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Protective Effect of Esculin Against Lead-Induced Brain Oxidative Stress in C57BL/6 Mice

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ABS TRA C T

Due to oxidative stress caused by reactive oxygen species, heavy metal exposure to lead is linked to significant neuronal damage. This research examined esculin's possible neuroprotective effects using the C57bl/6 model of lead (Pb)-induced brain damage. The research used four mouse groups: 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 used to detect changes brought on by lead in the levels of lipid peroxidation, nitric oxide, protein carbonyl, and enzymatic and non-enzymatic activity. Additionally, histological alterations in the cortex and hippocampal regions were investigated. The findings showed that PbAc dramatically reduced glutathione content, superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity while increasing nitrite and hippocampus and cortical lipid peroxidation levels. The hippocampus and cortex showed significant damage and a decrease in neuronal density, according to histological findings of lead-induced neurotoxicity. However, by reestablishing the equilibrium between oxidants and antioxidants, esculin therapy saved hippocampal and cortical neurons from PbAc-induced neurotoxicity and improved memory and motor coordination. Esculin also reduces the amount of neuronal density and morphological damage in the C57bl/6 mice's brain and hippocampus. Esculin may thus be helpful in preventing lead acetate-induced neuronal damage, according to the research.
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INTRODUCTION

to antioxidant enzymes, causing conformational changes

The term "neurotoxicity" describes the changes in the nervous system caused by exposure to dangerous chemicals. Mood swings, memory issues, cognitive deficits, or the beginnings of mental diseases may all result from these changes. [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 affect an animal's or human's neurobehavioral and functional performance is lead. According to research, lead causes oxidative stress and interacts with the antioxidant defense system, potentially leading to oxidative damage to brain systems. [5] The capacity to bind to sulfur-containing groups found in the cysteine molecule associated to

lead is the mechanism of lead neurotoxicity. that make them dormant. [6] These situations make the cell very susceptible and may result in cell death or apoptosis. Pb may block a variety of enzymes because of its strong affinity for a number of essential functional groups, including sulfhydryl, carboxyl, and amino groups. [7] These include superoxide dismutase (SOD), reduced glutathione, and catalase, which are elements of the antioxidant defense system. This will result in oxidative stress (OS), which will upset the equilibrium of the antioxidant system and raise the risk of neuronal damage. [8] Ocimum sanctum, or holy basil, contains a coumarin derivative called esculin (6,7-dihydroxy coumarin-6-o-glucoside). [9] Bursaria spinosa (prickly box), Aesculus california (California buckeye), and Aesculus hippocastanum L. (horse chestnut). Additionally, it has been discovered in dandelion.

coffee. [10] Antioxidant, antistress, anti-inflammatory, hepatoprotective, antidiabetic, antipsychotic, anticancer, and anticoagulant are only a few of its pharmacological actions. It also has neuroprotective qualities. Previous research indicates that esculin demonstrated antioxidative stress and anti-inflammatory properties, perhaps via the MAPK, and improved cognitive impairment in experimental diabetic nephropathy.

Group 4: For 14 days, mice received an oral dose of esulin (15 mg/kg of body weight).

Measurement of Body Weight

On the first and end days of the trial, body weight was measured. Body weight (1st day–14th day) was used to compute the percentage changes in body weight.

signaling chain. [11] Furthermore, F. sielboldiana esculin demonstrated anti-apoptotic actions in dopamine-

weight on the first day × 100

prevented the release of apoptosis-inducing factor, increased SOD activity, decreased GSH levels, and protected mitochondria to cause cytotoxicity in the SH-SY5Y cell. [12] The goal of the current investigation was to ascertain if esculin improves motor and cognitive deficits in the hippocampus and cortical areas of the brain caused by lead acetate-induced neurotoxicity.

Chemicals, Materials, and Procedures

Sigma Aldrich was the supplier of lead acetate. Sigma Chemicals provided the esculin. Every additional chemical that was used was analytical grade.

Animals Male C57BL/6 mice weighing 20–25 g each were used in the studies. Dr. ALM PGIBMS's central animal house facility at the University of Madras' Taramani campus, Chennai 113, Tamil Nadu, India, is where they were acquired. The animals were kept on a natural light and dark cycle (14 ± 1h: 10 ± 1h) and housed in regular laboratory settings (25 ± 2°C). They have unlimited access to food and drink. Prior to the trial, the animals were acclimated to the lab environment. The institutional animal ethics committee (IEAC no. 02/22/2020) of Dr. ALM PGIBMS, University of Madras, Taramani campus, Chennai-113, Tamil Nadu, India, approved the experimental procedures.

Administration for Drugs

For 14 days, 10 mg/kg b.wt. of lead acetate (PbAc) was administered intraperitoneally after being diluted with saline (pH-7.4). Esculin was administered orally for 14 days at a dosage of 15 mg/kg b.wt. after being dissolved in tween 20.

Animal Grouping 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 to the mice. inside the peritoneum

Group 3: For 14 days, mice given lead acetate (10 mg/kg) were given esculin (15 mg/kg of body weight) orally.

Behavioral Evaluations

Rotarod examination

A rotarod device was used to measure grip strength and motor coordination. Before beginning the actual evaluation of the pharmacological treatments, the animals were subjected to a training session to get them used to the rotarod. The animals were positioned on a 3 cm-diameter rotating rod that rotated at 20 rpm. The deadline was 120 seconds. Each rat was given three different trials separated by five minutes. On day 14, the average time drop was noted and shown as a count every two minutes. [13]

Morris Water Maze Test (memory impairment assessment)

The Morris water maze test was used to assess the spatial navigation task's acquisition and retention. [14] In the test room, the animals were taught to swim on a platform in a circular pool that measured 180 cm in diameter by 60 cm. A moveable circular

platform (9 cm in diameter) supported on a column was positioned in the pool 1 cm above the water level for the test after the pool had been filled with water down to a depth of 40 cm. The animals went through four trials in a training session prior to the injection of lead acetate. The starting positions were different for each of the four trials. For a maximum of two minutes, the latency to locate the escape platform was recorded. For the duration of the experiment, the platform stayed stationary in the middle of one of the four quadrants. The mice were released at random at any of the edges (North, South, East, and West) facing the pool wall, and the retention of the reaction was assessed. The acquisition latency was the amount of time it took the mice to get to the platform. The transfer latency was defined as the amount of time it took to get to the concealed platform on day 14 after esculin treatment. On day 14, the amount of time spent in the target quadrant was also computed.
An open-field test (OFT) was administered to each and every mouse. Every mouse was positioned in the middle of the open-field device. A 90-cm-diameter wooden circle served as the open-field device. The exam was administered from 9:00 to 12:00. A 60 W light bulb, which is estimated to provide 750 lumens and 375.38 lux;

The single source of light in the resting chamber was lux = lumen/m2, which was placed 90 to 100 cm above the center. On day 14, each animal was positioned in the middle of the open field, and for three minutes, the number of squares traversed, rearings, and head dips were counted by direct eye observation. [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 50 cm above the cushion support and grasped by the forepaws of every mouse. The amount of time the rat could grip the wire was noted. Ninety seconds was chosen as the latency to grip loss, which is regarded as an indirect indicator of the cut-off time for grip strength. [16]

Biochemical Characteristics

Cortex and hippocampal homogenization

The animals were employed for biochemical estimates on day fifteen. Decapitation was used to remove the animals' brains during the sacrificial process. Each isolated brain tissue was divided into the cortex and hippocampus. A tissue homogenate containing 10% (w/v) was made in 0.1 M phosphate buffer (pH 7.4). At 10,000 × g, homogenates were centrifuged. Separated aliquots of the supernatant were used for biochemical examinations. The cytosolic fraction was used for all biochemical assays in this investigation.

Biochemical characteristics

The total protein content was calculated using the technique outlined by [17], using bovine serum albumin as a reference. Using the technique of [18], malondialdehyde (MDA), a byproduct 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 where 2, 4-dinitrophenylhydrazine reacts with the carbonyl groups of oxidized proteins to form 2, 4-dinitrophenylhydrazone. The result was expressed as nmol/mg protein. The technique of [19] was used to quantify the production of nitric oxide (NO), and the findings were expressed as nmol/mg protein. Pyrogallol auto-oxidation inhibition was used to measure the enzyme-based antioxidant superoxide dismutase (SOD) [20], and catalase (CAT) activity was used to measure H2O2 consumption. [21] The reduced glutathione (GSH) level was determined by reducing 5,5'-dithiobis-2-nitrobenzoic acid to a yellow-colored sulfhydryl molecule, which was detected at 412 nm and reported as mol of GSH/min/mg protein. [22] Glutathione peroxidase (GPx) activity was assessed using the test. [23] Using GR as a catalyst, GSH was oxidized with NADPH, and the result was detected at 340 nm. Oxidative damage to proteins was measured using

The enzyme activity was expressed as mol NADPH oxidized/min/mg protein, which was determined by detecting the amount of oxidized NADPH in the reaction mixture at 340 nm. The activity of glutathione reductase (GR) was assessed. [24] Glutathione-S-transferase (GST) catalyzes the formation of glutathione-CDNB couples; its expression was evaluated and expressed as nmol CDNB conjugate formed/min/mg protein. [25] The non-enzymatic antioxidant activity of acetylcholine esterase (AChE) was measured [26] and represented as moles of substrate hydrolyzed/L/min/mg protein. The activity of the enzymes Na/K+ and Ca2+ ATPase was measured [27] and represented as µmol of phosphorus liberated/min/mg of protein.

Histological analysis of the cortex and hippocampal regions

On the fourteenth day, animals were sacrificed in order to conduct cervical decapitation. After the skull was opened, the brain was gently extracted without causing any harm. After that, the cortex and hippocampus areas were extracted and preserved in 10% buffered formalin. Ethanol and xylazine were used to dehydrate the samples. After that, they spent a whole day immersed in paraffin at 56°C in hot water. Blocks of paraffin wax tissue were produced for sectioning using a slide microtome at a thickness

of 4 µm. Following their collection on glass slides, tissue slices underwent deparaffinization, hematoxylin and eosin staining, and light microscopy examination.

RESULTS

Behavioral Parameters

Effect of esculin on lead acetate-induced alterations in experimental and control C57BL/6 mice's body weight 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.) resulted in a considerable ($p<0.05$) increase in weight 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. 1).

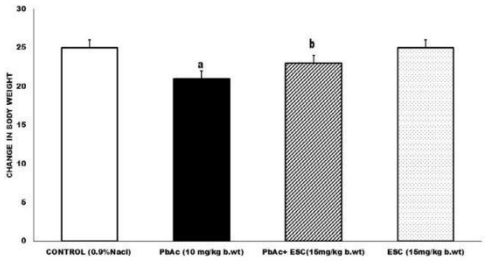

Fig. 1 Impact of esculin on lead acetate-induced alterations in control and experimental subjects' body weight C57BL/6 Mice The mean ± SD of six mice per group is shown in the data. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); Tukey's post-hoc test revealed aP<0.01 compared to the control group and bP<0.05 compared to the PbAc-induced group.

Impact of esculin on alterations caused by lead acetate in the rotarod test of experimental and control C57BL/6 mice Mice treated with intraperitoneal PbAc showed a substantial ($p <0.01$) loss in muscle grip strength when compared to untreated mice. By reducing fall-off time, esculin (15 mg/kg b.w.) therapy considerably ($p<0.05$) increased muscular strength in comparison to the PbAc-induced group. There was no discernible difference between the esculin (15 mg/kg)-treated and control groups (Fig. 2).

Impact of esculin on lead acetate-induced modifications in experimental and control C57BL/6 mice's Morris water maze test Memory recall was assessed using the Morris water maze test. In the normal control group, trained mice's transfer latency progressively decreased over training sessions. On days 7 and 14, however, mice given PbAc exhibited a much longer escape latency in the Morris water maze than the control group ($p<0.01$). The esculin (15 mg/kg) therapy showed a significant ($p<0.05$) reduction in transfer latency when compared to the PbAc administered group. There was no appreciable difference between the control group and the esculin (15 mg/kg) alone group (Fig. 3).

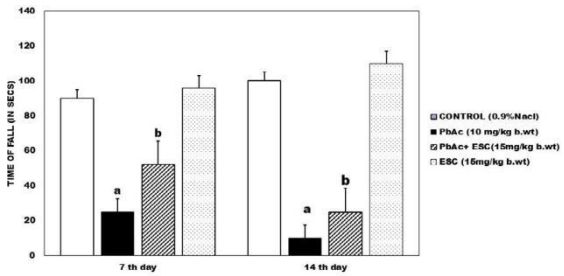

Fig. 2: Impact of Esculin on Lead Acetate-Induced Alterations in the Rotarod Test of Experimental and Control C57BL/6 Mice The mean ± SD of six mice per group is shown by the data. Group I: Control; Group II: PbAc (10 mg/kg b.w.); Group III: PbAc (10 mg/kg b.w.) + ESL (15 mg/kg b.w.); Group IV: ESL (15 mg/kg b.w.); Tukey's post-hoc test revealed aP<0.01

compared to the control group and bP<0.05 compared to the PbAc-induced group.

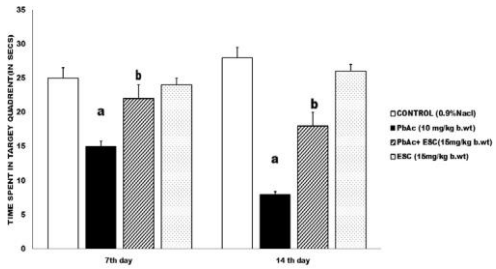

Fig. 3: Impact of Esculin on Lead Acetate-Induced Alterations in Control and Experimental C57BL/6 Mice's Morris Watermaze Test

The mean ± SD of six mice per group is shown by the data. 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.); Tukey's post-hoc test revealed aP<0.01 compared to the control group and bP<0.05 compared to the PbAc-induced group.

Impact of esculin on lead acetate-induced alterations in locomotor activity in experimental and control C57BL/6 mice in an open field test

Compared to mice that were not treated, intraperitoneal PbAc therapy resulted in a substantial ($p < 0.01$) decrease in animal mobility. Animal movement was considerably ($p < 0.05$) enhanced by esculin (15 mg/kg b.w.) therapy. There was no appreciable difference between the esculin (15 mg/kg b.w.) treatment group and the control group (Fig. 4).

Esculin's impact on grip strength tests in C57BL/6 experimental and control mice given pbac

Compared to the control mice, the PbAc-treated animals exhibited significantly ($p < 0.01$) shorter hanging periods, suggesting a greater loss of grip. Grip strength significantly improved after esculin (15 mg/kg b.w.) therapy in the PbAc-induced group, and a clear difference was seen between the control group and the group treated with esculin (15 mg/kg b.w.) alone (Fig. 5).

Biochemical Parameters

Effect of esculin on lead acetate-induced alterations in LPO, NO, and protein carbonyl levels in the cortical and hippocampal regions of experimental and control C57BL/6 mice

The levels of peroxidation, nitric oxidation, and protein carbonyl content were significantly ($p<0.01$) higher in mice given PbAc than in the control group. In the PbAc-induced group, concurrent administration of esculin (15 mg/kg b.w.) resulted in a substantial ($p<0.05$) reduction in LPO, NO, and protein carbonyl levels, whereas the esculin-only (15 mg/kg) treated group showed no discernible change in comparison to the control group (Table 1).

Impact of esculin on lead acetate-induced alterations in SOD, CAT, GPX, GR, GSH, and GST activity in the control and experimental hippocampal and cortical regions Mice C57BL/6

PbAc-treated animals had considerably ($p < 0.01$) lower levels of SOD, CAT, GPX, GR, GSH, and GST than control animals.

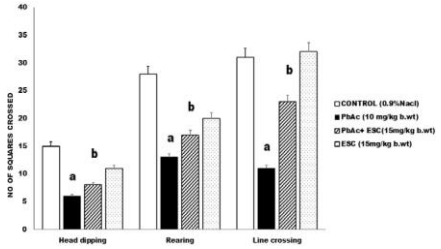

Fig. 4: Impact of Esculin on Lead Acetate-Induced Alterations in Control and Experimental C57BL/6 Mice Open Field Test

The mean ± SD of six mice per group is shown by the data. 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.); Tukey's post-hoc test revealed aP<0.01 compared to the control

group and bP<0.05 compared to the PbAc-induced group.

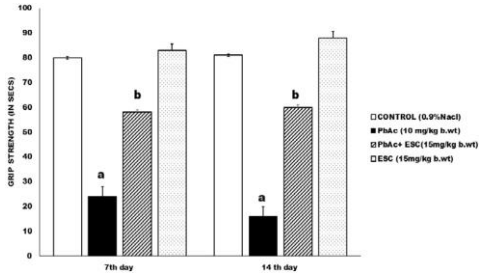

Fig. 5: Impact of Esculin on Lead Acetate-Induced Modifications in Control and Experimental Grip Strength Tests Mice C57BL/6

The mean ± SD of six mice per group is shown by the data. aP<0.01 vs the control group, bP<0.05 versus the PbAc-induced group (one-way ANOVA followed by Tukey's post-hoc test); 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.).

mice. In mice treated with PbAc, esculin (15 mg/kg b.w.) significantly (p<0.05) increased the levels of all these enzymes. There was no discernible difference between the esculin-only (15 mg/kg) group and the control group (Table 2).

Esculin's impact on lead acetate-induced alterations in the activities of Mg2+ ATPase, Ca2+ ATPase, and Na/K ATPase in the normal and experimental hippocampal and cortical Mice C57BL/6

The PbAc-induced group had considerably (p<0.01) higher levels of Na/K ATPase, Ca ATPase, and Mg2+ ATPase than the control group. Concurrent administration of esculin (15 mg/kg) significantly (p<0.05) increased ATPase levels. There was no appreciable change between the control group and the Esculin (15 mg/kg) alone group (Fig. 6). Impact of esculin on lead acetate-induced alterations in acetylcholine esterase levels in the control and experimental hippocampal and cortical regions Mice C57BL/6

Acetylcholine activity was significantly (p<0.01) lower in the PbAc-induced group than in the control group. Acetylcholine levels were considerably (p<0.05) raised by concurrent esculin (15 mg/kg) therapy. When comparing the esculin (15 mg/kg) alone group to the control, no discernible changes occurred (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	Hippocampus	4.34 ± 0.11	12.16 ± 0.17[a]	8.08 ± 0.26[b]	2.08 ± 0.04
(Units/mg of protein)	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+ mg/ kg b.wt)*	*ESC(15*	*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).

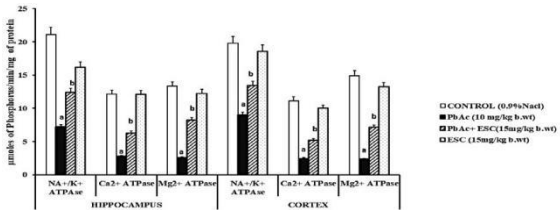

Fig. 6: Effect of Esculin on Lead Acetate induced induced alternations in the levels of membrane bound ATPases in the hippocampus and cortex of control and experimental mice

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).

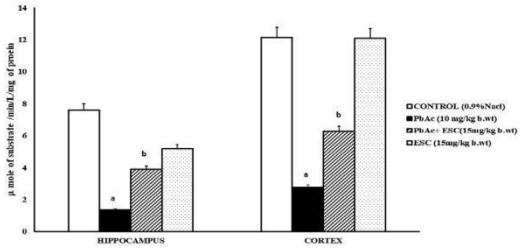

Fig. 7: Effect of Esculin on Lead Acetate induced alternations in the levels of Acetylcholine esterase activity in the hippocampus and cortex of control and experimental mice

The mean ± SD of six mice per group is shown in the data. 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.); Tukey's post-hoc test revealed aP<0.01 compared to the control group and bP<0.05 compared to the PbAc-induced group.
Esculin's impact on lead acetate-induced histological alterations in the control and experimental hippocampal and cortical regions C57BL/6 The hippocampus and cortical areas of the mice's brain tissues from the control, PbAc-induced neurotoxicity, and therapy groups were examined for abnormal structures using H&E staining. A&E) The cortical and hippocampal regions of control mice had normal neuronal tissue. B&F) The PbAc-induced group showed more inflammatory, degenerative cells than the other mice that had undergone esculin therapy. This C & G is characterized by acute inflammation, vacuolation with condensed nuclei, and neuronal edema. Mice treated with esculin (15 mg/kg) showed more normal neuronal cells and less degenerating neurons in their cortex and

hippocampus. D&H) The neuroprotective effect of esculin against lead acetate-induced neurotoxicity was shown by the healthy neurons in the cortex and hippocampal regions of animals treated with esculin (15 mg/kg) alone (Fig. 8).

DISCUSSION

The current research shown that consuming lead acetate causes oxidative damage and notable alterations in the antioxidant system in the mouse brain. The government

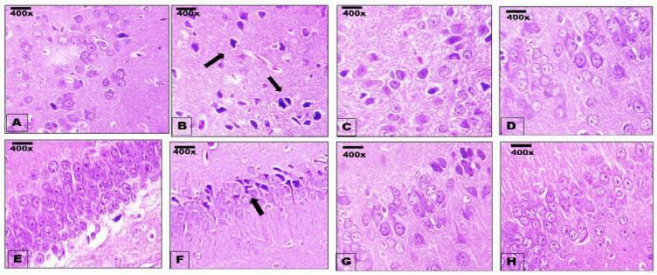

Fig. 8: Impact of Esculin on Histological Alterations Caused by Lead Acetate in the Control and Experimental Mice's Hippocampus and Cortex: Sections stained with hematoxylin and eosin were seen under a 400x magnification microscope. Figures A and E: Control mice displaying the typical hippocampal and cortical neuronal organization. Figure B&F: The animal cortex and hippocampal regions affected by PbAc exhibit a higher degree of neurodegenerative and inflammatory cells (shown by the arrow). 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. Figure D&H: When ESL (15 mg/kg bw) is administered alone, the hippocampal and cortical regions exhibit positive, healthy neurons that are identical to the control histology. The mean ± SD of six mice per group is shown by the data. 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.); Tukey's post-hoc test revealed $^{a}P<0.01$ compared to the control group and $^{b}P<0.05$ compared to the PbAc-induced group.

Adding esculin to lead acetate ingestion mitigated neurobehavioral, biochemical, and histological changes, therefore altering the antioxidant response. First, throughout the induction phase of our current investigation, the animals' body weight was routinely measured. The animals intoxicated with lead exhibit a considerably lower body weight ($p<0.01$) than the usual, healthy group on the last day of our experiment. Our findings suggest that mice's overall body weight increase is negatively impacted by lead acetate. Low food intake, hormone imbalances, and decreased protein levels might be the cause. Growth retardation caused by lead has long been documented. [28] Additionally, lead (Pb) results in acute gastritis and malabsorption, which lowers body weight and reduces calorie intake. The results obtained are consistent with those of the earlier research, which found that lead induction reduced the growth rate of mice. [29] Animals given esculin (15 mg/kg) exhibit increased body weight when compared to the group given lead acetate. This is because esculin has a strong antioxidant impact.

Rota rod activity and grip strength were used to measure motor activity in neurobehavioral studies. Our research on lead-intoxicated mice shows that rotarod performance is compromised, which accounts for deficiencies in balance and motor coordination. The current study's findings were consistent with those of earlier research. [30, 31] The open field test also showed that lead poisoning reduced exploratory and locomotor activities. The impaired exploratory behavior that accompanies these locomotor impairments is comparable to previous observations [32, 33]. One may argue that the lack of exploratory activity is due to the lead-

treated animals' hippocampal and cortical functional defects, which encourage them to explore the open field. Because of its possible antioxidative effect on neurons, esculin (15 mg/kg) therapy markedly increased the mice's motor and muscular activity. 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] Because of their hippocampus-related cognitive loss, the lead-intoxicated rats in our current investigation spent less time in the target quadrants. The findings of other authors' publications are in line with the learning and memory test results of lead-induced impairments. [36, 37] Compared to the lead-induced animal, the esculin-treated animal spent a considerably longer amount of time in the target quadrant.

In living cells, aerobic metabolism continually produces reactive substances, including free radicals. The development of certain

diseases, such neurodegenerative disorders, is significantly influenced by free radicals and other reactive chemicals. [38] Antioxidants and phenolics may be able to chelate heavy metals by overcoming the blood-brain barrier. Esculin, a promising coumarin with strong antioxidative qualities, was used in this work to reduce lead-induced oxidative stress in the cortical tissue and hippocampal regions after PbAc injection. Following PbAc exposure, esculin treatment dramatically decreased the raised level of MDA in the cortical tissue and hippocampal regions, preventing enhanced lipid peroxidation by squelching peroxide radicals. Lipid peroxidation was therefore considerably decreased by esculin therapy. In a different experimental setting, esculin inhibited the elevated lipid peroxidation that arsenic exposure caused in the cortical cortex. Esculin's capacity 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. According to a number of experimental models, esculin may reduce excessive NO release, and CoQ10 may inhibit iNOS production, which is consistent with the findings described here. Additionally, it was shown that esculin increased the activities of GSH, SOD, CAT, GPx, and GR, therefore counteracting the oxidative burst that was created after Pb exposure. These antioxidants' upregulation may be the cause of the elevated levels we observed. These findings are consistent with earlier research on the protective properties and antioxidant activity of esculin in various tissues. [39] By interfering with the action of Na+/K+-ATPase, Pb also suppresses energy metabolism. [38] According to experimental research, Pb suppresses the production of ATP in the brain as well as Na+ and K+, which are ATPases that are highly concentrated at nerve ending membranes and are in charge of creating and maintaining the ionic gradient required for neuronal excitability. [40, 41] Additionally, lead's capacity to flow through the

Its capacity to replace calcium ions (Ca^{2+}) is primarily responsible for the blood–brain barrier (BBB). In vitro experiments on brain capillary endothelial cells, the main component of the blood-brain barrier, have directly shown the function of the Ca-ATPase pump in the transport of lead into the brain. [42] According to our research, esculin preserves synaptic activity in neurons by controlling their neurobehavioral activity and markedly boosted the membrane-bound ATPase activity in the cortex and hippocampus tissues.

The current work demonstrates that co-treatment with esculin restored decreases in AChE expression in Pb-treated brains, suggesting improvements in cholinergic circuitry. AChE hydrolyzes the neurotransmitter acetylcholine in cholinergic synapses. Neurological impairment results from AChE malfunction. The prefrontal cortex's working memory function is hampered by an overactive cholinergic system. [43] Therefore, working memory and spatial encoding are supported by the hippocampus-prefrontal cortex. [44] Conversely, acetylcholine deficiency affects brain function. Therefore, the current study showed that esculin effectively restored AChE expression, which might help Pb-intoxicated mice regain equilibrium to 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 were encircled by haloes, looked deteriorated, and lost their distinctive forms. Similar results on the neurotoxic impact of lead acetate have previously been published utilizing other lead exposure procedures. [45–47] Nevertheless, the histological changes in every brain have been preserved with little cell death when esculin (15 mg/kg) has been given to the lead-induced mice. Lead acetate causes oxidation and a lot of free radicals. Increased oxidation processes cause the brain's neuronal networks, synaptic connections, cell death, and neural destruction—all of which are essential for sustaining and regulating behavioral responses. According to our research, esculin significantly reduced the harmful effects of lead when it was administered, indicating that it has high antioxidant and neuroprotective properties. This reverses all changes by eliminating and balancing free radicals.

References

1. Han DY, Hoelzle JB, Dennis BC, and Hoffmann M. A brief review of cognitive assessment in neurotoxicology. Neurologic Clinics. 2011; 29(3): 581–590.
2. Caban-Holt M, Mattingly G, Cooper, and Schmitt FA. Neurodegenerative memory disorders: a potential role of environmental toxins. Neurologic Clinics. 2005; vol. 23(2): 485–521.
3. Mason LH, Mathews MJ, and Han DY.Neuropsychiatric symptom assessments in toxic exposure.Psychiatric Clinics of North America. 2013;36(2): 201–208.
4. Dobbs MR. Clinical Neurotoxicology: Syndromes, Substances, Environments, Saunders, 2009.
5. Patra RC, Swarup D, Dwivedi SK. Antioxidant effects of α tocopherol,

ascorbic acid and L-methionine on lead-induced oxidative stress to the liver, kidney and brain in rats. *Toxicology*. 2001;162(2):81–88.

6. Bellinger DC. Very low lead exposures and children's neurodevelopment. Curr Opin Pediatr 2008;20: 172–177.
7. Ercal N, Gurer-Orhan H and Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage, Curr. Trends Med. Chem., 2001; 1: 529–539.

8. Ding Y, Gonick HC and Vaziri ND. Lead promotes hydroxyl radical generation and lipid peroxidation in cultured aortic endothelial cells, Am. J. Hypertens 2000; 13: 552–555.
9. Kaushik Vilas Kulkarni and BelvotagiVenkatraoAdavirao. A review on:Indian traditional shrub Tulsi (ocimum sanctum): The unique medicinal plant.2018;6(2): 106-110
10. C.Michael Hogan, California buckeye:Aesculus californica,2008.
11. Yu Song, Xiaochun Wang, Shengkai Qin, Siheng Zhou, Jiaolun Li, Yue Gao Esculin ameliorates cognitive impairment in experimental diabetic nephropathy and induces anti-oxidative stress and anti- inflammatory effects via the MAPK pathway. Molecular Medicine Reports.2018: 7395-7402
12. Zhao DL, Zou LB, Lin S, Shi JG, Zhu HB. Neuropharmacology.2003;53 ,724–732.
13. Deacon RM. Measuring motor coordination in mice. J Vis Exp. 2013;75: e2609.
14. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods. 1984; 11:47–60.
15. Rodrigues ALS, Rocha JBT, Mello CF & Souza DOG. Effect of perinatal lead exposure on rat behavior in open field and two-way avoidance tasks. Pharmacology and Toxicology.1996. 79: 150-156
16. Tariq M, Khan HA, Deeb SAl, et al.Neuroprotective Effect of Nicotine Against 3-Nitropropionic Acid (3-Np)-Induced Experimental Huntington's Disease in Rats. Brain Res Bull.2005. 67: 161-168.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 1951;193: 265-275.
18. Utley HC, Bernheim F, Hochslein P. Effect of sulfhydryl reagent on peroxidation inmicrosome, Arch. Biochem. Biophys. 1967;260: 521–531.
19. L.C. Green, D.A. Wagner, J.Glogowski, P.L. Skipper, J.S.Wishnok, S.R.Tannebaum,Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids, Anal. Biochem. 1982; 126:131–138.
20. Marklund S, Marklund G, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem. 1947;47: 469–474.
21. Aebi H, Catalase in vitro, Methods Enzymol.1984; 105: 121–126.
22. Ellman GL, Tissue sulfhydryl groups, Arch. Biochem. Biophys. 1959: 82 :70–77.
23. Wheeler CR,.Salzman JA, Elsayed NM, Omaye ST, Korte DW, Automated assaysfor superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activity, Anal. Biochem.1990; 184 ;193-199.
24. Carlberg I, Mannervik B, Purification and characterization of the flavoenzyme glutathione reductase from rat liver, J. Biol. Chem.1975; 250: 5475-5480.
25. Habig WH, Jakoby WB, Assays for differentiation of glutathione–S– Transferases,Meth. Enzymol. 1981;77: 398–405.
26. Ellmann GE, Courtney KD, Andersen V, Featherstone RM, A new and rapid colorimetric determination of acetyl cholinesterase activity, Biochem. Pharmacol. 1961;7 :88–95.
27. Chan KM, Delfert D, Junger KD. A direct colorimetric assay for Ca2+ -stimulated ATPase activity. Anal Biochem. 1986; 157:375-80
28. Allouche L, Hamadouche M, Touabti A. Effect of long-term exposure to low or moderate lead concentrations on growth, lipid profile and liver function in albino rats. Adv Biol Res 2011; 5:339-47
29. Seddik L, Bah TM, Aoues A, Brnderdour M, Silmani M. Dried leaf extract protects against lead-induced neurotoxicity in Wistar rats. Eur J Sci Res. 2010;42(1):139–151.
30. Rogers DC, Campbell CA, Stretton JL, Mackay KB. Correlation between motor impairment and infarct volume after permanent and transient middle cerebral artery occlusion in the rat. Stroke.1997; 28: 2060–2065.
31. Rozas G, Guerra MJ, Labandeira-Garcia JL. An automated rotarod method for quantitative drug-free evaluation of overall motor deficits in rat models of parkinsonism. Brain Res. Brain Res. Protoc. 1997; 2:75–84.
32. Reckziegel P. Dias VT. Benvegnu D, Boufleur N, Silva Barcelos RC, Segat HJ, et al. Locomotor damage and brain oxidative stress induced by lead exposure are attenuated by gallic acid treatment. Toxicol. Lett. 2011; 203:74–81.
33. Sansar W, Ahboucha S, Gamrani H. Chronic lead intoxication affects glial and neural systems and induces hypoactivity in adult rat. Acta Histochem.. 2011; 113:601–60.
34. Leon WC, Bruno MA, Allard S, Nader K, Cuello AC. Engagement of the PFC in consolidation and recall of recent spatial memory. Cereb Cortex.2010; 17:297-3
35. Silachev DN, Shram SI, Shakova FM, Romanova GA, Myasoedov NF. Formation of spatial memory in rats with ischemic lesions to the prefrontal cortex; effects of a synthetic analog of ACTH (4-7). Neurosci Behav Physiol. 2009; 39:749-56.
36. Anderson DW, Mettil WA, Schneider JS. Rearing environment, sex and developmental lead exposure modify gene expression in the hippocampus of behaviorally naïve animals. Neurochem Int. 2013;62:510–520.

37. Schneider JS, Anderson DW, Talsania K, Mettil WA, Vadigepalli R. Effects of developmental lead exposure on the hippocampal transcriptome: influences of sex, developmental period, and lead exposure level. Toxicol Sci. 2012; 129:108–125.
38. Zheng L, Yang L, Wang Z, Chen C and Su Y.Protective effect of esculin in adjuvantinduced arthritic (AIA) rats via attenuating proinflammatory cytokines and oxidative stress. Cell Mol Biol. 2015;61: 15.
39. Niu X, Wang Y, Li W, Zhang H, Wang X, Mu Q, He Z and Yao H: Esculin exhibited antiinflammatory activities in vivo and regulated TNFα and IL6 production in LPSstimulated mouse peritoneal macrophages in vitro through MAPK pathway. Int Immunopharmacol 2015;29: 779786.
40. Erecinska M, Silver IA. Ions and energy in mammalian brain. Prog Neurobiol. 1994; 43:37-71.
41. Xie Z, Askari A. Na (+)/K (+)-ATPase as a signal transducer. Eur J Biochem. 2002; 269:2434-9.
42. Kerper LE, Hinkle PM. Cell ular uptake of lead is activated by depletion of intracellular calcium stores. J Biol Chem.1997b; 272: 8346–52.
43. Vijayraghavan S, Major AJ, Everling S. Muscarinic M1 receptor overstimulation disrupts working memory activity for rules in primate prefrontal cortex. Neuron. 2018; 98:1256–1268.
44. Spellman T, Rigotti M, Ahmari SE, Fusi S, Gogos JA, Gordon JA. Hippocampal–prefrontal input supports spatial en-coding in working memory. Nature. 2015; 522:309–314.
45. Amal EA, Mona HM. Protective effect of some antioxidants on the brain of adult male albino rats, Rattus rattus , exposed to heavy metals. Biosci. Res. 2009; 6: 12 – 19.
46. Barkur RR, Bairy LK. Histological study on hippocampus, amygdala and cerebellum following low lead exposure during prenatal and postnatal brain development in rats. Toxicol. Ind. Health. 2014
47. Hsiang J, D í az E. Lead and developmental neurotoxicity of the central nervous system. Curr. Neurobiol.. 2011; 2: 35 – 42.