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RESEARCH PAPER

Protective roles of melatonin on Hematological Parameters and Thyroid Hormone Levels in rats treated with Aluminum Chloride

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A B S T R A C T:

Background: Aluminum exists in numerous produced foods, medicines and likewise added to drinking water for refining purpose. Its existence has so heavily contaminated with the surroundings that exposed to it is almost inescapable.

Goal: This survey was aimed at evaluating effect of Melatonin for inhibiting effect of Aluminum Chloride.

Methods: Randomly selected rats were grouped separately into three groups for 40 days: (n = 5/group): healthy key group, Aluminum Chloride ($AlCl_3$) group (Urged with 1000mg/L of $AlCl_3$ in administrated water) and The last group were awarded (1000mg/L) of $AlCl_3$ in water plus melatonin (50mg/kg diet). The estimated haematological parameters were leukocytes, erythrocytes, haemoglobin concentration and thrombocytes count. Also serum and brain supernatant beta amyloid and serum levels of T_3, T_4 and TSH were also measured.

Results: At the end of this study, statistical analysis showed that the some haematological parameters (RBC count Hb, PCV, WBC count, granulocyte and lymphocyte differential leucocyte counts, platelet count) serum and brain beta amyloid and thyroid hormone values in $AlCl_3$ with melatonin at a dosage of 50mg/kg diet has aroused the rats to stay approximately near to the standards.

Conclusion: From the results of this study it was discovered that melatonin has a therapeutic effect on $AlCl_3$ induced in albino rats.

KEY WORDS: Melatonin, $AlCl_3$, Hematology, β amyloid, Thyroid Hormones.
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1. INTRODUCTION

The toxic effects of Aluminum have been studied on a variety of different body organs, including brain and circulatory system. It effects, as an environmental factor, also contributes to some neurodegenerative disease and effects on several enzymes and biomolecules relevant to Alzheimer's disease (Kadhum, 2017). Melatonin, as an eminent endogenous antioxidant, suggest that defended to the central nervous system (CNS) by propelling free radicals and consequently enhances the employment of further antioxidants.

Additionally, its metabolites have the capability to shield tissues versus oxidative destruction created by a diversity of toxic agents and metabolic behaviours (Peyrot and Ducrocq, 2008).

Previous in *vivo* and in *vitro* investigations have proposed the protective effects of melatonin against metal-induced oxidative damage, with high lipophilicity, if supplied exogenously it can swiftly cross the blood brain barrier to reach neurons and glial cells (Daniel et al., 2004). It is concluded that Melatonin acts as a hunter of free radicals, that is especially important in the brain, the most sensitive organ for oxidative stress and have ability to limit the generation of free radicals at the mitochondrial level by a mechanism which not fully understood (Reiter et al., 2005).

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One strategy to restrict or diminish the advancement of AD is completely the attack in the Aβ gathering process. Melatonin, by communicating beside Aβ peptide, can inhibit the progressive creation or displacement of β-sheet and/or amyloid fibrils (Poeggeler et al., 2001). The mechanization of this activity is that melatonin could boost the conversion of β-sheets into random coils by obstructing the imidazole-carboxylate salt bridges thus, block Aβ fibrillogenesis and gathering. In the aforementioned design, executes moulding the formation of the secondary β-sheet compatibility possible. It, hence, is not diminishing neurotoxicity alone but further benefits the clearance of the peptide via enhanced proteolytic degradation (Masilamoni et al., 2008).

On the other hand, the occurrence of remarkable variations in the blood of the cases with AD and influence the blood hemostasis assigns to the development of Aβ plaques in the circulatory vessels and brain parenchyma (Smith and Greenberg, 2009). Abnormal haematological factors such as lower levels of erythrocyt, haemoglobin , and hematocrit also have been recognized as vied to ordinary individuals. This survey was aimed at evaluating the possible effects that Aluminum chloride could have on some blood parameters and thyroid hormones and the role of melatonin for inhibiting persuaded Aluminum Chloride of adult Albino rats.

1. Materials and Methods

2.1 Animals and Housing

This study consisted of fifteen adult male albino rats (Rattus norvegicus) each weighing of 190-240 g B.W. and 10-12 weeks old. The study was conducted in the animal house in Biology Department / Education College / Salahaddin University-Erbil, between October 2019 to January 2020. The rodents lived in plastic enclosures bedded with wooden chips supporting by official lab specifications, bout 12:12 light/dark photoperiod (LD) at 22±4 ºC (Coskun et al., 2004). An automatic light-switching pattern was used for preserving conventional 12-hours daytime cycles.

1.2 Experimental Design

This study involved fifteen adult male rats. They implied randomly categorized into three cohorts each consisting of five animals. The first cohort was supplied with standard rat chow and tap water which was considered as a controller cohort. The second ones were supplied (1000mg/L) of $AlCl_3$ in water and the latest ones were given (1000mg/L) of AlCl3 in water plus melatonin (50mg/kg diet). The treatments were engrossed for 40 days and then the data were earned.

1.3 Anesthesia, Dissection and Removal of Brain

Following the procedure of Laird et al., (1996), Ketamine (35mg/kg B.W.) and xylazine (5mg/kg B.W.) were used to anesthetize all animals then they sacrificed at the end of experiment. Brain of each rat was divided into two equal parts, in which one part cut into small pieces (less than $0.5cm^3$ thickness) then kept in fixative, while the other part stored at -80 ºC in freezer until they were needed for estimation of Aβ (1-42) peptide levels.

1.4 Tissue Homogenate Preparation

Brain tissues washed with cold saline, dried then weighed as asserted to a modified method elaborated by Dhuha and Ali (2020). Half of each brain used for homogenization by 10 mm cold phosphate buffer saline pH 7.4. The brain tissues homogenized (10 % w/v) using an electrical homogenizer at 20000 rpm for 6 seconds, while healthy cells and cell rubble were separated by centrifugation at 3000 rpm for 20 minutes by practicing a refrigerated centrifuge at (4°C). The supernatants were utilized for Aβ (1-42) peptide analyses

1.5 Blood Collection

After 40 days of continuous treatment, blood specimens were obtained from the heart of the animals and then anaesthetize by diethyl ether. Tubes have ethylene diamine trichloroacetic acid (EDTA) were utilised for accumulating blood and directly were applied for estimating haematological determinants. Tubes without

anticoagulants were also utilized for serum preparation and centrifuged at 3000 rpm for 15 minutes for Aβ (1-42) peptide and hormonal estimation (Cheng, 2002).

2.6 Determination of Serum and Brain Supernatant Beta Amyloid (1-42) Peptide Level

The kit assay, Aβ (1-42) peptide (model No. SL0049Ra), was used to purify rat Aβ (1-42) antibody to coat microtiter plate wells and to make a solid-phase antibody. Then Aβ (1-42) added to the wells to combine Aβ (1-42) antibody with Horse Radish Peroxidase (HRP) labeled to form antibody - antigen - enzyme-antibody complex. After thoroughly washing, the Tetra Methyl Benzene (TMB) substrate solution was added turning the TMB substrate into blue and then the HRP enzyme-catalyzed reaction was terminated by the addition of sulphuric acid solution. The colure change measured with the plate reader at a wavelength of 450 nm. The concentrations of the peptide for the samples were determined by comparing their optical density with the standard curve.

***2.7.* Estimation of haematological parameters**

Automated parameter haematology analyzer (Mythic 22) was used to analyze erythrocyte count and additional haematological data.

2.8. Estimation of Hormone

Automated Immunoassay Analyzer (Biomerieux, France) nominated applied to appraise serum values of triiodothyronine, thyroxine and thyroid stimulating hormone beside a chemiluminescence immunoassay.

3. Statistical Analysis

Statistical software (Graph pad Prizm, version 8) was used. Data are represented as mean ± standard errors of means (SEM) of the number of animals used in each group. To compare the individual means in each group with the control group, ordinary one-way analysis of variation (ANOVA) for compared within control and studied treatments were applied and Tukey post hoc test was used to compare individual means. After analysis of variance (ANOVA). $P<0.05$ was considered statistically significant.

Results

Effect of melatonin on serum and brain β amyloid level

As illustrated in Fig. 1 and Table 1, Urged of $AlCl_3$ in a dosage of (1000mg/L) generated a significant increase ($P<0.001$) in serum and brain β-Amyloid in compared to standard and melatonin with $ALCL_3$ cohort rats, while no important ($p\leq 0.05$) alteration in the serum and brain β Amyloid values mentioned in melatonin with $ALCL_3$ cohort's rats.

Table 1: Effect of melatonin on serum and brain β amyloid parameters:

Parameters	Control	$AlCl_3$	$AlCl_3$ + Melatonin
Serum β Amyloid ug/L	24.780 ± 0.871	44.83 ± 1.100***	25.440 ± 1.307###
Brain β Amyloid ug/L	73.66 ± 0.886	92.510 ± 2.092***	78.410 ± 1.994###

All data represent means ± standard errors (SE),
n = 5,
*** = P<0.001
= P<0.0001 significant differences between induced $AlCl_3$ and melatonin treated parameters

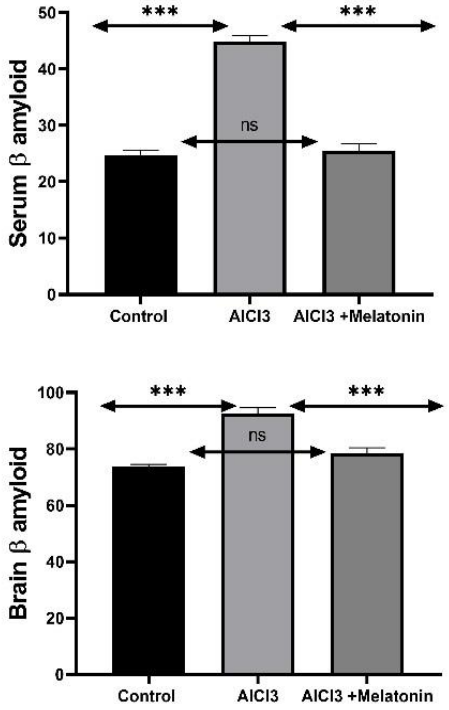

Figure 1. Effect of melatonin on the serum and brain β amyloid level

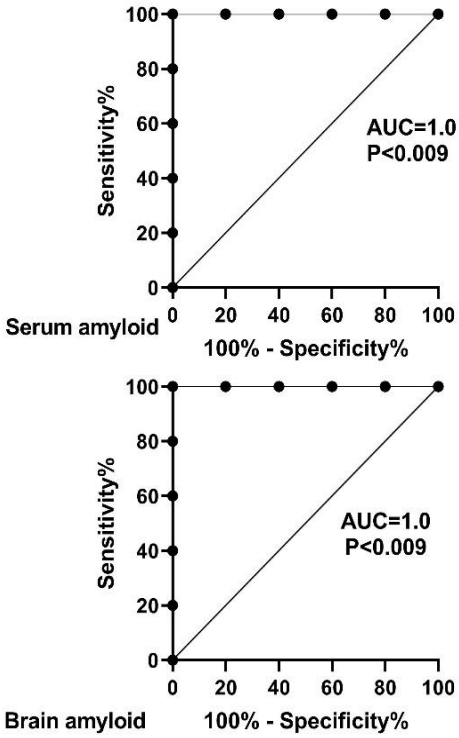

Figure 2. ROC curve shows the sensitivity of melatonin as a bio marker

The results in figure 2 showed improves in the ROC curve sensitivity of β amyloid in both serum and brain $AlCl_3$ induced rats, (with the value of AUC= 1.0 and P<0.009).

Effect of melatonin on serum T_3, T_4 and TSH levels:

The effects of $AlCl_3$ in albino induced rats on T_3, T_4 and TSH secretion levels were displayed in Table 2 and figure 3. The outcomes appear that T_3 is altered improved (P<0.01) between control and $AlCl_3$ induced rats, while there are obvious changes between the values of $AlCl_3$ melatonin treated and both control and $AlCl_3$ induced rats, nevertheless there were no statistical ($p>0.05$) changes occur among them. The T4 values of $AlCl_3$ induced rats are affected significantly (P<0.001) with both control and $AlCl_3$ melatonin treated rats, with none notable changes happen between control and $AlCl_3$ with melatonin treated rat values. TSH values showed, that there are no statistical changes among control and both $Alcl_3$ induced and $AlCl_3$ melatonin treated rats.

Table 2. Effect of melatonin on serum T3, T4 and TSH levels:

Parameters	Control mean ± S.E n = 5	$AlCl_3$ mean ± S.E n = 5	$AlCl_3$ + Melatonin mean ± S.E n = 5
T3 nmol/l	141.195 ± 10.692	96.451 ± 6.524**	123.800 ± 5.892
T4 nmol/l	1.996 ± 0.365	16.200 ± 1.689***	4.404 ± 0.633$^{###}$

TSH uIU/ml	**2.488 ± 0.309**	**2.488 ± 0.337**	**2.400 ± 0.387**

- **Data presented as mean ± S.E**
- **n = number of observation**
- **** = P<0.01 *** = P<0.001**
- **### = P<0.0001 significant differences between induced $AlCl_3$ and melatonin treated parameters**

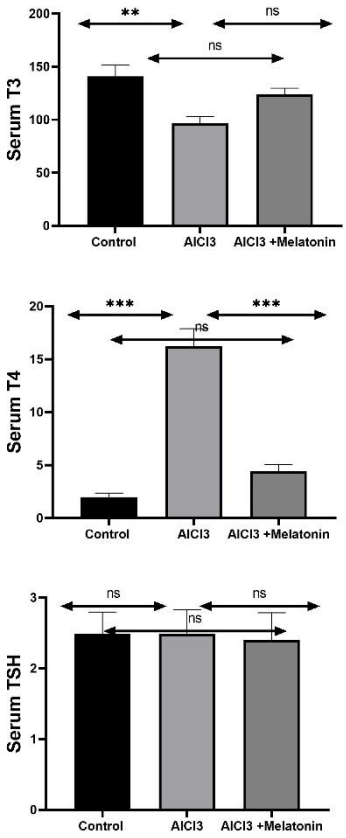

Figure 3 Effect melatonin on serum T3, T4 and TSH parameters

Effect of melatonin on some hematological parameters:

a-Effect of melatonin on RBC indices parameters

From Table 3, revealed a meaningful (P<0.05) decrease in RBC count within AlCl3 rat's cohort during vied with of normal cohort. After administration of melatonin, the improvement in the count of RBC is observed, but statistically (P<0.05) not changed. Otherwise, the Hb values of $AlCl_3$ cohort diminished statistically (P<0.001) when viewed with the control cohort. Also, the Hb values of melatonin treated cohort were significantly (P<0.05) raised during viewed with AlCl3 effected rat cohort. However, PCV values of $AlCl_3$ stimulated rats were lowered at (P<0.001) if observed with the normal cohort. Moreover, the melatonin treated rats were statistically (P<0.01) improved when compared with AlCl3 stimulated rat cohorts. On the other hands, the other inquired RBC indices data of the current study, particularly MCV, MCH and MCHC values, were not altered at (P<0.05) if

viewed in stimulated rats with control cohort and $AlCl_3$ with melatonin treated animals.

Table 3: Effect of melatonin on RBC indices parameters:

Parameters	Control mean ± S.E n = 5	$AlCl_3$ mean ± S.E n = 5	$AlCl_3$ + Melatonin mean ± S.E n = 5
R.B.C. $\times 10^{12}$/L	5.460 ± 0.258	4.525 ± 0.131*	5.109 ± 0.903
Hb g/dL	13.510 ± 0.258	10.721 ± 0.386***	12.180 ± 0.883#
PCV %	40.841 ± 1.496	33.060 ± 1.491***	36.967 ± 1.373##
MCV fL	75.095 ± 3.137	69.364 ± 1.109	72.583 ± 1.879
MCH Pg	24.883 ± 0.825	22.811 ± 1.347	23.732 ± 1.184
MCHC g/dL	33.159 ± 0.594	32.553 ± 1.031	32.758 ± 0.875

Data presented as mean ± S.E.

- **n = number of observations.**
- *** =P<0.05 ** = P<0.01 *** = P<0.001.**
- **# =P<0.05 ## = P<0.01 significant between $AlCl_3$ induced and melatonin treated rats.**

b- Effect of melatonin on WBC, differential and platelet count parameters:

The results obtained from the table (4) revealed important (P<0.05) improvement in the WBC, Granulocyte differential count and platelet counts, with the notable(P<0.05) reduction in the lymphocyte differential counts rates of AlCl3 induced rat's cohort when compared with a control cohort. But the values of WBC, Granulocyte differential Count platelet count and lymphocyte differential counts from melatonin treated cohorts were not affected at (P<0.05) with both control and AlCl3 stimulated cohorts. Finally, the monocyte values not changed within AlCl3 influenced rats, melatonin treated and normal cohorts' rats

Table 4: Effect of melatonin on WBC, differential and platelet counts levels:

Parameters	Control mean ± S.E n =5	AlCl3 mean ± S.E n = 5	AlCl3 + Melatonin mean ± S.E n = 5
W.B.C. $\times 10^9$/L	766.465 ± 22.239	807.613 ± 27.410*	785.807 ± 19.128
Granulocyte Count %	50.600 ±5.749	55.000 ± 4.871*	54.300 ± 3.920
Lymphocyte %	47.800± 6.010	43.200 ± 5.43*	44.100 ± 4.970

Monocyte %	**1.600 ± 1.244**	**1.800 ± 0.200**	**1.600 ± 0.258**
Platelet $\times 10^9$/L	**422.573 ± 19.452**	**544.310 ± 23.078***	**458.213 ± 23.421**

- **Data presented as mean ± S.E**
- **n = number of observation**

*** =P<0.05**

Discussion:

Effect of melatonin on serum and brain β amyloid levels

As indicated by the current study, AlCl3 caused a significant increase in β amyloid level both in serum and brain supernatant of male rats which is supported by Castronia et al., (2010), they published that extreme consumption of aluminium (Al) may provoke the deposition of amyloids in the neurons and deficits memory as well as learning confusions in rats.

Increased β amyloid level in serum and brain supernatant of AlCl3 cohort may be associated with the point that Al may attack the nucleus and may create nuclear vacuolation as proved by the current investigation creating gene mutation consequently starting to the disposition of this peptide which is firmly adhered with Al and deposited there. (Castronia et al., 2010).

In this analysis melatonin decreased β-amyloid values in serum and brain supernatant of male rats, the finding agreed with the data sustained by Millan-Plano et al. (2003), they documented that melatonin inhibits Al stimulated disposition of β amyloid and oxidative end products in the synaptosomal membranes, by merging with Al such adhesive may shed light into the role of this factor in the a etiology of AD (Lack et al., 2001).

Melatonin could advance the exchange of β-sheets into random coils by breaking the imidazole-carboxylate salt bridges thus, obstruct Aβ fibrillogenesis and aggregation. Therefore, it does that achievable by preventing the creation of the secondary β-sheet conformation; it is not only diminishing neurotoxicity but also help the withdrawal of the peptide via enhanced proteolytic degradation (Kurhaluk et al.,2017).

Effect of melatonin on serum T3, T4 and TSH levels:

Our study, examined the relationships between serum levels of thyroid hormones and TSH in $AlCl_3$ induced and melatonin treated rats. Serum T_3 levels were decreased, while having high serum T4 levels were connected with an increase in cerebral β amyloid deposition. These results supported by the findings of Christopher et al., (2016), in which they proved that in patients with AD, T_4 levels are about 10 folds higher than normal, showing that a reduction in expression of isoform of the enzyme type 2 deiodinase (D2) or oxidative neurodegeneration cholinergic accompanying this disease occurs alongside the development, which contributes to a reduction of the enzymatic activity D2. Until today there isn't a full understanding of the mechanisms underlying the relationship between serum levels of T4 and cerebral β amyloid (Choi et al., 2017). The conversion of serum T4 to T3 by type 2 deiodinase occurs after serum T4 crosses BBB (Blood-brain barrier) by mono carboxylate transporter 8 and reaches the astrocytes (Morte & Bernal, 2014). The cerebral gene expression of β amyloid precursor protein are suppressed by brain T3 (Belakavadi et al., 2011). In the present study, due to small contribution to brain T3, the serum T3 was not associated with brain amyloid burden. Serum T3 seems to be degraded by tyrosyl ring deiodinase before it reaches the neuronal space that is why in the cerebral cortex, active T3 is predominantly derived from serum T4 rather than serum T3 (Choi et al., 2017). Negative association between brain T3 and β amyloid

precursor protein expression was found from the preclinical studies by using a transgenic mouse model of AD (Contreras-Jurado, and Pascual, 2012). On the other hand, there was a decrease in the $AlCl_3$ melatonin treated albino rat cohorts T4 levels of our study, and it is because of the potent antioxidant role of melatonin which decreases the effects of $AlCl_3$ in deposition of β amyloid (Khidhir, and Ismail, 2016).

Effect of melatonin on some hematological parameters:

a- Effect of melatonin on RBC indices parameters:

The results of current study showed changes in RBC indices parameters of rat's exposure to $AlCl_3$. A decreased number of RBCs, Hb concentration and PCV value after 40 days of $AlCl_3$ exposure were observed. There was a development of normocytic normochromic anemia in the rat's RBC in our study. This kind of anemia has occurred in most of the studies regarding the effects of $AlCl_3$ on erythrocyte parameters (Farina et al., 2002). Our results show improvement of rat's RBC indices after treating them with the melatonin plus $AlCl_3$. In the study achieved by Turgut et al. (2004), noted the alterations of RBC parameters were noticed after 3 months of the oral exposure to aluminum sulfate in mice.

In the biological system, the hem synthesis is disturbed as the aluminum Ions replace Iron and magnesium Ions then reduce Fe^{+2} binding to ferritin (Yakubu; 2017). The other inhibition cause of hem-oxygenase is that this enzyme which is necessary for hemoglobin formation series, would be stopped by the toxicity of Aluminum and increase the destruction of RBCs then RBCs are transformed to bilirubin (Kalaiselve et al., 2015). As a result of Aluminum toxicity, the free radicals are accumulated in the target organs such as liver and it causes the inhibition of glutathione enzyme there. This is an important way of maintaining the hemoglobin in red blood cell and increase the removal of hydrogen peroxide (H_2O_2), also increases the lifetime of red blood cell (Buraimoh et al., 2011). The reduction of some enzyme such as glutathione reductase, catalase, glucose-6-phosphate dehydrogenase were lead to accumulate of toxins inside red cells (Kalaiselve et al., 2015). da Rosa et al., (2010) showed that melatonin decreases the oxidative stress by acting as scavenger of free radicals and provides antioxidant protection of biomolecules. Melatonin reduces the lipoperoxidation levels in liver and erythrocytes and increases the antioxidant enzyme Superoxide dismutase activity in erythrocytes.

b- Effect of melatonin on WBC, differential and platelet count:

Current study showed that $AlCl_3$ has a significant effect in increasing white blood cell, differential granulocyte counts and platelet counts, also a significant effect in decreasing lymphocyte differential counts. This is because of the effect of Aluminum chloride in inducing infections in the target organs such as liver, brain, kidney, spleen and smooth muscle (Kadhum, 2017). In a histopathological study of spleen of $AlCl_3$ induced rats which was conducted by (Buraimoh et al., 2012) showed the same results as it noted the increase in white pulp, bleeding and degeneration of spleen tissues. It's known as this destruction is caused by the effects of free radicals and increase in lipid peroxidation which is the process of initiating tumor formation in target organs.

The antioxidant properties of melatonin against $AlCl_3$ induced toxicity in the WBC and platelet count of rats can be explained by two different mechanisms. The Melatonin scavenges hydroxyl radicals (Kim et al., 2005), which are considered as the highest reactive ROS and, thus, diminish damage of cell structures, including DNA. The second method shows that the Melatonin also reduces oxidative stress by stimulating antioxidant enzymes (Reiter et al., 2003).

Monocytes have shown playing critical roles in the pathogenesis of AD (Gu et al., 2016). However, we could not find any significant discrepancy between AD patients and NCs. Given that, we can say that the effect of monocyte playing in pathogenesis of

AD is mainly determined by its functions rather than its quantity. The defective phagocytosis of monocytes in AD have been confirmed in previous studies, and enrichment of levels of functionally normal monocytes resulted in substantial remission of disease progression in animal models of AD (Fiala et al., 2005).
The decreased number of lymphocytes in AD were recorded by Shad et al., (2013) in which the mechanisms related to the decreased lymphocyte count in AD is the entry of peripheral lymphocytes into the brain and it was induced by elevated neuro inflammations, production of inflammatory mediators, and facilitated by increasing permeability of blood-brain barrier in AD (Chen et al., 2017). From the results of this study it was discovered that melatonin has a therapeutic effect on $AlCl_3$ induced in albino rats which it decreased amount of brain β amyloid, improved, some hematological parameters (RBC count Hb, PCV, WBC count, granulocyte and lymphocyte differential leucocyte counts, platelet count) and T_4 parameters.

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