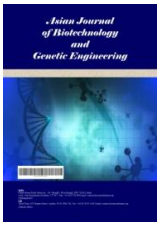

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Acute Lead (II) Acetate 3-Hydrate Neurotoxicity Alters Neurocognition and Induced Depressive-like Behavior Via 5-Hydroxytryphtamine, Neurohormone Melatonin and (Na^+,K^+)-ATPase Activity in Female Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author OGA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OGA and IW managed laboratory experiments and the analyses of the study. Author BBA managed the literature searches and methodology. Authors WA and ORA managed the literature searches. Author OUN managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Lead (II) acetate 3-hydrate also known as lead acetate is a neurotoxin that accumulates in soft tissues and bones causing damage to the nervous system of the human body. Hence, this study investigated the effect of short-term administration of Lead (II) Acetate 3-hydrate on serotonin, melatonin, (Na^+, K^+)-ATPase enzyme activity and neurocognition.
Methodology: Female Wistar rats (150-200 g b.wgt) were divided into groups (n=14). Control group (n=7) received 0.5 ml of normal saline and the treated group (n=7) were administered lead (II) acetate 3-hydrate at 100mg/kg for seven (7) days intraperitoneally (IP). Serotonin (5-HT), Melatonin and Sodium/Potassium-Adenosine Triphosphate [(Na^+, K^+)-ATPase] enzyme was investigated in the brain tissue and neurobehavior: Tail suspension test (TST), Forced swimming test (FST) and Novel object recognition test (NORT) were investigated. Body weight of the rats was also taken daily.
Results: The result obtained revealed significant ($P<0.05$) decrease in body weight in the treated group. Neurobehavioral results investigated showed a significant ($P<0.05$) increase immobility time both in FST and TST and a significant ($P<0.05$) decrease in retention latency in the NORT. The biochemical analysis reveals a significant ($P<0.05$) increase level of serotonin (5HT), but melatonin and (Na^+, K^+)-ATPase enzyme activity decreased significantly ($P<0.05$) when compared with the control group.
Conclusion: This current study shows that lead (II) acetate 3-hydrate affect neurocognition and can possibly initiate depressive-like behavior via melatonin and serotonin imbalance and also tampering with the (Na^+, K^+)-ATPase mechanism in the brain mitochondria.

Keywords: Lead; serotonin (5HT); melatonin; Adenosine Triphosphate (ATP); neurocognition and neuerotoxin.

1. INTRODUCTION

Lead is a heavy post-transition metal found in the environment. Its existence is due to its natural origin and the industrialized use. It is a heavy non- radioactive element that accumulates both in soft tissues and the bones [1]. Lead poisoning can damage the nervous system, and cause blood and brain disorders. Lead interferes with a variety of body processes, organs and tissues including the heart, bones, intestines, kidneys, and reproductive system. Its interference with the nervous system has been seen to cause potentially permanent learning and behavior disorders [2]. Exposure to lead includes contaminated air, water, soil, food, and consumer products but most importantly occupational exposure is a common cause of lead poisoning in adults [3].

Research studies suggested that cholinergic dysfunction, alteration in glutamate receptor and increase oxidative stress levels were all associated with lead-induced neurotoxicity [4,5]. Acute lead poisoning have revealed typical neurological disorders, gastrointestinal problem, blood disorder, kidney disorder [6], and cardiovascular disorder such as shock [7]. Chronic poisoning usually presents with symptoms affecting multiple systems [8]. Epidemiological studies have shown that excessive consumption of lead may cause CNS injury in young children affecting their growth, decreased intelligence and short-term memory, hearing loss, permanent brain damage and even death [9].

Various experimental studies also reveal that lead interferes with neurotransmitter release, disrupting the function of GABAergic, dopaminergic, and cholinergic systems as well as inhibiting NMDA-ion channels [10]. Cognitive symptoms of depression, such as poor attention and concentration as well as impaired memory and information processing, reveals deficits in cholinergic function. There are clarities that an imbalance of multiple neurobiological systems underlying depression results in cholinergic dysfunction which, in turn, causes further disruption in the body [11].

Among this neurotransmitters and neuro-hormones studied are: serotonin and melatonin. Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter also produced in CNS, specifically in the raphe nuclei located in the brain stem where they are secreted by the serotonergic neurons [12]. It contributes to modulating cognition, reward, learning, memory, mood, appetite, sleep and numerous physiological processes [13,14]. Serotonin deficiency is thought to be associated with

several psychological symptoms and increased level can lead to serotonin syndrome ranging from mild to severe cases such agitation or relentlessness, confusion, rapid heart rate and high blood pressure, muscle rigidity, headache, seizures, unconsciousness and high fever. Endogenous melatonin; another important body chemical is also released by the pineal gland which synchronizes the circadian rhythm including sleep–wake timing, blood pressure regulation, and seasonal reproduction [15,16,17]. It produces its effects through activation of the melatonin receptors, while others are due to its role as an antioxidant in regulating oxidative stress in the body [18,19]. Since the brain is very rich in mitochondria, in vitro studies have shown that lead activates protein kinase C in capillary cells and inhibits Na+/K+-ATPase in the cell membrane, interfering with energy metabolism [20]. Within the cell, lead appears to interfere with calcium release from the mitochondria, resulting in formation of reactive oxygen species, speeding mitochondrial self-destruction through formation of the permeability transition pore, and priming activation of programmed cell death processes [21]. Abnormal mitochondrial respiration can also result in uncoupling of the oxidative pathways from mitochondrial ATP synthesis, and subsequent failure of cellular energetic processes [22,23].

The aim of this present study investigated the effect of lead (II) acetate 3-hydrate neuro-toxicity as it alters neurocognition and possibly induced depressive-like behaviour via 5-hydroxytryphtamine, neurohormone melatonin and (Na^+,K^+)-ATPase activity in female Wistar rats.

2. MATERIALS AND METHODS

2.1 Drugs and Reagents

The lead (II) acetate 3-hydrate, melatonin (IBL Elisa kit), serotonin-assay (Abnova Serotonin Kit) and ATP colorimetric-assay (gene tex elisa kit) were products of Sigma Chemicals Co., USA. Other reagents were of analytical grade and the purest quality available.

2.2 Experimental Animals/Groupings

Female Wistar (n=14) weighing between 150-200g used for this study were obtained from the Central Animal House, PAMO University of Medical Sciences, Port-Harcourt, River State. The animals were maintained under standard laboratory conditions and housed in well ventilated plastic cages at room temperature (26-28^{o}C) and relative humidity (60-70%), light-dark cycles (12hr/12hr) respectively. The animals were acclimated for 2 weeks and allow free access to standard diet. Animals were randomly divided into two groups as follows:

Control group (n=7): given 0.5 ml normal saline intraperitoneally for seven days and

Treated group (n=7): given lead (II) acetate 3-hydrate at 100mg/kg intraperitoneally for seven days respectively.

2.3 Experimental Protocol for Depression Performance Task

2.3.1 Forced Swimming Test (FST)

A 2L glass cylinder was filled with water at temperature 23 ± 1°C. The cylinder is 19-cm tall, 10-cm in diameter and filled to 13-cm (the 1600-mL point) with tap water and allowed to sit overnight to achieve room temperature [24].

Rats touching the bottom of the cylinder with their tails were prevented, as this may alter their behavior. One hour after treatment with Lead (II) Acetate 3-hydrate, the rats were individually picked up from their home cages in a plastic container with holes in the bottom to let out water, and are individually dropped (placed in, head downward, trying to ensure that the rat's head does not go underwater) into the glass cylinder and observed for immobility for one 6-min trial. A stopwatch was used to record immobility. Immobility is defined as the cessation of all movements except those necessary to stay afloat, such as paddling lightly with one.

2.3.2 Tail Suspension Test (TST)

The tail suspension test (TST) was performed [25]. The method is based on the observation that a rat suspended by the tail shows alternate periods of agitation and immobility. One hour after treatment with Lead (II) Acetate 3-hydrate, the rat were individually suspended 50 cm above the floor approximately by 1 cm from the tip of the tail with an adhesive tape. The rat was 150mm away from the nearest object and was both acoustically and visually isolated. All of the experiments were video-taped, and the total duration of inactivity was scored for the last 6 min of the 10 min-long sessions. Immobility was measured when the rat did not make any

struggle to get rid of the tape for at least 1 minute [26].

2.4 Experimental Protocol for Memory Performance Task

2.4.1 Novel Object Recognition Test (NORT)

The novel object recognition test is done in an open-field chamber (60cm × 50 cm × 40cm) as previously described [27]. It consists of two phases: the training phase and the test phase. The training phase for both groups was carried out on the last 6th day of the treatment. During the training phase, the rats were placed in the middle of the two identical objects (A and B) on opposite, each side, at a distance of 8cm from the walls, and 34cm from each other. The animals were first acclimated to the experimental condition for a period of 5 minutes during which they explored the environment. The amount of time spent exploring the objects (in seconds) was recorded. Exploration included behaviors such as touching, climbing and snuffing at the object. Thereafter, the animals were returned to their home cages for an interval of 24 hours each. Following a retention interval of 24 hours, rats were placed in the open field for 3 minutes (testing phase-7th day). In the test phase, object B (one of the blue painted tin cans) was replaced with object C (the red painted tin can), which was novel to the rats and different from either object A or B. Rats were then left to explore objects and C for a period of 3 minutes.

The discrimination index, which was used as a measure of non-spatial memory function, was calculated as the difference in time exploring the novel and familiar object divided by the total amount of time spent with both objects. The percentages exploratory preference (PEP) score was calculated as the time spent exploring the novel object divided by the total time spent exploring both objects multiplied by 100. The DI and PEP was thus used as index of memory in this present study.

$$DI = \frac{Difference\ in\ time\ exploring\ the\ novel\ and\ familiar\ object (C-A)}{Total\ time\ spent\ exploring\ both\ objects\ (A+C)}$$

$$PEP = \frac{Time\ spent\ exploring\ the\ novel\ object\ (C)}{Time\ with\ novel\ object\ (C) + Time\ with\ familiar\ object\ (A)} \times 100$$

2.4.2 Biochemical estimation in the brain tissue

After the lead exposed treatment, the animals of the treated group and the control groups were anaesthetized and sacrificed and the brain tissues were extracted for serotonin [28], melatonin [29], and ATP synthase estimation [30].

2.5 Statistical Analysis

All the data are presented as Mean ± Standard Error of Mean (SEM). Statistical analysis was done using paired t-test (two-tailed t-test) and two-way ANOVA followed by Bonferroni post hoc test. All statistical analyses were done by using Prism software, version (Diego, CA, USA). The statistical difference at level *$p<0.05$ and **$p<0.01$ was considered significant.

3. RESULTS

3.1 Effect of Lead (II) Acetate 3-hydrate Neurotoxicity on the Body Weight in Female Wistar Rats

There was significantly ($p < 0.05$) decrease in weight gained in the rats treated with lead (II) acetate 3-hydrate at 100mg/kg from day 3 to day 7 when compared to the control group (Fig. 1).

3.2 The Effect of Lead (II) Acetate 3-hydrate Neurotoxicity on Immobility Time in Forced Swimming and Tail Suspension Test in Female Wistar Rat

The administration of lead (II) acetate 3-hydrate produces an increase in immobility time in the treated rat group. There was significant ($p<0.01$) increase in immobility time for forced swimming test (immobility × treatment: $F(3,4) = 10.44$; $p=0.0019$) and a significant ($p<0.05$) increase in immobility time for tail suspension test (immobility × treatment: $F(3,4) = 5.502$; $p=0.0118$) as determined by paired t test when compared to the control group (Fig. 2A and 2B).

3.3 The Effect of Lead (II) Acetate 3-Hydrate Neurotoxicity on Percentage Exploration Preference & Discrimination Index in Female Wistar Rat

The administration of lead (II) acetate 3-hydrate produces a decrease in retention latency in the treated rat group. There was significant ($p<0.05$) decrease retention latency for percentage exploratory preference (retention× treatment:

F(3,4) = 4.612; p=0.0192) and a significant (p<0.05) decrease in retention latency for discrimination index (retention × treatment: F(3,4) = 5.414; p=0.0124) as determined by paired t test when compared to the control group (Fig. 3A and 3B).

3.4 The Effect of Lead (II) Acetate 3-hydrate Neurotoxicity on Serotonin in Female Wistar Rat

The result shows that the level of serotonin (5HT) significantly (p < 0.05) increase in the rats treated with lead (II) acetate 3-hydrate at 100mg/kg when compared to the control group (Fig. 4A).

3.5 The Effect of Lead (II) Acetate 3-hydrate Neurotoxicity on Melatonin in Female Wistar Rat

The rats treated with lead (II) acetate 3-hydrate at 100 mg/kg shows a significantly (p < 0.05) decrease in the level of melatonin when compared to the control group (Fig. 4B).

3.6 The Effect of Lead (II) Acetate 3-hydrate Neurotoxicity on (Na^+, K^+)-ATPase in Female Wistar Rat

The rats treated with lead (II) acetate 3-hydrate at 100mg/kg show significant (p < 0.05) decrease in the activity of (Na^+, K^+)-ATPase when compared to the control group (Fig. 4C).

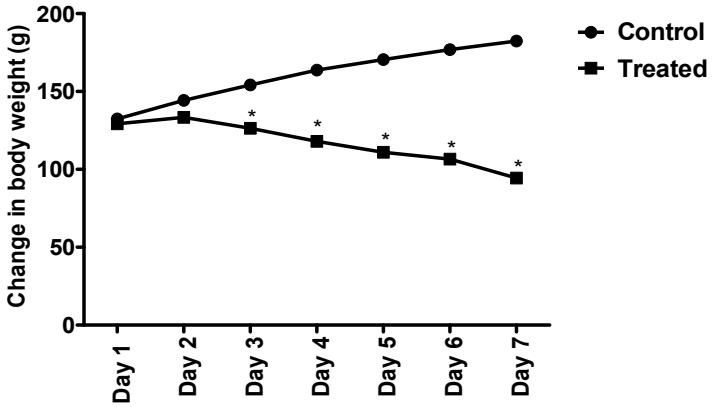

Fig. 1. Effect of lead (II) acetate 3-hydrate neurotoxicity on the body weight in rats. Values are expressed as mean ± SEM, n=7. The level of significance was expressed as *p<0.05 compared with the control group and the treated group

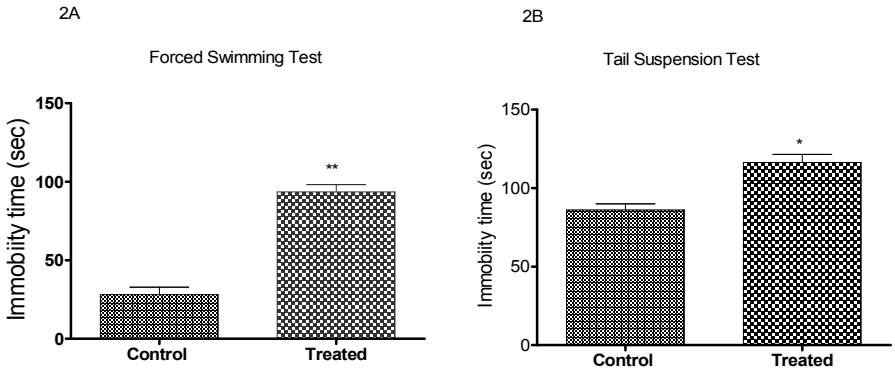

Fig. 2 (A and B). Effect of lead (II) acetate 3-hydrate neurotoxicity on the immobility time in rats. A= forced swimming test. B= Tail suspension test. Values are expressed as mean ± SEM, n=4. The level of significance was expressed as *p<0.05 and **p<0.01 compared with the control group and the treated group

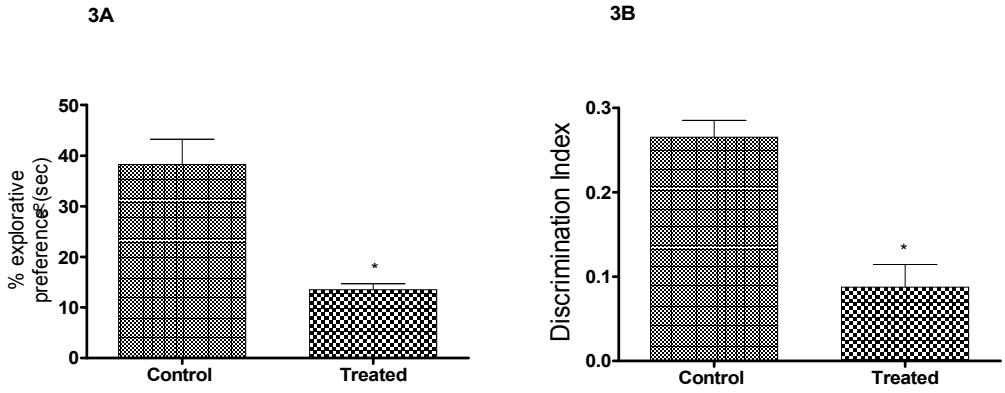

Fig. 3 (A and B). Effect of lead (II) acetate 3-hydrate neurotoxicity on novel object recognition test in rats. A= % exploration preference. B= Discrimination index. Values are expressed as mean ± SEM, n=4. The level of significance was expressed as *p<0.05 compared with the control group and the treated group

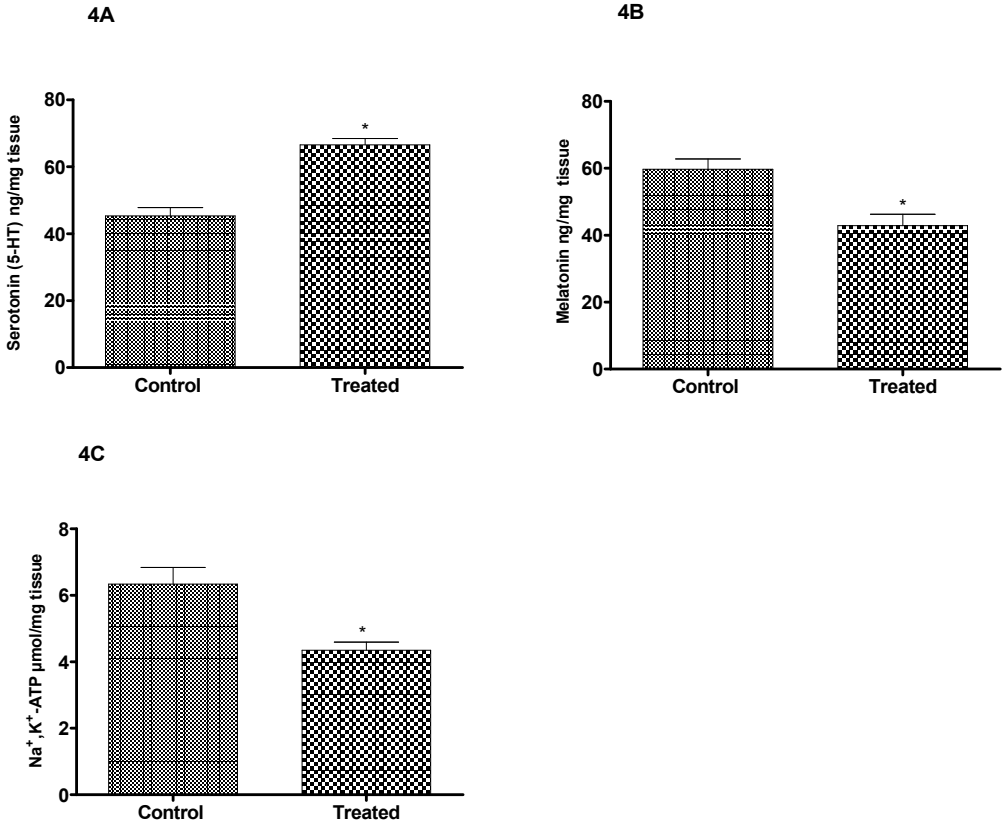

Fig. 4 (A, B and C). Effect of lead (II) acetate 3-hydrate neurotoxicity in the brain tissues in rats. A= Serotonin (5HT), B= Melatonin, and C= (Na^+, K^+)-ATPase activity. Values are expressed as mean ± SEM, n=4. The level of significance was expressed as *p<0.05 compared with the control group and the treated group

4. DISCUSSION

The maintenance of neurotransmitters in the brain is essential for minimizing cerebral dysfunction in the body. The administrations of lead (II) acetate 3-hydrate to rats in this study have shown to increase cognitive impairment, weight loss, and initiate depressive-like behavior; thereby depicting a possible neurodegenerative effects. Cognitive decline emanating from neurotransmitter reduction or excess in the central nervous system (CNS) is considered a major neurobehavioral disorder cause, associated with depression and Dementia of the Alzheimer's type (DAT), and is a major condition that reduces the quality of life of sufferers [31]. Maintaining and regulating the brain neurotransmitters have previously shown to reduce the risk of developing cognitive dysfunctions or neurodegenration [32].

The administration of lead (II) acetate 3-hydrate to the treated rats for 7 days showed significant weight loss. Decrease in body weight is an index of reduced activeness in rodents. An earlier study reveals that the consumption or administration of lead (II) acetate 3-hydrate will lead to a significant decrease in body weight [33].

Three well-established memory task models were used to assess neurobehavior in the rats: Forced swimming test, Tail suspension test and Novel object recognition test [24,25,26,27]. The neurobehavioral results obtained in the study reveals significant increase in immobility time in the treated rats after seven days of intraperitoneal administration with 100mg/kg lead (II) acetate 3-hydrate. Increase in immobility time is an index of depression and previous investigations on the effect of lead toxicity showed altered monoaminergic system and lead to neurobehavioral deficit such as memory decline, anxiety, depression and reduce motor coordination [34]. The novel object recognition test performed on the rat also reveals reduced memory retention. There was significant decrease in retention latency in the percentage exploration preference and discrimination index measured in the rats. Increase retention latency of the novel object introduced to the rats in the testing phase is an index of improved short/long-term memory and spatial/non-spatial memory retention. The prefrontal cortex regulating visual and working memory involving attention have been earlier reported to contain high level of muscarinic (M_1) acetylcholine receptors [35,36].

Neurotransmitters like Serotonin (5HT) which can point to the level of neurogenesis in the brain cells were reported in this study. Intraperitoneal administrations of 100mg/kg of lead (II) acetate 3-hydrate lead to an increase in serotonin production in the brain of the treated rats. Previous researches on lead (II) acetate 3-hydrate have shown that long-term administration will lead to imbalanced serotonin secretion and this effect is due to an increase in monoamine oxidase inhibitor (MOI) activity and a possible development of serotoninergic neuron in the central nervous system (CNS) [37]. These findings suggest that lead exposure may possibly induce increased cognitive decline, anxiety and depressive-like state as a consequence of changes in neuronal 5-HT content in the dorsal raphe nucleus (DRN) [37]. The result in this study corroborate with previous work done on the effect of lead on serotonin secretion [38,39].

The endogenous melatonin is a hormone that synchronizes the circadian rhythm including sleep–wake timing, regulate blood pressure, and initiate seasonal reproduction [15]. From the result obtained in this study, it was observed that endogenous melatonin decreases in the rat treated with lead (II) acetate 3-hydrate. The fall in endogenous melatonin could be due to tampering effect of the lead (II) acetate 3-hydrate on the pineal gland or serotonin imbalance. Melatonin and serotonin imbalance will synergistically trigger depressive-like behavior; therefore, administration of melatonin will compensate the drastic changes in the animal to normal within a period of few weeks [40,41,42, 43]. From our finding, the significant reduction in the endogenous melatonin level in the treated group may be associated with lead induced stress leading to the generation of excessive free radicals, down-regulation of melatonin receptors-complex mechanism, and increase night sympathetic activity which can alter the neurobehavior in the rat [44].

To investigate the effects of lead (II) acetate 3-hydrate on neurotransmitter production in the brain, the ATPase synthase activity were examined. The result reveals a significant reduction in the (Na^+, K^+)-ATPase activity in the brain mitochondria of the treated rat. This results into lead interfering with the ion transport mechanism and cellular energy metabolism of the treated rat's brain [44]. Adenosine triphosphate is produced by the enzyme ATP synthase in the mitochondria and decrease (Na^+, K^+)-ATPase activity observed can therefore deduce that the administration of lead (II) acetate

3-hydrate reduces the enzyme ATP synthase. Reduced activity of the ATPase synthase causes fluctuations in energy metabolism, prevents muscular contractility and decreases the mental response to the body functioning system. Previous studies have shown that lead when administer to animals interferes with cellular energy metabolism by inhibiting ATP (Adenosine triphosphate) synthesis and hydrolysis [45].

5. CONCLUSION

This present study shows that the intraperitoneal administration of lead (II) acetate 3-hydrate to female Wistar rat attenuates neurocognition by producing depressive-like effect via serotonin and endogenous melatonin imbalance and also down-regulating ATPase synthase activity.

ETHICAL APPROVAL

All procedures and treatment of the animals used for the experimental design were in accordance with the guidelines of the University research ethics which agreed with the internationally accepted principles for animal handling and care.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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