Original Article

Evaluation of the Ameliorative Effect of Green Tea Extract Versus Berberine on Methyl Mercury Toxicity of Cerebellum in Adult Male Albino Rats: Histological & Immunohistochemical Study

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ABSTRACT:

Background: Methyl mercury (MeHg) is one form of organic mercury. Brain tissue is the most susceptible for its toxicity. Both berberine (BBR) and green tea extract (GTex) exhibit antioxidant capabilities. **Aim:** The current study assessed biochemical and histological changes to compare between impact of BBR and GTex on MeHg-induced cerebellar toxicity in rats. Methods: forty eight male albino rats were used. Control group; Subgroup Ia: received no treatment. Subgroup Ib: received 1 mg of L-cysteine powder dissolved in 1 ml distilled water. Subgroup Ic: recieved 1 ml of distilled water. Group II(BBR) group: received 100 mg/kg of BBR orally daily for 30 days. Group III (GTex) group: received GTex solution as their only source of drinking water for 30 days. Group IV(MeHg) group: Rats received MeHg orally once daily for 30 days at a dose of 10 mg/kg. Group V(MeHg + BBR) group: received 100 mg/kg of BBR by orally daily along with MeHg for 30 days. Group VI(MeHg+ GTex) group: received GTex solution as their only source of drinking water, combined with MeHg. The cerebellum was taken out of animals under anaesthesia after 30 days. Biochemical analysis and light microscopic inspection of cerebellar tissues were conducted. **Results:** MeHg significantly raised MDA and NO levels, while significantly lowering GSH levels in comparison to control subgroup Ia. Histologically, Purkinje cells in MeHg group were destroyed, others had pyknotic nuclei. Mean area% of positive cells for bax immunostain significantly increased, the optical density of calbindin immunopositive cells dramatically reduced . When compared to the MeHg group, both BBR and GTex treatments significantly reduced MDA and NO levels and significantly improved GSH levels , improved histological cerebellar architecture, and reversed calbindin and bax immunoexpressions. **Conclusions:** Following MeHg poisoning, BBR had more beneficial effect on the rat cerebellum than GTex but without significant difference.

Keywords: MeHg, berberine, green tea, toxicity, cerebellum.

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I. INTRODUCTION

Mercury (Hg) is employed in many fields, including agriculture, painting, medicine, and industry. However, stored mercury in sea food signifies the most prevalent source for human intake (Jha et al., 2019). It serves as a component in a variety of devices including barometers and thermometers, a catalyst in the creation of plastic, and a filling material in dental procedures (Ozbolat and Tuli 2016; Kumari and Chand 2021). There are three different types of Hg: metallic, inorganic, and organic. The degree of toxicity of Hg varies with its chemical form (Liu et al. 2021). The most dangerous form of mercury is organic mercury. Methyl mercury (MeHg) is one form of organic Hg, is created naturally by bacteria like Desulfovibrio desulfuricans or chemically by methylation of inorganic mercury (Ozbolat and Tuli 2016). The central nervous system (CNS) is the organ most susceptible to MeHg-induced toxicity (Costa et al., 2004, Hassan et al.,; Johansson et al., 2007 and Heimfarth et al.,2012). Because of the high concentration of polyunsaturated fatty acids in CNS, it is vulnerable to lipid peroxidation and to oxidative damage (Teixeira et al., 2018).

Protoberberine, a naturally arising quaternary benzylisoquinoline alkaloid, is the representative primary active component in totally portions of the Berberis species. Its chemical name is BBR (Imenshahidi & Hosseinzadeh, 2019). Through increasing the expression of the antioxidant defence system, BBR has been proven in various experimental models to reduce oxidative damages (Saleh et al., 2018; Hassani-Bafrani et al., 2019). Previous research demonstrated that BBR's anti-inflammatory effects were achieved via suppressing pro-inflammatory mediators (Li et al., 2019; Zhao et al., 2019 and Kumar et al., 2020).

Green tea extracts are gotten from the plant Camellia sinensis (Mukhtar and Ahmed, 2000). Flavonoids, such as catechins and their derivatives, are particularly abundant in green tea. Most of the catechins found in green tea are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), and epicatechin-3-gallate (ECG) (Shirakami and Shimizu, 2018). Additional phenolic acids that are frequently found in green tea include gallic acid, chlorogenic acid, neochlorogenic acid, and p-coumaryl quinic acid (Ahin and Ozdemir 2006). Many of polyphenols in green tea have antioxidant properties (Yu et al., 2007; Abib et al., 2011; Wang et al., 2012). Quercetin, Kaempferol, and Myricetin are the primary flavonols found in green tea (Maiti et al., 2019).

The current study assessed biochemical and histological changes to compare between the impact of BBR and GTex on Methyl Mercury-induced cerebellar toxicity in rats.

II. MATERIAL &METHODS

Animals and Housing:

In this experiment, forty eight mature male albino rats weighed between 180

and 220 g were employed. They were acquired from the Benha University Faculty of Veterinary Medicine's animal house. Rats were given to them with a 12-hour cycle of light and darkness. The rats were kept at room temperature in 8 cages according to their group, given access to free water, and fed commercial laboratory food.

Materials:

1. Me Hg was purchased from Sigma Aldrich Company with the CAS number 115-09-03. It was a white, crystalline powder that was melted with L-cysteine powder (as an organic solvent) in distilled water (Han et al.,2020).

2. BBR chloride hydrate with (CAS Number: 141433-60-5) was purchased from Sigma (St. Louis, MO, USA) in the form of powder.

3. Green tea extract (GTex) was bought from Sigma Aldrich in the United States. 15 g of powder of instant green tea were dissolved in 1 L of boiling distilled water for 5 minutes to create GTex. 1.5% GTex solution was created by filtering the solution. Rats were given this solution for free consumption for 30 days as their only source of drinking water.

Experimental design:

Six groups of rats were used in the experiment (The control group was 18 rats, rest of groups were 6 rats each).

Group I, the control group: 18rats were split into three smaller subgroups. Each one contained 6 rats.

Subgroup Ia: Rats were received nothing but only food and water, For a period of 30 days.

Subgroup Ib: For a period of 30 days, 1 mg of L-cysteine powder was dissolved in 1 milliliter of distilled water and administered orally by gavage to rats each day(Han et al.,2020).

Subgroup Ic: For a period of 30 days, 1 ml of distilled water was administered orally by gavage to rats each day.

Group II, (BBR)-treated group: oral supplements of 100 mg/kg of berberine (BBR) were administered by gavage daily for 30 days (Akhzari et al., 2019).

Group III, (GTex)-treated group: Rats in this group were given GTex solution as their only supply of water for 30 days (Usharaniet al.,2019).

Group IV (MeHg-treated group): This group's rats were given MeHg solution orally once daily for 30 days at a dose of 10 mg/kg body weight. 10 mg of MeHg and 10 mg of L-cysteine powder were dissolved in 10 ml of distilled water to create the MeHg solution (Sugianto et al., 2019) & (Fadhila et al., 2020).

Group V (MeHg+ BBR)-treated group: Rats in group were given oral supplements of 100 mg/kg of berberine (BBR) by gavage daily for 30 days, along with MeHg solution at a level of 10 mg/kg body weight via oral gavage once daily for 30 days.

Group VI (MeHg+GTex)-treated group : Rats in this group were given

GTex as their only supply of water for 30 days along with MeHg at a concentration of 10 mg/kg body weight by oral gavage once daily for 30 days. Each day, the contents of drinking glasses were changed. The entire volume of tea solution consumed by each cage was tracked daily at home. We divided the number of rats in each cage in order to determine the average daily intake for each rat.

Ethical approval

Every step of this study was carried out in accordance with the ethics committee's rules and regulations for the faculty of medicine at Benha University , Benha, Egypt.

The study complied with the US National Institutes of Health's "Guide for the Care and Use of Laboratory Animals" for the treatment and wellbeing of experimental animals (NIH publication No. 85–23).

Sample Collection:

The animals were chloroformed-anesthetized in the sacrificial chamber after 30 days. A cut was made through the skull's muscle and skin. The cerebellum was taken out of the skull after a mid-sagittal incision. Each cerebellum was cut in half mid-sagittally, and each portion was maintained at -80° C for biochemical analysis. In Bouin's fluid, other cerebellar tissue halves were fixed. To prepare the tissues for light microscopy, they were treated.

Biochemical analysis:

According to a technique published by (Ohkawa et al. 1979) to determine lipid peroxidation, level of malondialdehyde (MDA) was measured.

Techniques were used to measure glutathione (GSH) and nitrite/nitrate (nitric oxide; NO) according to Green et al. (1982) and Ellman (1959), respectively. (Kim et al., 2003)

Procedure for processing tissues

Ascending alcohol concentrations were used to dry the fixed tissues after they had been taken out of the bouin's fluid. The melted paraffin wax was used to submerge the tissues. The tissues were divided into 5µm sections coronally using a rotary microtome. To assist in the spreading of the paraffin ribbons, the tissue sections were let to float in a water bath set at 30°C. The tissues were removed from the warm water bath using the clean slides. The slides were allowed to dry before being H&E stained.

Immunohistochemistry:

Primary rabbit anti-CBD-28k (diluted 1:1100; Cell Signaling Technology, Danvers, MA, USA), was used to immunoreact on paraffin-embedded sections. The sections were then developed with Vectastain ABC after being treated with biotinylated goat anti-mouse IgG (diluted 1:200; Vector Laboratories Inc., Burlingame, CA, USA) (Vector Laboratories Inc., Burlingame, CA, USA). The slices

were then observed under a microscope while being visualised with 3,30-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich Co., St. Louis, MO, USA). After each immunoreaction was identified, it was immediately halted by washing the sections with graded ethyl alcohol and PBS. Finally, Mayer's haematoxylin was added to the immunostained slices as a counterstain. (Mohammadi et al., 2018)

Overnight, the primary anti-Bax antibody (rabbit polyclonal antibody, 1/50 dilution, Abcam) was incubated with paraffin-embedded sections in a humid atmosphere. Sections were then exposed to the matching biotinylated secondary antibody for an hour before being cleaned once more in phosphate buffered saline (PBS). Streptavidin peroxidase was used for 10 minutes, followed by a PBS wash. As a chromogen, 3, 3'-diaminobenzidine (DAB)-hydrogen peroxide was used to localise and highlight the immunoreaction. Finally, Mayer's haematoxylin was added to the immunostained slices as a counterstain (Ramos-Vara et al., 2008).

Morphometric analysis

The image analysis system was employed by the Central Research Lab at the Faculty of Medicine at Benha University in Egypt (Leica Qwin 500 C Image analyzer computer system). The DAB-stained slides were used to evaluate the color intensity of the Calbindin-positive immunoreaction. Additionally, it was determined the percentage of the cerebellar cortex had a positive Bax immunoreaction. From each specimen of the cerebellum from each animal group, ten separate microscopic fields were studied.

Analytical Statistics:

One-way analysis of variance was used to assess the significance of the variations between these average values, and the Tukey's post-hoc test for multiple comparisons was then used, using statistical analysis software from the statistical package for social sciences. For each group, the data are expressed as means + standard errors of the mean (version 19; SPSS Inc., Chicago, Illinois, USA). $P \leq 0.05$ served as the threshold for significance.

III. Results

Survival and general look:

During the study time, rats were observed, and it was discovered that MeHg was well incured by the rats and that no mortality was found. All groups of rats displayed ordinary activity.

Biochemical parameters:

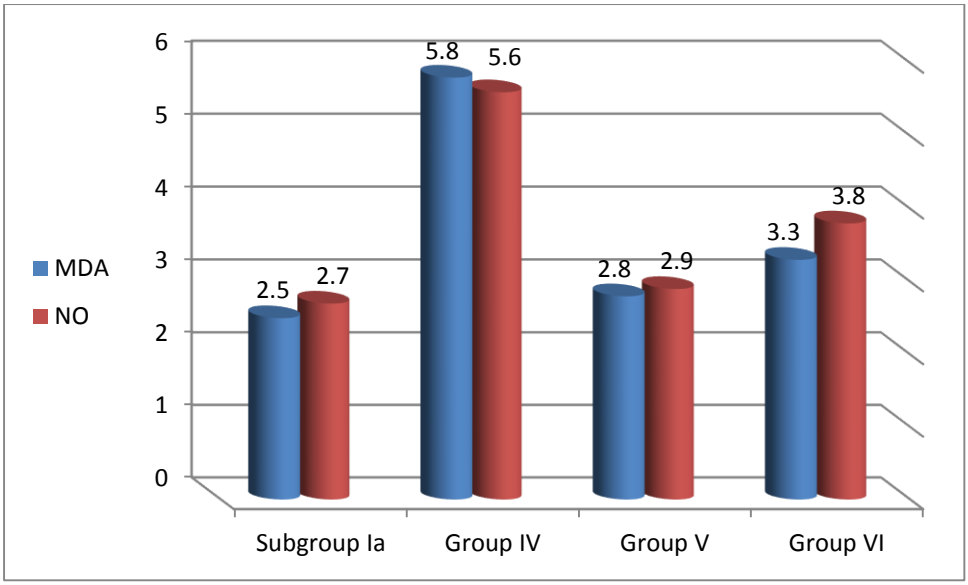
Biochemical data between control subgroups and between control subgroups**,** (BBR)-treated group & (GTex)-treated group were statistically analyzed using One way ANOVA followed by post-hoc Tukey's test and the difference of the results were found to be statistically insignificant, so control subgroup Ia was applied to them

According to the represented data in Table 1 and histograms1and 2, in group IV (MeHg-treated group), MDA and NO levels significantly raised ($p<0.05$) compared to control subgroup Ia. The level of GSH was also reduced in comparison to control subgroup Ia ($p< 0.05$). However, in group V (MeHg+ BBR)-treated group and group VI (MeHg+GTex)-treated group, When compared to group IV (MeHg-treated group), all of the produced alterations were significantly reverted to values close to subgroup Ia ($p \leq 0.05$).MDA and NO levels insignificantly reduced in Group V (MeHg+ BBR)-treated group compared to group VI (MeHg+GTex)-treated group ($p > 0.05$),While GSH level increased in Group V (MeHg+ BBR)-treated group in comparison to group VI (MeHg+GTex)-treated group without significant difference ($p > 0.05$).

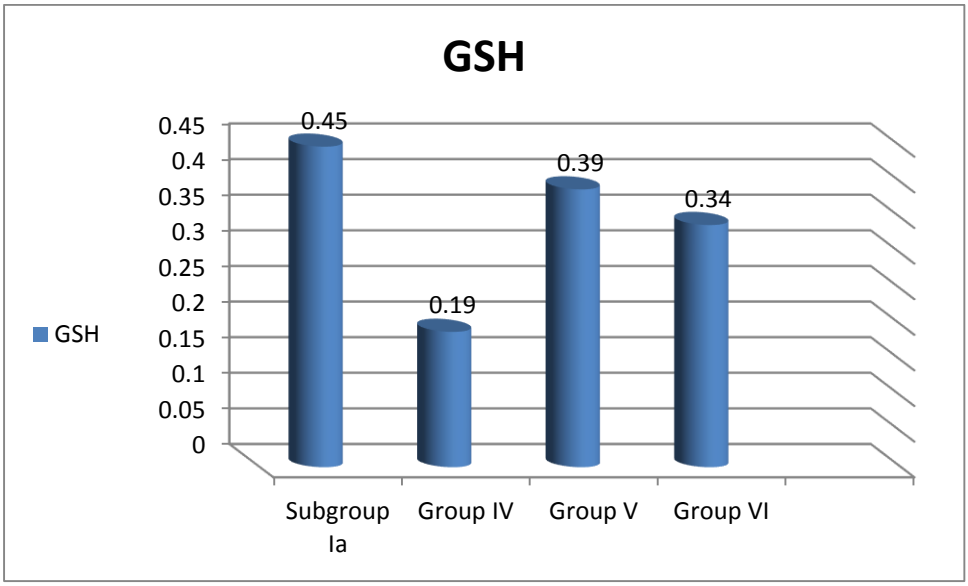
Table (1): Analysis of tissue MDA, NO and GSH among the studied groups using One way ANOVA followed by post-hoc Tukey's tests

	Control Subgroup Ia	**Group IV (MeHg-treated group)**	**Group V (MeHg+ BBR)-treated group**	**Group VI (MeHg+GTex)-treated group**
MDA (nmol/ mg protein)	2.5 ± 0.39	5.8 ± 1.4 [a,c &d]	2.8 ± 0.35 [b]	3.3 ± 0.5 [b]
NO(µmol/ mg protein)	2.7 ± 0.25	5.6 ± 0.85 [a,c & d]	2.9 ± 0.15 [b]	3.8 ± 0.27 [b]
GSH (mmol/mg protein)	0.45 ± 0.05	0.19 ± 0.02 [a,c &d]	0.39 ± 0.012 [b]	0.34 ±0.02 [b]

Data expressed as mean± SD, *: significance ≤ 0.05, a: Significance vs Control, b: Significance vs MeHg, c: Significance vs MeHg +BBR, d:Significance vs MeHg +GTex, MDA: Malondialdehyde, GSH :Glutathione, NO:Nitric oxide



Histogram(1) showing average values of tissue MDA & NO across the studied groups.



Histogram(2): showing average values of tissue GSH across the studied groups.

Histological results

Haematoxylin and eosin analysis:

H&E stained cerebellar sections of control subgroups, BBR and GTex groups showed no difference in the standard histological structure of cerebellar cortex, so control subgroup Ia was applied to them. The cerebellar cortex was composed of three layers; external molecular, middle Purkinje cell layer and internal granular layer. The molecular layer is composed primarily of "neuropil" (unmyelinated fibres), basket cells, and stellate cells. Between the granular and molecular layers, a single row of middle Purkinje cells was present. Purkinje cells appeared large pyriform in shape with large rounded vesicular nuclei with

noticeable nucleoli, pale acidophilic cytoplasm and large processes. The internal granular layer presented collection of small deeply stained granular cells with dark spherical nuclei and large vesicular Golgi type II cells among cerebellar islands which were pale non cellular areas. (Figure 1)

H&E stained cerebellar sections of rats of group IV (MeHg-treated group) revealed stellate and basket cells were surrounded by prominent perinuclear spaces. Shrunken irregularly shaped purkinje cells with darkly stained eosinophilic cytoplasm and pyknotic nuclei were seen. Several Purkinje cells were destroyed, leaving spaces. Spaces between cellular aggregations of granule cells were present in granular cell layer. Congested dilated blood vessel was noticed in the white matter (Figure 2)

H&E stained cerebellar sections of group V (MeHg+ BBR)-treated group showed some cells in the molecular cell layer were surrounded by perinuclear spaces. Purkinje cell layer appeared with relative normal appearance of some Purkinje cells having vesicular nuclei and noticeable nucleolus, apart from it lost Purkinje cells which were replaced by neuroglia cells, and some of Purkinje cells had pyknotic nuclei .Granular cell layer appeared with normal aggregated granule cells (Figure 3).

H&E stained cerebellar sections of group VI (MeHg+GTex)-treated group revealed that some Purkinje cells in the Purkinje cell layer were relatively normal, with vesicular nuclei and noticeable nucleoli. However, other Purkinje cells were destroyed, leaving spaces, and some Purkinje cells were shrunken and darkly stained pyknotic nuclei. Some cells in the molecular layer are surrounded by perinuclear spaces. Granular cell layer showed spaces between cellular aggregations of granule cells (Figure 4).

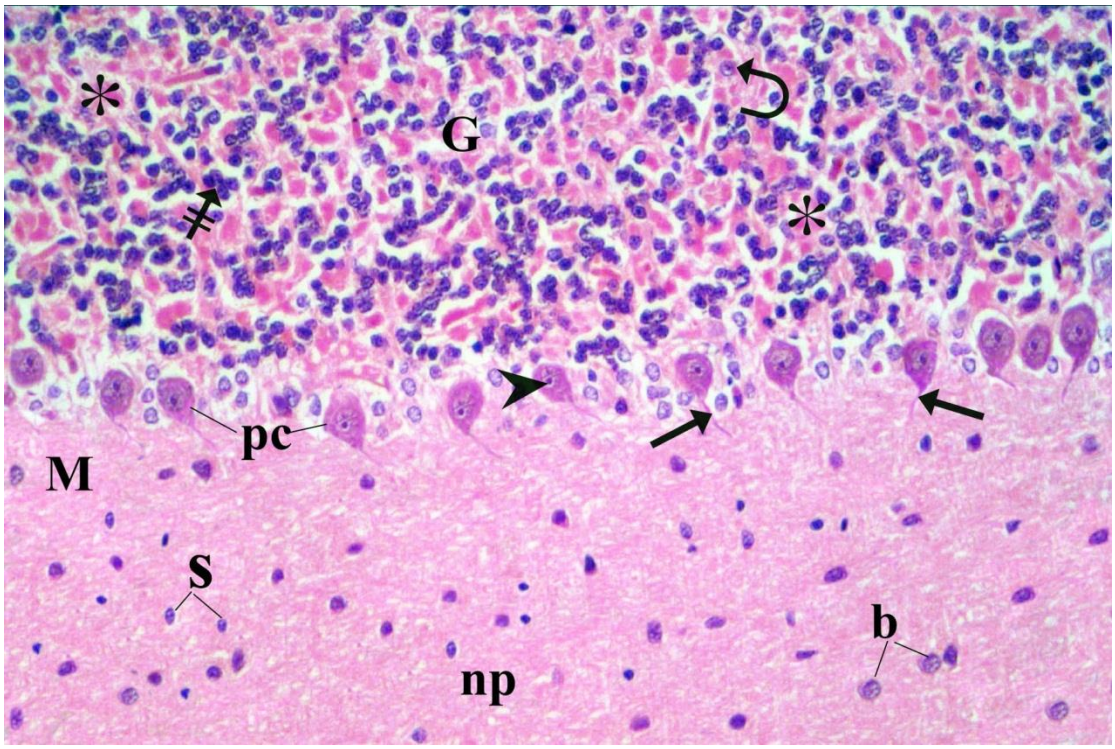


Fig. 1: photomicrographs of cerebellar cortex' sections of rats of Control subgroup Ia stained with H&E, (X200) showing: Normal arrangement of three cerebellar cortex layers; external molecular layer (M), middle Purkinje cell layer (pc), and internal granular layer (G). Molecular cell layer contains unmyelinated fibers "neuropil" (np), basket cells (b) and stellate cells (s) .Purkinje cells (pc) arranged in one row .They are large "pyriform shaped" cells with vesicular nucleus , prominent nucleolus (head arrow) and pale acidophilic cytoplasm and large process (arrow) and . Granular layer contains small tightly packed granule cells (crossed arrow) with highly pigmented nuclei, more large vesicular Golgi type II cells (curved arrow) and non-cellular pale cerebellar islands (asterisk).

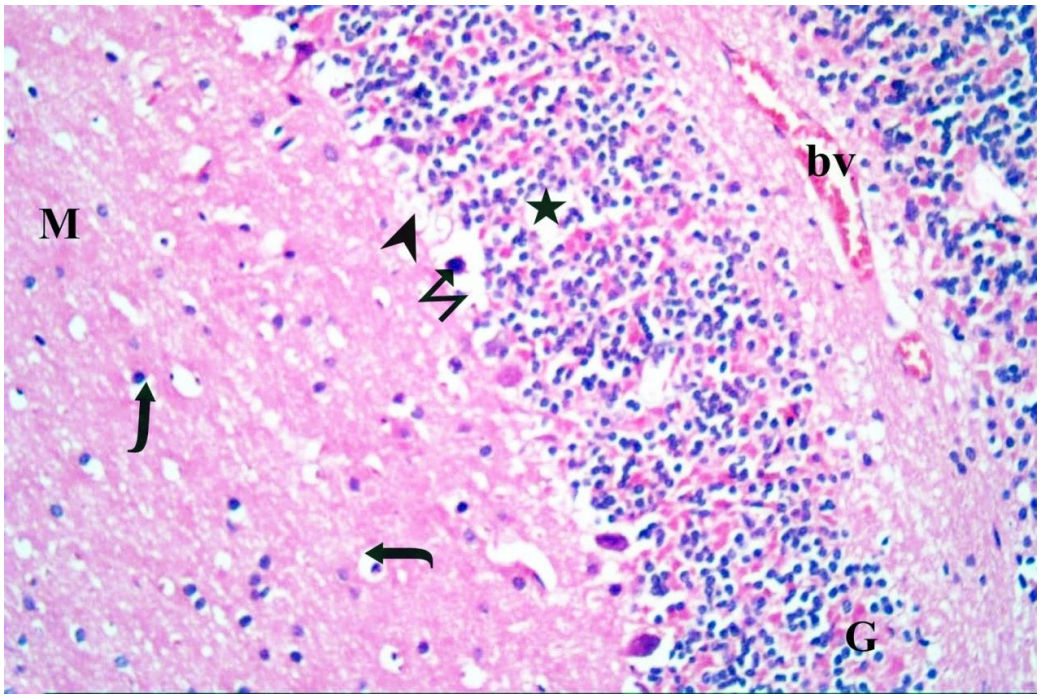


Figure 2: photomicrographs of cerebellar cortex' sections of rats treated with MeHg stained with H&E, (X200) showing**:** prominent perinuclear spaces (tailed arrow) surrounds basket and stellate cells in the molecular layer(M) .Purkinje cells are shrunken and irregularly shaped with dark stained eosinophilic cytoplasm and pyknotic nuclei (zigzag arrow) . Most of Purkinje cells are destroyed, leaving spaces (head arrow). Granular cell layer (G) is showing spaces (star) between granule cells. Additionally, white matter has dilated blood vessel (bv)

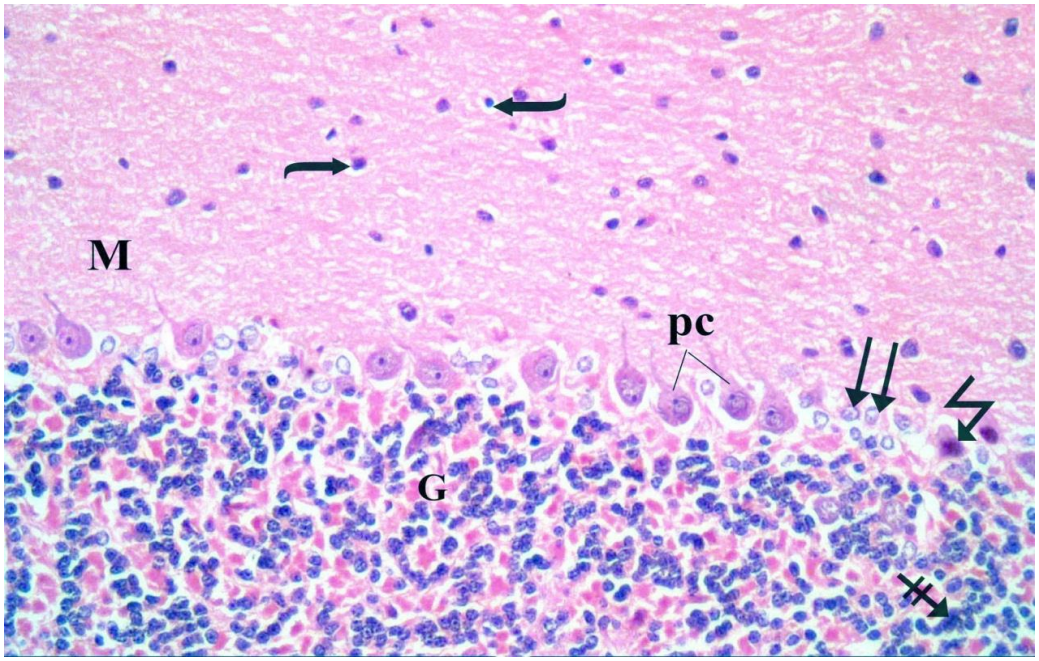


Figure 3: photomicrographs of cerebellar cortex' sections of rats treated with MeHg plus BBR stained with H&E, (X200) showing: Some cells in the molecular cell layer (M) are surrounded by perinuclear spaces (tailed arrows). Purkinje cell layer (pc) cells appear relatively normal with their vesicular nuclei and noticeable nucleolus with a part of this row lost Purkinje cells are replaced by neuroglia cells(double arrow) , some of Purkinje cells have pyknotic nuclei (zigzag arrow). Granular cell layer (G) showing normal aggregated granule cells (crossed arrow).

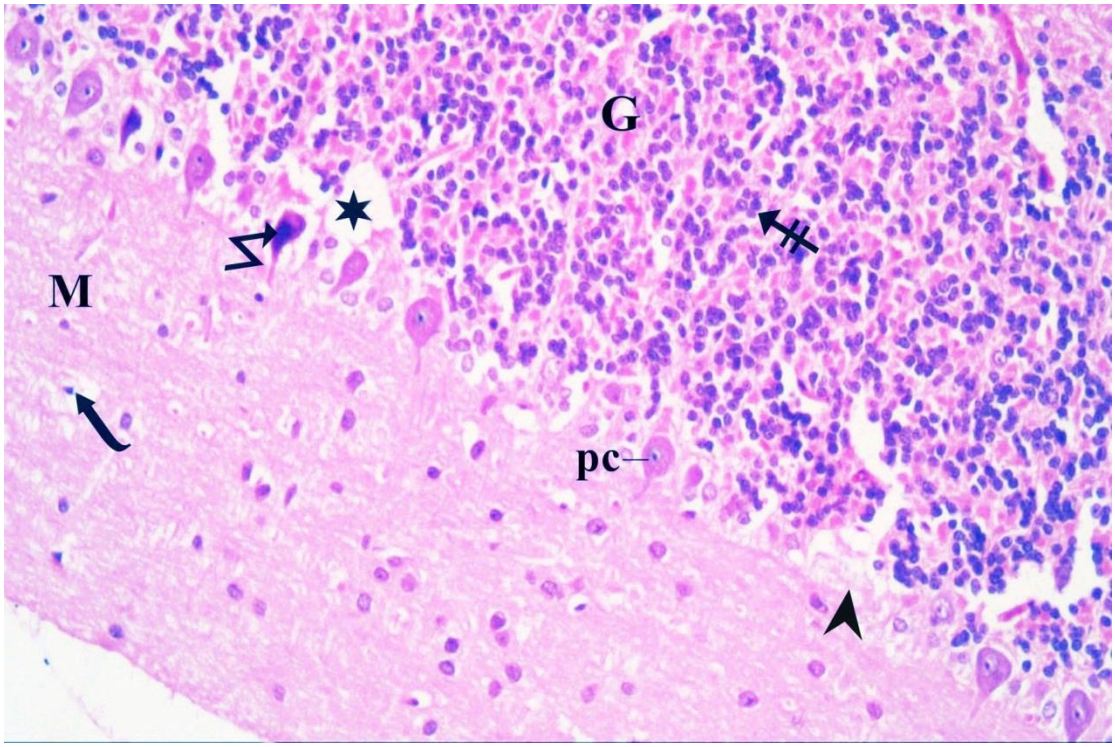


Figure 4: photomicrographs of cerebellar cortex' sections of rats treated with MeHg plus GTex stained with H&E, (X200) showing: Purkinje cell layer (pc) showing relatively normal appearance of some Purkinje cells having vesicular nuclei and noticeable nucleolus, apart from this layer showing spaces (head arrow) due to lost Purkinje cells, and some Purkinje cells are shrunken with darkly stained pyknotic nuclei (zigzag arrow). Some cells in the molecular layer (M) are surrounded by perinuclear spaces (tailed arrows). Granular cell layer (G) is showing spaces (star) between cellular aggregations of granule cells (crossed arrow).

Immunostaining results:

Immunostained cerebellar sections of control subgroups, BBR and GTex groups showed no difference in the standard histological structure of cerebellar cortex, so control subgroup Ia was applied to them.

- **Calbindin immunostaining :**

Control subgroup Ia presented multiple Purkinje cells with strongly +ve immunoreactivity for Calbindin protein. MeHg group presented few Purkinje cells with weakly +ve immunoreactivity for Calbindin protein. Both MeHg plus BBR group and MeHg plus GTex group showed many Purkinje cells with moderately +ve immunoreactivity for Calbindin protein (Fig. 5).

- **Bax immunostaining :**

Control subgroup Ia group showed negative immunoreactivity for Bax protein in all cerebellar cortex layers. MeHg group showed strong immunoreactivity for Bax protein in all cerebellar cortex layers. Both MeHg plus BBR group and MeHg plus GTex group showed mild cytoplasmic immunoreactivity for Bax protein in all cerebellar cortex layers. (Fig. 6).

Morphometric and statistical results:

Morphometric results between control subgroups and between control , (BBR)-treated group & (GTex)-treated group were statistically analyzed using One way ANOVA followed by post-hoc Tukey's test and the difference of the results were found to be statistically insignificant, so control subgroup Ia was applied to them

The statistical data were represented in table 2 and histograms 3 and 4.

The optical density of positive cells of calbindin immunostain was significantly lessened (P≤ 0.05) in group IV (MeHg-treated group) in comparison to Subgroup Ia, group V (MeHg+ BBR)-treated group and group VI (MeHg+ BBR)-treated group. While the optical density of positive cells of calbindin immunostain was higher in group V (MeHg+ BBR)-treated group compared to group VI (MeHg+ BBR)-treated group with no significant difference (P > 0.05).

The mean area % of positive cells of bax immunostain was significantly raised (P≤ 0.05) in group IV (MeHg-treated group) in comparison to Subgroup Ia, group V (MeHg+ BBR)-treated group and group VI (MeHg+ BBR)-treated group. While the mean area % of positive cells of bax immunostain was lower in group V (MeHg+ BBR)-treated group compared to group VI (MeHg+ BBR)-treated group with no significant difference (P > 0.05).

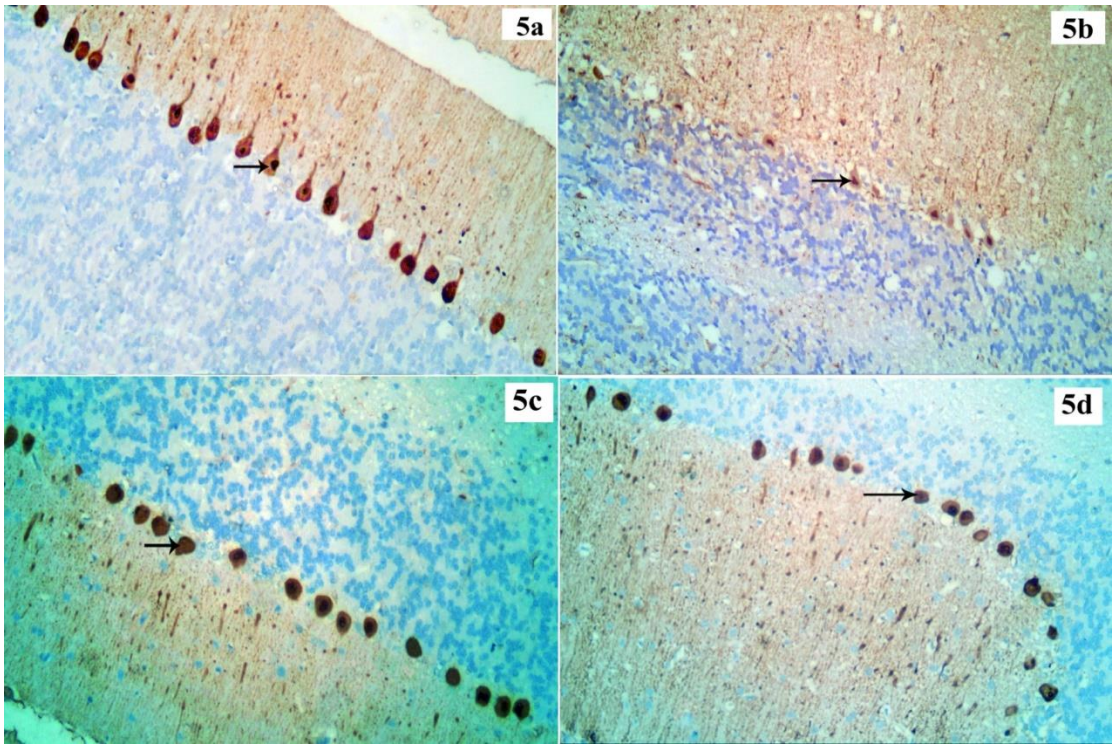


Fig.5 : photomicrographs of cerebellar cortex' sections of rats stained with Anti-Calbindin antibody (X200) showing: **(5a) Control subgroup Ia:** Multiple Purkinje cells with strongly positive immunoreactivity for Calbindin protein (arrows), **(5b) MeHg group:** Few small Purkinje cells with weakly positive immunoreactivity for Calbindin protein (arrows), **(5c) MeHg plus BBR group:** Many Purkinje cells with moderately positive immunoreactivity for Calbindin protein (arrows), (**5d**) **MeHg plus GTex group:** Many Purkinje cells with moderately positive immunoreactivity for Calbindin protein (arrows).

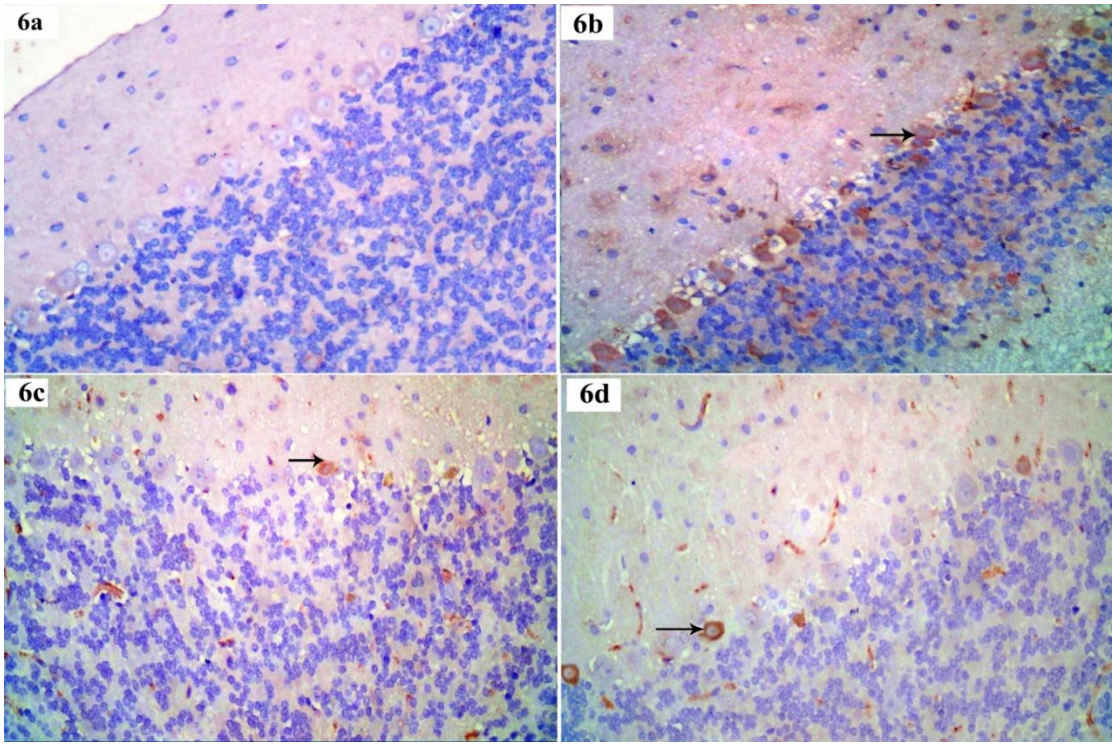
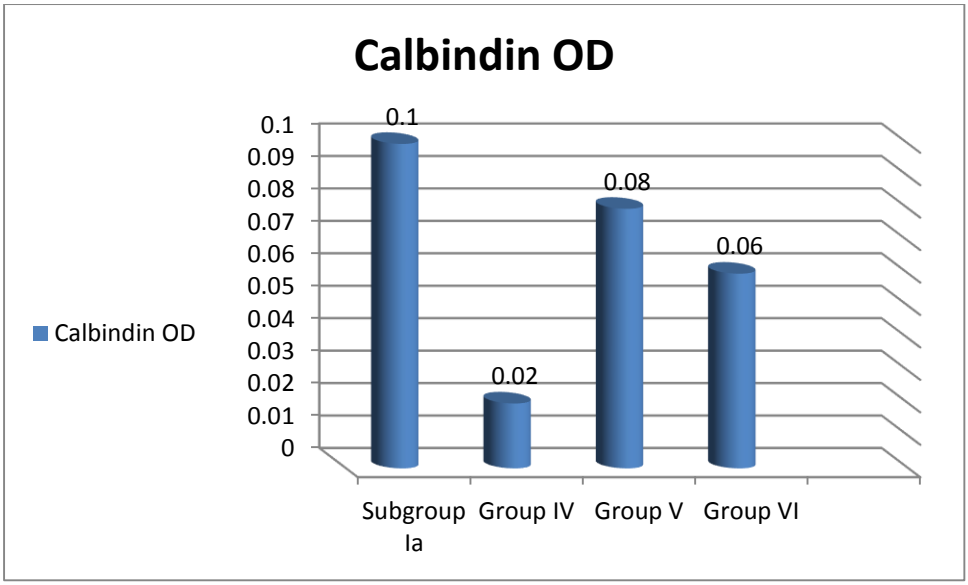


Fig. 6: photomicrographs of cerebellar cortex' sections of rats stained with Anti-Bax antibody (X400) showing: **(6a) Control subgroup Ia:** Negative cytoplasmic immunoreactivity of Bax protein in all layers, **(6b) MeHg group:** Strong cytoplasmic immunoreactivity of Bax protein (arrows), especially in Purkinje cells, **(6c) MeHg plus BBR group:** Mild cytoplasmic immunoreactivity of Bax protein (arrows), **(6d) MeHg plus GTex group:**Mild cytoplasmic immunoreactivity of Bax protein (arrows).

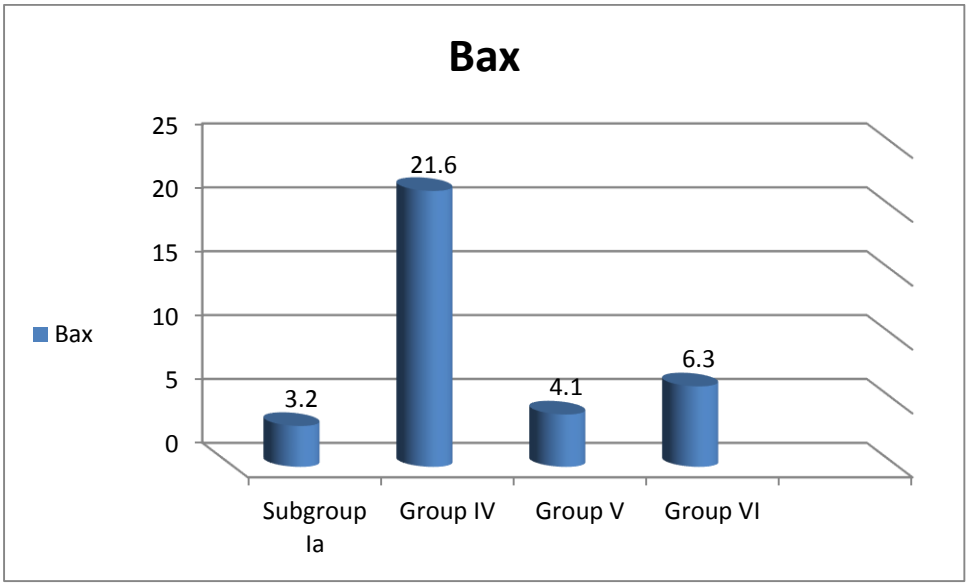
Table(2) comparison between average values of optical density of calbindin immunostain and average values of area % immunoreactivity of bax in the studied groups using One way ANOVA followed by post-hoc Tukey .

	Control Subgroup Ia	Group IV (MeHg-treated group)	Group V (MeHg+ BBR)-treated group	Group VI (MeHg+GTex)-treated group
Calbindin OD	**0.1± 0.04**	**0.02 ± 0.006** [a, c & d]	**0.08 ± 0.009** [b]	**0.06 ± 0.01** [b]
Bax area %	**3.2 ± 0.4**	**21.6 ± 2.04** [a ,c & d]	**4.1 ± 0.16** [b]	**6.3 ± 1.6** [b]

Data expressed as mean±SD, *:significance ≤ 0.05, a: Significance vs Control, b: Significance vs MeHg, c: Significance vs MeHg +BBR, d:Significance vs MeHg +GTex, Calbindin OD: Calbindin optical density



Histogram (3) showing average values of optical density of calbindin immunostain in the studied groups.



Histogram (4) showing average values of area % immunoreactivity of Bax in the studied groups.

IV. DISCUSSION

In this work, we provided evidence demonstrating that giving methylmercury (MeHg) to rats recorded alterations in oxidative and histopathological parameters. These alterations were lessened by giving these rats berberine or green tea extract .The effect of berberine was better than green tea extracted but without significant difference.

In the present study, When compared to the Control group, the MeHg group's MDA and NO levels increased significantly, while the GSH content significantly dropped .These results were consistent with (Ibegbu et al.,2014). A decrease in the activity of antioxidant enzymes like superoxide dismutase and an increase of lipid peroxidation are two mechanisms by which mercury's detrimental effects are mediated, this generates free radicals and produces oxidative cell injury (Owoeye et al.,2019). The rise of lipid peroxidation causes changes to cellular membrane structure and function that may result in cell damage in the target organs (Manju and Jagadeesan , 2019). Yang et al.,(2020) revealed that MeHg interfered with cellular GSH synthesis, whether it was enzymatic by decreasing glutathione peroxidase(GPx) activity or non-enzymatic through glutamate dyshomeostasis results in glutamate accumulation which leads to cysteine uptake inhibition results in decreased GSH synthesis ,since the rate-limiting factor for GSH synthesis is the intake of cysteine . An antioxidant enzyme called GPx provides two electrons to convert H2O2 and lipid peroxides to water and lipid alcohols, respectively, in presence of GSH (Antunes et al., 2018). Mercury interacts with a macromolecule carrying sulfhydryl group to cause irreversible cell membrane damage and enzyme inhibition (Zhu et al. 2021).

The administration of HgCl2 produced increased production of free radicals was indicated by the higher level of MDA (a biomarker of lipid peroxidation) in cerebellum of HgCl2-treated rats that could damage and alter the lipid bilayers of membranes, resulting in cellular dysfunction (Aragão et ai.,2018). HgCl2 may have increased inducible NO synthase activity, the enzyme that controls generation of NO, producing an rise in the level of NO. High levels of NO cause cell death by compromising a number of cellular processes through the generation of peroxynitrite radicals (Albasher et al., 2020).

Numerous researches have shown that the generation of reactive oxygen species (ROS) and depletion of antioxidant enzymes including GPx, superoxide dismutase (SOD), and catalase enzymes are two ways of mercury toxicity. In addition to causing apoptosis and neurodegenerative diseases, ROS also causes mitochondrial malfunction, neuroinflammation and neuronal cell death. (Rao et al., 2010;Jakaria et al.,2018 and Jha et al.,2019)

Lipid peroxidation of the membrane distorted its integrity; this decreases its

elasticity and permeability. Free radicals cause cytotoxicity in cells by inhibiting mitochondrial respiration, decreasing ATP generation, and activating enzymes that produce radicals, which raise the level of Ca2 + in the cell (Manju& Jagadeesan ,2019).

These results are further supported by the light microscopic evaluation of the cerebellar cortex of MeHg treated group of rats revealed degenerative features observed in shrunken Purkinje cells with pyknotic ill-defined nuclei and several cells were destroyed leaving spaces, prominent perinuclear spaces around basket and stellate cells and congested blood vessels in the white matter with areas of degenerations in granular layer. These results agreed with numerous studies **(**Abdel-Salam et al., 2013; El-Azab et al., 2018). According to another researcher, the Purkinje cells were the cerebellar cortex's most vulnerable layer to MeHg poisoning. In response to these toxic substances, the Purkinje cells exhibited degeneration and eventually lost their position in the Purkinje cell layer (Ibegbu et al.,2014; Sherin &Sumathi ,2016) . According to earlier research, MeHg can cross the blood-brain barrier (BBB) and accumulate in nervous tissue, having neurotoxic effects. (Ranjan et al.,2015). Other researchers suggested that MeHg may cause BBB damage (Takahashi et al.,2017). Overproduction of ROS and a weakening of the antioxidant defence system, which results in oxidative stress, are linked to MeHg-induced neurotoxicity (Othman et al., 2014).

In the current study, apoptosis in MeHg group was indicated by significant rise in the mean area percent of positive of Bax immunoreactivity in cerebellar cortex. Other authors also found that mercury increased the brain's Bax/Bcl-2 ratio (Abdel Moneim ,2015; Venkatesan& Sadiq ,2017). Fujimura & Usuki (2018) attributed up-regulation of Bax induced MeHg due to overload Ca^{2+} that activated mitochondrial apoptotic pathways

Calbindin-D_{28k} belongs to types of high-affinity proteins that achieves numerous functions in neuronal cell, as it controls calcium homeostasis, neuronal survival, and saves cells from apoptosis by blocking numerous pro-apoptotic pathways (Ouh et al.,2013). In the cerebellum, Purkinje cells can be identified by their expression of the marker calbindin. Calbindin protein overexpression in the cerebellar cortex may be a sign of Purkinje cell function in neuroprotection. (Karelina et al.,2016). The optical density of Purkinje cells inside Calbindin immunostain significantly decreased in the current study, demonstrating features of Purkinje cell loss. Impaired intracellular Ca^{2+} homeostasis due to decrease of calbindin content in the Purkinje cells induces neuronal degeneration or even cell death(Dhar et al.,2018).Mohamed et al., (2021) stated that increase intracellular Ca^{2+} as a consequence of decrease Calbindin content leads to mitochondrial degeneration.

In this study, BBR treatment with MeHg inverted the imbalance in the

oxidative stress index prompted by MeHg by significant reduction of MDA and NO levels as well as significant elevation of GSH level in cerebellar tissue. This was in accordance with Albasher et al.,(2020) who suggested that BBR intake restored the disruption in the oxidative stress index in the testicular tissue initiated by mercury through inhibition of LPO and NO production, as well as rise levels and activity of antioxidant molecules and their gene expression. The capacity of berberine to scavenge free radicals may be the cause of its antioxidant properties. According to studies, berberine has a potent reductive ability and a powerful effect on reducing radicals, particularly those caused by NO, superoxide anions, and hydroxyl radicals (Akhzari et al., 2019). Brebarine therapy suppresses cytochrome C and prevents ROS production, also it reduces the damage to neurons caused by hydrogen peroxide by promoting the PI3k/Akt/Nrf-2 pathway (Mohi-Ud-Din et al.,2022).

In this study, the BBR plus MeHg group's area percent of bax expression significantly decreased as compared to the MeHg group .This was in same line with Guna et al., (2018) who observed that Antiapoptotic protein (Bcl2) expression was increased by BBR treatment, while proapoptotic protein expression was decreased (caspase 3 and Bax) in pentylenetetrazole-induced kindling rat model. Berberine reduced apoptotic neuronal cell death through lowering Bax/Bcl-2 ratio and amount of cleaved caspase 3. Additionally, its anti-inflammatory effects were mediated through a reduction in TNF-level and NO generation (Mohammadzadeh et al.,2017).

In the present study, GTex had decreased MDA and No levels, as well as increased GSH in comparison to the MeHg group. By microscopic examination , there was improvement in the histopathological findings after co-administration of GTex with MeHg. This was in accordance with Imam and Gadallah (2019) who stated that GTex MDA levels significantly dropped and GSH significantly increased after co-administration of GTex with Acrylamide-induced cerebellar toxicity. The catechins content in GTex have a significant role in scavenging free radicals. GTex showed a significant scavenging effect against superoxide radicals and H2O2, which lead to oxidation in the cell, particularly in the lipids of the cell membrane. So, the antioxidant/oxidant balance improved (Çavuşoğlu et al.,2022). Catechins contain antioxidant, anti-inflammatory, anti-apoptotic, and neurotogenic activities, especially epigallocatechin gallate (EGCG) and epicatechin gallate (ECG). By regulating the amounts of antioxidants like GSH, SOD, and CAT, elevating inflammatory markers like TNF-, IL-6, NF-kB, and NO, and reducing lipid peroxidation by increasing Nrf2 protein expression (Afzal et al., 2022).

In the current work, both BBR and GTex significantly improved in the optical density of Purkinje cells with Calbindin immunostain as compared to

MeHg-treated rats. According to other researchers findings's, CBD-28k-Purkinje cells dramatically enhanced and lipid peroxidation in cerebellar Purkinje cells was reduced, protecting the cerebellar Purkinje cells from damage (Kim et al .,2022).

V. CONCLUSIONS

Following MeHg poisoning, BBR had more beneficial effect on the rat cerebellum than GTex but without significant difference**.**

Morever, the current study reveals that MeHg -induced lipid peroxidation and oxidative stress that play a crucial role in the cerebellar toxicity through elevating MDA and NO and lowering the antioxidant GSH. Lipid peroxidation and oxidative stress were greatly reduced by BBR's and GTex's direct antioxidative action by lowering MDA and NO and elevating the antioxidant GSH, BBR has a greater potential for treatment of Purkinje cell's apoptosis than GTex.

VI. RECOMMENDATIONWe

recommend further studies on the use of berberine and green tea extract in different doses or as a therapeutic method after a period of exposure to methylmercury.

VII. CONFLICTS OF INTEREST AND SOURCE OF FUNDING

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تقييم التأثيرالتحسيني للشاي الأخضر مقابل البربرين على سمية ميثيل الزئبق في المخيخ في ذكور الجرذان البيضاء البالغة: دراسة نسيجية وكيميائية مناعية

هند رجب موسى، مى حسن إبراهيم

قسم التشريح وعلم الأجنة ، كلية الطب ببنها ، جامعة بنها ، بنها ، مصر.

الخلفية: ميثيل الزئبق هو أحد أشكال الزئبق العضوي.أنسجة المخ هو الأكثر عرضة للسمية التي يسببها ميثيل الزئبق. يُظهركل من البربرين و مستخلص الشاي الأخضر قدرات مضادة للأكسدة

الهدف: قيمت الدراسة الحالية التغيرات الكيميائية الحيوية والنسيجية للمقارنة بين تأثير البربرين و مستخلص الشاى الأخضر على السمية المخيخية التي يسببها ميثيل الزئبق في الجرذان.

الطريقة: تم استخدام ست مجموعات من ثمانية وأربعين من ذكور الجرذان البيضاء. المجموعة الضابطة: لم تتلق الجرذان أي دواء. المجموعة المعالجة بالبربرين: تلقت الجرذان 100 مجم / كجم من البربرين يوميًا لمدة 30 يومًا. المجموعة المعالجة بمستخلص الشاى الأخضر: تلقت الجرذان في هذه المجموعة محلول مستخلص الشاى الأخضر كمصدر وحيد لمياه الشرب لمدة 30 يومًا. مجموعة معالجة بميثيل الزئبق: تم تلقي الجرذان ميثيل الزئبق بجرعة 10 مجم / كجم من وزن الجسم عن طريق الحقن الفموي مرة واحدة يوميًا لمدة 30 يومًا. المجموعة المعالجة بـ ميثيل الزئبق مع البربرين: تمت اعطاء الجرذان عن طريق الفم بـ 100 مجم / كجم من البربرين يوميًا في وقت واحد مع ميثيل الزئبق لمدة 30 يومًا. المجموعة المعالجة بميثيل الزئبق والشاى الأخضر: تلقت جرذان هذه المجموعة محلول مستخلص الشاي الأخضر كمصدر وحيد لمياه الشرب ، بالتزامن مع ميثيل الزئبق يوميًا لمدة 30 يومًا. بعد 30 يومًا ، تم تخدير الجرذان ثم إزالة المخيخ. تمت معالجة أنسجة المخيخ من أجل الفحص الكيميائي الحيوي والفحص المجهري الضوئي.

النتائج: ميثيل الزئبق يمكن أن يزيد بشكل كبير من مستويات MDA و أكسيد النيتريك لكن مستوى الجلوتاثيون انخفض مقارنة بالمجموعة الضابطة.نسيجيا، أظهرت المجموعة المعالجة بميثيل الزئبق خلايا بركنجى منكمشة مع نوى حنية. مع تدمير خلايا بركنجي الأخرى. انخفضت الكثافة الضوئية للخلايا الإيجابية لمناعة الكالبيندين بشكل ملحوظ بينما زاد متوسط المساحة لنسبة الخلايا الإيجابية للمناعة bax معنويا في المجموعة المعالجة بميثيل الزئبق. نتج عن كل من علاج البربرين و الشاى الأخضر انخفاضًا كبيرًا في مستويات MDA و أكسيد النيتريك وزيادة ملحوظة في مستوى الجلوتاثيون بالمقارنة مع المجموعة المعالجة بميثيل الزئبق. مع تحسن في البنية النسيجية للمخيخ وانعكاس التعبير المناعي للكالبيندين والباكس.

الاستنتاج: بعد التسمم بزئبق الميثيل ، كان للبربرين تأثير مفيد على مخيخ الجرذان أكثر من مستخلص الشاى الأخضر ولكن الفرق كان ليس دلالة احصائية.

التوصيات:

نوصى بمزيد من الدراسات حول اسخدام البربرين و مستخلص الشاى الأخضر بجرعات مختلفه أو كوسيلة علاجية بعد فترة التعرض لميثيل الزئبق.