Acetate meliorates depressive-like behaviour in a rat model of PCOS through suppression of HDAC2 expression and DNA methylation

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Abstract

Objective

Polycystic Ovarian Syndrome (PCOS) is the most common endocrine disorder among women of reproductive age. PCOS has been demonstrated to induce depressive-like behaviour. Epigenetic alterations such as histone deacetylation (HDAC) and DNA methylation have been suggested in major depression. However, their effects with respect to neuroinflammation are not clear. This study therefore investigated the pathogenic role of epigenetic modification in PCOS-associated depression and the therapeutic potential of HDACi, acetate.

Methods

Adult female Wistar rats (120-150 g) were allotted into groups (n = 6/group) namely: control (vehicle; p.o.), acetate-treated (200 mg/kg), letrozole (LET)-treated (1 mg/kg) and LET+Acetate-treated. Letrozole was administered for 21 days to induce PCOS.

Results

Treatment with letrozole caused hyperandrogenism, hyperinsulinemia and disrupted ovarian morphology with evidence of degenerated follicles. In addition, these animals showed depressive-like behaviours and increased expression of HDAC2 and DNA methyltransferase in PFC and hippocampal tissues. Biochemical analyses showed elevated NF-κB and acetylcholine (ACH) levels in PFC and hippocampus as well as plasma lipid peroxidation and impaired antioxidant system in LET-treated animals. Histological analysis of PFC and hippocampus showed neurodegeneration in LET-treated animals compared with control. However, these alterations were attenuated when treated with acetate.

Conclusion

The study demonstrates that PCOS-associated depression is characterised by neuroinflammation and elevated ACH levels, and this is associated with increased expression of HDAC2 and DNA hypermethylaction in PFC and hippocampus. Besides, the study suggests that acetate ameliorates PCOS-associated depression through the suppression of prefrontal and hippocampal DNA methyltransferase and prefrontal but not hippocampal HDAC2 expression.

Introduction

Polycystic ovarian syndrome (PCOS) has become the most prevalent gynecological disorder among women of child-bearing age in the recent years (Gnanadass et al., 2021). Global reports indicate that between 6–20% of women are affected by PCOS (Escobar-Morreale, 2018). Moreover, the disease etiology is heterogeneous and complex, as it is influenced by the interaction of several susceptibility factors including environmental, lifestyle and genomic determinants (Anjmal et al., 2019; Zhang et al., 2020). The clinical/biochemical manifestations of PCOS are defined by menstrual irregularities,
hyperandrogenism, metabolic disturbances and infertility (Witchel et al., 2019). PCOS' reproductive and metabolic co-morbidities have been linked with psychophysiological and behavioral derangements that are highlighted by anxiety and depression, with estimated prevalence of 34 to 57%, and 28 to 64% respectively (Dokras et al., 2012). Mainly, studies have attributed these adverse mental outcomes to hirsutism, androgenic alopecia, infertility and overweight/obesity (Bishop et al., 2009; Kirimizi et al., 2020), which indeed could engender psychological distress and reduce overall quality of life of patients, however, the exact mechanism(s) underlying mood disorders in the PCOS subpopulation remains to be clearly understood.

The hippocampal formation coordinates nerve regeneration and encodes memory experiences as well as interacts via neural connectivity with key brain regions including the amygdala, cingulate cortex and prefrontal cortex (PFC) to modulate cognitive functions, mood and behavior (MacQueen and Frodl, 2011). Structural and functional alterations in the hippocampus and PFC could lead to cognitive deficits and depression (MacQueen and Frodl, 2011; Sun et al. 2013). Deregulated autonomic stress response and/or impaired neurotransmitter signaling underlie mood abnormalities like depression. Studies show that stress and several related disorders may affect neurotransmission components including nerve fiber tone, neurotransmitter level and synaptic/post-synaptic activities (Mineur et al. 2013; Hamon et al. 2013). The monoamine neurotransmitter, dopamine regulates motivation and reward-related movements, and also serves as a precursor in the biosynthesis of norepinephrine and epinephrine, which are other neuromodulatory monoamines important in the maintenance of mood balance (Belujon and Grace, 2017). Studies in experimental PCOS models have shown that dopamine deficiency with depression (Yu et al. 2016; Chaudhari and Nampoothiri, 2017). Similarly, cholinergic pathway alterations are linked with mood and emotional disturbances, with reports indicating a causal role for increased central acetylcholine in the development of depression (Mineur et al. 2013). With the increasing global prevalence of PCOS, elucidation of PFC-hippocampal function in relation to depression in PCOS is critical, as this could necessitate the exploration of this axis for treatment of PCOS-linked psychological disorders.

Furthermore, metabolic dysregulations, such as oxidative stress and low-grade inflammation could increase the risk of neural injury, causing impaired neurotransmitter signaling and neurocognitive dysfunctions (Chan et al. 2019). Neuroinflammation hallmarks the complex relationship between metabolic disorder-induced immune activation and neurological damage that might evolve in PCOS (Kirimizi et al. 2020). The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) family of transcription factors plays critical role in cellular immune homeostasis through mediation of inflammatory response (Zhou et al. 2020). NF-κB activity is regulated by its interaction with cytoplasmic IkB proteins which in turn determines its nuclear accessibility. Stress and dysmetabolism-associated metabolites like free radicals and/or reactive oxygen species (ROS) could facilitate IkB kinase (IKK) phosphorylation of IkB, resulting in its disintegration and nuclear translocation and subsequent DNA binding (Liu et al. 2017). In the CNS, NF-κB plays significant immuno-regulatory role that includes resolution of nerve cell injury and neurogenesis (Koo et al. 2010). Moreover, transcriptional regulation by NF-κB triggers expression of immune response genes and results in immoderate liberation of chemokines
and pro-inflammatory cytokines which might modulate neuronal activity and survival (Liu et al., 2017). Elevated circulating and tissue NF-κB is a mediator and/or aggravator of insulin resistance in PCOS (González et al. 2006). Plausibly, PCOS-associated CNS inflammation could precipitate neurodegeneration to cause cognitive loss and emotional imbalance.

The pathogenesis of PCOS is influenced by genomic alterations involving modification of the epigenome leading to non-DNA encoded protein expression (Cao et al. 2021). Since the first study over a decade ago (Xu et al. 2010), several genome-wide sequencing assays have identified small non-coding micro-RNA levels, histone acetylation and histone/DNA methylation patterns as critical epigenetic mechanisms in PCOS-associated cardiometabolic and neuropsychological pathologies (Cui et al. 2018; Wang et al. 2019; Chen et al. 2021). DNA methylation is an enzymatic reaction involving the addition of a methyl group to the DNA promoter region by methyltransferases causing transcriptional gene repression (Illingworth et al. 2008). In PCOS, DNA methylation differentially affects genes that regulate steroid synthesis, inflammation and metabolism, and these patterns have been recorded in various tissues including ovary, adipose tissue, skeletal muscle and hypothalamus (Shorakae et al. 2018; Cui et al. 2018; Cao et al. 2021). Moreso, histone alterations (involving removal or addition of acetyl or methyl functional groups to/from chromatin histone proteins) determines DNA accessibility and gene expression (Tsankova et al. 2006). Histone hypoacetylation [particularly through histone deacetylases (HDACs)] cause compression of chromatin and transcriptional gene repression (Sun et al. 2013). Previous studies have demonstrated the involvement of HDAC in hippocampal cholinergic (Sailaja et al. 2012) and neurotrophic (Tsankova et al. 2006) gene expressions. Further, histone modification footprints were reported in hippocampal NF-κB transcriptional activity in healthy (Yang et al. 2012; Lopez-Atalaya et al. 2013) and disease states including in neuro-pathologies (Sharma et al. 2015; Sevastre-Berghian et al. 2020; Dudek et al. 2020).

HDAC inhibition has attracted significant therapeutic attention having recorded protective benefits in various pathological conditions (Sevastre-Berghian et al. 2020). Interestingly, gut microbiome-derived short chain fatty acids (SCFAs) have demonstrated profound capacity to influence metabolic regulation through HDAC inhibition (Soliman et al. 2012; Cao et al. 2018). Additionally, there is evidence that gut microbiota is altered in women with PCOS (Chen et al. 2021). HDAC inhibitory effect of SCFA therapy projects their great potential as an anti-neuroinflammation candidate in the management of neurological and psychiatric disorders (Soliman et al. 2012). However, much information is not available regarding the role of SCFAs on epigenetic aberrations in PCOS-associated depression. In this study, we investigated the involvement of HDAC2 and DNA methylation in experimental PCOS-linked PFC/hippocampal neuroinflammation and depressive-like behavior and tested the potential ameliorative value of acetate supplementation.

**Materials And Methods**

**Experimental design and grouping**
Female Wistar rats weighing between 120-150 g were procured from the animal house of the College of Health Sciences, Afe Babalola University, Ado-Ekiti. These rats had unlimited 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, and the experimental protocol was approved by the Institutional Ethical Review Board of Afe Babalola University (Ado-Ekiti, Nigeria) with the number ABUADERC/15E/2021, and every effort was made to minimize the number of animals used and their suffering. All the animals used were with at least three consecutive regular estrous cycles and on the same estrous stage, which was determined via vaginal smear for each rat. Following the two weeks of acclimatization, the animals were randomly allotted into four groups (n=6/group) namely: Control (Ctl), Acetate-treated, Letrozole (LET)-treated and LET+Acetate-treated groups. Rats were maintained under standard environmental conditions of temperature (22-26°C), relative humidity (50-60%), and 12-hour dark/light cycle.

Treatment

The groups received vehicle (distilled water), acetate (200 mg/kg; Sigma-Aldrich, St Louis, MI), letrozole (1 mg/kg; Sigma-Aldrich, St Louis, MI) and letrozole plus acetate (200 mg/kg; Sigma-Aldrich, St Louis, MI) respectively by oral gavage for 21 days (Kafali et al. 2004; Adeyanju et al. 2020; Olaniyi et al. 2021).
Behavioural assessment

Open field test (OFT)

Locomotor activity was tested by placing each animal in the center of an open field box and left undisturbed for five minutes to explore the field arena. After each animal, the box was thoroughly cleaned with methylated spirit to eliminate all olfactory stimulant/cues present. The tests were recorded using a digital video camera and later scored for measures of locomotion which included number of line crossings and rearing. The open field apparatus used in this study is a wooden box that has 25 cm (width) and 25 cm (length) dimensional square floor surrounded by 20 cm walls.

Elevated plus maze (EPM) test

Each animal was placed in the center of an elevated plus maze and left undisturbed for five minutes whilst being recorded by a video camera. After each animal, the maze was cleaned with methylated spirit to remove the olfactory stimulant/cues. The recorded videos were later analyzed to record time spent in open arms and assessed the anxiety level of the animals.

Y-Maze test

Each animal was placed in a Y-shaped maze with three opaque arms and left undisturbed for five minutes whilst being recorded by a video camera. After each animal, the
maze was cleaned with methylated spirit to erase the olfactory stimulant/cues. The recorded videos were later analyzed to record the number of arm entries and triads to calculate spontaneous alternations and assessed spatial working memory of the animals.

Sucrose preference test

Each animal group was given two drinking water bottles, one containing water and the other containing 2% sucrose solution. This test was done on two consecutive days with the water and sucrose solution renewed on the second day. The bottle locations were switched every twelve hours to eliminate location preference. After each day, the volume of water and sucrose solution intake were taken and used to calculate sucrose preference and assessed anhedonia. Sucrose preference (percentage) was calculated as follows: Preference = \([\text{sucrose solution intake (ml)/total fluid intake (ml)}] \times 100\).

Collection of samples

After the treatment and behavioral assessment, 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.

Preparation of prefrontal cortex (PFC) and hippocampal tissue homogenates
The PFC and hippocampus were isolated, and 100 mg section of each 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 is required for biochemical assays.

Determination of hormonal profile

Plasma insulin, FSH, LH and insulin concentrations were determined with Rat ELISA kits obtained from Calbiotech Inc. (Cordell Ct., El Cajon, CA 92020, USA) and the manufacturer's procedures were followed. The ratio of LH/FSH was estimated.

Determination of malondialdehyde (MDA) and glutathione (GSH)

Malondialdehyde was determined from the plasma by standard non-enzymatic spectrophotometric method using assay kits from Randox Laboratory Ltd. (Co. Antrim, UK). This method involves the reaction of MDA in the sample with thiobarbituric acid (TBA) to generate an MDA-TBA adduct, which was quantified spectrophotometrically, while GSH was determined using a non-enzymatic spectrophotometric method with assay kits obtained from Oxford Biomedical Research Inc. (Oxford, USA).

Determination of NF-kB levels
The levels of NF-κB were determined in the supernatants of the PFC and hippocampal tissues by quantitative standard sandwich ELISA using a monoclonal antibody specific for this parameter with rat kit obtained from Elabscience Biotechnology Inc. (Wuhan, Hubei, P.R.C., China).

Determination of acetylcholine (ACH) levels

The levels of ACH were estimated from the supernatants of the PFC and hippocampus by Rat ELISA kits obtained from Elabscience Biotechnology Inc. (Wuhan, Hubei, P.R.C., China). This kit recognized Rat ACH in samples and this method uses the Sandwich-ELISA principle.

Histological assessment of ovaries

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

Histological assessment of prefrontal cortex and hippocampus

For hematoxylin and eosin (H & E) staining of PFC and hippocampus, a section of PFC and hippocampus was taken from each animal and fixed in 10% formolsaline overnight, dehydrated through graded alcohols series (50–100%) and cleared using two changes of xylene then
embedded in paraffin. The paraffin-embedded samples were sectioned at 5-μm thickness using the microtome then stained by H & E. The slides were examined using OPTO-Edu industrial camera light microscope and a computer (Nikon, Japan) and the images were assessed and captured.

Stereological evaluation of PFC and hippocampus

For the stereological evaluation of PFC and hippocampus, ten sections from each animal were analyzed in a serial section. Images were captured systematically using an OPTO-Edu industrial camera light microscope and a computer. The sections (5-μm widths) were captured and then processed with an image-processing and analysis software Image-J (Version 1.52). The mean total number of neuron per section was determined after counting every ten sections.

Reverse transcriptase polymerase chain reaction analysis

RNA was isolated from the supernatants of PFC and hippocampus using TRIzol Reagent (Thermo Fisher Scientific). Purified DNA-free RNA was converted to cDNA immediately using ProtoScript First Strand cDNA Synthesis Kit (NEB), and total cDNA was subjected to PCR amplification and quantification. The expressions of HDAC2 and DNA methyltransferase were determined using qPCR. Beta actin was used as a reference control gene. Primers used were:

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Exon junction 1041/1042 (forward primer) on template NM-053447.1

Products on intended targets

>NM-053447.1 Rattus norvegicus histone deacetylase 2 (HDAC2), mRNA

product length = 116

Forward primer 1  CCTAACTGTCAAGGTGCACGC  21
Template 1028 .................. 1048
Reverse primer 1  ACGTCCAACATCGAGCAACA  20
Template 1143 .................. 1124

Sequence (5’->3’) Template strand Length Start Stop Tm GC% Self complementarity Self 3’ complementarity
Forward primer 1.00 GGAGCAAGTCGGACAGTGAG Plus 20 490 509 60.39 60.00 5.00
Reverse primer 3.00 CGTTTAGCGGGACCCTTGAA Minus 20 601 582 60.32 55.00 5.00

Product length 112

Exon junction 589/590 (reverse primer) on template NM-053354.3

Products on intended targets

>NM-053354.3 Rattus norvegicus DNA methyltransferase 1 (DNMT), mRNA

product length = 112

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Template 490 .................. 509
Reverse primer 1  CGTTTAGCGGGACCCTTGAA  20
Template 601 ........................ 582

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Product length 121

Exon junction 72/73 (forward primer) on template NM-031144.3

Products on intended targets

>NM_031144.3 Rattus norvegicus actin, beta (Actb), mRNA

Product length = 121

Forward primer 1 CGCCACCAGTTCCGCCAT 17

Template 64 .......................... 80

Reverse primer 1 CCACGATGGAGGGGAAGAC 19

Template 184 .......................... 166

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**Data analysis and statistics**

All data were expressed as means ± SD. Statistical group analysis was performed using the Graphpad prism 5. One-way ANOVA was used to compare the mean values of variables and *post hoc* analysis was performed with Bonferroni’s test. Statistically significance was considered at p<0.05.

**Results**

Effects of acetate administration on plasma insulin, testosterone and LH-FSH ratio in LET-induced PCOS animal
model

The endocrine profile, including the plasma insulin, testosterone and LH-FSH ratio significantly increased in LET-treated group compared with control, and these were significantly decreased following the administration of acetate to LET-treated group compared to the non-acetate LET-treated group (Figure 1).

Effects of acetate administration on the histology of ovarian tissue in LET-induced PCOS animal model

The photomicrographs of ovarian tissues were characterized with degenerated follicles and disrupted antrum, granulosa cells, thecal cells and oocyte in LET-treated group compared with normal ovarian morphology in control and the ovarian tissues of LET+Acetate-treated group were characterized with preserved granulosa cells, thecal cells and oocyte with mild degeneration of antrum (Figure 2).

Effects of acetate administration on depressive-like behavioral assessments in LET-induced PCOS animal model

The animals treated with letrozole significantly reduced sucrose preference, number of rearing, line crossing in open field test, indicating anhedonic behaviour when compared to the control, which were significantly reversed following the administration of acetate to LET-treated group compared to the non-acetate LET-treated group. It was also observed that letrozole reduced the time spent in the open arm of elevated plus maze and spontaneous alternation in Y-maze compared with control group, indicating anxiety and disrupted spatial memory in LET-treated animals, and the administration of acetate to LET-treated animals reversed these alterations compared with non-acetate LET-treated group (Figure 3).

Effects of acetate administration on the levels of acetylcholine in PFC and hippocampus of LET-induced PCOS animal model

The levels of acetylcholine increased significantly in LET-treated group compared with control, and these were significantly decreased following the administration of acetate to LET-treated group compared to the non-acetate LET-treated group (Figure 4).

Effects of acetate administration on the plasma lipid peroxidation and glutathione concentration in LET-induced
PCOS animal model

The concentration of MDA increased significantly, while glutathione decreased in LET-treated group compared with control, and these were significantly reversed with decreased MDA and increased glutathione following the administration of acetate to LET-treated group compared to the non-acetate LET-treated group (Figure 5).

Effects of acetate administration on the levels of NF-kB in PFC and hippocampus of LET-induced PCOS animal model

The levels of NF-kB increased significantly in LET-treated group compared with control, and these were significantly decreased following the administration of acetate to LET-treated group compared to the non-acetate LET-treated group (Figure 6).

Effects of acetate administration on the histology of PFC in LET-induced PCOS animal model

The photomicrographs of PFC were characterized with neuronal degeneration in LET-treated group compared with normal neurons in control and the PFC of LET+Acetate-treated group were characterized with mild neuronal degeneration. Stereological analysis showed decreased neuronal count in LET-treated group compared with control, which was reversed in LET+Acetate-treated group compared with non-acetate LET-treated group (Figure 7).

Effects of acetate administration on the histology of hippocampal tissue in LET-induced PCOS animal model

The photomicrographs of hippocampus were characterized with neuronal degeneration with reduction of granular cell layer in LET-treated group compared with normal neuronal population in control and hippocampus of LET+Acetate-treated group showed mild neuronal degeneration. Stereological analysis showed decrease in neuronal count in LET-treated group compared with control, which was reversed in LET+Acetate-treated group compared with non-acetate LET-treated group (Figure 8).

Effects of acetate administration on the expression of HDAC2 and DNA methyltransferase in the PFC of LET-induced PCOS animal model
There was a significant elevated expression of HDAC2 and DNA methyltransferase in LET-treated group compared with control and these were significantly decreased following the administration of acetate in LET+Acetate-treated group compared with non-acetate LET-treated group (Figure 9).

**Effects of acetate administration on the expression of HDAC2 and DNA methyltransferase in the hippocampal tissue of LET-induced PCOS animal model**

There was a significant increased expression of HDAC2 and DNA methyltransferase in LET-treated group compared with control and DNA methyltransferase but not HDAC2 was significantly decreased following the administration of acetate in LET+Acetate-treated group compared with non-acetate LET-treated group (Figure 10).

**Discussion**

Key results from this study demonstrate that exposure of female rats to letrozole caused PCOS with hyperandrogenemia, systemic oxidative stress, and abnormal ovarian histomorphology. In addition, there was a significant increase in acetylcholine level in the PFC and hippocampus. Moreover, levels of PFC and hippocampal inflammatory mediator, NF-κB and neuronal loss were elevated in PCOS rats compared with controls. The central inflammatory-neurotransmitter alterations in this PCOS model were accompanied with increased HDAC2 and DNA methyltransferase expressions, while neurobehavioral assessments show depressive-like behavior. However, treatment with acetate ameliorated systemic and ovarian defects, with corresponding attenuation of PFC and hippocampal inflammation, abnormal neurotransmitter levels and neurobehavioral deficits through HDAC2/DNA methyltransferase inhibition in letrozole-induced PCOS.

Polycystic ovarian syndrome is a complex hormonal disorder affecting up to 20% of women around the world (Escobar-Morreale, 2018). Clinical and biochemical diagnosis of PCOS have revealed abnormal ovarian morphology with dysregulated hormone synthesis that is detectable by elevated circulating androgens (Ehrman et al. 1995). Data in this study show the presence of degenerated ovarian follicles, abnormal granulosa cells and a significant increase in plasma testosterone level, LH/FSH ratio and insulin level in PCOS rats compared with controls. Excessive ovarian androgen secretion has been attributed with elevated gonadotropin-releasing hormone (GnRH) pulse frequency, causing impaired follicular development, hyperleuteinization and high secretory activity of the granulosa cells (Blank et al. 2009). Alternatively, down-regulated aromatization of androgen (as a result of suppressed aromatase activity) which prevents conversion of testosterone to estrogen in the ovarian granulosa and theca cells could contribute to excess testosterone in PCOS (Suriyakalaa et al. 2021). Plausibly, administration of letrozole, a non-steroidal aromatase inhibitor, in this study, produced features typical of dysfunctional granulosa cells in PCOS.
Hormonal imbalance in PCOS is associated with metabolic derangements causing systemic oxidative stress that could lead to tissue dysfunction or injury (Duleba and Dokras, 2012). Oxidative stress ensues when the concentration of cellular reactive oxygen species (ROS) is unmatched by innate antioxidants defense systems leading to the accumulation of highly reactive superoxide and free radical species which can damage different cellular components (Blair et al. 2013). As an antioxidant mechanism, GSH reacts with ROS via its thiol groups to detoxify ROS and prevent activities like peroxidation and cellular damage (Lee et al. 2010). The present results show elevation in plasma MDA level that is accompanied with reduced glutathione (GSH) level in PCOS group compared with control. This observation is consistent with a recent study in PCOS patients that found elevated circulating MDA (Uçkan et al. 2022) and in experimental animals (Haslan et al. 2021). Usually, PCOS present with higher levels of blood lipids (Klimczak et al., 2015). The interaction of oxidizing radicals with blood lipids generates membrane-damaging end products, including malondialdehyde (MDA) which is a common marker of oxidative stress (Lee et al., 2010). Increased plasma level of MDA might have caused correspondent depletion of GSH in PCOS group. It could also be suggested that systemic oxidative stress and/or lipid peroxidation in this study progressed with elevated testosterone-led metabolic dysregulations of PCOS.

About two-third of PCOS women have mood disorders, although with unascertained underlying causes (Dokras et al. 2011). Studies hypothesized that mood disturbances in PCOS women occur due to high level of psychophysiological stress from associated reproductive-metabolic comorbidities and poor disease management (Kurjula et al. 2017; Chen et al. 2021). Stress triggers chronic alterations in neuronal functions especially neurotransmitter activities. In addition, dysmetabolism-related systemic oxidative stress (as evident in our PCOS model) may perturb the blood-brain-barrier resulting in leakage of oxidizing/inflammatory mediators into the brain thus, affecting neurotransmission (Richa et al. 2017). Importantly, neurochemical alterations like abnormal neurotransmitters level and/or signaling hampers neuro-modulatory processes involving cognition and mood (Ruhé et al., 2007). Our data show increased acetylcholine level in the PFC and hippocampus of PCOS rats compared with control. This finding is corroborated by previous studies that show hippocampal cholinergic hyperactivity in anxiety and depressive disorder (Mineur et al. 2013). In contrast to our observation on acetylcholine, the study of Chaudhari et al documented elevated acetylcholinesterase activity in the pituitary gland, hypothalamus, hippocampus and frontal cortex of PCOS rats and suggested that acetylcholine level was lowered with depression (Chaudhari and Nampoothiri, 2017).

Evidence persist that neuroinflammation is a critical phenomenon in PCOS-linked depression (Kirimizi et al., 2020). In this respect, we found that NF-κB concentration was elevated in the PFC and hippocampus of PCOS rats. NF-κB transcriptionally regulates the production of tumor necrosis factor-α (TNF-α) and other pro-inflammatory cytokines which would in turn initiate injury and nerve death in the brain (Koo et al. 2010; Wang et al. 2019). Histopathological staining of the PFC and hippocampal regions of PCOS rats’ brain in the present study revealed marked neuronal degeneration. Depression has been shown to correlate with reduced neural number/density (Goldwater et al. 2009; Fan et al. 2018). In addition, neurobehavioral tests were performed to monitor behavioral changes in PCOS rats. To evaluate fear and impaired working memory, open field and Y-maze tests were performed. PCOS rats expressed reduced
locomotor/exploratory activity and number of spontaneous alternations. Furthermore, the loss of ability to experience pleasure (anhedonia), and anxiolytic behavior observed from the sucrose preference and elevated plus maze tests confirm overt depression in our model. These behavioral findings in PCOS animals agree with other research works where similar cognitive and behavioral deficits in PCOS animals were reported (Yu et al. 2016; Cui et al. 2018; Mohammadi et al. 2021).

Epigenetic remodeling is increasingly recognized as a crucial mechanism in several important brain (dys)functions, including neurotransmission and depression (Sun et al. 2013). The role of DNA methylation and HDAC in PCOS-linked depression is still elusive. Subsequently, we determined whether DNA methylation and histone deacetylation were involved in neuroinflammation and depression in the current model. Intriguingly, higher expressions of DNA methyltransferase and HDAC2 were observed in the PFC and hippocampus of depressed PCOS rats when compared with controls. Histone deacetylation could co-express with DNA hypermethylation in certain diseases (Weaver et al. 2004). In the context of our observation, a recent study observed elevated Methyl-CpG binding protein 2 (MeCP2, a DNA methylation reader) and HDAC expressions in the frontal lobe and hippocampus in an animal model of depression (Sevastre-Berghian et al. 2020). Besides, global methylation was suggested to underlie increased DNA methyltransferase expression of candidate genes in the hypothalamus of PCOS rats (Cui et al. 2020). Put together, our data reveal that aberrant epigenetic modifications correlate with chronic inflammation in key areas of PCOS rats’ brain. This inference is further supported by previous evidence of higher HDAC expression with activated NF-κB/TNF-α pro-inflammatory signaling in the nucleus accumbens of stress-depressed animals and patients (Dudek et al. 2019).

Epigenetic changes are reversible, unlike genetic mutations. Hence, epigenetic alterations underpinning neuroinflammation could be therapeutically targeted to alleviate depression. Robust preclinical evidence revealed the efficacy of histone deacetylase inhibitors in reducing chronic inflammation, neurodegeneration and depression (Machado-Vieira et al. 2011; Cao et al. 2018). Our observations in the present study show that suppression of neuroinflammation by acetate could be an attractive therapeutic approach against depression in PCOS women. Acetate treatment of PCOS rats significantly diminished PFC HDAC2 and DNA methyltransferase expressions with corresponding reduction in NF-κB level as well as decreased hippocampal DNA methyltransferase but not HDAC2 expression. Enhanced histone acetylation and DNA demethylation could re-organize chromatin architecture and thereby restore DNA accessibility and gene expression (Machado-Vieira et al. 2011). That acetate decreased brain HDAC/NF-κB in depressed PCOS rats in this study, gains support from an in vitro experiment that demonstrated increased histone acetylation and decreased NF-κB expression with acetate treatment in LPS-induced microglia inflammation (Soliman et al. 2012). Moreover, treatment with butyrate, a SCFA like acetate, was associated with elevated DNA demethylation-facilitating enzyme (Wei et al. 2015) and reduced NF-κB expression (Lu et al. 2015) in the brain of animal models of depressive and neurodegenerative disorders. These previous works affirm that acetate as an HDACi would reverse epigenetic dysfunction and neuroinflammation in PCOS condition. Moreover, in the present study, acetate-treated PCOS rats exhibit decreased PFC/hippocampal acetylcholine level, suggesting that acetate further recover depressed PCOS animals from cholinergic dysfunction. Histology of the PFC and hippocampus of acetate-treated PCOS
rats show minimal nerve degeneration compared with the untreated group. In addition, acetate significantly reduced follicular and granulosa degeneration, plasma testosterone LH-FSH ratio, insulin and malondialdehyde levels, while glutathione level was restored in PCOS rats. Neurobehavioral results of the acetate-treated PCOS group show improved memory, increased exploratory activity and overall mood stability compared to the untreated group. These results reveal that HDAC/DNA methyltransferase inhibition by acetate exerted neuroprotection in PCOS-linked depression.

Conclusion

The study demonstrates that PCOS-associated depression is characterised by neuroinflammation and elevated ACH levels, and this is associated with increased expression of HDAC2 and DNA hypermethylation in PFC and hippocampus. Besides, the study suggests that acetate ameliorates PCOS-associated depression through the suppression of prefrontal and hippocampal DNA methyltransferase and prefrontal but not hippocampal HDAC2 expression.

Declarations

Acknowledgements and consent to participate

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Competing interest

The authors declare no conflicts of interest.

Authors’ contributions

The study was conceived and designed by KSO. The experiment was conducted by JAW and SEA. The data were analyzed and interpreted by JAW and KSO. All the authors drafted, read, revised and approved the manuscript for submission.

Data availability

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

Ethical approval
This study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the Institutional Ethical Review Board of Afe Babalola University (Ado-Ekiti, Nigeria) with the number ABUADERC/15E/2021.

Consent to participate

Not applicable

Consent to publish

All authors have given consent for publication

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**Figures**
Figure 1

Effect of acetate on plasma testosterone (a), LH-FSH ratio (b) and insulin (c) in LET-induced PCOS animal model. Values are presented as mean ± SD. n=6 and analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.01, **p<0.001, ***p<0.0001). Follicle stimulating hormone (FSH); Luteinizing hormone (LH).

Figure 2

Effect of acetate on the histology of ovaries in LET-induced PCOS animal model with H&E x800. Thecal cell (T); Oocyte (O); Granulosa cell (G); Antrum (A).
Figure 3

Effect of acetate on behavioral assessment (a-f) in LET-induced PCOS animal model. Values are presented as mean ± SD. n=6 and analyzed by one-way ANOVA followed by Bonferroni *post hoc test* (*p*<0.01, **p**<0.001, ***p***<0.0001).
Figure 4

Effect of acetate on PFC ACH (a) and hippocampal ACH (b) in LET-induced PCOS animal model. Values are presented as mean ± SD. n=6 and analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.01, **p<0.001, ***p<0.0001). Prefrontal cortex (PFC); Acetylcholine (ACH).
Figure 5

Effect of acetate on plasma MDA (a) and GSH (b) in LET-induced PCOS animal model. Values are presented as mean ± SD. n=6 and analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.01, **p<0.001, ***p<0.0001). Malondialdehyde (MDA); Glutathione (GSH).
Figure 6
Effect of acetate on PFC NF-κB (a) and hippocampal NF-κB (b) in LET-induced PCOS animal model. Values are presented as mean ± SD. n=6 and analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.01, **p<0.001, ***p<0.0001). Prefrontal cortex (PFC); Nuclear factor-kabbaB (NF-κB).

Figure 7
Effect of acetate on the histology of prefrontal cortex with H&E x800 (a) and neuronal cell count in LET-induced PCOS animal model. Values are presented as mean ± SD. n=6 and analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.01, **p<0.001, ***p<0.0001). Prefrontal cortex (PFC).

Figure 8
Effect of acetate on the histology of hippocampus with H&E x800 (a) and neuronal cell count in LET-induced PCOS animal model. Values are presented as mean ± SD. n=6 and analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.01, **p<0.001, ***p<0.0001). Molecular layer (M); Polymorphic layer; Granular layer of dentate gyrus (DG).
Figure 9

Effect of acetate on PFC DNA methyltransferase (a) and HDAC2 expression (b) in LET-induced PCOS animal model. Values are presented as mean ± SD. n=6 and analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.01, **p<0.001, ***p<0.0001). Prefrontal cortex (PFC); DNA methyltransferase (DNMT); Histone deacetylase (HDAC).
Figure 10

Effect of acetate on hippocampal DNA methyltransferase (a) and HDAC2 expression (b) in LET-induced PCOS animal model. Values are presented as mean ± SD. n=6 and analyzed by one-way ANOVA followed by Bonferroni post hoc test. (*p<0.01, **p<0.001, ***p<0.0001). DNA methyltransferase (DNMT); Histone deacetylase (HDAC).