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Ameliorating Effect of Black Chia (*Salvia Hispanica L.*) Oil and/ or Peppermint (*Mentha Piperita L.*) Oil on Neurological Effects Induced by Lead Acetate in Growing Albino Rats

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Abstract

Lead poisoning has been classified as a non-traumatic brain injury. Exposure to lead during childhood may result in cognitive changes, memory disorders and requires neurodevelopmental monitoring till early adulthood. The present study investigated the ameliorative effect of black chia oil (COE) peppermint oil (POE) and their mixture against the neurological effects induced by lead acetate (LA) in growing albino rats. Eighty male rats were divided into eight groups, HCG: healthy control group; COCG: Chia oil group; POCG: peppermint oil group; COE+POE: Chia & peppermint oils mixture group; LACG: Lead acetate group; LA+COE: lead acetate + chia oil group; LA+POE: lead acetate+ peppermint oil group; LA+MOsE: lead acetate + chia and peppermint oils mixture group. Biochemical parameters including neural cell markers (BTChe, 5-HT and DA), antioxidant (SOD, CAT, TAC, GST and GPx), inflammatory (TNF-α and IL-6), oxidative stress (MDA), liver enzymes and kidney functions were investigated. Histopathology changes in brain and liver tissues was also detected. Supplementation of COE, POE and their mixture have proved to exerts an ameliorative effect against the neurological and histological disorders resulted from oral lead administration in growing rats. The synergistic effect of peppermint oil with chia oil resulted in a strong ameliorating effect against lead-intoxication.

Keywords: Neurological disorders, Black chia oil, Peppermint volatile oil, Lead acetate, Biochemical changes, Synergistic action

1. Introduction

Neurons represent the main unit of the central nervus system (CNS). Its plays a very important function in organization of neural and non-neural cells. Neurons also responsible for controlling many behaviors including motor and sensory functions. Neurons have different types differentiated according to its morphology and function and each neuronal cell have its own genomic profile (Jové et al., 2014). Exposure to toxic agents may resulted in neurophysiological changes in the CNS, which causes in turn cognitive changes, memory disorders, and changes in mood or onset of psychiatric disturbances and finally lead to degenerative disorders as Alzheimer's diease (Mason et al., 2013). Recent researches stated that exposure to environmental toxins as neurotoxic metals (Lead, Mercury, Arsenic, Cadmium and Aluminum) during childhood may lead to neurodegenerative ailments in older ages (Nabi & Tabassum 2022)

Lead is a common heavy metal found naturally in the Earth's crust and widely used in industry, particularly in products such as construction materials, paint, batteries, and piping. Lead have been classified as neurotoxin that can easily accumulate inside soft tissue and bones. It affects nervous system and interferes with biological enzymes functions. Lead may also cause a sever health problems in other organs as liver, kidneys and hematopoietic system. Moreover, the IARC has classified lead as carcinogenic compound to humans (Al-Naimi et al., 2011).

Chia (Salvia hispanica L.) and Peppermint (Mentha piperita L.) are belonging to Lamiaceae family. Chia is a small seed that comes from an annual herbaceous plant, Salvia hispanica L. It known with its high nutritional and medicinal values as it contains healthy omega-3 fatty acids, polyunsaturated fatty acids, proteins, minerals, dietary fiber, and vitamins. The seeds are an excellent source of polyphenols and antioxidants, such as rosmarinic acid, caffeic acid, quercetin and myricetin (Hrnčič et al., 2020). Omega

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fatty acids were reported for their high protecting effect against lead toxicity. They also known to play a very important role in brain development during childhood stage and vital for the maintenance of normal brain function throughout life (Abdou and Hassan, 2014).

Peppermint oil is one of the most widely used essential oil. That used in food, as herbal tea or for medicinal uses as it includes carminative, anti-inflammatory, antiemetic,antispasmodic, diaphoretic, analgesic and as stimulant. It is also used against nausea, flatulence, bronchitis, anorexia, ulcerative colitis, and liver complaints (Bellassoued et al., 2018).

The present study focused on the ameliorative effect of CO, PO or their mixture on neurological disorder induced experimentally via several biochemical and histopathological investigations. Moreover, studying the antioxidant and anti-inflammatory effect of the tested extract oils.

2. Materials and methods

2.1 Tested oils Preparation, Extraction

Black chia seeds were extracted by Soxhlet apparatus using hexane to obtain the highest linolic acid content *(Ishak et al., 2020)*. Chia seeds were obtained from abo auf company Industrial Zone, No. 31 - 33 - Third Settlement - New Cairo – Egypt. The method of *Freedman et al., (1979)* was adopted with a minor modification. Samples of 100 g of grounded black chia seeds was inserted in the Soxhlet thimble, using hexane at a rate of 3 ml/g plant material and extracted for 8 hrs. The solvent was evaporated till dryness under vacuum using rotavapor apparatus using a water bath adjusted to 40°C. The crude residues were then weighed for estimating the yield percentages and kept in a deep freezer (-4ºC) till used. Peppermint oil was extracted by hydro distillation method using Clevenger apparatus *(Mansour et al., 2016).* Peppermint leaves were collected from the field, washed with tap water, shad dried and crushed gently by hands. The leaves powder was mixed with distilled water and extracted for 3 hrs.

The total % yield was calculated according to *Khajeh (2011)* as follow:

% yield= weight of crude extract/ weight of plant powder x 100.

2.2. Analysis of plant oils

The chemical composition of black chia seeds and peppermint oils were identified using GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The oven temperature was initially held at 50°C and then increased by 5°C /min to 250°C withhold 2 min. The injector temperature was kept at 270°C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 µl were injected using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source and transfer line were set at 200 °C and 280 °C respectively. The components were identified by comparing their mass spectra with those of WILEY 09 and NIST14 mass spectral database.

2.3. Animals

Eighty growing male albino rats *(Rattus norvegicus)* weighing 90±5 g was precured for the present study. Tested rats were 3 weeks old to represent childhood stage (***Andreollo et al., 2012*).** Animals were obtained from the National Research Centre animal house and kept in good ventilated cages, fed on commercial pellet diet with drinking water *ad-libitum.* Animals were divided into groups and adapted for 7 days.

2.4. Dosing and Treatments

Growing rats were segregated into 8 groups (10 for each) as illustrated in **table (1).** Lead acetate and tested oils were orally administrated. Lead acetate was administrated at 75 mg/kg b.wt while chia oil and peppermint oils were orally applied at 0.9g/ kg b.wt (*(Institute of Mwdicine, 2005)* and 0.04 g/kg b.wt (*Bellassoued et al., 2018*), respectively. Mixture groups were administrated a mixed dose of chia at (0.45g/ kg b.wt.) and peppermint at (0.02g/ kg b.wt.). A uniform volume of 1 ml was used for all rats and control group received an equivalent volume of distilled water.

2.5. Blood sampling and tissue preparation

At the end of experimental period (4 weeks) blood samples were collected under sodium barbiturate anesthesia *(Flecknell,2009)* in non-heparinized tubes and centrifuged at 3500 round per minute (rpm) (600 g) for 10 min at - 4°C using Hereaeus Labofuge 400R, Kendro Laboratory Products GmbH, Germany. The separated serum was kept at –20 °C until analyzed. After blood collection, rats were euthanized by cervical dislocation and dissected for removing internal organs. Isolated organs (liver, brain, kidney, spleen and heart) were weighed to calculate relative organs weight and brain was divided into two parts. The first part (1gm) was homogenized in Nacl buffer, centrifuged for 15 min at 1800 rpm at -4°C and the resulted supernatants were kept at – 20°C till used in biochemical analysis. The second part was preserved in 10% buffered formaldehyde solution until used in histopathological examination.

Table (1): Chart bars illustrating the dosing plan of lead acetate and tested oils orally administrated for growing albino rats

Treatment groups	Days		
	1	7	28
HCG	Distilled water		
COCG	Chia oil		
POCG	Peppermint oil		
MOsCG	Chia + peppermint oil mixture		
LACG	Lead acetate	Distilled water	
LA+CO	Chia oil	Lead acetate	
LA+PO	Lead acetate	peppermint oil	
LA+MOs	Lead acetate	Chia &peppermint oil mixture	

2.6. Biochemical analysis

2.6.1. Estimation of Neural Cell Markers

Brain markers were measured to identify lead-neural changes and the effect of tested oils and their mixture**.**

Butryil Choline esterase (BTChe) was determined by using quantitative colorimetric kinetic assay kit (BEN-Biochemical Enterprise, Italy) according to ***Young et al. (2000)***. Levels of serotonin (5-HT), and dopamine (DA) were measured in brain tissue homogenate using (Mybiosource, USA), CAT.NO (MBS725497) kits by competitive enzyme immunoassay technique according to ***Kittler et al. (2010).***

2.6.2. Estimation of Neural Oxidant/Antioaidant Markers

Glutathione peroxidase (GPx) and Glutathion S-Transferases (GST) Superoxide dismutase (SOD), Catalase (CAT), Total antioxidant capacity (TAC) and Malondialdehyde (MDA) were measured in brain tissue homogenate using colorimetric method in accordance to enclosed manufacture pamphlet.

2.6.3. Estimation of Pro-inflammatory Markers

Tumor necrosis factor -α (TNF-α) and Interlukine-6 (IL-6) were determined in brain tissue homogenate by colorimetric method using their related ELISA kits according ***Dowlati et al. (2010)*** and ***Hirano, (1998)***, respectively.

2.6.4. Estimation of Liver Enzymes activity

Coulometric method was used to determine Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in liver serum using kits of Spectrum Diagnosis, Egypt. Serum Alkaline phosphatase (ALP) was measured by kinetic method according to **Zawta *et al. (1994*)** .

2.6.5. Estimation of Kidney Functions activity

Serum Urea and Creatinine were determined by colorimetric method using commercial kits obtained from Biodiagnosis, Egypt according to ***Fawcett and Soctt (1960)***, and ***Schirmeister et al. (1964*)**,* respectively.

2.7. Histopathological studies

Brain and liver Tissue samples were fixed in 10% neutral buffered formalin for 72 hrs. stained by Hematoxylin and Eosin and examined with light microscopic under magnification X100 and X400 by using Full HD microscopic imaging system (Leica Microsystems GmbH, Germany) ***(Culling, C.F.A. , 2013)*** .

2.8. Statistical analysis

Data were using one-way analysis of variance ANOVA using Statistical Package for Social Science ―SPSS‖ version 16.0 Microsoft Windows, SPSS Inc. Values were expressed as mean ± standard error (SE) and the mean difference was significant at the ($P \leq 0.05$) level according to ***Levesque, (2007).***

3. Results

3.1. Phytochemical Constituents of Black Chia andPeppermint oils

The percent yield for chia seeds (CO) and peppermint oil (PO) was, respectively. Tables (2, 3) represent the concentration of the main constituents in black chia oil and peppermint volatile oil analyzed by GC-MS analysis. Fatty acid content represents 37.81% in CO and Z-citral represent the main constituent (19.59%). CG/MS analysis identification of PO revealed the presence of enthol, menthone, menthofuran, menthyl acetate, camphene, D-limonene, eucalyptol, 1-Hexade canol,2-methyl, geranyl acetate, myrcene as active constituents, where Menthol represents the highest concentration (35.47%). **Figures 1 & 2** represented the chromatograms of CO and PO, respectively.

Table (2): The Chemical Components of Black Chia Oil Extract by GC-MS and their Retention Time (min)

Compound name	Peak area %	RT (min)
Z-Citral	19.59	9.87
α- Citral	18.82	10.61
Oleic acid	8.88	29.74
n-Hexadecanoid acid	6.28	26.49
Estragole	5.61	8.86
Erucic acid	5.2	36.21
β- Sitosterol	4.27	42.58
Yamogi alcohol	3.58	12.87
2,3-Epoxyneral	3.00	12.01
Geranyl acetate	2.79	13.53
Glycidyl oleate	2.77	34.89
9,12-Octadecadienoic acid- (Z,Z)	2.58	29.49
Neric acid	2.21	13.29
d-Linalool	1.87	6.75
1H-Benzocycloheptan-7-ol	1.58	29.11
Octadecanoic acid	1.54	30.16
Geraniol	1.48	10.43
Linoleic acid ethyl ester	1.29	34.00
D-8-Hydroxycarvotan acetone	1.25	12.59
9-Octadecenoic acid (Z)-2,3-dihydroxypropyl ester	1.25	34.14
D-Limonene	1.11	5.32
9,12,15-Octadecenoic acid 2,3-dihydroxypropyl ester,(Z,Z,Z)-	1.04	34.76
Hernandulcin	0.90	29.28
9,12 - Octadecenoic acid (Z,Z)-,2-hydroxy-1-1-(hydroxymethyl) ethyl ester	0.84	31.32
Total	99.73%	
Sesquiterpenoids	37.81%	
Monoterpenoids	61.02%	
Fatty acids	0.90%	
Others	0.27%	

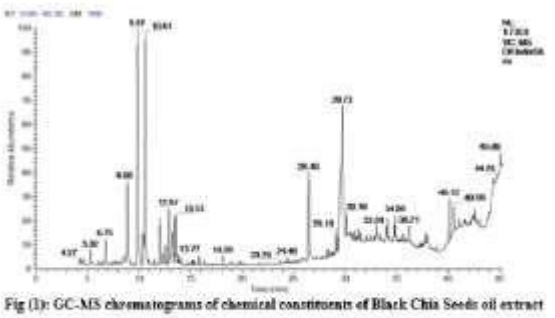

Fig (1): GC-MS chromatograms of chemical constituents of Black Chia Seeds oil extract

Table (3): The chemical components of Peppermint Oil Extract by GC-MS and their Retention Time (min)

Compound name	Peak area (%)	RT (min)
Menthol	35.47	19.02
Menthone	20.25	18.39
Menthofuran	7.81	18.72
Menthyl acetate	6.23	22.24
Camphene	5.92	4.10
D-Limonene	3.68	10.18
Eucalyptol	2.32	20.04
1-Hexadecanol,2-methyl	2.04	23.28
Geranyl acetate	1.99	17.31
Myrcene	1.48	4.76
a-Citral	1.46	22.54
Alpha-pinene	1.39	4.32
Octanol	1.37	6.60
a-Curcumene	1.11	17.05
β- Eunebrene	1.08	17.84
Neo-iso-menthyl acetate	1.06	13.91
P-Cymene	0.85	6.23
Cis-Carane	0.83	14.48
Thymol	0.68	25.84
Cedrene	0.67	17.41
β -Bisabolene	0.35	17.98
1,8 Cineole	0.24	11.85
Neryl acetate	0.18	24.36
Trans-Caryophllene	0.16	12.34
Germacrene	0.02	10.56
Total %	93.12%	
Sesquiterpenoids	7.17%	
Monoterpenoids	87.95%	
Others	4.88%	

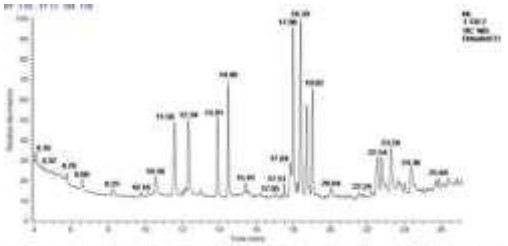

Fig (2): GC-MS chromatograms of chemical constituents of peppermint volatile oil extract

3.2. Effect of Black Chia, peppermint oils and Their Mixture on Brain Neurotransmitters and Brain Inflammatory Markers in Lead acetate-treated rats

Growing male rats treated with lead acetate showed a significant decline ($P \leq 0.05$) in the levels of BuChE, serotonin and dopamine when compared with control group **(Table 4)**. This effect was ameliorated after the administration of COE, POE and their mixture. The pest ameliorative effect was recorded in oils mixture group (MOsE) as 7764.50±21.26 U/L, 38.152±0.29 ng / g and 44.86±0.91 ng / g for BuChE, serotonin and dopamine, respectively when compared with COE and POE groups. Lead acetate toxicity induces massive increase in the levels of brain TNF-α and IL-6 in the LACG when compared with control groups. Oral administration of COE, POE and MOsE to intoxicated rats caused improvement in the levels of inflammatory markers where mixture treatment group showed the highest ameliorative effect **(Table 5).**

Table (4): Effect of Black Chia Oil and / or Peppermint Volatile Oil Extracts and their Mixture Supplementation on Brain butyryl cholinesterase (BuChE), Brain serotonine (5-HT) level and Brain dopamine (DA) level in lead acetate intoxicated Rats

Parameters / Groups	BuChE (U/L)	5-HT (ng/g)	DA (ng/g)
G1: HCG	7687.80±8.99 ª	40.643±0.41 ª	50.06±0.58ª
G2: COECG	7614.70±8.99 ª	37.038±0.28 ª	49.98±0.4526ª
G3: POECG	7660.1±8.89 ª	34.804±0.53ª	48.78±0.63 ª
G4: MOsECG	7787.80±14.83 ª	39.186±0.43 ª	50.06±0.77ª
G5: LACG	4368.40±47.06 ª	9.394±0.25ª	12.52±0.25 ª
G6: LA+COE	5495.50±26.87 ª	36.83±0.23 ª	32.66±0.66ª
G7: LA+POE	5938.60±43.97ª	28.75±1.09ª	34.07±0.19ª
G8:LA+MOsE	7784.10±21.26 ª	38.152±0.29 ª	44.86±0.91ª
LSD	75.8469	1.4512	1.63255

There was no significant difference between mean have the same alphabetical superscripts letter in the same column ($P \leq 0.05$).

Table (5): Effect of black chia and / or peppermint essential oils extracts and their mixture on TNF-α and IL-6 in different experimental groups (mean + SE)

Parameters / Groups	TNF-α (pg/mL)	IL-6 (pg/mL)
G1: HCG	15.17±0.07 ª	47.40±0.54 ª
G2: COECG	21.06±0.51 ª	62.47±0.56 ª
G3: POECG	19.17±0.12 ª	57.80±0.15 ª
G4: MOsECG	17.75±0.25 ª	54.27±0.22 ª
G5: LACG	72.41±0.74ª	101.18±0.51ª
G6: LA+COE	40.50±0.36 ª	97.18±1.14 ª
G7: LA+POE	37.47±0.10 ª	88.33±0.72 ª
G8:LA+MOsE	25.04±0.01 ª	78.47±1.57 ª
LSD	3.0243	2.2825

There was no significant difference between mean have the same alphabetical superscripts letter in the same column ($P \leq 0.05$).

3.3. Effect of Black Chia, peppermint oils and Their Mixture on Antioxidant System and Lipid Peroxidation in Lead-intoxicated Growing Rats

Compared with control group, oral administration with LA resulted in a significant decrease in antioxidant markers; SOD, CAT, TAC, GPX as well as GST as illustrated in **table (6)**. Treatment with COE, POE ameliorated the LA-intoxication effect while supplementation of chia and peppermint oils mixture bring them back to normal levels. injection with Lead acetate caused a significant increase ($P \leq 0.05$) in MDA level to record (7.06±0.01 nmol/g) in LACG, this value decreased significantly ($P \leq 0.05$) to reach (6.28±0.02 nmol/g), (5.93±0.02 nmol/g) and (4.74±0.04 nmol/g) in (LA+COE), (LA + POE) and (LA + MOsE), where the administration of COE caused the most significant improvement in brain MDA level followed by (POE) and finally (MOsE) supplemented groups **(Fig.3)**.

Table (6): Effect of black chia and/or peppermint essential oils extracts and their mixture on antioxidant and oxidative stress in different experimental groups (mean ± SE)

Parameters / Groups	SOD (U/g)	CAT (U/g)	TAC (mM/L)	GPx (U/g)	GST (U/g)
G1: HCG					
G2: COECG					
G3: POECG					
G4: MOsECG					
G5: LACG					
G6: LA+COE					
G7: LA+POE					
G8: LA+MosE					
LSD	21.8799	Zero	0.0244	34.1965	0.3322

There was no significant difference between mean have the same alphabetical superscripts letter in the same column ($P_{\leq} 0.05$)

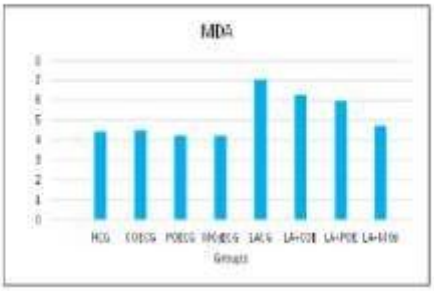

Fig. (3): Effect of COE, POE and MOsE administration on MDA (nmol/ g) level in LA intoxicated rats.

3.4. Effect of CO, PO and their mixture on Liver Enzymes level and Kidney functions in lead-treated rats

Results in **table (7)** indicated a significant increment in serum AST, ALT and ALP enzymes activities in groups of rats injected with LA by (98.42±0.14 U/L), (97.61±0.54 U/L) and (425.53±0.05 U/L), respectively when compared with control group. As illustrated in **fig. 4 & 5**, oral administration with LA resulted in a significant alteration in serum creatinine and urea levels. This effect was significantly decrease in LA+CO, LA+POE and reached the healthy control level in mixture group (LA+ MOsE).

Table (7): Effect of black chia and/or peppermint essential oils extracts and their mixture on AST, ALT, and ALP in different experimental groups (mean ± SE):

Parameters / Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
G1: HCG			
G2: COECG			
G3: POECG			
G4: MOsECG			
G5: LACG			
G6: LA+COE			
G7: LA+POE			
G8:LA+MOsE			
LSD	0.8329	1.2914	99.9777

There was no significant difference between mean have the same alphabetical superscripts letter in the same column ($P_{\leq} 0.05$)

3.5. Ameliorative Effect of CO & PO and their mixture against Histological alteration in Lead-treated rats

3.5.1. Brain Cerebral Cortex

Cerebral cortex of LACG revealed diffuse neuronal damage and loss of in outer cerebral cortical layers with abundant figures of shrunken and pyknotic perikarea with ill distinct subcellular details accompanied with moderate perineuronal edema. Many records of reactive astroglial cells and microglial cells infiltrates were found **(fig. 6)**. LA + COE group demonstrated minimal neuroprotective efficacy with more or less records as Model diseased group 5 (LACG) samples. As well as, cerebral cortex of rats from group co-treated with LA + POE showed high ameliorative efficacy with persistent outer cortical focal areas of neuronal damage and degenerative changes**.** The best histological feature was noticed in cerebral cortex of rats from group eight (co-treated with LA+MOsE) , with abundant records of apparent intact neurons allover cortical layers and single sporadic records of neuronal degenerative changes and minimal reactive glial cells infiltrates.

3.5.2. Liver tissue

As illustrated in **figure (7)**, LA group showed diffused moderate records of hepatocellular damage with alternated figures of vacuolar degenerative changes, necrotic hepatocytes and apparent intact cells. However; marked dilatation of hepatic vasculatures including hepatic sinusoids were showed with multiple records of perivascular inflammatory cells infiltrates. Post-treatment of LA-intoxicated rats with chia oil slightly improved the histological image. LA and peppermint group showed more organized histological features of hepatic parenchyma with abundant records of apparent intact hepatocytes

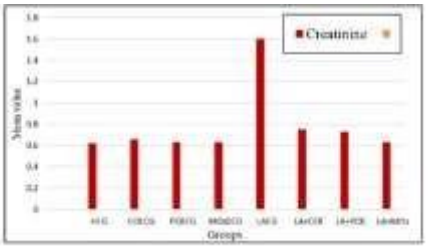

Fig. (4): Effect of COE, POE and MOsE administration on creatinine (mg/dl) levels on LA intoxicated rats

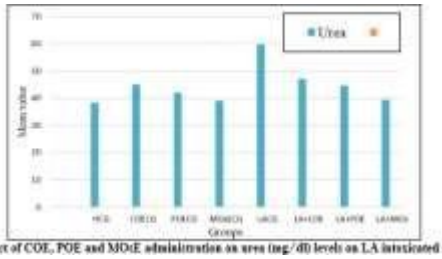

Fig. (5): Effect of COE, POE and MOsE administration on urea (mg/ dl) levels on LA intoxicated rats

allover different zones of hepatic lobules except of mild pericentral records of hepatocellular necrosis. Post-treatment with chia & peppermint mixture resulted in normal histological feature as control model.

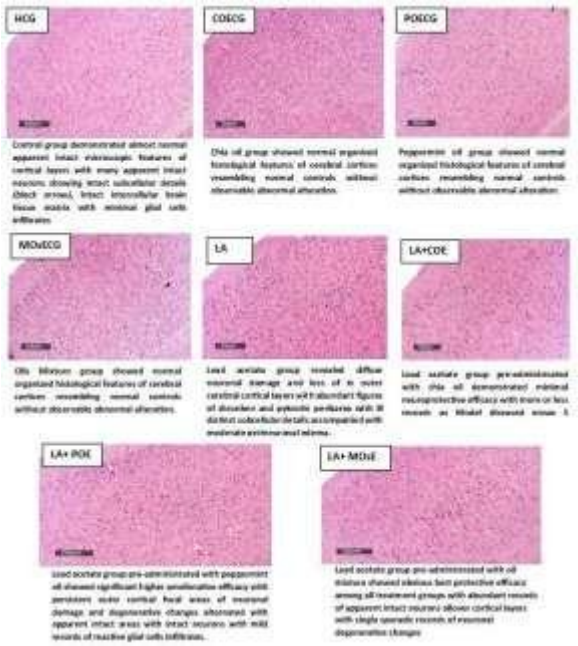

Fig. (6): Microscopic examination of cerebral cortex in the experimental groups. (1) HCG, (2) COECG, (3) POECG and (4) MOsECG showed normal features of cortical layers. (5) LACG showed diffuse neuronal damage and loss of in outer cerebral cortex (6) LA+COE group showed minimal neuroprotictive efficacy. (7) LA+POE group showed persistent outer cortical focal areas of neuronal damage. (8) LA+MOsE group showed abundant records of apparent intact neurons allover cortical layers.

4. Discussion

Lead is commonly known as developmental neurotoxins. Exposure to lead through contaminated air, soil or water can cause a serious harm to the child's health, including brain and nervous system damage, slowing growth and brain development, learning and behavior problems, and speech and hearing problems ***(Abelsohn AR and Sanborn, 2010)***. This harmful effect could be extended to brain serious diseases in adulthood and was reported to be a major cause of decreases in brain volume which associated with childhood exposure ***(Cecil et al., 2010)***. The results of the current study showed that oral administrated with lead acetate serves as a potent oxidant and caused a state of oxidative stress that significantly ($P \leq 0.05$) decreased the levels of neural markers activity by decreasing brain BuChE, 5-HT and DA levels when compared with other control groups. Furthermore, inflammatory biomarkers including (IL-6 and TNF-α), liver enzymes (AST, AIT and ALP) and kidney functions (creatinine and urea) showed a significant increase in lead acetate induced group. This alteration in biochemical parameters may be related to the ability of lead to cross blood brain barriers and affects their structural components by casing an injury in the brain glial cells and a remarkable damage in the cerebral cortex ***(Abdel Moneim et al., 2011)***. Moreover, the observed alteration could also be attributed to the interference of lead with calcium in the activation of protein kinase C (PKCs) which play an important role in cell growth ***(Gracia et al., 2007)***.

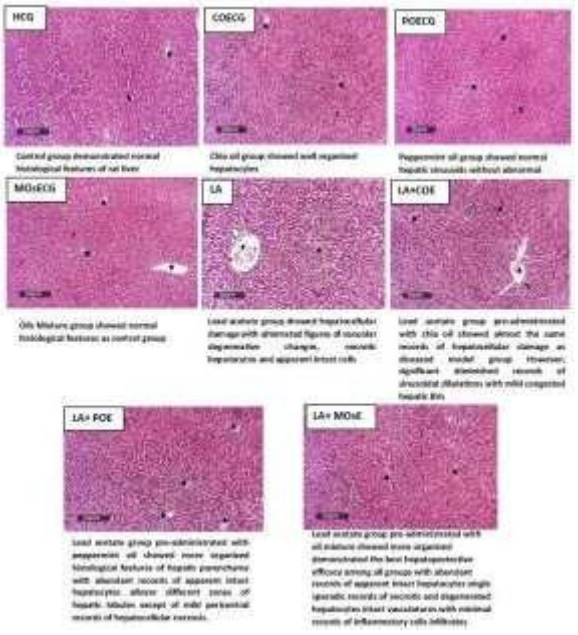

Fig. (7): Microscopic examination of liver tissues in the experimental groups. (1) HCG, (2) COECG, (3) POECG and (4) MOsECG showed normal features of liver parenchyma. (5) LACG showed diffuse hepatocellular damage with multiple perivascular inflammatory cells infiltrates. (6) LA+COE group showed significant diminished records of sinusoidal dilatations with mild congested hepatic BVs. (7) LA+POE group showed more organized features of hepatic parachyma. (8) LA+MOsE group showed the best hepatoprotective efficacy with apparent intact hepatocytes.

The oral administration of lead resulted in a significant decrease in GPx and GST accompanied by significant decline ($p \leq 0.05$) and antioxidant parameters (SOD, CAT and TAC). Also, a significant elevation in oxidative stress biomarkers (MDA) was observed. Such findings may be related to role of lead in producing reactive oxygen species (ROS) which increasing the cytotoxicity in endothelial cells ***(Ramesh and Jadhav 2001)***.

Supplementation with black chia seeds and peppermint oil resulted in a significant amelioration in biochemical parameters which may be related to the high antioxidant level in both oils ***(Rahman et al., 2017)***. Our results were in agreement with ***Marineli et al. (2015)*** who found that among rats consuming chia seeds or seed oil, there was statistically significant increase in the activity of plasma antioxidant enzymes as and glutathione peroxidase (GPx) concentration, compared to the control group. In rat livers, glutathione reductase (GRd) activity was increased while GPx activities were unchanged. ***Marineli et al. (2015)*** have also reported that dietary chia seeds oil reduced oxidative stress *in vivo*, since it improved antioxidant status by increasing GSH, TAC, SOD and catalase levels associated with reducing lipid peroxidation indicated by decreased MDA level reported that phenolic compounds of chia seeds oil are potent antioxidants in foods and are essential for biological systems because of their redox properties. ***Gazem et al. (2016)*** found that in vitro application of CSO increases the anti-inflammatory activity with an increase in the dose of oil from 10 to 40 µl/ml. ***da Silva et al. (2019)*** found that wistar rats fed a high-fat diet (HFD) enriched with 41.3% of chia flour for five weeks increased the SOD (liver) and CAT (plasma) activities.

Co-administration of lead-intoxicated rat with peppermint oil resulted in a remarkable amelioration especially in liver enzymes level. Such findings are in line with ***Ogaly et al. (2018)*** who confirmed that peppermint leaves have significant anti-hepatotoxicity effect by improving lipid peroxidation, transforming growth factor -β1 (TGF-β1) and was found to suppress desmin. This effect may be related to active components of peppermint oil as menthol or menthone which were reported to significantly increased the absolute and relative liver weights and the vacuolization of hepatocytes at all doses ***(Marjani et al. 2012).***

Histopathological studies of the present research on brain tissue have proved some neurodegenerative changes as cytoarchitectural distortions including Purkinje cell layer-related perineuronal vacoulations and karyopyknotic necrosis observed in cerebellar sections of the PbA-treated rats. Such results are in line with the reports on heavy metal compounds demonstrated to induce nervous tissue damage with Purkinje cells most sensitive elements of the cerebellar cortex to these neurotoxins ***(Yusuf et al., 2017)***. Co-administration of chia seeds and peppermint oils improved the histological image of brain tissue. Such findings may be attributed to presence of some components as vitamin E and carvol which give a long-lasting protection against oxidative damage induced by ions by preventing entry to neuronal cells (***Crouzin et al. 2010 and Alvi et al., 2021).***

Oral adminstration of lead have resulted in abnormal histopathological changes in rat liver tissues. Consumption of chia seeds and peppermint oil improved this alteration in liver tissue. Our results were supported by ***Al-Hayder et al. (2020)*** who noticed that liver tissue showed good restoration of the hepatic parenchymal cells with only scattered degenerated and necrotic ones as well as congested central veins and dilated and congested sinusoids after chia oil supplementation.

Administration of black chia oil and peppermint oil mixture have ameliorated the alteration in lead-intoxicated rats and nearly bring them to control group levels. Chia and peppermint mixture have also reduced the neurodegeneration, inflammatory response and hepatotoxicity and resulted in normal histological image in brain and liver tissue. Such findings may be related to the synergistic effect resulting from mixing COE and POE oil together as illustrated by ***Pezzani et al. (2019)***.

5. Conclusion

The present research demonstrated that mixture of black chia and peppermint oils multiple their beneficial effects in ameliorating lead acetate neurotoxicity in growing rats. This effect was evidenced by decreased neural and antioxidants markers levels, as well as enhanced liver enzyme and kidney function levels in pretreated rats. The observed neuroprotective effect of the oil extracts could also be mediated by suppressing the oxidative stress, inflammatory biomarkers. The protective effect is confirmed by histopathological examination of brain and liver tissues. However, Future studies are needed to investigate the mechanism of action of chia and peppermint oil.

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Institutional Review Board Statement

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Conflicts of Interest

The authors declare no conflict of interest.

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