



Beneficial Role of *Echinacea* Against Lead Acetate-Induced Brain Toxicity Through Reducing Inflammation, Oxidative Stress and Apoptosis in Rats

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ABSTRACT

Background: Lead is widely distributed in the environment and has been associated with a variety of negative health impacts. The present study investigated the use of *Echinacea* with lead acetate in decreasing or preventing the serious side effects of lead on the brains of male rats. **Methods:** Twenty-four rats were distributed into four groups randomly. Group1: Rats orally administered 1ml of distilled water followed by an intraperitoneal (I.P) injection of 1ml of physiological saline (control group). Group2: Rats I.P injected with lead acetate (50 mg/kg b.w/day, 3 days a week for 4 weeks). Group3: Rats orally administered *Echinacea* (150 mg/kg b.w./day for 4 weeks). Group4: Rats administrated with *Echinacea* plus lead acetate with the same doses of G2 and G3. **Results:** Lead exposure resulted in a significant elevation in nitric oxide (NO) production and malondialdehyde (MDA), along with a decline in reduced glutathione (GSH) and enzyme activities of superoxide dismutase (SOD) and catalase (CAT) in the rats' brain. These results were linked with significant increases in aminotransferase enzymes, lactate dehydrogenase (LDH), alkaline phosphatase (ALP) in brain tissues and serum thyroid stimulating hormone (TSH) levels. Abnormal histopathological changes and increased caspase-3 immunopositivity were also observed in comparison to control. The co-treatment with *Echinacea* in lead-exposed rats improved all of these changes. **Conclusions:** This study pointed out that *Echinacea* could be used as an alternative treatment to counteract the negative effects of lead acetate.

INTRODUCTION

Lead (Pb) is a very poisonous heavy metal that can be found in a variety of industrial and environmental settings, including soils, rocks, water, and aquatic environments [1]. Humans are exposed to lead mostly through petroleum products, leaded paints, and drinking water. Therefore, lead can enter the body in different ways (inhalation, ingestion, and dermal contact), where it is then distributed across red blood cells (RBCs), soft tissues (central nervous system, liver, lungs, intestine, spleen, and kidney) and bones [2]. Several reports have revealed that lead can induce haematological, neurological, immunological, and gastrointestinal diseases [3]. The most exposed organ to lead poisoning is the central nervous system. Neuropathic pain, irritability, memory loss, and tingling in the facial skin are all symptoms of lead-induced neurotoxicity [4,5].

Furthermore, Pb has a strong affinity for key functional groups such as carboxyl, amino, and especially sulfhydryl groups, so it can interfere with a variety of biomolecules and enzymes [6] such as reduced glutathione (GSH), glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD) which are involved in the antioxidant defense system [1]. As a result, the antioxidant system's hemostasis will be disrupted, resulting in the production of reactive oxygen species (ROS), which can contribute to the development of neurological illnesses including Alzheimer's, Parkinson's, and Schizophrenia [7]. Also, Pb induces neurotoxicity by replacing some essential ions mainly Ca²⁺ in brains [8] this leads to disturbance in ions transport, inter- and intracellular signaling, neurotransmitter releases, and apoptosis [9]. Therefore, searching for natural agents with high anti-inflammatory and antioxidant activities may have a neuro-ameliorative effect against neuronal injury induced by Pb.

Echinacea purpurea (family: Asteraceae) is a plant of medical importance used by Native American Indians and Europeans with several biological actions, such as anti-oxidation, anti-inflammation, immunomodulation properties [10,11] and antimicrobial activity [12]. It has bioactive constituents including phenolic compounds that comprise active components, such as Caffeic acid derivatives (Caftaric acid, Cichoric acid, Chlorogenic acid, Echinacoside and cynarin), alkamides, essential oils, and lipophilic poly acetylenes compounds with glycoproteins and polysaccharides [13,14], and many valuable minerals (Ca, Na, B, P, S, and K) [15].

In view of the harmful effects of lead acetate on the brain due to free radical production, and the role of *Echinacea* as antioxidant, anti-inflammatory, and immunomodulatory in several diseases, such as atherosclerosis, hypertension, cardiovascular diseases, hepatotoxicity and type2 diabetes [16, 17]. The main objective of the current study was to investigate the ameliorative effect of *Echinacea* against the negative impact of lead acetate in brain rats.

Material and Methods

Chemicals

Lead acetate (Pb) was obtained from NATCO, Laboratory Chemical Reagents, Egypt. *Echinacea* (**Echn. pu**) was supplied by Puritan's Pridet (Holbrook, NY11741 USA)as capsules each containing 400 mg of extracted natural whole herb. The content of the capsules was dissolved in distal water and freshly prepared for oral administration depending on the weight of each rat. Based on the conversion of a therapeutic human dose to an animal dose according to Zink and Chaffin [18], the experimental dose for *Echinacea* was selected.

Animals

The twenty- four male albino rats, weighting about 180-190 gm (10 weeks old) were obtained from the laboratory animal house of the Egyptian Atomic Energy Authority, Biological Application Department at Nuclear Research Center, Inches. Animal experiments were done in accordance with the criteria of investigation and ethics committe of Atomic Energy authority laws govering the use of experimental animals. All animals were housed in wire-mesh-covered stainless steel cages with access to water at all times and a balanced standard meal for seven days prior to the trial. Rats subjected to a 25°C room temperature 12 h light/12 h dark cycle.

Experimental design

Twenty- four rats were distributed into four groups randomly (6 rats/group):

Group1 (G1): Rats were orally administrated with 1ml distilled water followed by intraperitoneal(I.P) injection of 1ml physiological saline daily during the whole experiment period , served as control. Group2 (G2): Rats were I.P injected with lead acetate (50 mg/kg b.w /day- 3days in week for 4 weeks), the dose of lead acetate was chosen based on a former study [19]. Group3 (G3): Rats were orally administrated with *Echinacea* (150 mg/kg b.w / day for 4 weeks) according to Khalaf et al.[20] . Group4 (G4) : Rats were I.P injected with the above-mentioned doses of lead acetate, as in G2, plus given orally of *Echinacea* 150 mg/kg / day for 4 weeks , as animals in G 3.

Biochemical analysis

Twenty -four hours after the last treatment, all rats were sacrificed, and serum was obtained by centrifuging blood samples for TSH analysis. The brain of each animal was quickly removed after killing, washed in normal saline and deionized water, blotted with filter paper, weighed, and split into two portions. The first portion was weighed and rapidly homogenized in sodium phosphate buffer, and the second portion was put in 10% formalin for the histological examination. All samples were kept at- 20 °C waiting for analysis.

Then, brain tissues from the first portion were homogenized to produce a 10% (W/V) homogenate in an ice-cold 50mM sodium phosphate buffer (pH 7.4) containing 0.1mM ethylene diamine tetraacetic acid (EDTA). The homogenate was centrifuged at 10,000 rpm for 20 min at 4C°, and the collected supernatant of the brain was used to measure some biochemical parameters.

Reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), catalase (CAT) activity, and superoxide dismutase activity (SOD) in brain supernatant were estimated by using assay kits from Biodiagnostic Co, Egypt by the methods of Beutler et al. [21], Ohkawa et al. [22], Green et al.[23], Aebi [24], and Sun et al.[25] respectively. Total protein, total cholesterol (TC), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in the supernatant of brain homogenate were estimated by using colorimetric assay

kits from Biodiagnostic Co, Egypt. Serum TSH was assessed by radioimmunoassay using kits purchased from (DIA source Immuno Assay S.A.-Rue du Bosquet, 2-B 1348 Louvain- La- Neuve- Belgium).

Histological analysis

Rat brain tissue samples were fixed in buffered formalin at a 10 percent concentration, and the fixative was then removed for 30 minutes in distilled water. The tissues were then put through a graded sequence of alcohol to dehydrate them (70 percent, 90 percent, and 100 percent). The samples were then cleared in numerous changes of xylene after dehydration. The samples were then imbedded and blocked out after being impregnated with molten paraffin wax. Hematoxylin and eosin were used to stain the paraffin slices (4-5 um) [26].

Immuno-histochemical examination

According to the standard immunohistochemical methods [27], five micron paraffin segments were fixed on glass slides with positively charged (Biogenex, USA), deparaffinization by xylene. Slices were put in a plastic vessel full of antigen retrieval solution and placed in a microwave for 5 min cooled, then washed with deionized water and phosphate buffer saline (PBS). Sections were incubated with DAKO peroxidase blocking reagent (Cat.No.S 2001). Then, slides were keep warm at 60 C° with primary monoclonal antibody against Caspase3 (R&D system abiotecne brand, Catalog #MAB835, USA), washed with PBS buffer. Diaminobenzidine was used as a chromogen, washed with distilled water. Mayer's hematoxylin (Hx) was used for staining. Morphometric analysis was carried out to measure the area % of Caspase-3 positive expression by image analysis software (JID801D).

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) test .Duncan's Range Test was used to compare between groups using COSTAT. Program 3.03,198.

RESULTS

Oxidative stress and Antioxidant status of rat brains

The I.P injection of lead acetate (G2) to rats yielded a significant elevation ($p<0.05$) in MDA (Lipid peroxidation marker), NO (inflammatory marker) and a significant decline ($p<0.05$) in GSH content , CAT and SOD activities in brain tissues compared to the G1 group (control group), while rats treated with *Echinacea*(G3) showed non significant changes compared to the control group. Group 4 (Echinacea plus Pb) exhibited a significant ($p>0.05$) decrease in MDA, and NO and a significant increase in GSH content, CAT and SOD compared to the animals in (G2) (Table 1&Figure 1).

Biochemical parameters

Table 2 and Figure 2 display mean values \pm SD and percentage of the changes compared to the normal control group for levels of the brain ALT, AST, ALP, LDH, TC, TG, and serum TSH in response to lead acetate, Echinacea, and both in male rats. These data showed the destructive effect of lead acetate on the brains of lead treated animals.The significant ($p>0.05$) elevation in ALT, AST, ALP, LDH, TC and TG, and a significant decrease in serum TSH hormone. Data also revealed that *Echinacea* improved the adverse effect of lead acetate on these parameters to the same extent compared to the Pb group but not reaching the G1 (control). Like in Table 1, this data revealed insignificant changes between the Echinacea group and the control group.

Table (1): Effect of *Echinacea* capsules administration on CAT and SOD activities and MDA, NO & GSH contents the in brain homogenate of the four rat groups.

Parameters \ Groups	Control (G1)	Pb (G2)	Echn.pur (G3)	Pb +Echn.pur (G4)
CAT (U/ mg tissue)	0.94 \pm 0.04 ^a	0.65 \pm 0.03 ^c	0.90 \pm 0.04 ^a	0.77 \pm 0.02 ^b
SOD (U/g wet tissue)	232.46 \pm 21.63 ^a	163.46 \pm 10.80 ^c	273.40 \pm 30.45 ^a	203.79 \pm 7.66 ^b
MDA (nmol/g tissue)	29.85 \pm 4.85 ^c	46.41 \pm 3.13 ^a	28.67 \pm 2.92 ^c	36.83 \pm 3.28 ^b
NO (μ mol/g tissues)	33.54 \pm 5.12 ^c	104.35 \pm 9. 75 ^a	34.47 \pm 3.77 ^c	74.56 \pm 8.01 ^b
GSH (mg/g tissue)	16.37 \pm 1.21 ^a	10.79 \pm 1.36 ^c	17.12 \pm 0.92 ^a	12.37 \pm 1.51 ^b

1-Values expressed as the means \pm SD.

2- Different superscripts in the same row indicate significant difference between groups at $P<0.05$.

Histopathological Results.

The brain tissues of control rats exhibited a normal histological architecture (Fig 3, G1). Exposure to lead acetate, caused Pathognomonic neuro-toxic changes involving the pyramidal, stellate/granular cells, fusiform cells, superficial spindle cells, and cells of Mortinotti (small triangular cells) beside the glial cells (astrocytes, oligodendroglia, and microglia). These cells were markedly degenerated, apoptotic, and necrotic. A characteristic focal degeneration with intracellular accumulation of homogenous eosinophilic amorphous materials and consequence loss of Nissl's granules were seen in the pyramidal, stellate/granular cells. The intra and -periventricular tissue were edematous, and the cells of the Hippocampus were focally degenerated and apoptotic. The white matter tissue (neuropils) showed axonal degeneration, vacuolation, and demyelination (Fig 3, G2). Furthermore, the *Echinacea* group did not show any morphological changes comparable to those of the control group (Fig 3, G3). Co-administration of *Echinacea* Plus with lead acetate for rats in G4 demonstrated a remarkable ameliorative effect of the anti-oxidant compounds used, with normalisation of previously destructed cerebral tissue cells. However, focal cellular degenerative and apoptotic changes were seen in some of the cortical pyramidal, fusiform, stellate, cells of Mortinotti (small triangular cells) and hippocampal cells, beside some glial cells

(astrocytes, oligodendroglia, and microglia). Moreover, vacuolations and demyelination were also seen in some of the neuropils of the white matter (Fig 3, G4).

Immuno-histochemical Results

Examined sections from rat's brain tissue of the control group denoted very little number of cells' (0.5-1%) cytoplasmic and or nuclear reactivates to Caspase -3 apoptotic marker in brain tissues (Fig.4, G1) . On the other hand, sections from brain tissue of group 2 (lead acetate group) revealed intense cytoplasmic and nuclear staining reactivates (brownish staining reaction) to the apoptotic marker caspase-3, Positive cells were calculated to be approximately 20-30% of the examined tissue cells per 5 high power fields (HPF) (Fig.4, G2). Examined immune-stained brain tissue sections of group 3(*Echinacea* group) pointed out almost negative cytoplasmic and or nuclear staining reactions to the used apoptotic marker caspase-3. Very small numbers of cells in both the cerebral cortex and hippocampus were weakly reactive. They were about 1–3% of the examined tissue cells/5 HPF. (Fig.4, G3). The immuno-stained brain tissue sections of the treated group by lead acetate plus *Echinacea* showed mild cytoplasmic reaction to the apoptotic marker caspase-3. Positive cells showed weak brownish staining reactivity. Calculated positive cells were about 8-10 % of the examined tissue cells /5 HP (Fig.4, G4).

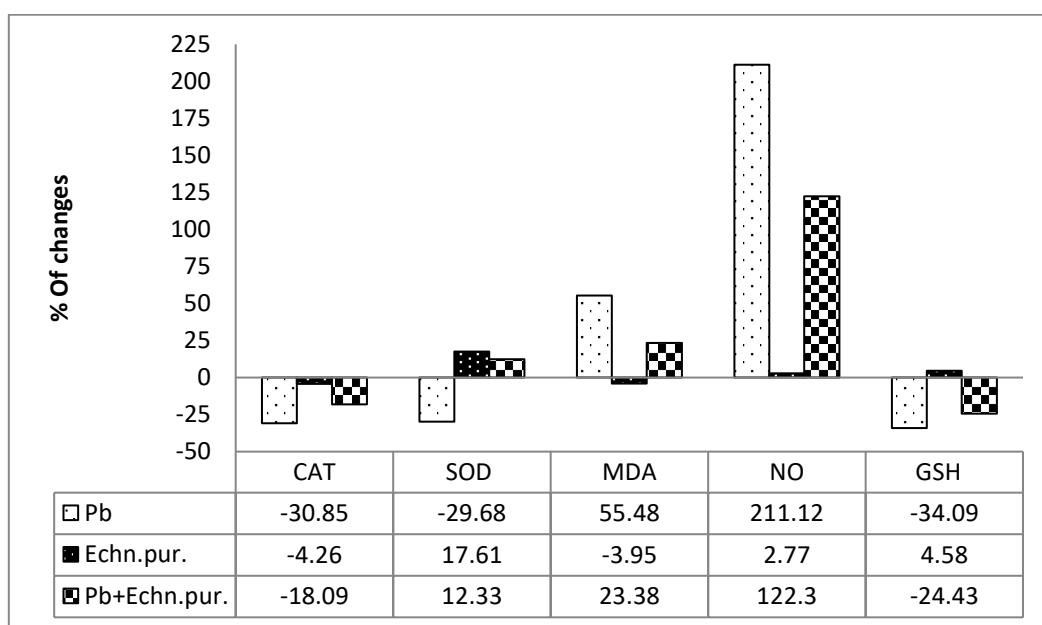


Fig. (1): The change in percentages of reduced glutathione (GSH), Catalase (CAT), Superoxide dismutase (SOD), Malondialdehyde (MDA), and Nitric oxide (NO) in all the tested groups in comparison to the corresponding control group .

Table (2): Effect of *Echinacea capsules administration* on some brain biochemical parameters and serum TSH in the treated rats in the four groups²

Groups Parameters	Control (G1)	Pb (G2)	Echn.pur (G3)	Pb + Echn.pur (G4)
TP mg/g tissue	0.53±0.09 ^a	0.28±0.02 ^c	0.51±0.06 ^a	0.42±0.08 ^b
LDH(U/g tissue)	225.67±23.55 ^b	393.67±60.80 ^a	234.83±24.29 ^b	264.83±11.94 ^b
ALT (U/g tissue)	12.93±1.34 ^c	52.17±8.61 ^a	12.5±1.05 ^c	30.67±4.50 ^b
AST (U/g tissue)	25.5±14.61 ^c	90.50±18.78 ^a	26.83±3.60 ^c	43.33±12.95 ^b
ALP (U/g tissue)	91.50±14.61 ^c	418.33±35.45 ^a	99.16±14.28 ^c	287.5±43.78 ^b
TC (mg/g tissue)	15.14±2.12 ^c	36.30±2.51 ^a	15.65±1.73 ^c	24.16±2.82 ^b
TG (mg/g tissue)	51.41±5.45 ^c	143.54±10.37 ^a	51.65±4.57 ^c	86.22±10.56 ^b
Serum TSH (μIU/ml)	0.30±0.02 ^c	0.62±0.10 ^a	0.32±0.04 ^c	0.40±0.03 ^b

1-Values expressed as the means ± SD.

2-Different superscripts in the same row indicate significant difference between groups at P< 0.05.

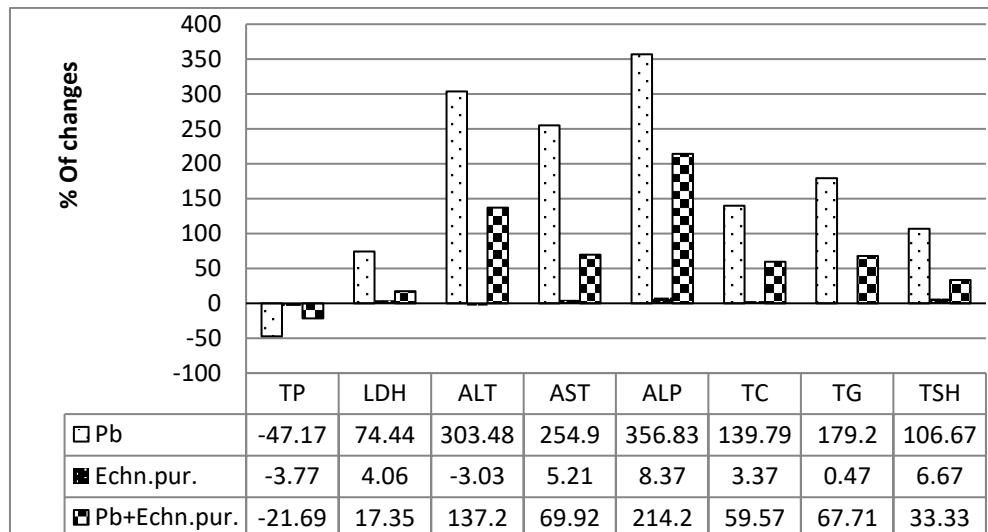


Fig.(2): The change in percentages of total protein (TP), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglyceride (TG) and thyroid stimulating hormone (TSH) in all the tested groups in comparison to the corresponding control group.

DISCUSSION

Lead is a major public health issue that affects all industrialized countries, and its occupational or accidental exposure is hazardous. The brain is a vulnerable organ to the poisonous effects of lead that may be attributed to inducing oxidative damage and lipid peroxidation in the cell membranes, and so compromising cellular functions and enhancing programmed cell death [28].

In addition, this disturbance in the antioxidant system may be attributed to the fact that the brain may have low antioxidant capacity, rich in lipids, and a high need for oxygen [29]. Thus, Pb enhances reactive oxygen species

generation (ex. hydrogen peroxide, hydroxyl radical, single oxygen) that causes the overproduction of malondialdehyde. MDA, a significant end product of polyunsaturated fatty acid oxidation, has been regarded as a key biomarker of lipid peroxidation resulting from the interaction of reactive oxygen species (ROS) with the lipid in cellular membranes, which induces disturbance in brain mitochondrial structure and function, increases membrane permeability, and alters Ca⁺² homeostasis [30]. Increasing the entry of Ca⁺² into the mitochondria enhances other electron transport across it and increases the production of ROS like O₂ [31].

NO has been associated with the mechanisms of cell harm and long-term physiological alterations in cellular excitability. A number of searches have revealed that NO may promote inflammation-induced cell and tissue dysfunction [32]. The significant increase in brain NO in the lead acetate treated group may be due to the fact that Pb enhances the inflammation in brain cells by stimulating nuclear factor kappa B (transcription factors), which activates nitric oxide synthase [32]. Calcium also activates nitric oxide synthase, increasing NO production in neurons [33]. The high NO levels interact with super oxide anion (O₂⁻) to form highly reactive peroxinitrite (ONOO⁻), a strong oxidising agent which induces oxidation of lipid, protein, and DNA. This oxidation plays an important role in brain cell death that has the characteristics of apoptosis [34].

GSH is a vital antioxidant formed in cells that, by conjugating with molecules, guards against the harm caused by free radicals and facilitates detoxification of xenobiotic compounds. Increasing oxidative stress was accompanied by a decline in GSH level. The significant decline of GSH in the group treated with Pb may be related to its binding with the SH group of GSH, which can be excreted, thus preventing the nucleophilic scavenger properties of GSH [35]. Previous studies found that exposure to lead significantly decreased the level of SOD, a free radical scavenger and metalloenzyme (zinc/copper) [36], and this is attributed to the high lead concentration in these tissues, which react with it. Catalase is a potent decomposer of H₂O₂ and also susceptible to lead toxicity [37]. Our results agree with Al-Quraishi et al. [38] and Akande et al. [39], who reported that Pb causes disturbances in antioxidant status by increasing MDA and NO, and decreasing CAT, SOD, and GPX in brain tissues.

Echinacea diminished oxidative stress induced by Pb through a significant decline in MDA and NO, restored GSH content, and improved the activities of CAT and SOD enzymes in brain tissues compared to the lead acetate group. This may be attributed to that *Echinacea* can preserve the structural integrity of brain cells from harmful effects of Pb due to the presence of bioactive ingredients in *Echinacea* such as cichoric acid, alkaloids, flavonoids, and phenolic acids which scavenge free radicals and inhibit nitration[13], thus *Echinacea* plays an important role in ameliorating neurotoxicity. These are in line with Ibrahim et al., [40] who found that *Echinacea* improves the antioxidant defense system in rabbits treated by dexamethasone. Additionally, it was discovered by

Sharif et al. [41] that *Echinacea* has high levels of antioxidant components such as caffeic acid, phenols, and echinacosides that can protect DNA from oxidation. Moreover, Elufioye et al [42] demonstrated that alkylamides in *Echinacea* exhibited anti-inflammatory action by decreasing nitric oxide, TNF- α , IL-2, and cyclooxygenase (COX)-dependent prostaglandin E2 production.

Lead acetate significantly decreases total protein in brain tissues this may be related to a decline in GSH content, increase in protein oxidation, and altered protein synthesis and degradation [35]. Additionally, Yousef et al. [43] observed that aluminum, a heavy metal like Pb, can interfere with protein or bind with some metal binding proteins and remove them through detoxification. However, *Echinacea* administration increased total protein in brain tissues, which may be due to *Echinacea*'s antioxidant properties, which reduce oxidative stress, improve GSH content, and decrease peroxinitrite compounds, which cause protein oxidation [42]. Our results are in agreement with Sadigh-Eteghad et al. [44], who observed that remediation by *Echinacea* (500 mg /4weeks) improved the effect of lead on total protein and albumin. Radwan et al. [45] also found that administration of *Echinacea* along with dexamethasone leads to normalization of the liver's protein levels.

The elevated brain levels of ALP, LDH, ALT, and AST after lead acetate intake reflect neuro-inflammation and damage of brain cells. ALP has a role in developmental neuro plasticity and activity-dependent cortical functions through contributing in γ -aminobutyric acid (GABA) metabolism [46]. LDH is an important metabolic enzyme in the neural cells which is involved in energy production by transformation of lactate to pyruvate.

Animal tissues require AST and ALT enzymes, whose activities are linked to the conservation of amino acid homeostasis. Disruption of their activities may be a sign of mitochondrial destruction and damage to the functional integrity of the cell membrane [47]. The elevated activities of AST, ALT, and ALP represent hippocampal injury and were accompanied by neuro inflammation and remarkable cell necrosis [48]. Increased permeability of the nerve cells' membrane leads to an increase in these enzymes in the soluble portion of nerve cells. This finding agreed with another study observed by Osman et al., [49] who recorded that the increase in LDH, ALP, ALT and AST in the brain of rats administrated aluminum another heavy metals similar to Pb. Baghshani and Shahsavani[50] revealed

that the LDH and ALT activities were elevated significantly in brain homogenates of common carp following Pb exposure.

The elevation in the levels of TC and TG in the lead group may be attributed to the Pb producing oxidative stress, causing an imbalance in brain lipid metabolism and elevation in TC, TG levels, thus causing neural degeneration and synaptic dysfunction. This result is consistent with Ghareeb et al [51] who found elevated levels of TC and TG in brain rats exposed to lead acetate in water 200 mg/l. Culter et al. [52] showed that oxidative stress in brain tissues induced accumulation of TC in membranes, which may have an effect on amyloid B peptide, thus increasing the risk of Alzheimer's. The *Echinacea* has the ability to improve the elevation of LDH, ALP, ALT, AST, TC, and TG in lead acetate treated rats. This amelioration might reflect the capability of *Echinacea* to avoid the injury effect caused by Pb in the cellular tissues of the brain, which may be related to the anti-inflammatory and antioxidant properties of *Echinacea* ingredients as scavenging free radicals. Furthermore, Radwan et al [45] found that *Echinacea purpurea* has antioxidant properties which cause a decline in the liver enzymes in dexamethasone treated rats. In addition, Nematalla et al. [53] concluded that administration of aged rats with *Echinacea* ethanolic and water extracts caused a significant improvement in the levels of lipid profiles (total cholesterol LDL and VLDL) and significantly elevated the level of HDL-C significantly in comparison with control aged rats.

The increase in serum TSH levels produced by lead may be linked to structural injury of thyroid follicular cells due to the accumulation of lead in the thyroid gland. This is similar to Algefare et al. [54], who observed that the elevation in serum TSH level induced by lead acetate is likely a response to the decrease in serum T4 and T3 levels in rats. Thyroid hormones play essential roles in the development of the central nervous system (CNS). Rieben et al.[55] and Elbadawy et al. [56] found a direct relationship between the increase in TSH level and an increased risk of dementia and Alzheimer's disease. Administration of *Echinacea* decreased TSH levels compared to the Pb group.

The histological alteration in the brain architecture of rats treated with Pb supported the biochemical finding. Highbab et al. [57] discovered that lead acetate caused histological changes in the brain. Also in our study, lead acetate displays neuro-toxic changes as well

as prominent caspase-3 positive immunoreactivity. This indicates that lead acetate enhanced MDA and NO lead to structural injury of the mitochondria and stimulation of cytochrome-c release into the cytosol, enhancing the activity of caspase-3 and facilitating apoptosis, proposing that lead-induced neurotoxicity [58]. However, *Echinacea* administration diminished the apoptotic effect of lead acetate through a significant decline in caspase 3 levels in brain immunohistochemical sections; this result supports the anti-apoptotic effects of these herbs. The antiapoptotic effect of *Echinacea* could be attributed to its antioxidant properties of several constituents such as polysaccharides, flavonoids, and Echinacoside [13]. Zhu et al. [59] reported that echinacoside inhibits the rotenone-induced release of cytochrome c and activation of caspase-3 through activating the Trk-extracellular signal-regulated kinase (Erk) pathway in neuronal cells. Because echinacoside can freely cross the blood-brain barrier, it may have a promising beneficial effect in neurodegenerative diseases. Moreover, according to Lu et al. [60], aloe polysaccharides are able to suppress caspase-3 expression and prevent neuronal death after ischemic brain damage. The mechanism underlying this could involve controlling the production of cytokines or cellular proteins to inhibit the activation of the caspase cascade. It might possibly be linked to lower brain neuronal apoptosis and controlling the suppression of caspase3 production.

CONCLUSION

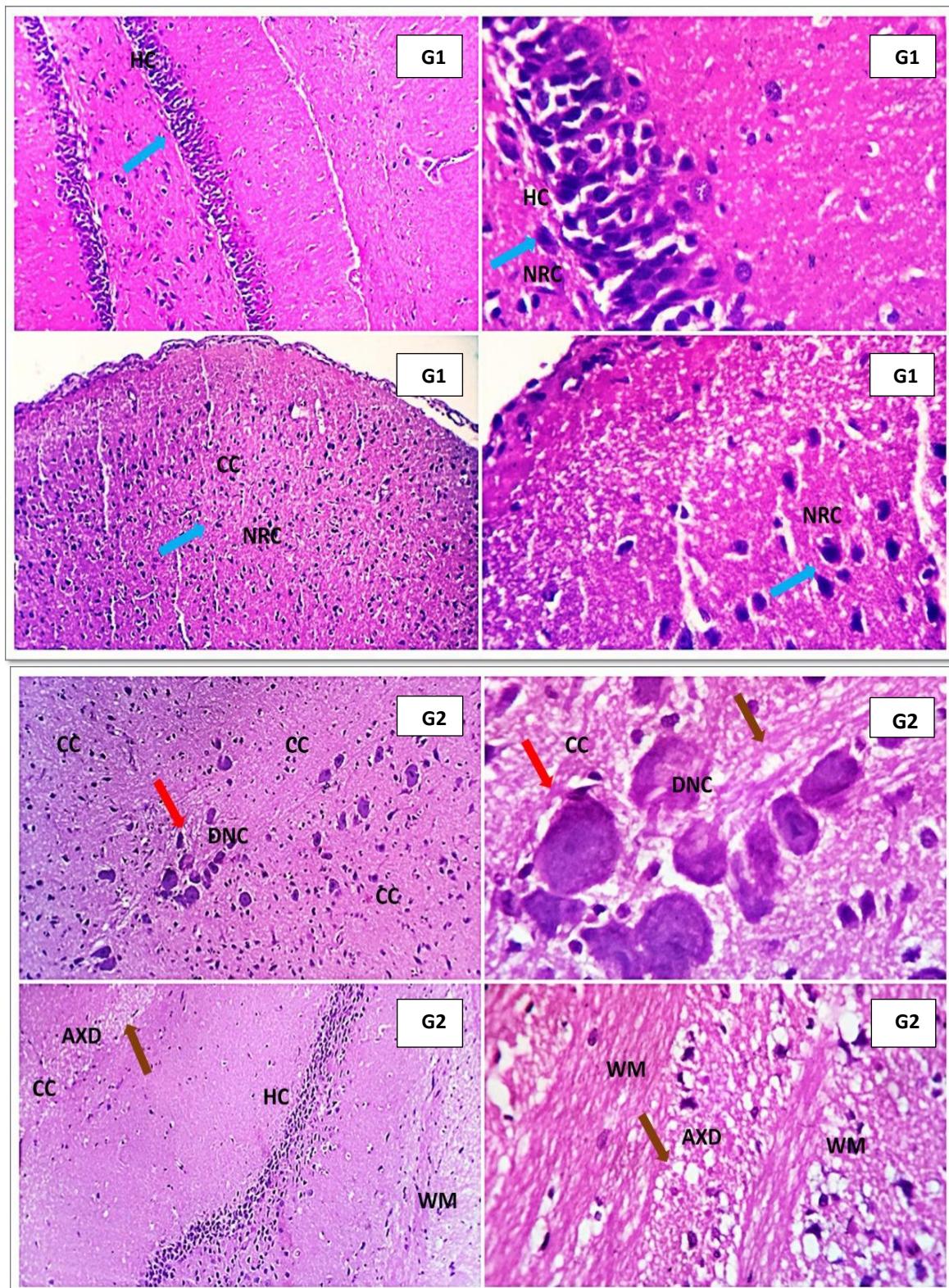
The current investigation demonstrates the potential effect of *Echinacea* in minimizing oxidative stress, apoptosis, inflammation, and histopathological alterations in the brain regions induced by lead acetate. The reasoning for its utilization is justified by biochemical and histological confirmation. Thus, this research can practically help to encourage the clinical application of *Echinacea* treatment for the risk effects of lead acetate on brain tissues.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.



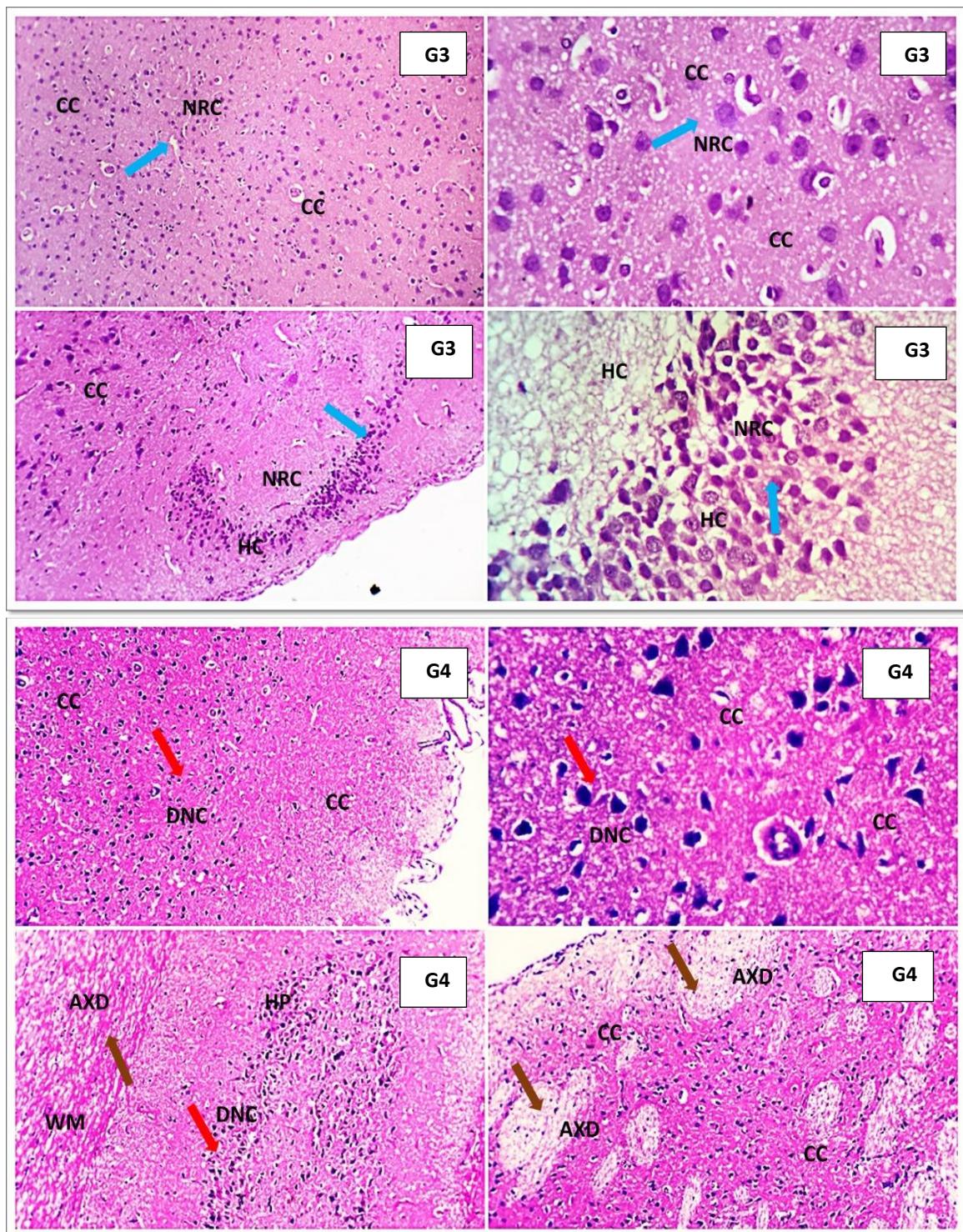


Fig. (3): Histological vertical section in the brain tissue of rat in the four different groups. (G1), Control, Showed normal histological structure. (G2), (Lead) showed mild disruption and degenerative changes in brain cell layer. G3, (*Echinacea*) showed no significant changes in the histological structure. G4 (Lead + *Echinacea*) showed regeneration and amelioration of brain cells. Key: NRC, Normal cells, WM, White matter, CC, Cerebral cortex, HC, Hippocampus, AXD, Anoxial degeneration, DNC, Degenerated nerve cells, (H&E, x100, x200, x400).

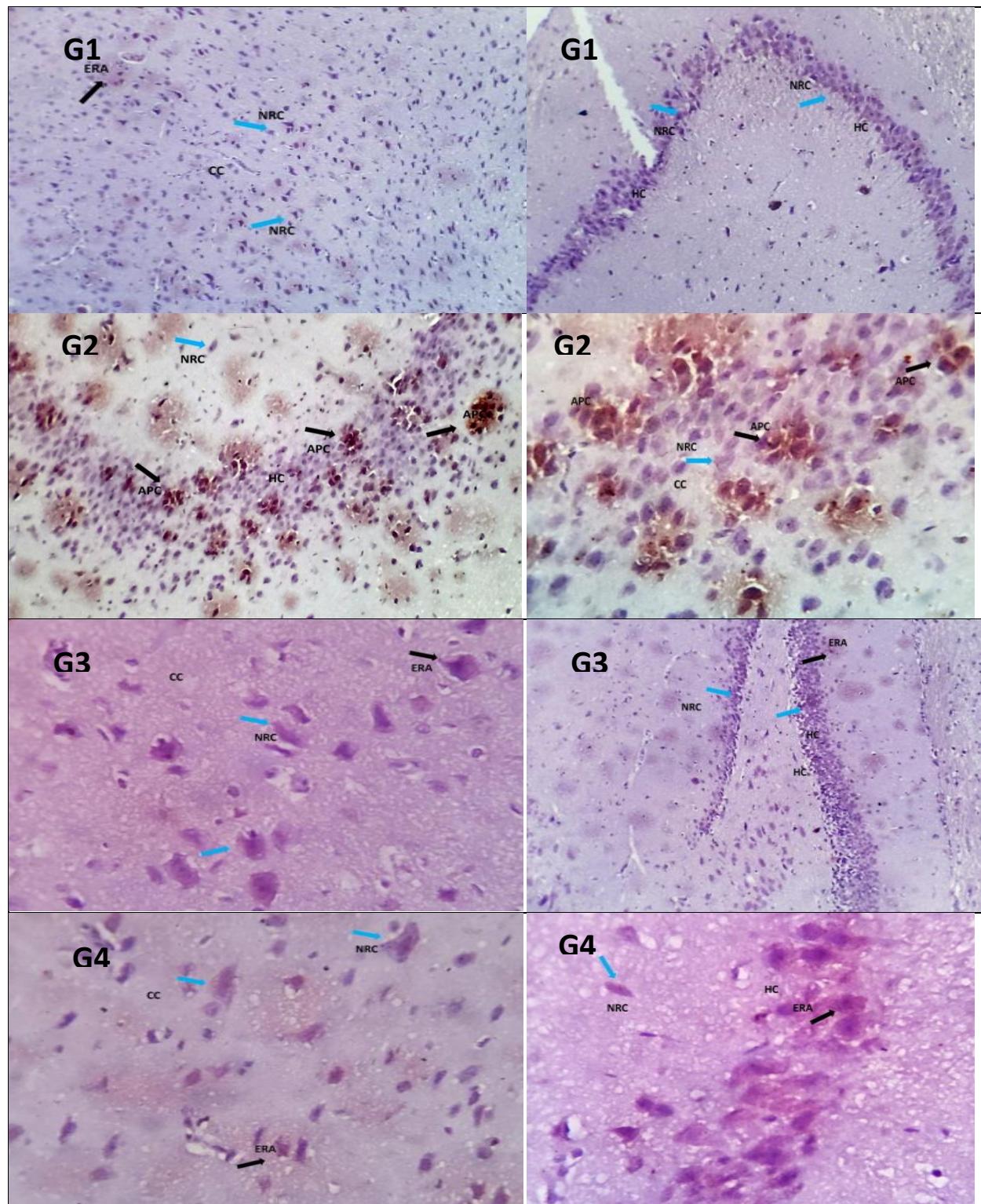


Fig. (4): Immunohistochemical staining of rat brain by caspase-3, the positive reaction of anti-calved caspase 3 antibodys were brown staining. Negative control group (G1). Lead acetate group (G2). *Echinacea* group (G3). Lead acetate treated with *Echinacea* (G4). NRC, normal cells; HC, hippocampus; APC, apoptotic cells; ERA, Early apoptotic cells; CC, Cerebral cortex, (H&E x200, x400).

REFERENCES

- [1] Lee, J.W., Choi, H., Hwang, U.K., Kang, J.C., Kang, Y.J., Kim, K.I. and Kim, J.H. (2019) "Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review," *Environmental toxicology and pharmacology*, **68**, 101-108.
- [2] Papanikolaou, N.C, Hatzidaki, E.G, Belivanis, S., Tzanakakis, G.N.and Tsatsakis, A.M (2005) Lead toxicity update. A brief review, *Medical science monitor*, **11**(10),RA329.
- [3] Patrick, L. (2006) Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment. *Altern Med Rev*, **11**, 2–22
- [4] Guidotti, T.L and Ragain, L. (2007) Protecting children from toxic exposure: three strategies, *Pediatr Clin North Am*, **54**, 227–235.
- [5] Liu, J., Liao, G., Tu, H., Huang, Y., Peng ,T., Xu, Y., Chen, X., Huang, Z., Zhang, Y., Meng X. and Zou, F. (2019) A protective role of autophagy in Pb-induced developmental neurotoxicity in zebrafish, *Chemosphere*, **235**, 1050–1058.
- [6] Kasprzyk, S., Dobrakowski, M., Kasprzyk, A., Ostalowska, A., and Birkner, E. (2013) The administration of N-acetylcysteine reduces oxidative stress and regulates glutathione metabolism in the blood cells of workers exposed to lead, *Clin. Toxicol. (Phila.)*, **51**,480–486.
- [7] Fan, Y., Zhao, X., Yu, J., Xie, J., Li, C., Liu, D., Tang, C.,and Wang, C. (2020) Lead-induced oxidative damage in rats/mice: a meta-analysis. *Journal of Trace Elements in Medicine and Biology*, **1**(58),126443.
- [8] Kalahasthi, R. and Tapu, B. (2018) Effect of Pb-exposure on serum calcium and phosphorus components among Pb-battery manufacturing workers, *Journal of Chemical Health Risks*, **8**(3), 183-190.
- [9] Rocha, A. and Trujillo, K. A. (2019) Neurotoxicity of low-level lead exposure: History, mechanisms of action, and behavioral effects in humans and preclinical models, *Neurotoxicology*, **73**,58–80
- [10] Khattab, H.A., Abounasef, S.K. and Bakheet, H.L. (2019) The biological and hematological effects of *Echinacea purpurea* L. Roots extract in the immunocompromised rats with cyclosporine, *Journal of Microscopy and Ultrastructure*, **7**(2), 65-71.
- [11] El-Sherbiny, E.M., Osman, H.F. and Taha, M.S. (2021). Effectiveness of *Echinacea purpurea* extract on immune deficiency induced by Azathioprine in male albino rats, *Bioscience Journal*, **1** (37), 1-12.
- [12] Coelho, J., Barros, L., Dias, M. I ., Finimundy, T. C. , Amaral, J.S. , Alves, M .J., Calhelha, R. C. and Santos, P. F. (2020) *Echinacea purpurea* (L.) Moench: Chemical Characterization and Bioactivity of Its Extracts and Fractions, *Pharmaceuticals*, **13**, 125:1-16.
- [13] Aarland, R.C., Bañuelos-Hernández, A.E., Fragoso-Serrano, M., Sierra-Palacios, E.D., Díaz de León-Sánchez, F., Pérez-Flores, L.J., Rivera-Cabrera, F.and Mendoza-Espinoza, J.A. (2017) Studies on phytochemical, antioxidant, anti-inflammatory, hypoglycaemic and antiproliferative activities of *Echinacea purpurea* and *Echinacea angustifolia* extracts, *Pharmaceutical biology*, **55**(1), 649-56.
- [14] Ahmdai, F., Samda, A., Sepehr, E., Rahimi, A. and Shabala, S. (2021) Increasing medicinal and phytochemical compounds of coneflower (*Echinacea purpurea* L.) as affected by NO₃–/NH₄⁺ ratio and perlite particle size in hydroponics, *Scientific Reports*, **11**(1), 1-11.
- [15] Çelik, S.A. and Kan, Y. (2018) The Determination of Mineral and Heavy Metal Contents of *Echinacea* Species Cultivated in Turkey, *International Journal of Secondary Metabolite*, **4**(3, Special Issue 2),363-71.
- [16] Shaban, N.Z., Abdelrahman, S.A., El-Kersh ,M.A., Mogahed, F.A., Talaat, I.M. and Habashy, N.H. (2020) The synergistic hepatoprotective potential of Beta vulgaris juice and 2, 3-dimercaptosuccinic acid in lead-intoxicated rats via improving the hepatic oxidative and inflammatory stress, *BMC Complementary Medicine and Therapies*, **20**(1),1-5.
- [17] Aladaileh, S.H., Khafaga, A.F., Abd El-Hack, M.E., Al-Gabri, N.A., Abukhalil, M.H., Alfwuaires, M.A., Bin-Jumah, M., Alkahtani, S., Abdel-Daim, M.M., Aleya, L. and Abdelnour, S. (2020) Spirulina platensis ameliorates the sub chronic toxicities of lead in rabbits via anti-oxidative, anti-inflammatory, and immune stimulatory properties, *Science of the Total Environment*, **701**,134879.

- [18] Zink, T. and Chaffin, J.(1998) Herbal ‘health’ products: what family physicians need to know, *Am Fam Physician*, **58**,1133–1140.
- [19] Al Zadjali, S., Nemmar, A., Fahim, M.A.A., Azimullah, S., Subramanian, D., Yasin, J., Amir, N., Hasan, M.Y. and Adem, A., (2015). Lead exposure causes thyroid abnormalities in diabetic rats. *International journal of clinical and experimental medicine*, **8**(5), p.7160.
- [20] Khalaf, A.A., Hussein, S., Tohamy A.F., Marouf, S., Yassa, H.D., Zaki, A.R. and Bishayee, A.(2019) Protective effect of Echinacea purpurea (Immulant) against cisplatin-induced immunotoxicity in rats, *Daru*,**27**(1),233-241.
- [21] Beutler, E., Duron, O. and Kefly, B.M. (1963) Improved method for the determination of blood glutathione, *J. Lab. Clin. Med.*, **61**,882-888.
- [22] Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Analytical biochemistry* , **95**(2),351-8.
- [23] Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R. (1982) Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids, *Anal Biochem*, **126**, 131–138
- [24] Aebi, H. (1984) Catalase in vitro, *Enzymol* , **105**,121–126.
- [25] Sun, Y., Oberley, L.W. and Li, Y. (1988)A simple method for clinical assay of superoxide dismutase, *Clin Chem* , **34**, 497–500.
- [26] Suvarna, S. K., Layton, C., and Bancroft, J. D. (2013). Theory and practice of histological techniques, *Pbl. London, Churchill Livingstone, Elsiver*, **173**,187.
- [27] Eissa, S. and Shoman, S. (1998) .Markers of invasion and metastasis and markers of tumor proliferation and apoptosis in: Tumors markers. Chapman and Hall, Lippincott- Raven Publisher Inc., London, UK, p. 131-153
- [28] Dkhil, M.A., Moneim, A.E. and Al-Quraishy, S. (2016) Indigofera oblongifolia ameliorates lead acetate-induced testicular oxidative damage and apoptosis in a rat model. *Biol Trace Elem Res*, **173**,354–361
- [29] Selim, N.M., Elgazar, A.A., Abdel-Hamid, N.M., Abu El-Magd, M.R., Yasri, A., El Hefnawy, H.M. and Sobeh, M. (2019) Chrysophanol, physcion, hesperidin and curcumin modulate the gene expression of pro-inflammatory mediators induced by lps in hepg2: In silico and molecular studies, *Antioxidants*, **8**(9),371.
- [30] Hulbert, A.J., Pamplona, R., Buffenstein, R. and Buttemer, W.A. (2007) Life and death: metabolic rate, membrane composition, and life span of animals, *Physiol Rev*, **87**,1175–1213.
- [31] Shaban, N.Z., El-Kader, A., Sara, E., Mogahed, F.A., El-Kersh, M.A. and Habashy, N.H. (2021) Synergistic protective effect of Beta vulgaris with meso-2, 3-dimercaptosuccinic acid against lead-induced neurotoxicity in male rats, *Scientific Reports*, **11**(1),1-8.
- [32] Adam-Viz, V. and Starkov A. A. (2010) Calcium and mitochondrial reactive oxygen species generation: how to read the facts, *Journal of Alzheimer's disease*, **20**(s2), S413-S426.
- [33] Al-Megrin, W.A., Alkhuriji, A.F., Yousef, A.O., Metwally, D.M., Habotta, O.A., Kassab, R.B., Abdel Moneim, A.E. and El-Khadragy, M.F. (2020) Antagonistic efficacy of luteolin against lead acetate exposure-associated with hepatotoxicity is mediated via antioxidant, anti-inflammatory, and anti-apoptotic activities, *Antioxidants*, **9**(1),10.
- [34] Garcin, E.D., Bruns, C.M., Lloyd S.J., Hosfield, D.J., Tiso M., Gachhui, R., Stuehr, D.J., Tainer, J.A. and Getzoff, E.D. (2004) Structural Basis for Isozyme-specific Regulation of Electron Transfer in Nitric-oxide Synthase*[boxs], *Journal of biological chemistry* , **279**(36),37918-27.
- [35] Aquilano, K., Baldelli, S. and Ciriolo, M.R., (2014). Glutathione: new roles in redox signaling for an old antioxidant. *Frontiers in pharmacology*, p.196.
- [36] Abdel Moneim, A.E. (2016) Indigofera oblongifolia prevents lead acetate-induced hepatotoxicity, oxidative stress, fibrosis and apoptosis in rats, *PloS One* **11**,e0158965
- [37] Chintapanti, S., Pratap Reddy, K. and Sreenivasula Reddy, P. (2018) Behavioral and neurochemical consequences of perinatal exposure to lead in adult male Wistar rats: protective effect by Centella asiatica, *Environ Sci Pollut Res Int* , **25**, 13173–13185
- [38] Al-Quraishy, S., Dkhil ,M.A., Ibrahim, S.R. and Abdel Moneim, A.E. (2016) Neuroprotective

- potential of *Indigofera oblongifolia* leaf methanolic extract against lead acetate-induced neurotoxicity, *Neural Regen Res.*, **11**(11), 1797–1803.
- [39] Akande, M.G., Aliu, Y.O., Ambali, S.F. and Ayo, J.O. (2016) Co-treatment of chlorpyrifos and lead induce serum lipid disorders in rats: alleviation by taurine, *Toxicol Ind Health*, **32**, 1328–1334.
- [40] Ibrahim, A.A., Saleh, R.M., Hassan, A.E. and Amer, M.S. (2020) Ameliorative effect of *Echinacea purpurea* and curcumin on dexamethasone-induced immunosuppressive rabbits, *KVMJ*, **18** (1), 10–16.
- [41] Sharif, K.O.M., Tufekci, E.F., Ustaoglu, B., Altunoglu, Y.C., Zengin, G., Llorente-Martínez, E.J., Guney, K. and Baloglu, M.C., (2021). Anticancer and biological properties of leaf and flower extracts of *Echinacea purpurea* (L.) Moench, *Food Bioscience*, **41**, p.101005.
- [42] Elufioye, T.O., Habtemariam, S. and Adejare, A. (2020) Chemistry and Pharmacology of Alkylamides from Natural Origin. Rev. Bras. Farmacogn, **30**, 622–640
- [43] Yousef, M.I. (2004) Aluminium induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of malarabbits: protective role of ascorbic acid, *Toxicology*, **199**(1), 47–57.
- [44] Sadigh-Eteghad, S., Abadi, N., Ghavami, S., Golabi, M., Shanebandi, D. (2011) Synergetic effects of oral administration of levamisole and *Echinacea purpurea* on immune response in Wistar rat, *Research in veterinary science*, **91**(1), 82–5.
- [45] Radwan, O.K., El-Nabarawy, S.K., El-Sayed, R.A., El-Sisi, S.F. and Abd El-Salam, F.F. (2019) The Protective Effect of Bee Venom and *Echinacea Purpurea* against Liver Injury Induced by Dexamethasone, *Pharm Chem J*, **6**, 25–38.
- [46] Fonta, C., Négyessy, L., Renaud, L. and Barone, P. (2004) Areal and subcellular localization of the ubiquitous alkaline phosphatase in the primate cerebral cortex: evidence for a role in neurotransmission, *Cereb Cortex*, **14**(6), 595–609.
- [47] Zhao, H., Liu, S., Zhao, H., Liu, Y., Xue, M., Zhang, H., Qiu, X., Sun, Z. and Liang, H., (2021). Protective effects of fucoidan against ethanol-induced liver injury through maintaining mitochondrial function and mitophagy balance in rats, *Food & Function*, **12**(9), pp.3842–3854.
- [48] Cattani, D., Cavalli, V.L.D.L.O., Rieg, C.E.H., Domingues, J.T., Dal-Cim, T., Tasca, C.I., Silva, F.R.M.B. and Zamoner, A., (2014). Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity, *Toxicology*, **320**, pp.34–45.
- [49] Osman, N.N., Alanbari, K.H. and Al-Shreef, H.A. (2018) Evaluation of the possible antioxidant effects of *Peganum Harmala* and *Ginkgo Biloba* in Ameliorating Alzheimer's disease in rat model *IJPSPR*, **9**(8), 3189–3198
- [50] Baghshani, H. and Shahsavani, D. (2013) Effects of lead acetate exposure on metabolic enzyme activities in selected tissues of common carp (*Cyprinus carpio*), *Comp Clin Pathol*, **22**, 903–907
- [51] Ghareeb, D.A., Hussien, H.M., Khalil, A.A., El-Saadani, M.A. and Ali, A.N. (2010) Toxic effects of lead exposure on the brain of rats: Involvement of oxidative stress, inflammation, acetylcholinesterase, and the beneficial role of flaxseed extract, *Toxicological & Environmental Chemistry*, **92**(1), 187–95.
- [52] Cutler, R.G., Kelly, J., Storie, K., Pedersen, W.A., Tammara, A., Hatanpaa, K., Troncoso, J.C. and Mattson, M.P. (2004) Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease, *Proceedings of the National Academy of Sciences*, **101**(7), 2070–5
- [53] Nematalla, Kh.M., Sahar, Arafa A., Ghada, Yousef M., and Zainb, Shabib A. (2011). Effect of chinacea as Antioxidant on Markers of Aging, *Australian Journal of Basic and Applied Sciences*, **5**, 18–26
- [54] Algefare, A., Sedky, A.M. and Alfwaires, M. (2021) Apigenin Ameliorates Lead Acetate induced Hyperlipidemia, Hypothyroidism and Hypogonadism in Male Rats, *researchsquare.com*, 1–15
- [55] Rieben, C., Segna, D., da Costa, B.R., Collet, T.H., Chaker, L., Aubert, C.E., Baumgartner, C., Almeida, O.P., Hogervorst, E., Trompet, S. and Masaki, K. (2016) Subclinical thyroid dysfunction and the risk of cognitive decline: a meta-analysis of prospective cohort studies, *The Journal of Clinical Endocrinology & Metabolism*, **101**(12), 4945–54.
- [56] Elbadawy, AM, Mansour AE, Abdelrassoul, IA, Abdelmoneim, RO. (2020) Relationship between

- thyroid dysfunction and dementia, *The Egyptian Journal of Internal Medicine*, **32**(1),1-9.
- [57] Highab, S. M., Magaji, R. A., and Muhammad, B. Y. (2018). Effect of Lead poisoning and antidepressant drug on the cerebral cortex of the Wistar rats. *Acta Scientific Pharmaceutical Sciences*, **2**(5), 16-21.
- [58] Hosseini, A., Sharifi, A.M., Abdollahi, M., Najafi, R., Baeeri, M., Rayegan, S., Cheshmehnour, J., Hassani, S., Bayrami, Z., Safa, M. (2015) Cerium and yttrium oxide nanoparticles against lead-induced oxidative stress and apoptosis in rat hippocampus, *Biological Trace Element Research*, **164**(1),80-9
- [59] Zhu, M., Lu, C. and Li, W., (2013). Transient exposure to echinacoside is sufficient to activate T rk signaling and protect neuronal cells from rotenone, *Journal of Neurochemistry*, **124**(4), pp.571-580.
- [60] Lu, Z.Q., Deng, Y.J. and Lu, J.X., (2012). Effect of aloe polysaccharide on caspase-3 expression following cerebral ischemia and reperfusion injury in rats. *Molecular Medicine Reports*, **6**(2), pp.371-374