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Research Article

The Antioxidative Effect of Esculin on Lead Acetate-induced Neurotoxicity in the Hippocampus and Cortex of C57BL/6 Mice

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ABSTRACT

Heavy metal exposure to lead is associated with severe neuronal impairment through oxidative stress mediated by reactive oxygen species. This study investigated the potential neuroprotective effect of esculin on the lead (Pb)-induced brain neurotoxicity C57bl/6 model. Four groups of mice were used for the study (control, lead acetate-treated (10 mg/kg), lead acetate and esculin (10 mg/kg +15 mg/kg) and esculin (15 mg/kg) alone treated for 14 consecutive days. Lead-induced alterations in the level of lipid peroxidation, nitric oxide, protein carbonyl, and enzymatic and non-enzymatic activity were measured in brain homogenates. Histological changes in the hippocampus and cortex were also examined. The results documented that PbAc significantly increased hippocampal and cortical lipid peroxidation and nitrite levels and decreased glutathione content, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. Histological observations of lead-induced neurotoxicity revealed severe damage and a reduction in neuronal density in the hippocampus and cortex. However, treatment with esculin rescued hippocampal and cortical neurons from PbAc-induced neurotoxicity by restoring the balance between oxidants and antioxidants and improvise motor coordination and memory activity. Esculin also attenuates the morphological damage and neuronal density in the hippocampal and cortex regions of C57bl/6 mice. Hence, the study suggests that esculin may be useful in combating lead acetate-induced neuronal injury.

Introduction

The word "neurotoxicity" refers to the neurophysiological alterations brought on by exposure to hazardous substances. These modifications may cause mood swings, memory problems, cognitive impairments, or the onset of mental disorders.[1–3] The most common toxicants include various heavy metals, pharmaceuticals, organophosphates, microbes, and animal neurotoxins.[4] Lead is one of the most prevalent heavy metal exposures that can seriously impair neurobehavioral and functional functioning in both people and animals. Research has shown that Pb generates oxidative stress and interacts with the antioxidant defense system, which might result in oxidative damage to brain systems.[5] The mechanism of lead neurotoxicity involves the ability to attach to sulfur-containing groups present in the cysteine molecule linked to antioxidant enzymes, causing conformational changes that render them inactive.[6] These instances render the cell exceedingly vulnerable and may cause apoptosis or cell death. Pb has a high affinity for several crucial functional groups, such as sulfhydryl, carboxyl, and amino groups, which allows it to inhibit a range of enzymes.[7] These comprise components of the antioxidant defense system, including catalase, reduced glutathione, and superoxide dismutase (SOD). This will cause oxidative stress (OS), disrupting the antioxidant system's homeostasis and increasing the likelihood of neuronal damage.[8]

Esculin (6,7-dihydroxy coumarin-6-o-glucoside) is a coumarin derivative found in *Ocimum sanctum* (Holy basil).[9] *Aesculus hippocastanum* L. (Horse-chestnut), *Aesculus california* (California buckeye) and *Bursaria spinosa* (prickly box). It has also been found in dandelion

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coffee.[10] It possesses various pharmacological effects, such as antioxidant, antistress, antiinflammatory, hepatoprotective, antidiabetic, antipsychotic, anticancer, and anticoagulant properties and also possesses neuroprotective properties. Earlier studies show that esculin ameliorated cognitive impairment in experimental diabetic nephropathy and exhibited antioxidative stress and antiinflammatory effects, potentially via the MAPK signaling pathway.[11] Additionally, esculin extracted from *F. sielboldiana* showed anti-apoptotic effects on dopamine-induced cytotoxicity in the SH-SY5Y cell by protecting mitochondria, elevating SOD activity and reducing GSH level, and also inhibiting the release of apoptosis-inducing factor.[12] The present study aimed to determine whether esculin ameliorates motor and cognitive impairments in lead acetate-induced neurotoxicity in the hippocampal and cortical regions of the brain.

Materials and Methods

Chemicals

Lead acetate was purchased from Sigma Aldrich. Esculin was obtained from sigma chemicals. All other chemicals used were of analytical grade.

Animals

Studies were carried out using male C57BL/6 mice weighing between 20 to 25 g each. They were obtained from the central animal house facility of Dr. ALM PGIBMS, University of Madras, Taramani campus, Chennai 113, Tamil nadu, India. The animals were housed under standard laboratory conditions (temperature 25 ± 2°C) and maintained on natural light and dark cycle (14 ± 1h: 10 ± 1h). They had access to food and water *ad libitium.* Animals were acclimatized to laboratory conditions before the experiment. The experimental protocols were approved by the institutional animal ethic committee (IEAC no. 02/22/2020) Dr. ALM PGIBMS, University of Madras, Taramani campus, Chennai-113, Tamil nadu, India.

Drug Administration

Lead acetate (PbAc) was diluted with saline (pH-7.4) and administrated intraperitoneally at the dose of 10 mg/kg b.wt. for 14 days. Esculin at the dose of 15 mg/kg b.wt was dissolved in tween 20 and administrated orally for 14 days.

Grouping of Animals

Animals were divided into four groups and each group comprised six animals.

Group 1: Control mice received physiological saline (0.9%) for 14 days

Group 2: Mice were administrated with lead acetate (10 mg/kg) intraperitoneally

Group 3: Mice induced with lead acetate (10 mg/kg) were treated orally with esculin (15 mg/kg of b.wt) for 14 days.

Group 4: Mice were orally treated with esulin (15 mg/kg of b.wt) for 14 days.

Measurement of Body Weight

Body weight was recorded on the first and last day of the experiment. Percent changes in
Body weight was calculated as

$$\frac{\text{Body weight } (1^{st} \text{ day-} 14^{th} \text{ day})}{\text{Body weight on } 1^{st} \text{ day}} \times 100$$

Behavioral Assessments

Rotarod test

Motor coordination and grip strength were assessed using a rotarod apparatus. The animals were exposed to a training session to acclimate them to the rotarod before starting the actual assessment of the drug treatments. Animals were placed on a rotating rod with a diameter of 3 cm (speed, 20 rpm). The cut-off time was 120s. Three separate trials after 5 minutes gap were given to each rat. The average fall in time was recorded on day 14 and expressed as a count per 2 minutes.[13]

Morris Water Maze Test

(Assessment of the memory impairment)

The acquisition and retention of the spatial navigation task were examined using the Morris water maze test.[14] The animals were trained to swim on a platform in a circular pool (180 cm diameter, 60 cm) located in the test room. The pool was filled with water to a depth of 40 cm, and a movable circular platform (9 cm in diameter) mounted on a column was placed in the pool 1-cm above the water level for the test. Before lead acetate administration, the animals underwent a training session of four trials. In all 4 trials the starting positions were different in all four trials. The latency to find the escape platform was recorded for a maximum of 2 minutes. The platform was fixed at the center of one of the four quadrants and remained in that location for the duration of the experiment. The time taken by the mice to reach the platform was recorded as acquisition latency, and the mice were randomly released individually at any one of the edges (North, South, East, West) facing the wall of the pool and tested for the retention of the response. The time taken to reach the hidden platform on day 14 following esculin treatment was recorded and termed as the transfer latency. The time spent in the target quadrant on day 14 was also calculated.

Open field test (OFT)

All mice were subjected to an open-field test (OFT). Each mouse was placed at the center of the open-field apparatus. The open-field apparatus consisted of a circle made of wood, 90 cm in diameter. The test was performed between 09.00 and 12.00 hours. A 60 W light bulb (estimated at approximately 750 lumens and approximately 375.38 lux;

lux = lumen/m^2 was positioned 90 to 100 cm above the center and provided the only source of illumination in the resting room. Each animal was placed in the center of the open field, and the number of squares crossed, number of rearing, and head dipping were measured on day 14 through direct visual observations for 3 minutes.[15] The floor was cleaned with a wet sponge and dry paper towel during each trial.

Grip strength test

All mice were allowed to hold with the forepaws a steel wire (2 mm in diameter and 80 cm in length) placed at a height of 50 cm over the cushion support. The length of time that the rat was able to hold the wire was recorded. This latency to grip loss considered an indirect measure of grip strength cut-off time, was taken as 90 seconds.[16]

Biochemical Parameters

Homogenization of hippocampus and cortex

On day 15, the animals were used for biochemical estimations. The animals were sacrificed and their brains were removed by decapitation. The hippocampus and cortex were separated from each isolated brain tissue. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). Homogenates were centrifuged at 10,000 × g. Aliquots of the supernatant were separated and used for biochemical analyses. In this study, all biochemical analyses were performed on the cytosolic fraction.

Biochemical parameters

Using bovine serum albumin as a reference, the method described by[17] was used to determine the total protein content. Malondialdehyde (MDA), a by-product of lipid peroxidation (LPO), was quantified at 535 nm using the method of[18] and expressed as nmol of MDA released/min/mg protein. Using a method in which 2, 4-dinitrophenylhydrazine interacts with the carbonyl groups of oxidized proteins to create 2, 4-dinitrophenylhydrazone, the quantity of protein carbonyl was estimated, and the result was expressed as nmol/mg protein. Nitric oxide (NO) synthesis was measured using the method of[19] and the results were reported as nmol/mg protein. Superoxide dismutase (SOD), an enzyme-based antioxidant, was quantified by pyrogallol auto-oxidation inhibition,[20] and H_2O_2 consumption was determined by catalase (CAT) activity.[21] By reducing 5,5'-dithiobis-2-nitrobenzoic acid to a yellow-colored sulfhydryl molecule, which was detected at 412 nm and expressed as mol of GSH/min/mg protein, the reduced glutathione (GSH) level).[22] The assay was used to measure the activity of glutathione peroxidase (GPx).[23] This method involved oxidizing GSH with NADPH, which was catalyzed by GR and measured at 340 nm. Protein oxidative damage was assessed using by measuring oxidized NADPH in the reaction mixture at 340 nm and the enzyme activity was reported as mol NADPH oxidized/min/mg protein. Glutathione reductase (GR) activity was evaluated.[24] The production of glutathione-CDNB couples is catalyzed by glutathione-S-transferase (GST), which was measured and expressed as nmol CDNB conjugate formed/min/mg protein.[25] Acetylcholine esterase (AChE) non-enzymatic antioxidant activity was assessed[26] and was expressed as moles of substrate hydrolyzed/L/min/mg protein. Na/K^+ and Ca^{2+} ATPase activity was measured[27] and the enzyme activity was expressed as µmol of phosphorus liberated/min/mg of protein.

Histological evaluation of the hippocampus and cortex

On day 14^{th}, cervical decapitation was performed by sacrificing animals. The brain was carefully removed without injury after opening the skull. The hippocampal and cortical regions were then removed and fixed in 10% buffered formalin. The samples were dehydrated using ethanol, followed by xylazine. They were then embedded in paraffin at 56°C in hot water for 24 hours. A slide microtome prepared paraffin wax tissue blocks for sectioning at 4 µm thickness. Tissue sections were collected on glass slides, deparaffinized, stained with hematoxylin and eosin, and examined under a light microscope.

Results

Behavioral Parameters

Effect of esculin on lead acetate induced changes in the body weight of control and experimental C57BL/6 Mice

PbAc (10 mg/kg) administered intraperitoneally caused a substantial ($p<0.01$) decrease in animal weight when compared to normal animals. When compared to the PbAc-induced group, treatment with esculin (15 mg/kg b.w.) significantly ($p<0.05$) increased weight. The group treated with esculin (15 mg/kg) alone did not differ significantly from the control group (Fig. 1).

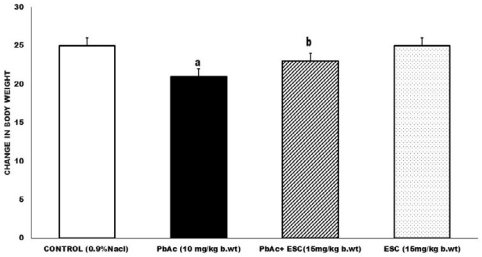

Fig. 1: Effect of esculin on induced lead acetate changes in the body weight of control and experimental C57BL/6Mice

Data represent the mean ± SD of 6 mice in each group. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); aP<0.01 versus control group, bP<0.05 versus PbAc induced group (one-way ANOVA followed by Tukey's post-hoc test).

Effect of esculin on lead acetate-induced changes in rotarod test of control and experimental C57BL/6 Mice

Mice muscular grip strength significantly (p <0.01) decreased after intraperitoneal PbAc treatment compared to untreated animals. When compared to the PbAc-induced group, treatment with esculin (15 mg/kg b.w.) significantly (p<0.05) enhanced muscle strength by decreasing fall-off time. The group treated with esculin (15 mg/kg) did not significantly differ from the control group (Fig. 2).

Effect of esculin on lead acetate-induced changes in morris water maze test of control and experimental C57BL/6 Mice

The Morris water maze test was used to gauge memory recall. During training sessions, trained mice transfer latency gradually decreased in the normal control group. However, PbAc-administered mice showed significantly longer escape latency in the Morris water maze on days 7 and 14 compared to the control group (p<0.01). In comparison to the PbAc given group, esculin (15 mg/kg) treatment demonstrated a substantial (p<0.05) improvement in transfer delay. Comparing the esculin (15 mg/kg) alone group to the control, there was no discernible difference (Fig. 3).

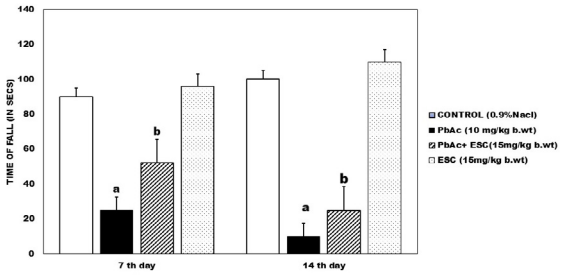

Fig. 2: Effect of Esculin on Lead Acetate induced changes in Rotarod test of control and experimental C57BL/6 Mice

Data represent mean ± SD of 6 mice in each group. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); aP<0.01 versus control group, bP<0.05 versus PbAc induced group (one-way ANOVA followed by Tukey's post-hoc test).

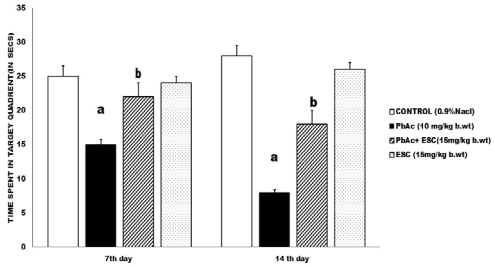

Fig. 3: Effect of Esculin on Lead Acetate induced changes in morris watermaze test of control and experimental C57BL/6 Mice

Data represent mean ± SD of 6 mice in each group. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); aP<0.01 versus control group, bP<0.05 versus PbAc induced group (one-way ANOVA followed by Tukey's post-hoc test).

Effect of esculin on lead acetate induced changes in locomotor activity in open field test of control and experimental C57BL/6 Mice

Animal mobility was significantly (p <0.01) reduced after intraperitoneal PbAc treatment when compared to untreated mice. Esculin (15 mg/kg b.w.) treatment significantly (p <0.05) improved animal mobility. When compared to the control group, the esculin (15 mg/kg b.w.) treated group showed no discernible difference (Fig. 4).

Effect of esculin on grip strength test in pbac induced control and experimental C57BL/6 Mice

The PbAc-treated mice showed noticeably (p < 0.01) shorter hanging times than the control mice, indicating a larger loss of grip. After treatment with esculin in PbAc induced group (15 mg/kg b.w.), a significant improvement was observed in grip strength, and there was a discernible difference between the control group and the Esulin (15 mg/kg b.w.) alone treated group (Fig. 5).

Biochemical Parameters

Effect of esculin on lead acetate induced changes in the levels of LPO, NO, and protein carbonyl in hippocampus and cortex of control and experimental C57BL/6 Mice

Mice administered with PbAc showed a significant (p<0.01) increase in the level of peroxidation, nitric oxidation, and protein carbonyl content compared to the control. Upon simultaneous treatment with esculin (15 mg/kg b.w.) in PbAc induced group, there was a significant (p<0.05) decrease in the levels of LPO, NO, and protein carbonyl, whereas no significant change was observed in esculin alone (15 mg/kg) treated group as compared to control (Table 1).

Effect of esculin on lead acetate induced changes in the activity of SOD, CAT, GPX, GR, GSH, and GST in the hippocampus and cortex of control and experimental C57BL/6 Mice

SOD, CAT, GPX, GR, GSH, and GST levels were significantly (p <0.01) lower in PbAc-treated animals than in control

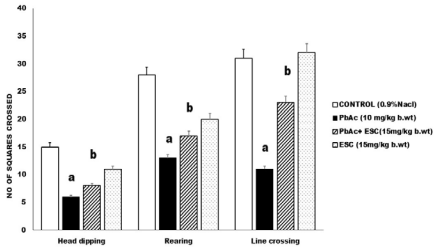

Fig. 4: Effect of Esculin on Lead Acetate induced changes in open field test of control and experimental C57BL/6 Mice

Data represent mean ± SD of 6 mice in each group. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); aP<0.01 versus control group, bP<0.05 versus PbAc induced group (one-way ANOVA followed by Tukey's post-hoc test).

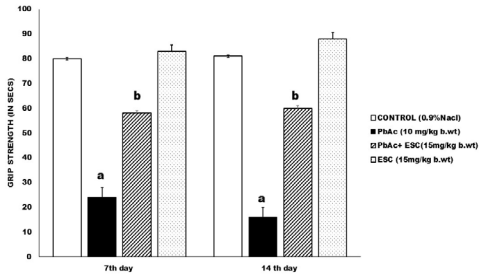

Fig. 5: Effect of Esculin on Lead Acetate induced changes in Grip strength test of control and experimental C57BL/6 Mice

Data represent mean ± SD of 6 mice in each group. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); aP<0.01 versus control group, bP<0.05 versus PbAc induced group (one-way ANOVA followed by Tukey's post-hoc test

mice. Esculin (15 mg/kg b.w.) treatment considerably (p< 0.05) boosted the levels of all these enzymes in PbAc-treated mice. The group treated with esculin alone (15 mg/kg) did not differ significantly from the control group (Table 2).

Effect of esculin on lead acetate induced changes in the activities of Na/K ATPase, Ca^{2+} ATPase, and Mg^{2+} ATPase in hippocampus and cortex of control and experimental C57BL/6 Mice

Compared to the control group, the Na/K ATPase, Ca ATPase, and Mg2+ ATPase levels were significantly (p <0.01) in the PbAc-induced group. Esculin (15 mg/kg) was administered concurrently, and this considerably (p< 0.05) raised the levels of ATPases. Comparing the Esculin (15 mg/kg) alone group to the control, there was no discernible difference (Fig. 6).

Effect of esculin on lead acetate induced changes in the level of acetylcholine esterase in hippocampus and cortex of control and experimental C57BL/6 Mice

PbAc induced group showed a significant (p<0.01) decrease in the level of acetylcholine activity as compared to the control group. Simultaneous treatment with esculin (15 mg/kg) significantly (p<0.05) increased the levels of acetylcholine. There was no significant change in the esculin (15 mg/kg) alone group compared to the control (Fig. 7).

Table 1: Effect of esculin on lead Acetate induced alternations in the levels of LPO, NO, and protein carbonyls in the hippocampus and cortex of control and experimental mice

Biochemical estimations	*Regions*	*CONTROL (0.9 %NaCl)*	*PbAc (10 mg/kg b.wt)*	*PbAc+ ESC (15 mg/ kg b.wt)*	*ESC (15 mg/kg b.wt)*
LPO (Units/mg of protein)	Hippocampus	8.32 ± 0.77	22.56 ± 0.71[a]	16.47 ± 0.14[b]	10.66 ± 0.22
	Cortex	10.23 ± 0.03	25.76 ± 0.01[a]	13.17 ± 0.11[b]	11.04 ± 0.02
NO (Units/mg of protein)	Hippocampus	1.14 ± 0.21	4.56 ± 0.37[a]	3.88 ± 0.26[b]	2.66 ± 0.35
	Cortex	0.28 ± 0.01	5.16 ± 0.06[a]	2.19 ± 0.14[b]	1.47 ± 0.07
PROTEIN CARBONYL (Units/mg of protein)	Hippocampus	4.34 ± 0.11	12.16 ± 0.17[a]	8.08 ± 0.26[b]	2.08 ± 0.04
	Cortex	3.28 ± 0.01	9.46 ± 0.03[a]	5.19 ± 0.14[b]	1.07 ± 0.03

Data represents mean ± SD of 6 mice in each group. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); aP<0.01 versus control group, bP<0.05 versus PbAc induced group (one-way ANOVA followed by Tukey's post-hoc test).

Table 2: Effect of esculin on lead acetate induced alternations in the level of SOD, CAT, GPX, GR, GST, GSH in the hippocampus and cortex of control and experimental mice

Biochemical estimations	*Regions*	*CONTROL (0.9 %Nacl)*	*PbAc (10 mg/kg b.wt)*	*PbAc+ ESC(15 mg/ kg b.wt)*	*ESC(15 mg/kg b.wt)*
SOD (Units/mg of protein)	Hippocampus	30.01 ± 0.13	05.13 ± 0.20[a]	12.37 ± 0.34[b]	28.14 ± 0.07
	Cortex	26.56 ± 0.19	06.24 ± 0.56[a]	16.29 ± 0.06[b]	25.84 ± 0.09
CAT (µmol of H2O2 reduced/min/mg protein)	Hippocampus	4.45 ± 0.26	1.02 ± 0.11[a]	2.10 ± 0.14[b]	4.06 ± 0.05
	Cortex	6.19 ± 0.36	0.52 ± 0.19[a]	3.30 ± 0.08[b]	5.76 ± 0.18
GPX (nmol of GSH consumed / min/mg protein)	Hippocampus	30.14 ± 0.04	12.99 ± 0.56[a]	22.63 ± 0.25[b]	29.33 ± 0.19
	Cortex	25.87 ± 0.02	10.54 ± 0.12[a]	18.43 ± 0.16[b]	23.45 ± 0.16
GR (µmol of NADPH oxidized/ min/mg protein)	Hippocampus	2.03 ± 0.04	0.14 ± 0.01[a]	1.80 ± 0.53[b]	1.95 ± 0.29
	Cortex	2.73 ± 0.24	0.75 ± 0.91[a]	1.30 ± 0.63[b]	1.55 ± 0.26
GST (nmol of CDNB conjugated/ min/mg protein)	Hippocampus	18.77 ± 0.39	3.00 ± 0.01[a]	11.85 ± 0.11[b]	15.54 ± 0.36
	Cortex	20.15 ± 0.43	2.20 ± 0.61[a]	14.45 ± 0.31[b]	18.54 ± 0.74
GSH (µmol of glutathione/min/mg protein)	Hippocampus	28.45 ± 0.32	12.41 ± 0.41[a]	20.71 ± 0.84[b]	25.67 ± 0.07
	Cortex	30.14 ± 0.12	10.24 ± 0.67[a]	21.81 ± 0.42[b]	28.76 ± 0.23

Data represent mean ± SD of 6 mice in each group. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); aP<0.01 versus control group, bP<0.05 versus PbAc induced group (one-way ANOVA followed by Tukey's post-hoc test).

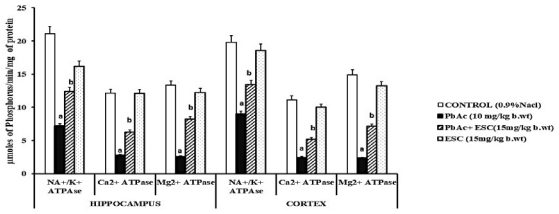

Fig. 6: Effect of Esculin on Lead Acetate induced induced alternations in the levels of membrane bound ATPases in the hippocampus and cortex of control and experimental mice

Data represent mean ± SD of 6 mice in each group. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); aP<0.01 versus control group, bP<0.05 versus PbAc induced group (one-way ANOVA followed by Tukey's post-hoc test).

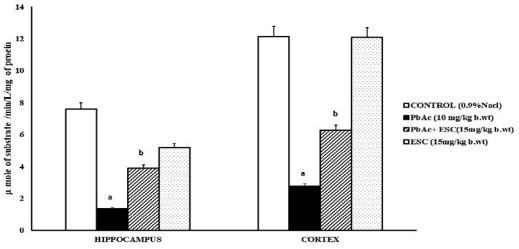

Fig. 7: Effect of Esculin on Lead Acetate induced alternations in the levels of Acetylcholine esterase activity in the hippocampus and cortex of control and experimental mice

Data represents mean ± SD of 6 mice in each group. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); aP<0.01 versus control group, bP<0.05 versus PbAc induced group (one-way ANOVA followed by Tukey's post-hoc test).

Effect of esculin on lead acetate induced histological changes in the hippocampus and cortex of control and experimental C57BL/6 Mice

H&E staining was used to look for aberrant structures in the brain tissues of the control, PbAc-induced neurotoxicity, and treatment groups in the hippocampal and cortical regions of the brain. A&E) Control mouse hippocampus and cortex had typical neuronal tissue. B&F) When compared to the other groups that had received esculin treatment, the PbAc-induced group displayed more inflammatory, degenerative cells. Neuronal swelling, vacuolation with condensed nuclei, and acute inflammation are signs of this C & G. In the cortex and hippocampus of esculin (15 mg/kg)-treated mice, there were fewer degenerating neurons and more normal neuronal cells. D&H) Animals treated with esculin (15 mg/kg) alone exhibited healthy neurons in the hippocampus and cortex, demonstrating the neuroprotective action of esculin against lead acetate-induced neurotoxicity (Fig. 8).

Discussion

The present study showed that lead acetate consumption induces significant changes in the antioxidant system and oxidative damage in the mouse brain. The administration

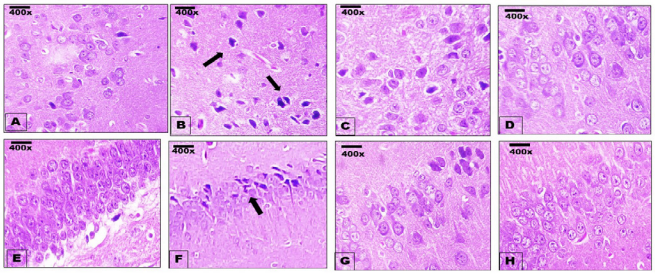

Fig. 8: *Effect of Esculin on Lead Acetate induced histological alternations in the hippocampus and cortex of control and experimental mice: Hematoxylin and eosin-stained* sections were visualized under the microscope at a magnification of 400x. Fig. A&E: Control mice showing the normal neuronal structure of the hippocampus and cortex region. Fig. B&F: PbAc-induced animal Hippocampus and Cortex showing the greater extent of inflamed (denoted by arrow) and neurodegenerative cells. Fig. C&G: PbAc+ESL (15 mg/kg bw) treated Hippocampus and Cortex showing mild recovery of inflammation and degenerative neurons. Fig. D&H: Hippocampus and Cortex of ESL (15mg/kg bw) alone treated showing positive healthy neurons with no alterations they resemble that of control histology. Data represent mean ± SD of 6 mice in each group. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); aP<0.01 versus control group, bP<0.05 versus PbAc induced group (one-way ANOVA followed by Tukey's post-hoc test).

of esculin to lead acetate consumption modified the antioxidant response by mitigating neurobehavioral, biochemical and histological alterations. Initially, in our present study, the body weight of the animals was regularly analyzed during the course of induction. On the final day of our experiment, the lead intoxicated animals show a significantly lower body weight ($p< 0.01$) than that of the healthy normal group. Our observation indicates that lead acetate has an adverse effect on mice's total body weight gain. It may be due to low food consumption, hormonal imbalance and reduction in protein levels. Lead induced growth retardation is already reported.[28] Moreover, lead (Pb) causes severe gastritis and malabsorption, leading to less calorie consumption and depressing body weight. The obtained results are in agreement with the findings in the previous study, where lead induction caused decreases in growth rate in rodents.[29] The animals treated with esculin (15 mg/kg) show a rise in body weight in comparison with lead acetate-induced group. This is due to the effective antioxidative effect of esculin.

In neurobehavioral analysis, the motor activity was assessed using rota rod activity and grip strength. Our findings on lead-intoxicated mice indicate that rotarod performance is impaired, which explains balance and motor coordination deficits. The results of the present study were in agreement with the results of previous studies.[30,31] In addition, the open field test revealed that lead poisoning decreased locomotor and exploratory activity. These locomotor abnormalities are similar to earlier findings[32,33] in that they are accompanied by poor exploratory behavior. It might be claimed that the low levels of exploratory behavior are a result of the lead-

treated animal's functional defect in the hippocampus and cortex region, which motivates them to explore the open field arena. The treatment with esculin (15 mg/kg) significantly enhanced the motor and muscular activity of the mice through its potential antioxidative activity to the neurons.

Morris water maze task depends on the hippocampus and related neuronal circuitry, including the prefrontal cortex which subserves attention and cognition.[34,35] In our present study, the lead-intoxicated animals spent lesser time in target quadrants due to the cognitive deficit in the hippocampal region. The results of lead-induced defects in the learning and memory tests are consistent with the reports of other authors.[36,37] Treatment with esculin significantly increased the time spent on the target quadrant when compared to the lead induced animal.

Free radicals and other reactive species are continuously produced by aerobic metabolism in living cells. Free radicals and other responsive substances play a significant role in the movement or inception of certain illnesses, such as neurodegenerative disorders.[38] It has been hypothesized that phenolics and antioxidants can cross the blood-brain barrier to chelate heavy metals. Our present study used esculin, a potential coumarin with significant antioxidative properties that can lower lead induced oxidative stress in the hippocampus and cortical tissue after PbAc injection. Treatment with esculin significantly reduced the elevated level of MDA in the hippocampus and cortical tissue following PbAc exposure, which prevented increased lipid peroxidation by quenching peroxide radicals. Thus, esculin treatment significantly reduced lipid peroxidation. In another experimental model, esculin prevented increased lipid peroxidation in the cortical tissue in response to arsenic exposure. The suppression of lipid peroxidation observed in this study may be due to the ability of esculin to scavenge ROS, particularly peroxide radicals, which also reduced the elevated level of cortical NO following PbAc exposure. Various experimental models have reported the ability of esculin to attenuate excessive NO release and have suggested that CoQ10 suppresses iNOS expression, corresponding with the results presented here. Furthermore, esculin was found to counteract the oxidative burst produced following Pb exposure by increasing the activity of GSH, SOD, CAT, GPx, and GR. We observed increased levels of these antioxidants, which may be due to their upregulation. These results concur with those of previous studies on the antioxidant activity and protective effects of esculin in different tissues. [39]

Pb also inhibits energy metabolism by disrupting Na+/K+-ATPase activity.[38] Experimental studies have shown that Pb inhibits Na+, K+ - ATPase enzyme highly concentered at nerve ending membranes, responsible for generating and maintaining the ionic gradient necessary for neuronal excitability, and ATP produced in the brain.[40,41] Further, the ability of lead to pass through the blood–brain barrier (BBB) is largely due to its ability to substitute for calcium ions (Ca^{2+}). Direct evidence for the role of the Ca-ATPase pump in the transport of lead into the brain has been provided by in vitro studies on brain capillary endothelial cells, the primary constituent of the BBB.[42] In our study, esculin significantly increased the membrane bound ATPase activity in the hippocampal and cortex tissues and protects the synaptic activity in neurons by regulating their neurobehavioral activity.

The present study shows reductions in AChE expression in Pb-treated brains were reversed via co-treatment with esculin indicating improvements in cholinergic circuitry. In the cholinergic synapses, AChE hydrolyzes the neurotransmitter acetylcholine. Dysfunctions in AChE cause neurological dysfunction. The over-activation of the cholinergic system hinders working memory activity in the prefrontal cortex.[43] Hence, the hippocampal–prefrontal cortex assists in spatial encoding and working memory.[44] On the other hand, diminished acetylcholine impairs cognition. As a result, the present investigation demonstrated that esculin efficiently restored AChE expression, which may aid in restoring equilibrium to regular acetylcholine build-up in Pb-intoxicated mice.

Histologically, in the present study lead acetate was neurotoxic; it caused disorganization of cell layers, loss of nerve cells and vacuolization in the regions of the regions of hippocampus and cortex. Moreover, the remaining neurons lost their characteristic shapes, appeared degenerated, and were surrounded by haloes. Similar findings for the neurotoxic effect of lead acetate have been reported earlier using different protocols for exposure to lead.[45-47] However, the administration of esculin (15 mg/kg) to the lead induced animals has protected the histological alterations in all brains with minimal loss of cells.

Lead acetate produces a high number of free radicals and oxidation. Increased oxidation mechanisms initiate neural destruction, cell death, synaptic connections, and neural networks in the brain which is crucial for maintaining and controlling behavioral responses. Our study concludes that the deleterious effects of Pb were greatly reduced upon administration with esculin, suggesting its strong antioxidant and neuroprotective potential. That eliminate and balance free radicals, thus reverting all alterations.

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