH and TT levels of LET-induced PCOS animals compared to that of the CTL group. However, upon administration of SAT, there was a significant increase ($p<0.05$) in the GnRH and FSH levels and a decrease in LH and TT levels of treated LET-induced PCOS animals (Fig. 1).

Acetate normalizes metabolic hormones in experimentally induced PCOS rat model

There was a significant increase ($p<0.05$) in the leptin levels and a decrease in the adiponectin level of LET-induced PCOS animals compared to that of CTL group. However, upon SAT administration, there was a significant decrease ($p<0.05$) in the leptin and increase of the adiponectin level in the LET-induced PCOS animals (Fig. 2).

Acetate decreases lipid indices in experimentally induced PCOS rat model

There was a significant increase ($p<0.05$) in the plasma TG, and hypothalamic TG of LET-induced PCOS animals compared to that of the CTL group. However, upon SAT administration, there was a significant decrease ($p<0.05$) in the plasma TG and hypothalamic TG of LET-induced PCOS animals. In addition, there was no significant change in plasma TyG but a significant increase was observed in hypothalamic TyG of LET-induced PCOS animals compared to the CTL group. However, administration of SAT significantly decreased ($p<0.05$) the hypothalamic TyG in LET-induced PCOS animals (Table 2).

Acetate decreases the protein levels of inflammatory biomarkers in experimentally induced PCOS rat model

There was a significant increase ($p<0.05$) in the NF-kB, TNF-α and SDF-1 and a decrease in the HIF-1α protein level in LET-induced PCOS animals compared to the CTL group. However, upon SAT administration, there was a significant decrease ($p<0.05$) in the NF-kB, TNF-α and SDF-1 and an increase in the HIF-1α protein level of LET-induced PCOS animals (Fig. 3).

Acetate decreases anti-oxidant regulatory and apoptotic markers in experimentally induced PCOS rat model

There was a significant decrease ($p<0.05$) in NrF2 and increase in the Caspase-6 levels of LET-induced PCOS animals compared to the CTL group. However, following SAT administration, there was a significant increase ($p<0.05$) in the NrF2 and a decrease in the Caspase-6 levels of LET-induced PCOS animals (Fig. 4).

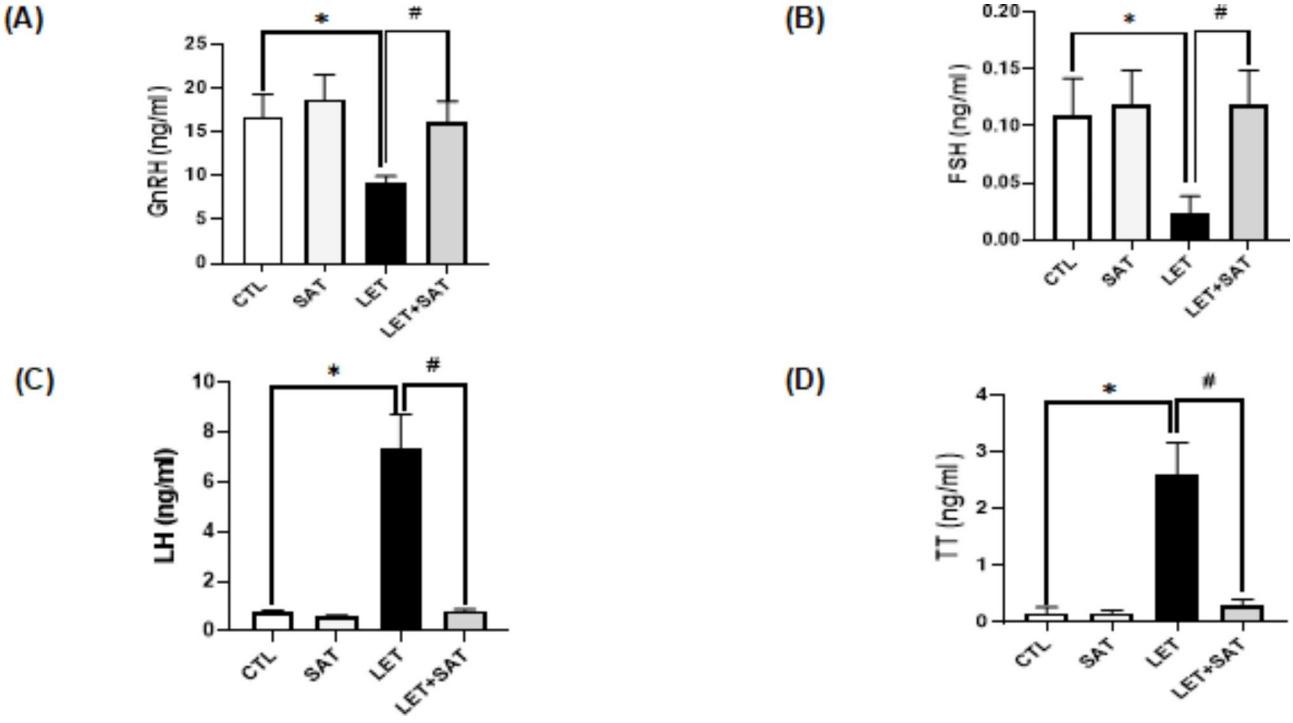

Fig. 1 Impact of acetate on GnRH (**a**), FSH (**b**), LH (**c**) and TT (**d**) in experimentally induced PCOS rat model. Data are expressed as mean ± SD. $n=5$. ($^{*}p<0.05$ vs. CTL; $^{\#}p<0.05$ vs. LET)

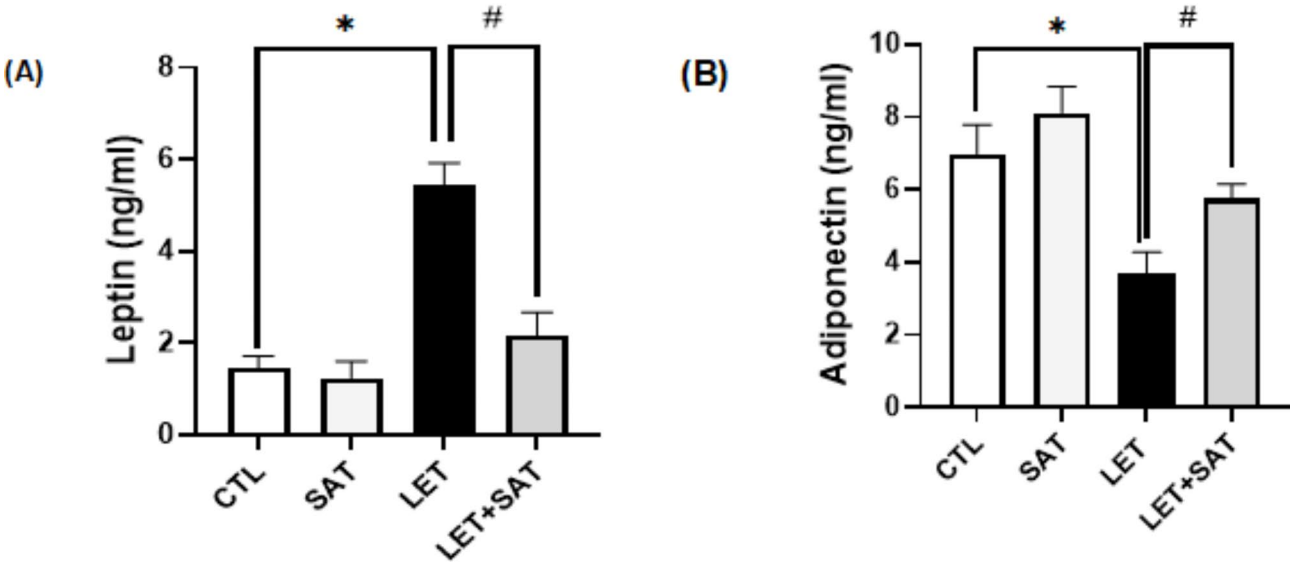

Fig. 2 Impact of acetate on leptin (**a**) and adiponectin (**b**) in experimentally induced PCOS rat model. Data are expressed as mean ± SD. $n = 5$. (*$p < 0.05$ vs. CTL; #$p < 0.05$ vs. LET)

Table 2 Effects of acetate on lipid indices in experimentally induced PCOS rat model

GROUPS	CTL	SAT	LET	LET + SAT
Plasma				
TG (mg/dL)	58.30 ± 3.44	56.81 ± 2.23	90.74 ± 2.53*	60.21 ± 1.20#
TyG	5.21 ± 0.10	5.40 ± 0.09	5.88 ± 0.08	5.44 ± 0.06
Hypothalamus				
TG (mg/dL)	0.78 ± 0.05	0.70 ± 0.07	2.11 ± 0.11*	0.89 ± 0.02#
TyG	0.13 ± 0.06	0.13 ± 0.07	2.00 ± 0.07*	0.49 ± 0.07#

Data are expressed as mean ± SD. $n = 5$. (*$p < 0.05$ vs. CTL; #$p < 0.05$ vs. LET)

Acetate decreases hypothalamic neurotransmitter in experimentally induced PCOS rat model

There was a significant decrease ($p < 0.05$) in GABA level of LET-induced PCOS animals compared to the CTL group. However, administration of SAT significantly increased ($p < 0.05$) GABA level of LET-induced PCOS animals (Fig. 5).

Acetate attenuates hypothalamic inflammation in experimentally induced PCOS rat model

Immunohistochemical analysis of hypothalamic tissue showed the significant expression of inflammasome ($p < 0.05$) in the neurons of the arcuate nucleus in LET-induced PCOS animals compared to the control group, which was significantly reduced ($p < 0.05$) in LET + SAT group compared with LET-induced PCOS animals (Fig. 6).

Discussion

Disrupted insulin signalling is a common dysmetabolism phenomenon in PCOS women. Insulin resistance is driven by the impaired capacity of the white adipose tissue to store excess energy as fat coupled with defective insulin receptor signalling [29]. Moreover, the endocrine function of the adipose tissue is compromised in IR [29]. Despite no change in plasma glucose level in PCOS animal model in this study, we observed elevated body weight gain and significant decreases in classical estimators of insulin sensitivity (QUICKI and TyG) and plasma adiponectin level. On the other hand, plasma insulin and leptin levels were elevated in PCOS group compared with control. This corroborates findings showing elevated insulinemia and leptinemia as twin indicators of systemic metabolic defects in PCOS [1, 30]. The decreased plasma adiponectin levels in this PCOS model plausibly suggest the presence of adipocyte endocrine dysfunction, while

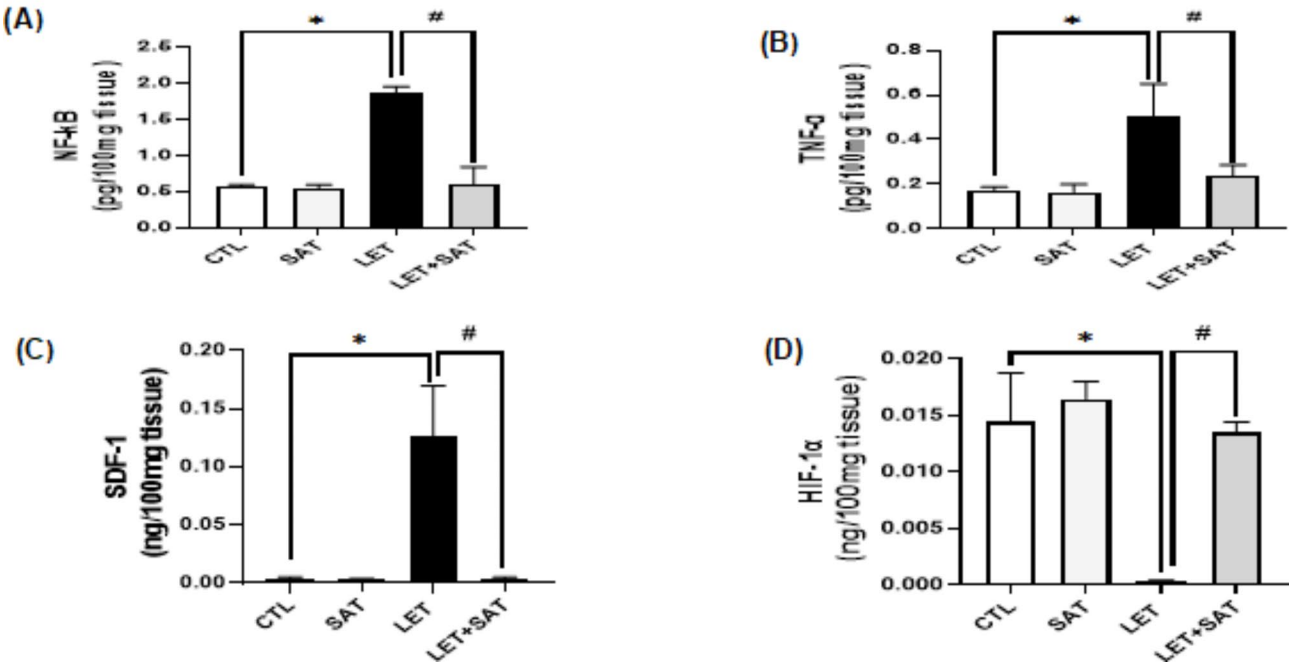

Fig. 3 Impact of acetate on hypothalamic NF-kB (**a**), TNF-α (**b**), SDF-1 (**c**) and HIF- 1α (**d**) in experimentally induced PCOS rat model. Data are expressed as mean ± SD. $n = 5$. (*$p < 0.05$ vs. CTL; $^{\#}p < 0.05$ vs. LET)

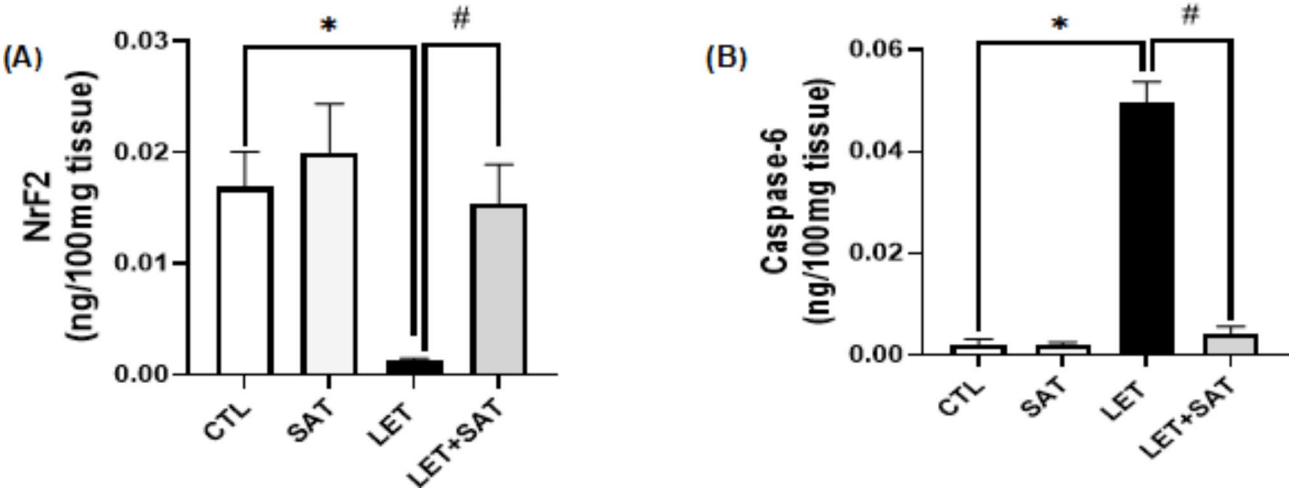

Fig. 4 Impact of acetate on NrF2 (**a**) and Caspase-6 (**b**) in experimentally induced PCOS rat model. Data are expressed as mean ± SD. $n = 5$. (*$p < 0.05$ vs. CTL; $^{\#}p < 0.05$ vs. LET)

elevated plasma leptin is sequel to hypothalamic leptin resistance [31, 32]. Compared with untreated PCOS rats, we observed that acetate administration decreased circulating LDL and TG levels, and hypothalamic triglyceride contents were attenuated, confirming the antilipidemic effect of acetate [15].

Polycystic ovarian syndrome (PCOS) patients are known to have malfunctioning

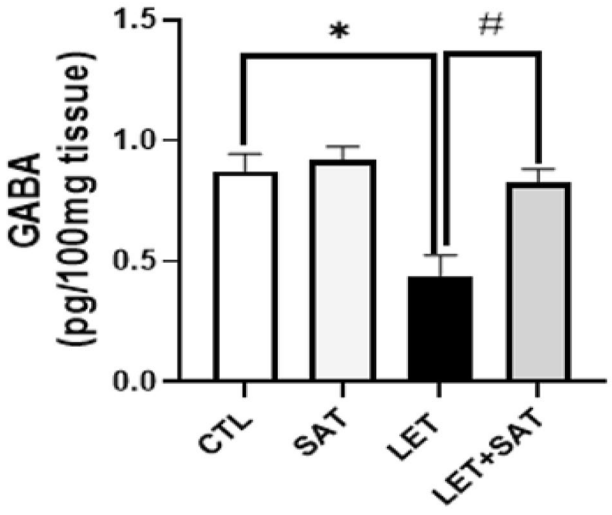

Fig. 5 Impact of acetate on GABA in experimentally induced PCOS rat model. Data are expressed as mean ± SD. $n = 5$. (*$p < 0.05$ vs. CTL; #$p < 0.05$ vs. LET)

hypothalamic-pituitary-ovarian axis characterized by persistent high levels of LH and androgens, including testosterone [5]. Hyperinsulinemia is linked to aberrant steroid and gonadotropin production [33]. As well, in PCOS, aromatase activity is suppressed, limiting the conversion of testosterone to estradiol, thereby increasing ovarian and circulating androgen levels [13, 34]. In this study, we observed a decrease in GnRH, and FSH levels, and a simultaneous increase in the LH and testosterone levels in Letrozole-treated animals, indicating an altered hypothalamic-pituitary-ovarian axis. Consistent with these findings perturbed repro-endocrine profile was seen in Letrozole [11, 25] and testosterone [35] induced PCOS animals. Our findings in acetate-treated rats in this study show reduced serum LH levels, and testosterone, whereas levels GnRH and FSH were elevated. These findings may emphasize the efficacy of acetate in alleviating letrozole-induced repro-hormonal disorder particularly, hyperandrogenism, which are in consonance with earlier studies [27].

Furthermore, insulin resistance is positively correlated with the development of atherogenic dyslipidemia. It is reported that women with PCOS exhibit elevated circulating low-density lipoprotein (LDL) and depleted high-density lipoprotein (HDL) [36]. Elevated circulating lipids species is primarily caused by adipocytes hypertrophy and overflow of non-esterified fatty acids into the blood. Nonetheless, excess pro-atherogenic lipoprotein could result from increased hepatic lipoprotein export [37]. Both scenarios have been shown to result in errant deposition of toxic lipids species in surrounding and distant non-adipose tissues. A significant increase in plasma triglyceride level was observed in PCOS model in the present study, which confirms IR-mediated dyslipidemia in letrozole-treated rats. In line with the likelihood of excessive lipid deposition in delicate tissues, we found hypothalamic triglyceride content to be markedly increased

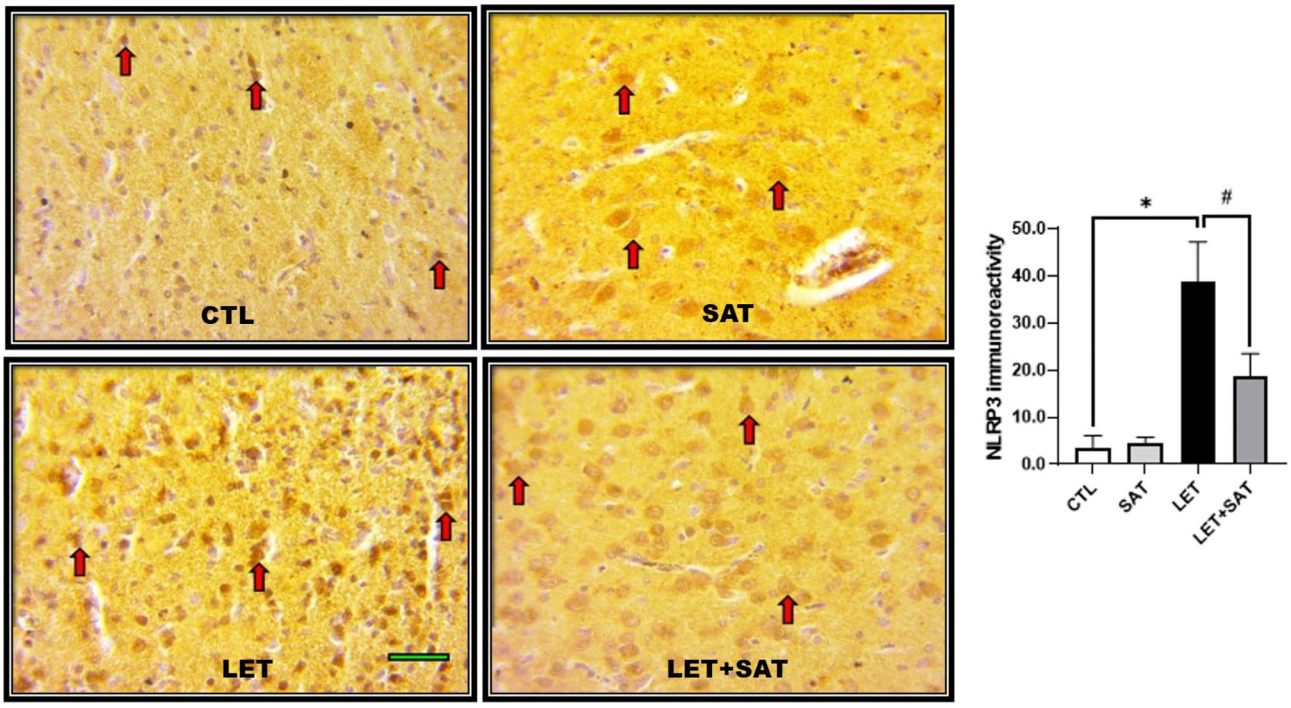

Fig. 6 Photomicrographs of hypothalamic tissue showing the response of the neurons in the arcuate nucleus to NLRP3 antibody in CTL (Control), SAT (Sodium acetate), LET (Letrozole) and LET + SAT groups. Scale bar, 51 µm. Data are expressed as mean ± SD. $n = 5$. (*$p < 0.05$ vs. CTL; #$p < 0.05$ vs. LET)

in PCOS rats compared with controls. Accumulation of excess lipid in the hypothalamus may negatively regulate local energy usage in this central nervous system area. Similarly, the central role of the arcuate nucleus neurons in whole body energy homeostasis could be impaired via decreased sensitivity of leptin receptors [38]. As such, impaired hypothalamic leptin signaling is reflected in the present model by elevated plasma leptin. Interestingly, acetate administration decreases plasma LDL, TG, and hypothalamic triglyceride in PCOS animals in line with previous reports [25, 27].

The pathology of hypothalamic neuro-cellular damage in this PCOS-associated metabolic phenotype relies on triglyceride-driven lipotoxicity and activation of inflammatory pathways [15, 25]. In congruence, we observed an increased levels of inflammation-associated cytokines and chemokine including NF-kB, TNF-α and SDF-1 in the hypothalamus of PCOS model in this study. In support of this chemokine profile, hypothalamus immunohistochemistry reveals higher expression of inflammasome in PCOS model compared with control group. Low-grade inflammatory response to perturbed lipid handling in non-adipose tissues is a vital signal in the activation of the pyroptosis cascade [17]. Although much is yet to be understood in terms of the possibly involved mechanism in PCOS-linked pyroptotic damage, oxidative stress, defined as increased generation of reactive oxygen species (ROS) with simultaneous depletion of resident antioxidant defense could profligately activate NLRP3-linked inflammasome, leading to the activation of the initiator caspase, pro-caspase-1 [39, 40]. Pro-caspase-1 notably mediates cleavage of preformed pro-inflammatory molecules especially pro-IL-1β and pro-IL-18 [39]. In the present study, NrF2 level in the hypothalamus was significantly reduced compared with control. An alteration in NrF2 signaling may downregulate antioxidants gene expression and possibly promote hypothalamic pyroptotic damage in this PCOS model. Previous studies have reported that initiator caspases subsequently process effector caspases to execute cell death. Caspase-6 plays a crucial role in executing pyroptotic cell death and inflammasome activation by regulating gasdermin D processing [40–42]. We found that marked chronic inflammation in the hypothalamus of the PCOS model in this study coexists with an increased level of caspase-6.

Considering that the hypothalamus is critical in nutrient sensing/signalling and whole-body metabolism, we thought that pyroptosis-driven chronic inflammation could mediate damage to microvascular function contributing to hampered intramural lipid status. Hypothalamic HIF-1α would majorly regulate blood-brain-barrier function such as nutrient transport and angiogenesis [43]. Hypoxia inducible factor-1 alpha (HIF-1α) level in the hypothalamus was found to be significantly decreased. In line with this observation, previous work by Varela and colleagues [14] revealed that HIF-1α deficiency promotes energy dysregulation that is underlined by defective synaptic patterns and transendothelial nutrient mishandling in the hypothalamus. Similarly, other investigators have linked the activation of HIF-1α to the prevention of gut inflammation [44] by reducing inflammatory gene expression through the inhibition of NLRP3 and its depletion has been demonstrated to exacerbate inflammation [45], in consonance with the present results, hypothalamic inflammation, which at least in part drives pyroptosis might possibly be mediated by HIF-1α deficiency. Interestingly, treatment of PCOS rats with acetate significantly reduced hypothalamic caspase-6 with concomitant reduction in NF-kB, TNF-α, SDF-1 levels. Uniquely, the level of NrF2 was markedly refurnished, suggestive of impact of acetate on antioxidant gene expression towards scavenging of pyroptotic and pro-inflammatory products in the hypothalamus of PCOS rats.

To determine whether HIF-1α deficiency in our PCOS model affected hypothalamic neurocircuitry, we assessed the level of key inhibitory neurotransmitter, GABA in the hypothalamus. Gamma aminobutyric acid (GABA)-ergic neurotransmission is necessary in tempering the excitability of neurons in the CNS by balancing neural circuit excitation with inhibition, and GABAergic dysfunction has been observed in several clinical and experimental cases of neurological and neurodevelopmental disorders [46, 47]. Without any surprise, hypothalamic GABA level in this study was markedly reduced, proving the deleterious implications of neuro-cellular and neurovascular damage on synaptic plasticity and neuromodulation. Besides, research has demonstrated that GABAergic neurons play a critical role as mediators of steroid feedback, controlling the activity of GnRH neurons during the reproductive cycle, suggesting one potential explanation for the hyperactivity of these brain cells in a hyperandrogenic state like PCOS and is supported by findings in previous studies [15, 48]. Data in the treatment group show significant increased hypothalamic GABA levels with acetate. Similar effect was previously observed in sodium butyrate, another SCFA in restoring hypothalamic GABA level in PCOS model [15]. Altogether, the present findings imply that SCFA, acetate ameliorates hypothalamic pyroptosis in PCOS model, and this is accompanied by enhanced HIF-1α and antioxidant defense (NrF2). Nevertheless, the present study is not without a caveat in such that the cause-effect relationships between HIF-1α and other biomarkers were not investigated. Nonetheless, the present data provide a justification for future study of molecular mechanisms underlying the beneficial effect of acetate on hypothalamic pyroptosis in PCOS models.

Conclusion

The data presented in this study suggests that acetate ameliorates hypothalamic lipotoxicity-driven pyroptotic damage in letrozole-induced PCOS model through modulation of NrF2/HIF1-α. The results in addition provide preclinical evidence for the beneficial effect of acetate against hypothalamic disruption in PCOS model.

Abbreviations

ANOVA	Analysis of variance
GABA	Gamma-amino butyric acid
ELISA	Enzyme-linked immunosorbent assay
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
HDAC	Histone deacetylase
HIF	Hypoxia-inducible factor-1
IR	Insulin resistance
LET	Letrozole
LH	Luteinizing hormone
NF-κ B	Nuclear factor-kappaB
NrF2	Nuclear factor erythroid 2-related factor 2
NLRP3	NOD-like receptors protein 3
PCOS	Polycystic ovarian syndrome
QUICKI	Quantitative check of insulin sensitivity
SAT	Sodium acetate
SCFA	short chain fatty acid
SD	Standard deviation
SDF-1	Stromal cell derived factor-1
TG	Triglyceride
TyG	Triglyceride-glucose index
TNF-α	Tumor necrosis factor-α

Author contributions

KSO conceived and designed the experiment. KSO, SUA and SEA conducted the experiment and analyzed the results. KSO, SEA, AAO, LAE, AOO, AOA and OAA contributed reagents for the study. KSO and IWS drafted the manuscript, while all the authors reviewed, revised and approved the manuscript for submission.

Funding

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

Data availability

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

Declarations

Ethics approval and consent to participate

The protocol was approved by the independent Institutional Ethical Review Board of Afe Babalola University, Nigeria with the approval number ABUADERC/10 C/2022 and the study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Consent for publication

Not applicable.

Consent to participate

is not applicable.

Competing interests

The authors declare no competing interests.

Received: 14 February 2024 / Accepted: 28 August 2024
Published online: 12 September 2024

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