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NEURO-PROTECTIVE POTENTIAL OF BETULINIC ACID AND ROTUNDIC ACID ON THE MERCURIC CHLORIDE INTOXICATED BRAIN OF ALBINO WISTAR RATS

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Keywords:

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ABSTRACT: Mercury is the more risky heavy metal for human health because of its ability to kill human nerve cells and its exposure to the environment through human activities, which implicate sewage drainage, agriculture, municipal, mining, incineration, and drainage of industrial wastewater. Mercury and its compounds cause toxic action in the body by several mechanisms, which mainly cause the nervous system through their molecular and cellular effects. The present research intended to investigate the ameliorative potential of betulinic acid and rotundic acid against mercuric chloride ($HgCl_2$) (1.29 mg/kg b. w.) induced neurotoxicity in adult male rats. The examination was implemented in male albino Wistar rats (n = 36). Which was partitioned into six gatherings as follows: Control, $HgCl_2$, $HgCl_2$ + betulinic acid, $HgCl_2$ + rotundic acid, betulinic acid alone, and rotundic acid alone. The results revealed that intense $HgCl_2$ regulation modified different biochemical specifications incorporated with the high volume of lipid peroxidation (LPO) portion and a significantly depleted level of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) pursuits in the brain tissue. Acetylcholinesterase AChE activity in the brain tissue was also brought down in $HgCl_2$ intoxicated rats. Betulinic acid and rotundic acid is a natural antioxidant that assists in safeguarding oxidative injury by diminishing oxidative stress. In contrast, the treatment of betulinic acid and rotundic acid (5 mg/kg B. W) in the brain tissue reveals a significant reduction in the degree of oxidant level and concurrently an elevated level of antioxidant properties and AChE activity via rehabilitation in brain tissues. Oxidant substance (LPO), AChE activity status, non-enzymatic antioxidant (GSH), and enzymatic antioxidants (GPx, SOD, CAT) reactions were additionally developed close to the normal (control) level when compared with mercury-treated groups.

INTRODUCTION: Mercury was poisonous in all forms that regulate cellular function by altering the tertiary and quaternary structure of proteins and binding with sulfhydryl and selenohydryl groups.

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Mercury can potentially diminish any organ's function or sub-cellular structure. The chief target organ of mercury vapors was the brain, whereas peripheral nerve function, renal function, immune function, endocrine and muscle function, and several types of dermatitis also included [1].

Tremendous acute exposure to mercury vapor leads to erosive bronchitis, and bronchiolitis prospectively leads to respiratory failure and is also accompanied by CNS symptoms such as tremor or erethism [2].

Chronic exposure to significant doses of mercury vapor results in neurological dysfunction. When low-level exposure, nonspecific symptoms like weakness, fatigue, anorexia, weight loss, and gastrointestinal disturbance have been occurs. Higher exposure levels were associated with mercurial tremor like fine muscle fasciculations punctuated every few minutes by coarse shaking. Exposure to mercury vapour causes erethism mercurial is of severe behavior and personality changes, emotional excitability, loss of memory, insomnia, depression, fatigue, delirium, and hallucination [3].

Mercury and its compounds mostly inhibit the activities of the free radical quenching enzymes such as catalase, superoxide dismutase, and glutathione peroxidase [4]. The affinity of mercury chloride towards the thiol-containing enzymes, a common factor in these mechanisms was the excessive generation of reactive oxygen species, which manifested as increased lipid peroxidation in the cells [5]. Significant reductions in the levels of glutathione, GSH-Px, GST, and catalase activities in the liver have been reported to accompany increased oxidative stress and lipid peroxidation in the liver [6]. Mercury has a fondness for thiol groups; on the other hand, organic Hg molecules affect essential enzymes such as the mitochondrial pyruvate dehydrogenase and also structural components present in the neuronal cytoskeleton system [7]. The brain was the major targeted organ of both the organic and elemental Hg. Hg exposure can bring about various other diseases, including hypertension, coronary heart disease, myocardial infarction, cardiac arrhythmias, generalized atherosclerosis, and renal dysfunction [8, 9].

The brain is an immensely heterogeneous organ with a numerous number of different neuronal and non-neuronal types of cells and broad morphological differentiation and biochemical compartmentalization inside the cell [10]. The nervous system comprises various greatly specialized cells including many different types of neurons and ganglia. Furthermore, the confinement and connectivity of individual neurons determine their structure and function in the central nervous system [11]. Mercury and its compounds damage the central nervous system and the accumulation of mercury in the brain leads to death [12, 13]. Heavy metal poisoning has primarily been treated with chelation therapy. About 80% of the world's population trusts traditional medicines for their primary health care, and most of them involve the use of plant extracts [14]. Chelation therapies mainly consider the solubility of the chelator in water or lipids [15]. They also may capable of aqueous solubility, which facilitates transport within the circulating blood and rapid excretion through the kidneys, whereas chelator may reveal greater penetration to the cellular membranes a barrier and within the central nervous system, and anti-oxidative agents will be able to chelate intracellular toxicants [16].

Betulinic acid (BA) is a habitually present pentacyclic triterpenoid, non-toxic and safe upto a dose of 500 mg/kg per body weight of animals, particularly in mice and rat [17, 18]. The birch tree (Betula spp., Betulaceae) is one of the best commonly announced wellsprings of betulinic acid. BA was also segregated across several geneses as follows Syzygium spp. (My- rtaceae), Ziziphus spp. (Rhamnaceae), Paeonia spp. (Paeoniaceae) and Diospyros spp. (Ebenaceae) [19]. BA has been proclaimed to possess antioxidant, antibacterial, anti-cancer, and anti-inflammatory activities followed by various researchers in different kinds of experiments [20, 21, 22, 23, 24, 25]. Rotundic acid, a plant-derived pentacyclic triterpene, is broadly present in edible and medicinal plants [26, 27]. RA is a typical bioactive pentacyclic triterpene that has been preferred as the quality control marker [28, 29]. RA has various and extensive pharmacological properties, including anticancer, anti-inflammatory, antidiabetic, antibacterial, antimycobacterial, lipid-lowering properties, *etc*. [30, 31, 32, 33, 34, 35]. With these points of view, the current investigation was designed to examine the protective potential of betulinic acid and rotundic acid as an oral administration in opposition to neurotoxicity generated through mercuric chloride in the brain tissue of albino rats.

MATERIALS AND METHODS:

Chemicals Utilized: Mercuric chloride ($HgCl_2$) and vital reagents for investigative evaluation were obtained from Hi-Media Laboratories Ltd, Mumbai, India. Betulinic acid and rotundic acid were acquired from Sigma Aldrich Laboratories Pvt. Ltd, Bangalore, India.

Adaptations of Animals: Healthful adult male albino rats, *Rattus norvegicus* of body weight between 180–200 g were acquired from the Central Animal House, Department of Experimental Medicine, Raja Muthiah Medical College and Hospital, Annamalai University, and kept up in a cool air conditioning room (25 ± 3°C) with a 12 h lighted and 12 h dull cycle. Feeds, water, and ad libitum were distributed to all the rats. The experimental protocols were examined and accepted by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (IAEC, Proposal Number: AU-IAEC/ 1228/1/19), Annamalai University, Annamalai Nagar.

Experimental Outline: 36 animals were acclimatized in the animal cages for 7 days. They were rifled and partitioned into six gatherings; each comprised of six rodents. The toxic dose of mercuric chloride has been resolved (sub-lethal dosage of $HgCl_2$ 1.29 mg/kg body weight) from our past examination conducted in our laboratory. Furthermore, it has adequate to evoke gentle or mediated oxidative stress in rodents [36].

Group I: Untreated control- just coursed (0.9% NaCl) was given to the animals and watched for 7 days.

Group II: Mercuric chloride treatment- the animals were managed with 1.29 mg of $HgCl_2$/kg body weight in 0.9% NaCl intraperitoneally for 7 days.

Group III: Mercuric chloride followed BA treatment- the animals were managed with betulinic acid (5 mg/kg body weight) after the inebriation of mercuric chloride for 7 days.

Group IV: Mercuric chloride followed RA treatment- the animals were managed with rotundic acid (5 mg/kg body weight) after the inebriation of mercuric chloride for 7 days.

Group V: BA treatment alone- the animals were given betulinic acid (5mg/kg body weight) alone for 7 days.

Group VI: RA treatment alone the animals were given rotundic acid (5mg/kg body weight) alone for 7 days.

Towards the end of the experiment, the animals were unconscious by intra-cutaneous infusion of ketamine hydrochloride (24 mg/kg body weight) and relinquished by cervical displacement. The entire brain tissue was immediately segregated from the animal, placed in super cold saline, and afterward utilized for the assessment of AChE, oxidant and antioxidant characters and for histological examination.

Determination of Lipid Peroxidation (TBARS): The LPO/TBARS quantity in the brain tissue was determined by following the procedure of Nichans and Samuelsen [37]. A notable measure of entire brain tissue homogenate was set up in a buffer of Tris-HCl (pH 7.5). From the homogenized tissue 1 ml was grabbed in a perfectly cleaned test tube, and 2 ml of TBA-TCA-HCL reagent were included and blended completely. The fusion was placed in a sizzling water bath (60°C) for up to 15 min and cooled under running tap water. After cooling, the mix was taken to study the chromophore absorption at 535 nm contra the reagent blank under a UV-visible spectrophotometer (Spectronic-20, Bausch, and Lamb). Around 1, 1', 3, 3' tetra methoxy propane was utilized to build the standard graph. The respective values are stated as n-moles of MDA delivered per 100 mg.

Determination of Reduced Glutathione (GSH) Activity: The proportion of reduced glutathione in the brain tissue was estimated by adopting Beutler and Kelley [38] method. The measured weight of tissue was homogenized with the help of phosphate buffer (0.1 M. pH 7.0) followed by centrifugation for 5 min at 2500 rpm. 0.2 ml of supernatant from the sample was grabbed, and 1.8 ml of EDTA solution was added to it. To this content, 3 ml of precipitating reagent was included and merged thoroughly and set aside for 5 min then centrifuged at 3000 rpm for10 min. 2 ml of the mixture was taken in a clean test tube and followed by adding 4 ml of 0.3 M disodium hydrogen phosphate suspension and 1 ml of DTNB reagents were included. The emergence of yellow colour was perused at 412 nm under a UV-visible spectro-photometer (Spectronic-20, Bausch, and Lamb). Group of standard solutions accommodated with 20–100 µg of reduced glutathione was correspondingly treated. The core values are illustrated by µg/100 mg protein.

Determination of Superoxide Dismutase (SOD) Activity: Superoxide dismutase take place in the brain tissue was analyzed using the Kakkar method [39]. The known amount of brain tissue was homogenized with 2 ml of 0.25 M sucrose solution and centrifuged at 10,000 rpm in a cold centrifuge for 30 min. After completion, the supernatant of the content was then grabbed in a clean test tube, dialyzed against Tris-HCl buffer, and mashed up thoroughly.

The combination was repeated centrifugation for 15 min at 3000 rpm. The supernatant was taken and 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of phenazine methosulphate and 0.3 ml of nitroblue tetrazolium reagents were introduced. The prepared enzyme sample remained in a water bath for 90 s at 30 ºC and the suitably diluted enzyme was prepared in 3 ml of twofold distilled water. The response was begun by adding 0.2 ml NADH. After completion of the incubation session, the response was halted by the addition of 1 m1 glacial acetic acid. The combination was vigorously stirred and wobbled with 4 ml n-butanol.

The combination was permitted to stay for 10 minutes and centrifuged for 5 minutes at 3000 rpm, and the n-butanol layer was differentiated. The shading thickness of the chromogen in n-butanol was estimated at 510 nm in a UV-visible spectrophotometer. A-frame without an enzyme acts as a control. The enzyme concentration needed to suppress the chromogen formed up to 50% for 1 min under standard background was considered one unit. The particular venture of the enzyme was indicated as unit/min/mg of protein for tissues.

Determination of Catalase (CAT) Activity: The catalase activity in the brain tissue was assayed with the help of Sinha [40] method. The tissue was homogenated by using the phosphate buffer (0.01 M. pH 7.0). 0.9 ml of phosphate buffer, 0.1 ml of homogenated tissue, and 0.4 ml of hydrogen peroxide were added to a clean test tube.

The changes were interrupted beside 15, 30, 45, and 60 s by adding 2.0 ml of dichromate acetic acid. The test tubes were allowed for 10 min in a boiling water bath and cooled under running tap water. The emerged colour was observed in a UV spectro-photometer at 620 nm. A fixed concentration scale of 20–100 µ moles was considered for further testing. The particular action was stated as µ moles of H_2O_2 absorbed per min/mg of protein for tissues.

Determination of Glutathione Peroxidase (GPx) Activity: The GPx activity in the brain tissue was estimated by adopting the Rotruck [41]. The weighted volume of brain tissue was homogenized with the help of tris buffer. The homogenate was centrifuged for 5 min at 2500 rpm. 0.2 ml of supernatant was grabbed into a clean test tube to that 0.2 ml of EDTA, and 0.1 ml of sodium azide reagents was also added. The content was mixed well through lateral shaking of the test tube. 0.2 ml of GSH followed by 0.1 ml of H2O2 reagents was added to the content. The composition was thoroughly mixed and incubated for 10 min at 37 ºC, and 0.5 ml of 10% TCA was added to it. At the same time, a reagent blank was likewise implemented with all the reagents without tissue homogenate. The medium was undergone centrifugation, and the supernatant was utilized for GSH analysis. The action was indicated as µ moles of GSH depleted per min/mg of protein tissues.

Determination of Acetylcholinesterase (AChE) Activity: Acetylcholinesterase (AChE) activity was estimated by the following method of Metcalf, [42]. The whole brain organ was isolated and homogenated in 2.0 ml of 0.25 N cold sucrose solutions. After completing the homogenisation process, the homogenate content was centrifuged at 3,000 rpm for 10 minutes. After centrifugation, the clear supernatant was taken in a clean test tube and used for enzyme assay.

In a clean test tube, 1.0 ml of clear supernatant was taken and 1.0 ml of reaction mixture (0.1 M Sodium potassium phosphate buffer and 0.008 M acetylcholine chloride in the ratio of 4:6) was added and then kept in incubation at 37 ºC for 30 minutes. After completing the incubation period, the reaction was stopped after adding 2.0 ml alkaline hydroxylamine hydrochloride followed by 1.0 ml of hydrochloric acid (1:1 ratio of HCl; water) and then the contents were shaken thoroughly and filtered with the help of Whatman no. 1 filter paper. From the filtrate, 2.5 ml of aliquot was taken in a clean dry test tube and then 0.5 ml of clear 0.37 M Ferric chloride solution was

added. The colour was developed. The intensity of the colour was measured at 545 nm in an UV–Spectrophotometer against a reagent blank. The enzyme activity was calculated from the standard graph. The values are expressed as µ moles hydrolyzed / mg wet wt. of tissue/ hr.

Histology and Histopathological Examination: The subjective examination of tissue histo-architexture was examined by selected brain tissue sample, fixed in 10% buffered formaldehyde for up to 48 h and dehydrated by processing effectively in various concentrations of ethyl alcohol and cleansed in xylene and embedded by paraffin wax. With the help of the rotary microtome, tissue sectioning (5–6 µm thick) was done and rehydrated. Then the samples were stained using hematoxylin and eosin dyes (H & E) and mounted in DPX medium for microscopic perceptions.

Statistical Analysis: Obtained values are expressed as mean ± S.D. for six animals in every group. T-test analysis was used to analyze various data acquired from assorted biochemical parameters, and the group means were correlated by Duncan's multiple range test (DMRT) [43]. Procure values were evaluated statistically significant when $p < 0.05$, and the values sharing a general superscript did not significantly differ.

RESULTS:

Estimation of Lipid Peroxidation (LPO) Level and Glutathione (GSH) Content: The proportion of lipid peroxidation was abundantly elevated, and concurrently the volume of reduced glutathione (GSH) content was lowered in the tissues of rats' brains intoxicated with mercuric chloride when compared against control animals **Table 1**. Under the post-treatment of betulinic acid and rotundic acid followed by mercuric chloride intoxication, the rat's brain tissues show restoration of LPO and GSH content close to the control animals **Table 1.**

TABLE 1: COMPARATIVE ANALYSIS OF BETULINIC ACID AND ROTUNDIC ACID ON LIPID PEROXIDATION (LPO) AND REDUCED GLUTATHIONE (GSH) CONTENT ON THE RAT'S BRAIN TISSUES INTOXICATED WITH MERCURIC CHLORIDE

Groups	LPO	GSH
Control	1.046 ± 0.01^{a}	48.11 ± 2.06^{a}
$HgCl_2$	1.985 ± 0.02^{b}	28.91 ± 2.82^{b}
$HgCl_2$+Betulinic acid	1.516 ± 0.02^{c}	42.67 ± 2.62^{c}
$HgCl_2$+Rotundic acid	1.694 ± 0.01^{d}	40.07 ± 3.02^{d}
Betulinic acid	1.041 ± 0.01^{a}	50.98 ± 2.38^{e}
Rotundic acid	1.044 ± 0.02^{a}	49.56 ± 4.02^{a}

Values are expressed as mean ± SD; values are taken as a mean of six separate experiments values not sharing a general superscript letter or differ significantly (DMRT). LPO values are expressed in *n*-moles of MDA released per 100 mg and GSH as µg/100 mg protein.

Analysis of Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) Content: The enzymatic antioxidants (SOD, CAT, GPx) levels were significantly decreased ($p < 0.05$) in the brain tissues of rats intoxicated with mercuric chloride when correlated with control rats **Table 2**. The post-treatment of betulinic acid and rotundic acid rehabilitated all the above enzymatic antioxidants directed ($p < 0.05$) nearly around to the control.

TABLE 2: COMPARATIVE ANALYSIS OF BETULINIC ACID AND ROTUNDIC ACID ON SUPEROXIDE DISMUTASE (SOD), CATALASE (CAT), GLUTATHIONE PEROXIDASE (GPX) CONTENT ON RAT'S BRAIN TISSUES INTOXICATED WITH MERCURIC CHLORIDE

Groups	SOD	CAT	GPx
Control	2.868 ± 0.37^{a}	31.43 ± 2.06^{a}	9.167 ± 0.42^{a}
$HgCl_2$	1.697 ± 0.42^{b}	20.76 ± 1.89^{b}	5.175 ± 0.38^{b}
$HgCl_2$+Betulinic acid	2.693 ± 0.35^{c}	28.57 ± 2.25^{c}	8.622 ± 0.44^{c}
$HgCl_2$+Rotundic acid	2.541 ± 0.34^{d}	27.01 ± 2.02^{d}	7.403 ± 0.39^{c}
Betulinic acid	2.996 ± 0.31^{a}	34.67 ± 1.91^{e}	9.042 ± 0.41^{a}
Rotundic acid	2.902 ± 0.32^{a}	32.80 ± 2.08^{a}	8.961 ± 0.35^{a}

Values are expressed as mean ± SD; values are taken as a mean of six separate experiments values not sharing a general superscript letter or differ significantly (DMRT). SOD values are expressed in unit/min/mg of protein for tissues, CAT as µ moles of H_2O_2 absorbed per min/mg of protein for tissues and GPx as µ moles of GSH depleted per min/mg of protein tissues.

Estimation of Acetylcholinesterase (AChE) Activity Status: The status of acetylcholinesterase activity was abundantly lowered in the tissues of rats' brain intoxicated with mercuric chloride when compared to control animals **Table 3**. Under the post-treatment of betulinic acid and rotundic acid followed by mercuric chloride intoxication, the rat's brain tissues show restoration of AChE activity levels close to the control animals **Table 3.**

TABLE 3: COMPARATIVE ANALYSIS OF BETULINIC ACID AND ROTUNDIC ACID ON ACETYLCHOLINESTERASE (ACHE) ACTIVITY STATUS ON THE RAT'S BRAIN TISSUES INTOXICATED WITH MERCURIC CHLORIDE

Groups	AChE
Control	7.489 ± 0.15^{a}
$HgCl_2$	3.943 ± 0.05^{b}
$HgCl_2$+Betulinic acid	6.596 ± 0.12^{c}
$HgCl_2$+Rotundic acid	5.752 ± 0.24^{d}
Betulinic acid	7.398 ± 0.16^{a}
Rotundic acid	7.246 ± 0.19^{a}

Values are expressed as mean ± SD; values are taken as a mean of six separate experiments values not sharing a general superscript letter or differ significantly (DMRT). AChE values are expressed in μ-moles of formazone formed/mg wet weight of tissues/hour.

Histological and Histopathological Observations in the Brain Tissue: The brain tissue **Fig. 1A** of the untreated control rat, *Rattus norvegicus*, shows the normal histo-architecture of Neuroglial cells (NG), Pyramidal cells (PY), and Pia mater (PM).

But the brain tissue treated with a sub-lethal dose of $HgCl_2$ for 7 days **Fig. 1B** shows irregular size and shape of Neuroglial cells (NG), Damaged Blood Vessels (DBV), Damaged Granule Cells (DGC), Damaged Pyramidal Cells (DPC) and increased vacuolated spaces.

Betulinic acid treated on $HgCl_2$ intoxicated rat brain tissue **Fig. 1C** shows the complete regenerated Neuroglial cells (NG), Regenerated Blood Vessels (RBV), Pyramidal cells (PY), and Granule Cells (GC).

Rotundic acid treated on $HgCl_2$ intoxicated rat brain tissue **Fig. 1D** shows the incomplete regeneration of histo-architecture. The number of vacuoles is reduced, and some vacuoles disappear.

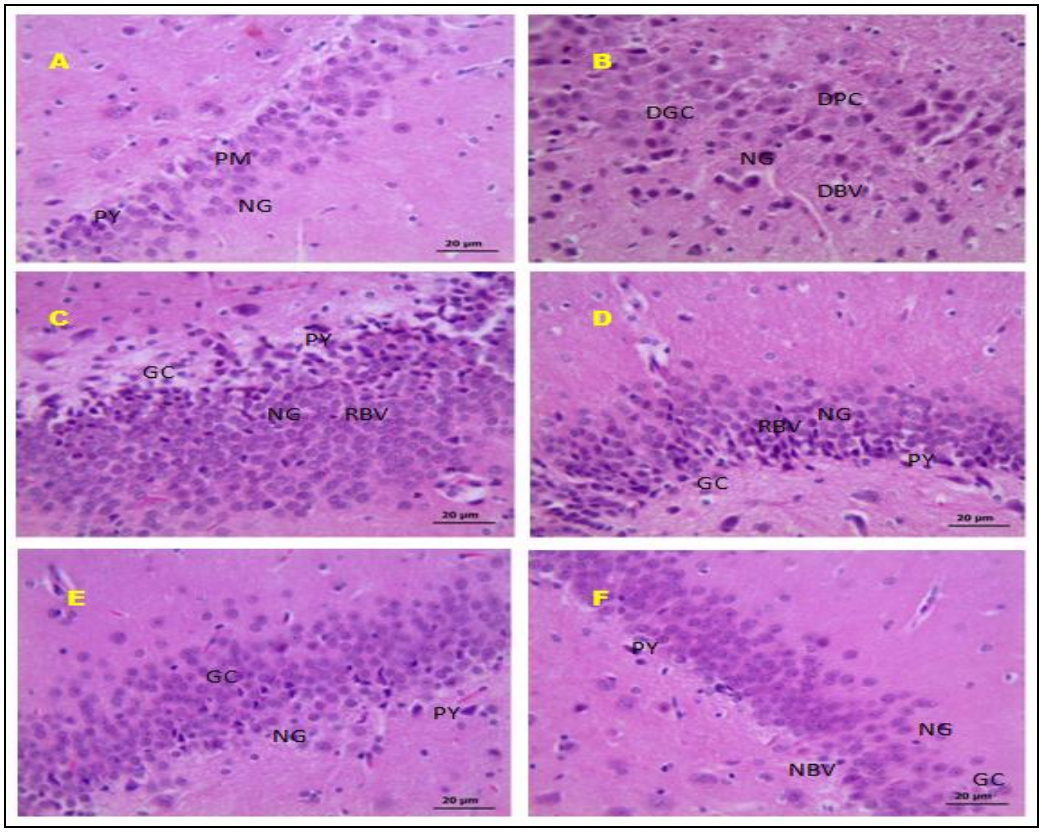

FIG. 1: HISTOLOGY OF INVESTIGATIONAL RAT BRAIN TISSUE UNDER LIGHT MICROSCOPE WITH H&E STAINING AT 10× AND 40× MAGNIFICATION. A, Control brain tissue of rat. B, Mercuric chloride treated brain tissue of rat. C, $HgCl_2$ followed with Betulinic acid brain tissue of rat. D, $HgCl_2$ followed with Rotundic acid brain tissue of rat. E and F, Betulinic acid and Rotundic acid alone treated brain tissue rat.

The regenerated Neuroglial cells and restoration of shape and size of the neuroglial cells are also noticed. The section shows Neuroglial cells (NG), Regenerating Blood Vessels (RBV), Pyramidal cells (PY), and Granule Cells (GC). Betulinic acid alone treated rat brain tissue **Fig. 1E** shows the remarkable size of neuroglial cells, their thick condition, and the complete normal untreated histo-architecture of the brain tissue. The section represented the increased number of Neuroglial cells (NG), Pyramidal cells (PY), and Granule Cells (GC). Rotundic acid alone treated rat brain tissue **Fig. 1F** shows the complete normal untreated histo-architecture of the brain tissue. The section represented the normal blood vessels (NBV), Neuroglial cells (NG), Pyramidal cells (PY), and Granule Cells (GC).

DISCUSSION: Mercury (Hg) pollution of the environment has gathered considerable attention due to its inherent toxicity to living forms [44, 45]. Heavy metal toxins cause deleterious effects in humans, may induce oxidative stress, and lipid peroxidation, and also promote a decline in cognitive performance are already documented in various studies [46, 47, 48]. A rise in the reactive oxygen species (ROS) is detected as a factor in toxicity, directly correlated to lipid peroxidation [49]. Lipid peroxidation is initiated by the mislaying of one hydrogen atom from the chains of unsaturated fatty acid within the membrane structure as a result of ROS synthesized in the organism. The membrane damage perceived as a result of lipid peroxidation is found to be irreversible [50].

The formation of lipid peroxidation is an autocatalytic free radical process whereby polyunsaturated fatty acids (PUFA) in cell membranes undergo degradation by a chain reaction to yield lipid hydroperoxides which subsequently decompose to form a variety of products in clouding malodialdehyde. Lipid peroxidation by Hg has been demonstrated in rat tissues, suggesting that the cell membrane permeability might be affected by this process [51, 52]. LPO was assessed by using TBARS methods [53]. Previous studies show that the accumulation of heavy metals causes to promote the production of LPO content in various tissues of animals [54, 55, 56]. LPO is a well-known mechanism of cellular damage in the animal body. LPO is a reactive 3-carbon dialdehyde and the chief oxidative product of unsaturated fatty acids in the membranes with toxic attributes; high levels of LPO have been assigned to variant disorders in humans [57]. Even though $HgCl_2$ toxicity produces reactive oxygen metabolites in many tissues, the brain is known to be the major target organ. In the present experimental study, the level of LPO content was enormously increased in rat brain tissue when treated with mercuric chloride for 7 days. LPO in the brain was identified by increased thiobarbituric acid reactive substances (TBARS) formation in Hg-treated rats. Thus, the level of LPO might be increased due to the adverse effect of mercuric chloride on the cell membrane. The end products of lipid peroxidation might result in the alteration of biological membranes; thus, these alterations generate serious cellular injury **Fig. 1B**.

The principal toxic impact of mercury involves interactions with many cellular processes, including the formation of complexes with free thiols and protein thiol groups by reducing its content which may lead to oxidative stress. Mercury produces oxidative damage *via* H_2O_2 generation, thereby leading to lipid peroxidation [58]. The elevated level of LPO in the $HgCl_2$ intoxicated rats indicates the increased free radicals formation, which could damage and disorganize the lipid bilayers of the membrane resulting in cellular dysfunction [59]. Ansar [58] also had been reported that administration of $HgCl_2$ caused a significant increase in the levels of LPO in the rat brain.

Furthermore, free radicals from the mercuric ion may also deactivate several enzymes by blocking the functional sites through binding to their sulfhydryl groups, which are part of catalytic or binding domains [60]. $HgCl_2$ also depletes defensive antioxidants, namely glutathione, and inhibits the activities of free radical scavenging systems, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) [61]. Primarily, oxidative damage occurs by developing reactive oxygen species such as superoxide anions, and peroxides, and it can damage the proteins, lipids, and DNA. Hence, it might cause a loss of enzymes' enzymatic activities and structural integrity along with activating inflammatory processes [62]. It was described that the toxic effects of mercury lead to alterations in the structural

stability of the mitochondrial inner membrane, resulting in the depletion of glutathione levels in mitochondria and enhancing the formation of hydrogen peroxide through the electron transport chain of mitochondria [63]. Glutathione is the most abundant low molecular weight thiol-containing compound in living cells. Its reduced form contributes to the stabilizing thiol groups of membrane enzymes and by acting as a protective agent for cells against oxidative stress and detoxification of ROS [64].

Glutathione (GSH) is a non-enzymatic antioxidant that plays a dominant role in protecting cells against oxidative stress, neutralizing free radicals and hydroperoxides, reducing H_2O_2, and detoxifying many xenobiotics [65]. The nucleophilic thiol groups afforded by cysteine residue of GSH play a vital role in detoxifying electrophilic metabolites and metabolically produced oxidizing agents [66]. GSH can scavenge free radicals by transferring hydrogen and also reducing peroxides to sustain protein thiols in reduced conditions [67]. Reduced Glutathione (GSH) is highly abundant in all cell chambers, and is the chief soluble antioxidant. The ratio between the reduced GSH and oxidized GSH is a major determinant of oxidative stress [68].

In the present experimental study, the level of reduced glutathione (GSH) content was drastically decreased in the brain tissue of rats when treated with a sub-lethal dose of mercuric chloride for 7 days. This result clearly shows that it is mainly due to the imbalance between oxidants and defense mechanisms in the intoxicated rats. This depletion of GSH leads to oxidative stress. Subsequently, it results in the loss of structural and functional integrity of cells **Fig. 1B**. Once mercuric chloride bonds with GSH were identified in the cells. The level of GSH, which is the primary line of cellular protection against toxic agents and major intracellular conjugation factors, reduced and displayed damaged function in Hg toxicity [69]. The binding of $HgCl_2$ to GSH reduced the level of GSH in the cells and reduced the antioxidant properties of the cells [70]. Because administration of $HgCl_2$ in normal rat tissues is getting significant reductions in GSH and decreases the GSH/GSSG ratio while markedly increasing MDA formation compared with that in the control group. The combination of raised LPO and reduction in the level of GSH support the presence of oxidative stress, which agrees with previous reports [71, 72]. The endogenous antioxidant enzymes such as SOD, CAT, and GPx address the principle caution step towards ROS [73]. Excessive oxidative stress brings a drastic imbalance between the antioxidant enzymes and pro-oxidative markers, namely superoxide anion, hydroxyl radical, H_2O_2, and singlet oxygen.

The excessive production of ROS exhausts the antioxidant defense capabilities and oxidatively modifies enzymatic proteins that become nonfunctional [74]. Enzymes such as SOD, CAT, and GPx also play important roles in protecting against free radical damage. They are considered the primary antioxidant enzymes since they are involved in the direct elimination of active oxygen species. A toxicant can be a nucleophile or converted to a nucleophile by microsomal enzymes. After administering toxicants, the enzymes responsible for protecting against nucleophilic damage are also affected [75]. Antioxidants exhibit a protective potential in ameliorating the metal-induced damages either by the activity of metal chelation or through increasing the activity of antioxidant enzymes [76, 77].

SOD's were described as common stress-receptive factor and could eliminate the cellular ROS induced by various physical, chemical, and biological signs [78]. Superoxide dismutase (SOD), as an important enzymatic antioxidant is generally considered a biomarker in evaluating oxidative stress for showing redox status [79]. Catalase (CAT) is a heme protein that decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals. The reduction in the activity of these enzymes may be due to oxidative stress exerted by the intoxication of different toxicants in animals [80]. The glutathione peroxidase (GPx) is a crucial antioxidant enzyme that is necessary for protection against the damaging effects of lipid peroxidation in organs [81]. Glutathione peroxidase (GPx) catalyzes the oxidation of GSH to GSSG; this oxidation reaction occurs at the expense of H_2O_2. Therefore it also helps in clearing the toxic compounds present in the cell like hydrogen peroxide [82, 83]. In opposition to free-radical damage, defense mechanisms induced endogenous antioxidant enzymes such as

SOD, CAT, and GPx [84]. High levels of ROS or any interruption in the oxidant and antioxidant status can result in oxidative damage to macromolecules like DNAs, proteins, and lipids, along with tissues and organs [85]. In the present experimental study, the level of antioxidant enzymes (SOD, CAT, and GPx) activities decreased in the brain tissue of HgCl2-induced rats compared to the control group. The present experimental result indicated that $HgCl_2$ could cause severe oxidative stress in the target organs of rats. Thus, these results are parallel to previous studies [86], which reported the significant depletion of GSH and a significant decrease in the activities of SOD, CAT, and GPx after the intoxication of $HgCl_2$ confirmed through the formation of oxidative stress [87, 88, 89].

The reduced level of SOD activity further indicated an increased superoxide radical production and, consequently, higher hydroxyl radical formation. CAT and GPx may provide a direct defense by eradicating the hydrogen peroxide, one of the leading hydroxyl radicals, a potential reactive structure [90]. Changes in these enzyme activities might finally lead to the depletion of GSH levels resulting in oxidative damage to the nerve tissues was also supported by histopathological observations **Fig. 1** [51, 60].

Acetylcholinesterase (AChE) is responsible for hydrolyzing and deactivating acetylcholine in the nervous system; it is also a good indicator of heavy metal toxicity [58, 91]. Among the heavy metals, mercury and its compounds are highly toxic. Neurotoxicity effects of mercuric chloride mainly depend upon the degree of alterations in the activity of different neurotransmitter enzymes in animals. Determining acetylcholinesterase (AChE) activity level in the brain tissue is a very good tool for mercuric chloride toxicity targets in intoxicated rats. The fluctuations and disturbance in AChE activity can explain some neurotoxicological characteristics of mercury and its compounds. In the present experimental work, AChE activity was drastically decreased in rat brain tissue when treated with a sub-lethal dose of mercuric chloride for 7 days. Because mercury and its compounds bind easily to the active site of AChE and reduce its activity. At this time, a neurotransmitter (ACh) is not hydrolyzed, and it might promptly be gathered in cholinergic regions of nerve endings in the intoxicated brain 92, 93, leading to impairment of numerous physiological functions [94, 95]. Most of the experimental work reported that AChE was significantly decreased in the brain of rats when treated with mercuric chloride [58, 91, 96, 97]. Coluccia *et al*., [98] have also reported behavioral and spatial learning defects in animals due to mercury treatment leading to cognitive impairment in rats. Motor neuron cognition functions and motor activities are supervised by normal levels of acetylcholine content and acetylcholinesterase (AChE) activity. The reduced level of AChE activity leads to an accumulation of acetylcholine, causing overstimulation of the receptors of neurons.

This mechanism can prompt undesirable effects in animals. The brain can generate the signal and pass it through the nerve junctions to promote muscular movements. Generally, a neurotransmitter (ACh) is liberated from one nerve to another nerve to promote the stimulation of body movements. During this process, ACh is split or hydrolyzed by AChE. This is an important enzyme found in various CNS junctions and nerve endings in various glands and nodes throughout the body.

In normal conditions, deposition of AChE and ACh consistently plays an important role in a delicate balance, interacting with the complex nervous system, which controls most of the body functions like learning and memory processes, as well as locomotor control and cerebral bloodstream [99, 100]. The cerebellum possesses various unique advantages as a model system for motor coordination. Abnormal behavior was noticed in an intoxicated animal mainly because exposure to toxicants may lead to cerebellum damage, and it may cause a reduction in the level of AChE activities. ROS has recently been indicated as an additional mechanism by which mercury exerts initial neurotoxic impact [101], accompanied by altered Na+/K+-ATPase and AChE activities in mammals [58]. In the present experimental study, the overproduction of reactive oxygen species (ROS) can also initiate undesirable biological responses, including tissue injury or cell death within the central and peripheral nervous systems. It has been shown that an increased production of ROS caused by mercury-induced toxic impacts might cause the altered brain or other cellular functions, at last

resulting in cell death and pathophysiological damages (Fig. 1). That occurrence is accompanied by depletion in enzymatic antioxidants. Several studies show decreased AChE activity in brain tissues after Hg exposure [101, 102, 103].

Additionally, AChE inhibitor probably promotes death in intoxicated animals by blocking neuro-transmission in the brain's respiratory center in neuromuscular function. Because during the intoxication process, inhibiting or suppressing the glutathione production in brain cells inhibits their ability to release mercury toxicants from the target tissues, especially brain tissue. So, mercury and its compounds mainly accumulate in the central nervous system, primarily in astrocytes, the cells that provide the first line of defense for the central nervous system against toxic compounds. Thus, they result in a loss of muscular coordination, convulsions, and finally, death [91, 101, 104].

Now a day's, massive attempts are being sought in the search for new drugs against the toxicity of different types of mercurial compounds; there is no effective treatment that totally eliminates the toxic impacts on the animals. Additionally, chelating compounds assist in diminishing mercury burdens in animals [105]. Previous studies have reported that plant-derived phenolic compounds mainly possess radical scavenging properties. The antioxidant (radical scavenging) activity of phenolics is majorly attributed to their potential ability to scavenge ROS like superoxide anions, hydrogen peroxides, hydroxyl radicals, and hypochlorous acids [106, 107]. Betulinic acid and Rotundic acid are the habitually present phenolic compound in many plants and have been proclaimed to possess antioxidant and anti-inflammatory activities [25, 32]. In the present investigation, mercury prompts oxidative stress in the brain tissue, manifested by increased lipid peroxidation formation. Simultaneously, it was also found to decrease the activity of three major enzymatic antioxidants, namely SOD, CAT, GPx, and non-enzymatic antioxidant (GSH), along with AChE activity, when they were intoxicated with mercuric chloride. These results indicated a significant oxidant burden on the brain of intoxicated mercury rats. Furthermore, in the present experimental study, BA, and RA improved the survival rate and prevented brain injury (Fig. 1C & D) in the mercury-intoxicated rat. BA and RA endeavor a protective effect against the toxicity induced by mercuric chloride by way of improving the non-enzymatic antioxidant (GSH) and enzymatic antioxidants (SOD, CAT, and GPx) along with AChE using its scavenging property like lowering the LPO levels as a meritorious antioxidant.

The antioxidant potential of betulinic acid and rotundic acid reduced the generation of free radicals in the respective organ of mercury-intoxicated rats. Thus the result suggested that both phytochemical compounds not only have the synthesis of antioxidant properties and also act as phyto-chelating substances. Different authors observed similar results in intoxicated rat and mice tissues when treated with Betulinic acid and Rotundic acid. Adeleke *et al*., [18], Betulinic acid enhanced the activities of superoxide dismutase, catalase and the levels of reduced glutathione in the liver and kidney of wistar rats treated with atrazine an herbicide.

Similarly, BA improves the activities of SOD, CAT, and GPx in a dose-dependent manner when compared with alcohol-treated liver of mice [108, 109]. To support the present study, Hsu *et al*., [32] found RA treatment reduces the production of ROS and GSSG and increases the GSH, GPx, and catalase in the heart and kidney of diabetic mice. Thus, these observations indicate that BA and RA exert prominent antioxidant effects against the toxicants' oxidative damage.

CONCLUSION: Therefore, the present study's finding indicated that exposure to $HgCl_2$ induced severe changes in the various activities of the brain and prompted neuro-toxicity. Betulinic acid and rotundic acid have a protective efficacy against $HgCl_2$-induced toxicity. This protection may be due to these antioxidants' free radical scavenging effects and/or their enhancing effects on the antioxidant properties. Our results suggest that betulinic acid and rotundic acid might be promising compounds for treating neuro-toxicity induced by mercuric chloride.

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REFERENCES:

1. Abbas BF, Al-Jubori WMK and Abdullah AM: Environmental Pollution with the Heavy Metal Compound. Research J. Pharm. and Tech 2018; 11(9): 4035-4041. DOI: 10.5958/0974-360X.2018.00743.6.
2. de Vos G, Abotaga S, Liao Z, Jerschow E and Rosenstreich D: Selective effect of mercury on Th2-type cytokine production in humans. Immunopharmacol Immunotoxicol 2007; 29 (3-4): 537–548, 2007.
3. Bernhoft RA: Mercury Toxicity and Treatment: A Review of the Literature. J. Environ. Public Health 2012; 460508, 10. doi:10.1155/2012/460508
4. Wusu AD, Ogunrinola OO, Ojekale AB, Olaitan S, Onwordi CT and Auta R: The interplay of antioxidant activities in rats exposed to sub chronic low level inorganic mercury is gender specific. J Biotech Biochem (IOSR-JBB) 2016; 2(4): 49-54.
5. Durak D, Kalender S, Uzun FG, Demır F and Kalender Y: Mercury chloride-induced oxidative stress in human erythrocytes and the effect of vitamins C and E *in-vitro*. Afr J Biotechnol 2010; 9: 488-495.
6. Temmar M, Watfa G, Joly L, Kearney-Schwartz A, Youcef M, Bensalah S, Mustapha Y, Salvi P, Gautier S, Safar M, Labat C and Benetos A: Elderly Algerian women lose their sex-advantage in terms of arterial stiffness and cardiovascular profile. J Hypertens 2013; 31: 2244-2250.
7. Syversen T and Kaur P: The toxicology of mercury and its compounds. J Trace Elem Med Biol 2012; 26: 215-226.
8. Houston MC. Role of mercury toxicity in hypertension, cardiovascular disease and stroke. J. Clin. Hypertens. (Greenwich). 2011; 13: 621-627. https://doi.org/10.1111/j.1751-7176.2011.00489.x.
9. Kim NH, Hyun YY, Lee KB, Chang Y, Rhu S, Oh KH and Ahn C: Environmental heavy metal exposure and chronic kidney disease in the general population. J Korean Med Sci 2015; 30: 272-277.
10. Bjorklund G, Dadar M, Mutter J and Aaseth J: The toxicology of mercury: current research and emerging trends. Environ Res 2017; 159: 545–554.
11. Deleve ID and Kaplowitz N: Importance and regulation of hepatic glutathione. Semin Liv Dis 1990; 10: 251-266.
12. Zulaikhah ST, Anies, Suwondo A and Santosa: Effects of Tender Coconut Water on Antioxidant Enzymatic Superoxida Dismutase (SOD), CATALASE (CAT), Glutathione Peroxidase (GPx) and Lipid Peroxidation In Mercury Exposure Workers. Int J Sci Res 2015; 4 (12): 515-524.
13. Bjørklund D: Toxic metal (loid)-based pollutants and their possible role in autism spectrum disorder. Environ Res 2018; 166: 234-25.
14. Ogbomida ET, Omofonmwan K, Aganmwonyi I, Fasipe IP, Enuneku A, Ezemonye LIN. Bioactive profiling and therapeutic potential of mushroom (Pleurotus tuberregium) extract on Wistar albino rats (Ratus norvegicus) exposed to arsenic and chromium toxicity. Toxicol Reports 2018; 5: 401–410.
15. Aaseth J, Ajsuvakova OP, Skalny AV, Skalnaya MG and Tinkov AA: Chelator combination as therapeutic strategy in mercury and lead poisonings. Coord Chem Rev 2018; 358: 1–12. https://doi.org/10.1016/j.ccr.2017.12.011.
16. Aaseth J, Skaug MA, Cao Y, Andersen O, Chelation in metal intoxication –Principles and paradigms. J Trace Elem Med Biol 2015; 31: 260–266. https://doi.org/10.1016/j.jtemb.2014.10.001.
17. Alakurtti S, Makela T, Koskimies S and YliKauhaluoma J: Pharmacological properties of the ubiquitous natural product betulin. Eur J Pharm Sci 2006; 29: 1–13.
18. Adeleke GE, Adedosu OT, Olajutemo TO, Ojetola OJ and Irekeola RA: Ameliorative potential of Betulinic Acid against Atrazine-induced Hepatic and Testicular Damage in Wistar rats. J Nat Sci Res 2018; 8: 8.
19. Moghaddam MG, Ahmad FBH and Kermani AS: Biological Activity of Betulinic Acid: A Review. Pharmacology & Pharmacy 2012; 3: 119-123.
20. Takada Y and Aggarwal BB: Betulinic acid suppresses carcinogen-induced NFkappa B activation through inhibition of I kappa B alpha kinase and p65 phosphorylation: abrogation of cyclooxygenase-2 and matrix metalloprotease- 9. J Immunol 2003; 171: 3278–3286.
21. Fontanay S, Grare M, Mayer J, Finance C and Duval RE: Ursolic, oleanolic and betulinic acids: antibacterial spectra and selectivity indexes. J Ethnopharmacol 2008; 120: 272–276.
22. Eksioglu-Demiralp E, Kardas ER, Ozgül S, Yagci T, Bilgin H, Sehirli O, Ercan F and Sener G: Betulinic acid protects against ischemia/reperfusion-induced renal damage and inhibits leukocyte apoptosis. Phytother Res 2010; 24: 325–332.
23. Viji V, Helen A and Luxmi VR: Betulinic acid inhibits endotoxin-stimulated phosphorylation cascade and pro-inflammatory prostaglandin E2 production in human peripheral blood mononuclear cells. Br J Pharmacol 2011; 162: 1291–1303.
24. Nader MA and Baraka HN: Effect of betulinic acid on neutrophil recruitment and inflammatory mediator expression in lipopolysaccharide-induced lung inflammation in rats. Eur J Pharm Sci 2012; 46: 106–113.
25. Lingaraju MC, Pathak NN, Begum J and Balaganur V: Betulinic acid attenuates lung injury by modulation of inflammatory cytokine response in experimentally-induced polymicrobial sepsis in mice. Cytokine 2015; 71: 101–108.
26. Saimaru H, Orihara Y, Tansakul P, Kang YH, Shibuya M and Ebizuka Y: Production of triterpene acids by cell suspension cultures of Olea europaea. Chem Pharm Bull 2007; 55(5): 784–788.
27. Yang B, Li H, Ruan QF, Tong Y, Liu ZQ, Xuan SX, Jin J and Zhao ZX: Rapid profiling and pharmacokinetic studies of multiple potential bioactive triterpenoids in rat plasma using UPLC/Q-TOF-MS/MS after oral administration of Ilicis Rotundae Cortex extract. Fitoterapia 2018; 129: 210–219.
28. Zhu JP, Yang B, Yang T, Tan QL, Tong JY, Tong PZ, Rong L and Zhao ZX: Simultaneous determination of four constituents in Cortex Ilicis Rotundae with HPLC. Tradit Chin Drug Res Pharmacol 2015; 26: 558–560.
29. Yang B, Li H, Ruan QF, Xue YY, Cao D, Zhou XH, Jiang SQ, Yi T, Jin J and Zhao ZX: A facile and selective approach to the qualitative and quantitative analysis of triterpenoids and phenylpropanoids by UPLC/QTOF-MS/MS for the quality control of Ilex rotunda. J Pharm Biomed Anal 2018; 157: 44–58.
30. Roy G, Guan S, Liu HX and Zhang L: Rotundic acid induces DNA damage and cell death in hepatocellular carcinoma through AKT/mTOR and MAPK pathways. Front Oncol 2019; 9: 545.

31. Han Y, Zhang L, Li W, Liu X, Xiao J, Chen G and Li N: Natural CAC chemopreventive agents from Ilex rotunda Thunb. J Nat Med 2019; 73(3): 456–467.
32. Hsu YM, Hung YC, Hu L, Lee YJ and Yin MC: Anti-diabetic effects of madecassic acid and rotundic acid. Nutrients 2015; 7(12): 10065–10075.
33. Aro AO, Dzoyem JP, Awouafack MD, Selepe MA, Eloff JN and McGaw LJ: Fractions and isolated compounds from *Oxyanthus speciosus* subsp. Stenocarpus (Rubiaceae) have promising antimycobacterial and intracellular activity. BMC Complement Altern Med 2019; 19(1): 108.
34. Nguyen HT, Ho DV, Vo HQ, Le AT, Nguyen HM, Kodama T, Ito T, Morita H and Raal A: Antibacterial activities of chemical constituents from the aerial parts of *Hedyotis pilulifera*. Pharm Biol 2017; 55(1): 787–791.
35. Yan ZH, Wu H, Yao HL, Pan WJ, Su MM, Chen TB, Su WW and Wang YG: Rotundic acid protects against metabolic disturbance and improves gut microbiota in Type 2 diabetes rats. Nutrients 2019; 12(1): 67.
36. Manju M and Jagadeesan G: *In-vivo* hepatoprotective effect of caffeic acid on mercuric chloride-induced biochemical changes in albino wistar rats. Asian J Pharm Clin Res 2019; 12(4): 119-124.
37. Nichens WG, Samuelson B, Fomulation of malondialdehyde from phospho-lipid arachidouate during microsomal lipid Peroxidation. Eur J Biochem 1968; 6: 126–30.
38. Beutler E and Kelley BM: The effect of disodium nitrate on RBC glutathione. Experintia 1963; 29: 97-101.
39. Kakkar P, Das B, Viswanathan PN, A modified spectrophotometric assay of SOD. Indian J Biochem Biophys 1984; 21: 131–2.
40. Sinha KA: Colorimetric assay of catalase. Anal Biochem 1972; 47: 389–94.
41. Rotruck JJ, Pope AL, Gauther HE, Swanson AB, Hateman DG and Hoekstra WG: Selenium biochemical role as a component at glutathione peroxidase. Science 1973; 179: 588–90.
42. Metcalf RL: Methods in biochemical analysis. (Glick D, Eds). Inter Sci Publication New York 1951; 44.
43. Duncan BD: Duncan's multiple range tests for correlated and hetroscedastic mean. Biometics 1957; 13: 354–64.
44. Khan F, Momtaz S and Abdollahi M: The relationship between mercury exposure and epigenetic alterations regarding human health, risk assessment and diagnostic strategies. J Trace Elem Med Biol 2019; 52: 37-47.
45. Budnik LT and Casteleyn L: Mercury pollution in modern times and its socio-medical consequences. Sci Total Environ 2019; 654: 720-34.
46. Lucena GM, Franco JL, Ribas CM, Azevedo MS, Meotti FC, Gadotti VM, Dafre AL, Santos AR and Farina M: Cipura paludosa extract prevents methyl mercury-induced neurotoxicity in mice. Basic Clin Pharmacol Toxicol 2007; 101: 127–131.
47. Taber KH and Hurley RA: Mercury exposure: effects across the lifespan. J Neuropsychiatry Clin Neurosci 2008; 20: 389.
48. Afriza D: The Effect of Mercury Vapour and the Role of Green Tea Extract on Brain cells. J Dentistry Indones 2013; 20(2): 39-45.
49. Astuti S: Soy Isoflavones and Their Potential as Capture of Free Radicals (In *Bahasa indonesia*). Jurnal Teknologi Industri dan Hasil Pertanian 2008; 13: 126-36.
50. Caner E and Değer Y: Lipid Peroxidation, Antioxidant Profile and Some Biochemical Parameters in Rat Applied Aminoglycozide. World Journal of Pharmacy and Pharmaceutical Sciences 2018; 7: 1.
51. Rao MV and Purohit AR: Neuroprotection by Melatonin on mercury induced toxicity in the rat brain. Pharmacol Pharmacy 2011; 2: 375–385.
52. Bharathi E, Jagadeesan G and Vijayakumar M: Hepato-ameliorative effect of hesperidin and ellagic acid on mercuric chloride intoxicated rats. Biomed & Ag Pathol 2014; 4: 17–21.
53. Qiao D, Seidler FJ and Slotkin TA: Oxidative mechanisms contributing to the developmental neurotoxicity of nicotine and chlorpyrifos. Toxicol Appl Pharmacol 2005; 206: 17–26.
54. Goering PL, Morgan DL and Ali SF: Effects of mercury vapor inhalation on reactive oxygen species and antioxidant enzymes in rat brain and kidney are minimal. J Appl Toxicol 2002; 22: 167–172.
55. Yang JM, Arnush M, Chen QY, Wu XD, Pang B and Jian XZ: Cadmium induced damage to primary cultures of rat Leydig cells. Reprod. Toxicol 2003; 17: 553–560.
56. Kalender S, Uzun FG, Demir F, Uzunhisarcıklı M and Aslanturk A: Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E. Food and Chemical Toxicology 2013; 55: 456–462.
57. Goudarzi M, Esmaeilizadeh M, Dolatshahi M, Kalantar H and Frouzandeh H: Protective Effect of Elaeagnus angustifolia L. Fruit Hydroalcoholic Extract on Cyclophosphamide-Induced Nephrotoxicity in Mice. Shiraz E-Med J 2018; 19(1): 55075. doi: 10.5812/semj.55075.
58. Ansar S: Pretreatment with diallylsulphide modulates mercury-induced neurotoxicity in male rats. Acta Biochimica Polonica 2015; 62(3): 599–603 http://dx.doi.org/10.18388/abp.2015_1064
59. Abolaji AO, Adesanoye OA, Awogbindin I and Farombi EO: Endocrine disruption and oxidative stress implications of artemether–lumefantrine combination therapy in the ovary and uterus of rats. Human and Experimental Toxicology 2016; 35: 1173-1182.
60. Ajsuvakova OP: Sulfhydryl groups as targets of mercury toxicity. Coord Chem Rev 2020; 417: 213343.
61. Zulaikhah ST, Wahyuwibowo J and Pratama AA: Mercury and its effect on human health: a review of the literature. International Journal of Public Health Science 2020; 9 (2): 103-114.
62. Genchi G, Sinicropi M, Carocci A, Lauria G and Catalano A: Int J Environ Res Public Health 2017; 14: 74.
63. Sener G, Sehirli O and Tozan A: Ginkgo biloba extract protects against mercury (II)-induced oxidative tissue damage in rats. Food Chem. Toxicol 2007; 45: 543–550.
64. Franco JL, Braga HC, Stringari J, Missau FC, Posser T, Mendes BG, Leal RB, Santos ARS, Dafre AL, Pizzolatti MG and Farina M: Comparative study of activities in reactive oxygen species production/defense system in mitochondria of rat brain and liver, and their susceptibility to methylmercury toxicity. Chem Res Toxicol 2007; 20: 1919–1926.
65. Rangi S, Dhatwalia SK, Bhardwaj P, Kumar M, Dhawan DK. Evidence of similar protective effects afforded by white tea and its active component 'EGCG' on oxidative-stress mediated hepatic dysfunction during benzo(a)pyrene induced toxicity. Food Chem Toxicol 2018; 116: 281–291.
66. Rahman I and MacNee W: Oxidative stress and regulation of glutathione in lung inflammation. Eur Respir J 2000; 16: 534–554.
67. Arslan C, Altan H, Beşirli K, Aydemir B, Kiziler AR and Denli S: The role of oxidative stress and antioxidant defenses in Buerger disease and atherosclerotic peripheral

arterial occlusive disease. Ann Vasc Surg 2010; 24: 455–460.
68. Mohan M: Pre-clinical toxicity study of tamrabhasma on albino wistar rats. Int Res J Pharm 2018; 9(1) 36.
69. Farina M and Aschner M: Glutathione antioxidant system and methylmercury-induced neurotoxicity: An intriguing interplay. Biochim. Biophys Acta 2019; 1863(12):129285.
70. Bashandy SA, El Awdan SA, Ebaid H and Alhazza IM: Antioxidant Potential of *Spirulina platensis* Mitigates Oxidative Stress and Reprotoxicity Induced by Sodium Arsenite in Male Rats. Oxid Med Cell Longev 2016; 7174351. doi: 10.1155/2016/7174351. [PubMed: 26881036].
71. Abdel MAE: The neuroprotective effect of berberine in mercury-induced neurotoxicity in rats. Metab Brain Dis 2015; 30: 935-942.
72. Gökçe G, Arun MZ and Ertuna E: Ergothioneine prevents endothelial dysfunction induced by mercury chloride. Exp. Ther. Med 2018; 15: 4697-4702.
73. Kim Y, You Y, Yoon HG, Lee YH, Kim K, Lee J, Kim MS, Kim JC and Jun W: Hepatoprotective effects of fermented Curcuma longa L. on carbon tetrachloride-induced oxidative stress in rats. Food Chem 2014; 151: 148-153.
74. Safhi MM: Nephroprotective effect of Zingerone against CCl4-induced renal toxicity in Swiss albino mice: molecular mechanism. Oxid Med Cell Longev 2018; 1-8.
75. Martin-Aragón S, Heras BDL, Sanchez-Reus MI and Benedi J: Pharmacological modification of endogenous antioxidant enzymes by ursolic acid on tetrachloride-induced liver damage in rats and primary cultures of rat hepatocytes. Exp Toxic Pathol 2001; 53: 199–206.
76. Moraes-Silva L, Siqueira LF, Oliveira VA, Oliveira CS, Ineu RP, Pedroso TF, Fonseca MM and Pereira ME: Preventive effect of CuCl2 on behavioral alterations and mercury accumulation in central nervous system induced by HgCl2 in newborn rats. J Biochem Mol Toxicol 2014; 28: 328–335.
77. Pires ENS, Frozza RL, Hoppe JB, Menezes Bde M and Salbego CG: Berberine was neuroprotective against an in vitro model of brain ischemia: survival and apoptosis pathways involved. Brain Res 2014; 1557: 26–33.
78. Reyes-Becerril M, Maldonado-Garcia M, Guluarte C, Leon-Gallo A, Rosales-Mendoza S, Ascencio F, Hirono I and Angulo C: Evaluation of ToxA and Vibrio parahaemolyticus lysate on humoral immune response and immune-related genes in Pacific red snapper. [Journal Article]. Fish Shellfish Immunol 2016; 56: 310-321. doi: 10.1016/j.fsi.2016.07.014
79. Teng Y and Liu RT: Insights into potentially toxic effects of 4-aminoantipyrine on the antioxidant enzyme copper–zinc superoxide dismutase. J Hazard Mater 2013; 262: 318-324. 10.1016/j.jhazmat.2013.08.047.
80. Nwani CD, Somdare PO, Ogueji EO, Nwani JC, Ukonze JA and Nwadinigwe AO: Genotoxicity assessment and oxidative stress responses in freshwater African catfish Clariasgariepinus exposed to fenthion formulations. Drug Chem Toxicol 2017; 40(3): 273–280.
81. Bharathi E, Jagadeesan G and Vijayakumar M: Hepato-ameliorative effect of hesperidin and ellagic acid on mercuric chloride intoxicated rats. Biomed & Ag Pathol 2014; 4: 17–21.
82. Bubber P: A Study on Prooxidative and Neurotoxic Effects of Mercury Chloride in Rats. EC Pharmacol Toxicol 2019; 7(2): 112-124.
83. Ogueji E, Nwani C, Mbah C, Iheanacho S and Nweke F: Oxidative stress, biochemical, lipid peroxidation and antioxidant responses in *Claria sgariepinus* exposed to acute concentrations of ivermectin. Environ. Sci Pollution Res 2020; 27: 16806–16815. https://doi.org/10.1007/s11356-019-07035-4.
84. Franciscato C, Moraes-Silva L, Duarte FA, Oliveira CS, Ineu RP, Flores EM, Dressler VL, Peixoto NC and Pereira ME: Delayed biochemical changes induced by mercury intoxication are prevented by zinc pre-exposure. Ecotoxicol Environ Saf 2011; 74: 480–486.
85. Kalantar M, Houshmand G, Kalantar H, Asadi M, Goudarzi M. Protective effect of hydroalcoholic extract of *Lavandula officinalis* L. on gentamicin induced nephrotoxicity in rats. J Babol Univ Med Sci 2016; 18(7): 62–7.
86. Goudarzi M, Kalantar M and Kalantari H: Protective effect of hydroalcoholic extract of *Lavandula officinalis* L. on gentamicin induced nephrotoxicity in rats. Toxicol Lett 2016; 258: 284–5. doi:10.1016/j.toxlet.2016.06.1991.
87. Jafari M, Rezaei M, Kalantari H and Hashemitabar M: Determination of Cell Death Induced by Lovastatin on Human Colon Cell Line HT29 Using the Comet Assay. Jundishapur J Nat Pharm Prod 2013; 8: 187–91.
88. Sheikh T, Patel B, Joshi D, Patel R and Jegoda M: Repeated dose oral toxicity of inorganic mercury in wistar rats, biochemical and morphological alterations. Vet World 2013; 6(8): 563.
89. Mehrzadi S, Fatemi I, Esmaeilizadeh M, Ghaznavi H, Kalantar H and Goudarzi M: Hepatoprotective effect of berberine against methotrexate induced liver toxicity in rats. Biomed Pharmacother 2018; 97: 233–9. doi:10.1016/j.biopha.2017.10.113. [PubMed: 29091871].
90. Cemek M, Çaksen H and Cemek F: Investigation of antioxidant status in children with acute otitis media and tonsillitis. Int J Pediatr Otorhinolaryngol 2004; 68(11): 1381-5.
91. Vanithasri V and Jagadeesan G: Comparative Beneficial Effect of N-Acetylcysteine and Gallic Acid on Antioxidant and Acetyl Choline Status of Mercury Intoxicated Rats. Int J Phytopharmacol 2013; 4(5): 293-300.
92. Dorman DC, Brenheman KA and Bolon B: Nervous system, hand book of toxicology pathology, edited by WM Haschek, CG Rousseaux and MA Walling, 2nd Edn. (Academic press, sandiego New York boston) 509, 2002.
93. Farina H, Brandao R, Lara FS, Soares F, Souza DO and Rocha JB. Mechanism of inhibitory effect of selenium and mercury on the activity of delta-aminolevulinate dehydratase from mouse liver, kidney and brain. Toxicol Let 2003; 139.
94. Margarat A and Jagadeesan G. Antidotal recovery in carbohydrate metabolism intoxicated mice. *Mus musculus*. U P Zool Soc 1999; 19(3): 193-196.
95. Bubber P: A Study on Prooxidative and Neurotoxic Effects of Mercury Chloride in Rats. EC Pharmacol Toxicol 2019; 7(2): 112-124.
96. El-Demerdash FM, Yousef MI and Elagamy EI: Influence of paraquat, glyphosate and cadmium on the activity of some serum enzymes and protein electrophoretic behaviour (*in-vitro*). J Environ Sci Health 2001; 36.
97. Sankarsamipillai S and Jagadeesan G: Protective role of Taurine on mercuric chloride induced neurotoxicity in rats. Poll Res 2006; 25(2): 353-356.
98. Coluccia A, Borracci P, Giustino A, Sakamoto M and Carratu MR: Effects of low dose methylmercury administration during the postnatal brain growth spurt in rats. Neurotoxicol. Teratol 2007; 29: 282–287.
99. Deiana S, Platt B and Riedel G: The cholinergic system and spatial learning. Behav Brain Res 2011; 221: 389–411.

100. Hut RA and Van der Zee EA: The cholinergic system, circadian rhythmicity and time memory. Behav Brain Res 2011; 221: 466–80.
101. Manju M and Jagadeesan G: Neuroprotective and Ameliorative Effect of Caffeic Acid on Antioxidant and Acetylcholine Status of Mercury Intoxicated Rats. Int J Pharm Biol Sci 2019; 9(2): 85-95.
102. Frasco MF, Fournier D, Carvalho F and Guilhermino L: Do metals inhibit acetylcholinesterase (AChE)? Implementation of assay conditions for the use of AChE activity as a biomarker of metal toxicity. Biomarkers 2005; 10: 360–375.
103. Kumar G, Srivastava A, Sharma SK and Guptaa YK: Safety evaluation of an Ayurvedic medicine, Arogyavardhini vati on brain, liver and kidney in rats. J Ethnopharmacol 2012; 140: 151-160.
104. Jagadeesan G and Sankar Samipillai S: Antidotal efficacy of Taurine on Acetylcholinesterase activity and Na+/K+ ATPase activity in the Brain tissue of mercury poisoned rats. Int J Curr Res 2011; 3(2): 054-060.
105. Franco JL, Posser T, Missau F, Pizzolatti MG, Santos AR, Souza DO, Aschner M, Rocha JB, Dafre AL and Farina M: Structure–activity relationship of flavonoids derived from medicinal plants in preventing methylmercury-induced mitochondrial dysfunction. Environ. Toxicol Pharmacol 2010; 30(3): 272–278.
106. Goudarzi M, Kalantar M and Kalantari H: Protective effect of hydroalcoholic extract of Lavandula officinalis L. on gentamicin induced nephrotoxicity in rats. Toxicol Lett 2016; 258: 284–5. doi:10.1016/j.toxlet.2016.06.1991.
107. Kalantar H, Sabetkasaei M, Shahriari A, Haj MollaHoseini M, Mansouri S and Kalantar M: The effect of rapamycin on oxidative stress in MCF 7 and MDA MB 231 human breast cancer cell lines. Jundishapur J Nat Pharm Prod 2016; 11(3). doi: 10.17795/jjnpp-38177.
108. Zheng Z, Song S, Wu Y, Lian L, Wan Y and Nan J: Betulinic acid prevention of Dgalactosamine/ lipopolysaccharide liver toxicity is triggered by activation of Bcl-2 and antioxidant mechanisms. J Pharm Pharmacol 2011; 63: 572–578.
109. Yi J, Xia W, Wu J, Yuan L, Wu J, Tu D, Fang J and Tan Z: Betulinic acid prevents alcohol-induced liver damage by improving the antioxidant system in mice. J Vet Sci 2014; 15(1): 141-148.

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