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Esculin's Antioxidant Effect on Lead Acetate-Induced Neurotoxicity in the C57BL/6 Mice's Hippocampus and Cortex

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ABSTARCT

Lead exposure to heavy metals is linked to significant neuronal damage due to reactive oxygen species-mediated oxidative stress. This research examined esculin's possible neuroprotective effects on the C57bl/6 model of lead (Pb)-induced brain damage. The experiment included four groups of mice: control, lead acetate-treated (10 mg/kg), lead acetate plus esculin (10 mg/kg + 15 mg/kg), and esculin (15 mg/kg) treated alone for 14 days in a row. Brain homogenates were subjected to lead-induced changes in lipid peroxidation, nitric oxide, protein carbonyl, and enzymatic and non-enzymatic activity levels. Examined were histological alterations in the cortex and hippocampal regions. The findings showed that PbAc dramatically reduced glutathione content, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reducase activity while increasing hippocampus and cortical lipid peroxidation and nitrite levels. In the hippocampus and cortex, histological examinations of lead-induced neurotoxicity showed significant damage and a decrease in neuronal density. However, by reestablishing the equilibrium between antioxidants and oxidants and improving motor coordination and memory function, esculin therapy protected hippocampal and cortical neurons against PbAc-induced neurotoxicity. Additionally, esculin reduces the amount of neuronal density and morphological damage in the C57bl/6 mice's cortex and hippocampus. Therefore, the findings implies that esculin could be helpful in preventing neuronal damage caused by lead acetate.

Introduction

The term "neurotoxicity" describes the changes in neurophysiology that result from exposure to dangerous chemicals. These changes might result in mood swings, memory issues, cognitive decline, or the start of mental illnesses.[1-3] Various heavy metals, medications, organophosphates, microorganisms, and animal neurotoxins are among the most prevalent toxicants.[4] One of the most common heavy metal exposures that may significantly harm an animal's or human's neurobehavioral and functional performance is lead. Pb has been linked to oxidative stress and interactions with the antioxidant defense system, both of which increase the risk of oxidative damage to brain systems, according to research.[5] Lead's capacity to bind to sulfur-containing groups in cysteine molecules connected to antioxidant enzymes results in conformational changes that make the enzymes inactive, which is the mechanism behind lead neurotoxicity.These conditions make the cell very susceptible and may result in cell death or apoptosis [6]. Pb may block a variety of enzymes because of its strong affinity for a number of essential functional groups, including amino, carboxyl, and sulfhydryl groups.[7] They include superoxide dismutase (SOD), catalase,

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and reduced glutathione, which are all parts of the antioxidant defense system. This will lead to oxidative stress (OS), which will upset the equilibrium of the antioxidant system and raise the risk of neuronal injury.[8] Ocimum sanctum, or holy basil, contains a coumarin derivative known as esculin (6,7-dihydroxy coumarin-6-o-glucoside).[9] Bursariaspinosa (prickly box), Aesculus california (California buckeye), and Aesculus hippocastanum L. (horse-chestnut). Also reported to include it is dandelion coffee.[10] It has a range of pharmacological actions, including neuroprotective, antidiabetic, antipsychotic, anticancer, antistress, antiinflammatory, hepatoprotective, and anticoagulant qualities. Previous research indicates that esculin had antioxidative stress and anti-inflammatory properties, perhaps via the MAPK signaling pathway, and improved cognitive impairment in experimental diabetic nephropathy.[11] Furthermore, on dopamine-induced cytotoxicity in the SH-SY5Y cell, esculin isolated from F. sielboldiana demonstrated anti-apoptotic actions by shielding mitochondria, increasing SOD activity, lowering GSH level, and also preventing the release of apoptosis-inducing factor.[12] The purpose of the current investigation was to ascertain if esculin may improve the motor and cognitive deficits caused by lead acetate-induced neurotoxicity in the brain's cortical and hippocampus areas.

Materials and Methods

Chemicals

Sigma Aldrich provided the lead acetate. Sigma Chemicals provided the esculin. Analytical grade compounds were employed for all other substances.

Animals

Male C57BL/6 mice weighing between 20 and 25 g apiece were used in the studies. They came from Dr. ALM PGIBMS's central animal house facility at the University of Madras' Taramani campus in Chennai 113, Tamil Nadu, India. The animals were kept on a natural light and dark cycle (14 ± 1h: 10 ± 1h) and housed in conventional laboratory settings (temperature 25 ± 2°C). They had unlimited access to food and drink.

Prior to the experiment, the animals were adjusted to the laboratory environment. The University of Madras, Taramani campus, Chennai-113, Tamil Nadu, India's institutional animal ethic committee (IEAC no. 02/22/2020) authorized the experimental procedures.

Administration of Drugs

Saline (pH-7.4) was used to dilute lead acetate (PbAc), which was then injected intraperitoneally at a dosage of 10 mg/kg b.wt. for a duration of 14 days. After being dissolved in Tween 20, esculin at a dosage of 15 mg/kg B.Wt was administered orally for 14 days.

Animal Classification

The animals were split up into four groups, with six animals in each group.

Group 1: For 14 days, control mice were given physiological saline (0.9%).

Group 2: Lead acetate (10 mg/kg) was given intraperitoneally to mice.

Group 3: For 14 days, mice given 10 mg/kg of lead acetate as an inducer were given 15 mg/kg of b.wt of esculin orally.

Behavior Evaluations

Rotarod experiment

Using a rotarod device, grip strength and motor coordination were evaluated. Before beginning the actual evaluation of the medication treatments, the animals were subjected to a training session to accustom them to the rotarod.

The animals were positioned on a 3 cm diameter, 20 rpm revolving rod. A 120-second time limit was set. Each rat received three distinct trials following a five-minute interval. On day 14, the average decline in time was measured and reported as a count every two minutes.[13]

Morris Water Maze Test: A memory impairment assessment The Morris water maze test was used to see at how well participants learned and retained the spatial navigation task.[14] The test room's 180 cm diameter by 60 cm circular pool served as the platform for the animals' swimming instruction.

A moveable circular platform (9 cm in diameter) set on a column was positioned in the pool 1-cm above the water level during the test. The pool was filled with water to a depth of 40 cm. The animals went through four trials in a training session prior to the injection of lead acetate. Every one of the four trials had a different starting position. For a maximum of two minutes, the latency to locate the escape platform was recorded. For the duration of the experiment, the platform was set in place at one of the four quadrants' centers. The mice were released one at a time at any of the four edges (North, South, East, and West) facing the pool wall, and their acquisition latency was measured by timing how long it took them to get to the platform. The mice's retention of the reaction was then assessed. The transfer latency was defined as the amount of time it took to get to the concealed platform on day 14 after starting esculin therapy. On day 14, the amount of time spent in the target quadrant was also computed.

Test in the open field (OFT)

An open-field test (OFT) was performed for every mouse. Every mouse was positioned in the middle of the open-field device.

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The 90-cm-diameter wooden circle that made up the open-field device. The exam was administered from 09:00 to 12:00. The sole source of light in the resting area was a 60 W light bulb, which was placed 90 to 100 cm above the center and is estimated to be 750 lumens and roughly 375.38 lux (lux = lumen/m2). On day 14, each animal was put in the middle of the open field, and for three minutes, direct visual observations were used to count the number of squares traversed, rearing, and head lowering.[15] During each experiment, the floor was cleaned using a dry paper towel and a wet sponge.

Test of grip strength

A steel wire, measuring 2 mm in diameter and 80 cm in length, was positioned at a height of 50 cm above the cushion support and was accessible for all mice to grasp with their forepaws. It was noted how long the rat was able to hang onto the wire.

Ninety seconds was chosen as the latency to grip loss, which is thought to be an indirect indicator of the grip strength cut-off time.[16]

Biochemical Characteristics

Cortex and hippocampal homogenization

The animals were used for biochemical estimates on day fifteen. The animals were beheaded in order to extract their brains during sacrifice. Both the cortex and the hippocampal regions were extracted from each individual brain tissue.

A homogenate of 10% (w/v) tissue was made using 0.1 M phosphate buffer (pH 7.4). At 10,000 × g, homogenates underwent centrifugation. For biochemical analysis, aliquots of the supernatant were taken and kept separate. The cytosolic fraction was used for all biochemical assays in this investigation.

Biochemical characteristics

The total protein content was calculated using the method outlined by[17], using bovine serum albumin as a reference. Using the technique of [18], malondialdehyde (MDA), a by-product of lipid peroxidation (LPO), was measured at 535 nm and expressed as nmol of MDA released/min/mg protein. The amount of protein carbonyl was calculated using a technique that involves 2, 4-dinitrophenylhydrazine interacting with the carbonyl groups of oxidized proteins to form 2, 4-dinitrophenylhydrazone. The result was represented as nmol/mg protein. The technique of[19] was used to quantify the production of nitric oxide (NO), and the findings were reported as nmol/mg protein. Catalase (CAT) activity was used to measure H2O2 consumption, while pyrogallol auto-oxidation inhibition was used to quantify the enzyme-based antioxidant superoxide dismutase (SOD)[20].[21] Reduced glutathione (GSH) level was determined by reducing 5,5'-dithiobis-2-nitrobenzoic acid to a yellow-colored sulfhydryl molecule, which was measured at 412 nm and expressed as mol of GSH/min/mg protein.[22] The glutathione peroxidase (GPx) activity was measured using this assay.[23] With the help of NADPH, which was detected at 340 nm and catalyzed by GR, GSH was oxidized using this technique. The enzyme activity was expressed as mol NADPH oxidized/min/mg protein, and the degree of protein oxidative damage was determined by measuring the amount of oxidized NADPH in the reaction mixture at 340 nm. The activity of glutathione reductase (GR) was assessed.[24] Glutathione-Stransferase (GST), which was quantified and expressed as nmol CDNB conjugate formed/min/mg protein, catalyzes the formation of glutathione-CDNB couples.[25]

The non-enzymatic antioxidant activity of acetylcholine esterase (AChE) was measured[26] and represented as moles of hydrolyzed substrate/L/min/mg protein. The activity of Na/K+ and Ca2+ ATPase was tested [27], and the result was represented as µmol of released phosphorus/min/mg of protein.

Results

Behavioral Parameters

The impact of esculin on lead acetate-induced alterations in the body weight of experimental and control C57BL/6 mice. Intraperitoneal administration of PbAc (10 mg/kg) resulted in a significant ($p<0.01$) reduction in animal weight in comparison to normal animals. Treatment with esculin (15 mg/kg b.w.) raised weight substantially ($p<0.05$) when compared to the PbAcinduced group. There was no discernible difference between the esculin (15 mg/kg) treatment group and the control group (Fig. 1).

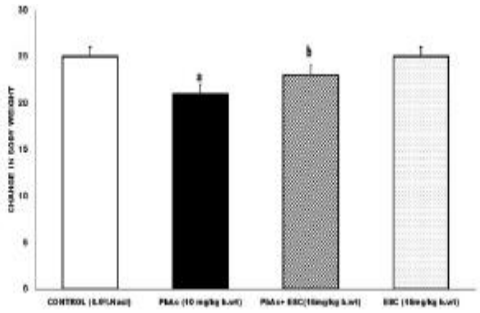

Fig. 1: Impact of esculin on lead acetate-induced alterations in control and experimental subjects' body weight C57BL/6Mice

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The data show the mean ± SD of six mice per group. First group: control; second group: PbAc (10 mg/kg b.w.); third group: PbAc (10 mg/kg b.w.) plus ESL (15 mg/kg b.w.); fourth group: ESL (15 mg/kg b.w.); one-way ANOVA with Tukey's post-hoc test performed to determine aP<0.01 vs control group and bP<0.05 versus PbAc-induced group.

Impact of esculin on lead acetate-induced modifications in the experimental and control rotarod tests C57BL/6 Mice

When compared to untreated animals, mice's muscle grip strength dramatically (p <0.01) diminished after intraperitoneal PbAc therapy. Esculin therapy (15 mg/kg b.w.) substantially (p<0.05) increased muscular strength by reducing fall-off time when compared to the PbAc-induced group. There was no discernible difference between the esculin (15 mg/kg)-treated group and the control group (Fig. 2).

Impact of esculin on lead acetate-induced modifications in the experimental and control trials of the Morris water maze C57BL/6 Mice

To measure memory recall, the Morris water maze test was used. In the normal control group, trained mice's transfer latency progressively reduced over training sessions. On days 7 and 14, the mice given PbAc exhibited a notably longer escape latency in the Morris water maze as compared to the control group (p<0.01). Esculin (15 mg/kg) therapy showed a significant (p<0.05) reduction in transfer latency compared to the PbAc administered group. The esculin (15 mg/kg) group in isolation was compared to

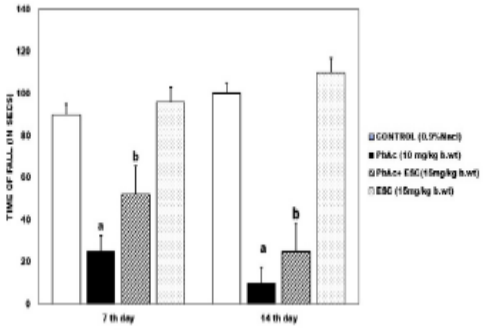

Fig. 2: Impact of Esculin on Lead Acetate-Induced Alterations in Control and Experimental C57BL/6 Mice Rotarod Tests

The data shows the mean ± SD of six mice per group. First group: control; second group: PbAc (10 mg/kg b.w.); third group: PbAc (10 mg/kg b.w.) plus ESL (15 mg/kg b.w.); fourth group: ESL (15 mg/kg b.w.); one-way ANOVA with Tukey's post-hoc test performed to determine aP<0.01 vs control group and bP<0.05 versus PbAc-induced group.

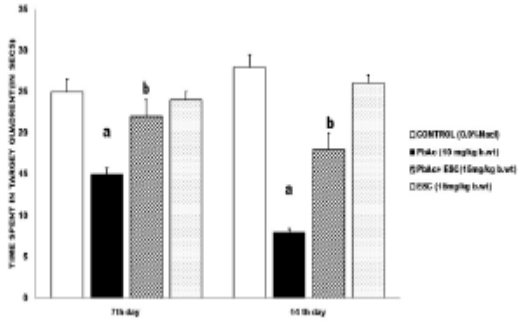

Fig. 3: Esculin's impact on lead acetate-induced modifications in the Morris watermaze test between experimental and control C57BL/6 mice

The data shows the mean ± SD of six mice per group. First group: control; second group: PbAc (10 mg/kg b.w.); third group: PbAc (10 mg/kg b.w.) plus ESL (15 mg/kg b.w.); fourth group: ESL (15 mg/kg b.w.); one-way ANOVA with Tukey's post-hoc test performed to determine aP<0.01 vs control group and bP<0.05 versus PbAc-induced group.

Discussion

The results of this research shown that eating lead acetate causes the antioxidant system to alter significantly and causes oxidative damage to the mouse brain. The executive branch

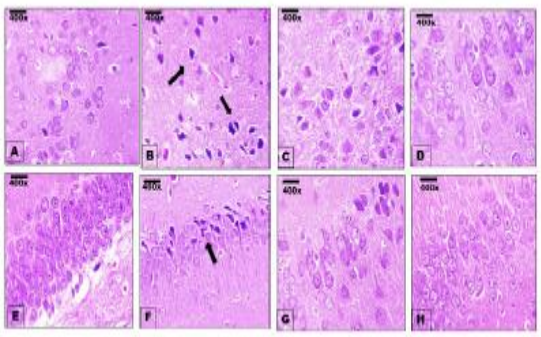

Fig. 8: Impact of Esculin on Histological Alterations Caused by Lead Acetate in the Hippocampus and Cortex of Experimental and Control Mice:

Sections stained with hematoxylin and eosin were seen at a 400x magnification using a microscope. Figures A and E depict the typical neuronal architecture of the hippocampal and cortical regions in control mice.

Figure B&F: Animal Hippocampus and Cortex Induced by PbAc, demonstrating the increased amount of inflammatory (arrow-denoted) and neurodegenerative cells. Figure C&G: The cortex and hippocampal regions treated with PbAc+ESL (15 mg/kg bw) exhibit a modest recovery of degenerative neurons and inflammation. Fig. D&H: The cortex and hippocampal regions of ESL (15 mg/kg bw) treated alone have positive, healthy neurons that do not change and resemble control histology. The data shows the mean ± SD of six mice per group. Group I is the control group; Group II is PbAc (10 mg/kg b.w.); Group III is PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV is ESL (15 mg/kg b.w.); Tukey's post-hoc analysis revealed that aP<0.01 was higher in the PbAc-induced group than in the control group. Adding esculin to lead acetate ingestion mitigated neurobehavioral, biochemical, and histological changes, therefore altering the antioxidant response. During the induction phase of our current investigation, the animals' body weight was initially monitored on a regular basis. The lead-intoxicated rats exhibit a considerably decreased body weight ($p<0.01$) on the last day of our experiment compared to the healthy normal group. According to our observations, lead acetate negatively affects the overall body weight increase of mice. Low food intake, hormone imbalances, and decreased protein levels might be the cause. Growth retardation caused by lead has long been documented.[28] Lead (Pb) also results in acute gastritis and malabsorption, which lowers body weight and reduces calorie intake. The acquired results are consistent with the results of the prior research, which showed that lead induction reduced the pace of growth in mice.[29] When comparing the esculin (15 mg/kg)-treated rats to the lead acetate-induced group, the former exhibit an increase in body weight. This is because esculin has a strong antioxidative impact.

Grip strength and rota rod activity were used in neurobehavioral analysis to measure motor activity. Our research on lead-intoxicated mice suggests that rotarod performance is compromised, which accounts for abnormalities in motor coordination and balance. The current study's findings concurred with those of earlier investigations.[30, 31] Furthermore, the open field test showed that lead poisoning reduced exploratory and locomotor activities. These locomotor anomalies are associated with poor exploratory behavior, which is comparable to previous observations [32, 33]. One may argue that the animals treated with lead have a functional impairment in the hippocampal and cortical regions, which drives them to explore the wide environment. This would explain the low levels of exploratory activity. Esculin (15 mg/kg) therapy markedly increased the mice's motor and muscular activity by perhaps protecting the neurons from oxidative stress.

The hippocampus and associated neural circuits, such as the prefrontal cortex, which supports attention and cognition, are essential for the Morris water maze test.[34, 35]

The hippocampus region's cognitive deficiency caused the lead-intoxicated rats in our research to spend less time in the target quadrants. The learning and memory test findings indicating lead-induced impairments agree with previous authors' reports.[36,37] Compared to the lead-induced animal, the esculin-treated animal spent a lot more time on the target quadrant.

Aerobic metabolism in living cells constantly produces free radicals and other reactive substances. Certain diseases, including neurodegenerative disorders, are largely influenced by free radicals and other reactive compounds during their first stages of development.38 It has been proposed that antioxidants and phenolics may chelate heavy metals via passing through the blood-brain barrier. In the current investigation, we used esculin, a putative coumarin with strong antioxidant qualities that may reduce lead-induced oxidative stress in the hippocampal and cortical tissue after PbAc injection. After exposure to PbAc, treatment with esculin dramatically decreased the high levels of MDA in the cortex and hippocampal tissue. This avoided enhanced lipid peroxidation by quenching peroxide radicals. As a result, esculin therapy greatly decreased lipid peroxidation. In a different experimental setting, esculin inhibited the cortical tissue's enhanced lipid peroxidation in response to exposure to arsenic. The capacity of esculin to scavenge ROS, especially peroxide radicals, may be the cause of the reduction of lipid peroxidation shown in this research. This ability also decreased the high level of cortical NO after PbAc exposure. The findings shown here are consistent with other experimental models that have shown esculin's capacity to reduce excessive NO release and that CoQ10 inhibits iNOS expression. Furthermore, it was shown that esculin increased the activities of GSH, SOD, CAT, GPx, and GR, therefore mitigating the oxidative burst caused by Pb exposure. These antioxidants were found at higher concentrations, which might be the result of their upregulation. These findings are consistent with earlier research on the protective properties and antioxidant activity of esculin in various tissues. [39]

Pb also inhibits energy metabolism by interfering with the activity of Na+/K+-ATPase.38 According to experimental research, Pb suppresses the production of ATP in the brain as well as the concentration of the

ATPase enzyme, Na+ and K+, at nerve ending membranes. This enzyme is in charge of creating and maintaining the ionic gradient required for neuronal excitability.[40, 41] Furthermore, lead's capacity to act as a calcium ion (Ca2+) replacement plays a major role in its ability to cross the blood–brain barrier (BBB). Studies conducted in vitro on brain capillary endothelial cells, the main building block of the blood-brain barrier, have directly shown the involvement of the Ca-ATPase pump in the transfer of lead into the brain.[42] According to our research, esculin preserves synaptic activity in neurons by controlling their neurobehavioral activity and dramatically raised membrane-bound ATPase activity in the tissues of the cortex and hippocampus regions.

According to the current research, co-treating esculin with AChE decreases in Pb-treated brains reversed the effects, suggesting improvements in the cholinergic circuitry. Acetylcholine is hydrolyzed by AChE in cholinergic synapses, a neurotransmitter. Neurological impairment is caused by abnormalities in AChE. Over-stimulation of the cholinergic system impairs prefrontal cortical working memory function.[43] Thus, the prefrontal cortex and hippocampus contribute to working memory and spatial encoding.[44] Conversely, less acetylcholine hinders thinking. Therefore, the current study showed that esculin effectively restored the production of AChE, which would help Pb-intoxicated mice regain equilibrium and normal acetylcholine build-up.

According to histology, lead acetate was neurotoxic in the current investigation; it resulted in cell layer disarray, nerve cell death, and vacuolization in the cortical and hippocampal regions. In addition, the surviving neurons had degenerative appearances, lost their distinctive forms, and were encircled by h aloes. Similar findings indicating the neurotoxic impact of lead acetate have been shown before with the use of various lead exposure techniques.[45–47]

Nevertheless, the histological changes in all of the animals' brains have been protected against lead-induced modifications with little cell death when esculin (15 mg/kg) has been administered.

Lead acetate causes a lot of oxidation and free radicals. Increased oxidation processes in the brain cause synaptic connections, neural networks, neuronal damage, and cell death, all of which are essential for sustaining and regulating behavioral responses. According to the results of our investigation, esculin significantly reduced the harmful effects of lead and *724–732.* may have substantial antioxidant and neuroprotective properties. that neutralize and balance free radicals, undoing all changes.

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