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AMELIORATIVE POTENTIAL OF BETULINIC ACID AND ROTUNDIC ACID ON NEPHROTOXICITY INDUCED BY MERCURY CHLORIDE IN RATS

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ABSTRACT: Mercury toxicity is the most hazardous problem emerging in the world as its accumulation is escalating persistently through the increased utility of mercury in medicinal, industrial, and domiciliary usage. Subjection to mercury illustrates a consequential provocation to humans and other living biomes. The intention of the present research was to investigate the shielding potential of betulinic acid and rotundic acid against mercury chloride ($HgCl_2$) (1.29 mg/kg b. w.) induced renal toxicity in adult males rats. The examination was implemented in male albino wistar rats (n = 36). Which was partition 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 HgCl2 regulation modified different biochemical specifications incorporated with the elevated 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 kidney tissue. Betulinic acid and rotundic acid is a natural antioxidant assist to safeguard oxidative injury by diminishing oxidative stress. In contrast, the treatment of betulinic acid and rotundic acid (5 mg/kg B. W) in the kidney tissue reveals a significant reduction in the degree of oxidant level and concurrently an elevated level of antioxidant properties via rehabilitation in kidney tissues. Oxidant substance (LPO), 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 [Hg] is known as the sixth most generous poisonous component existing in the earth's crust as an essential structure that is delivered into the habitat with the usance of both conventional in addition to anthropography origins [1]. Mercury is considered as one of the most unpredictable natural and industrial toxins established in diverse synthetic structures namely elemental, organic and inorganic mercury [2, 3].

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Mercury over any structure is harmful that genesis oxidative stress and exhaustion of the antioxidant network [4]. Toxicity of mercury was a proceeding complication to the earth as its usage is originating from industrial manufacture (fluorescent lamps, thermometers, thermostats, batteries, *etc*.,) and agriculture (pesticides, fungicides, and disinfectants) [5].

The main targeting sites of mercury toxicity are the cerebrospinal nervous system, gastrointestinal track, liver, and kidney [6, 7]. Headache, diarrhea, trembling, coordination impairment, dramatic disorders, stomach cramps, proteinuria, hepatic dysfunction and polyneuropathy also eventuate under the toxicity of mercury [5].

Acute tubular necrosis, acute nephritic disorder, or immunologic glomerulonephritis were triggered through the mercury chloride ($HgCl_2$); hence, it was known as vigorous nephrotoxic factor [7, 8]. Even though some investigations have reported that a significant molecular mechanism for nephrotoxicity is through oxidative stress caused by $HgCl_2$ prompted kidney damage, but not been perceived in elaborate manner [9, 10]. Thus, a raise in oxidative stress is presumably accompanied by the decrease in antioxidant enzyme activity, depletion of cellular cysteine thiols, depletion of ATP constitute, and formation of reactive oxygen species (ROS) [8, 11-14].

The kidneys assume a significant function in keeping up the homeostasis equilibrium by synchronizing the water-solute balance of the body and excrete waste products of metabolism. These tissues additionally provide shelter for mercury and its derivatives; they also aggregated in kidneys through crossing different organs [15]. Well-defined consternation about mercury exposure in the ethnic group is the need for a systematized remedy to tackle intoxication [8]. A chelating treatment of metals is the most efficient method of treatment that improves the discharge and ejection of cationic particles of metals [16]. Natural plant products have active elements that are the derivations of natural antioxidants that can able to safeguard cells, tissues and organs from oxidative pressure and play a remarkable function in detoxification of metals [17].

Betulinic acid (3β-hydroxy-lup-20(29)-en-28-oic acid, BA), a well known natural triterpenoid belongs to pentacyclic lupane- type that manifest a diversity of biotic and therapeutic values namely anti-bacterial, anti-inflammatory, hindrance of human immunodeficiency virus (HIV), anti-malarial, antinociceptive, anti-HSV-1, anthelmintic, and anti-cancer actions [18]. Betulinic acid is broadly scattered universally all over the botany kingdom [19]. The birch tree (Betula spp., Betulaceae) is one of the best commonly announced wellspring of betulinic acid. BA was also segregated across several genesis as follows Syzygium spp. (My- rtaceae), Ziziphus spp. (Rhamnaceae), Paeonia spp. (Paeoniaceae) and Diospyros spp. (Ebenaceae) [18]. Rotundic acid (3b, 19a, 23-trihydroxy-urs-12-en-28-oic acid, RA) is also a pentacyclic triterpene, which is isolated from the dry bark of I. rotunda [20].

RA reveals several biological activities such as anti-inflammatory, in vivo hepatoprotective, anti-diarrhoeal, anti-oxidant, anti-malarial, neuro-protective, anti-microbial, anti-hyperglycemic, and anti-nociceptive [21]. The current investigation was designed to examine the protective potential of betulinic acid and rotundic acid as an oral administration in opposition to nephrotoxicity generated through mercuric chloride in albino rats.

MATERIALS AND METHODS:

Chemicals Utilized: Mercuric chloride (HgCl2) and further more 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 between180–200 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: A total of 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 bodyweight) from our past examination conducted in our laboratory. Furthermore, it has adequate to evoke gentle or mediated oxidative stress in rodents [22].

- **Group I:** Untreated control- just coursed (0.9% NaCl) was given to the animals and watched for 7 days.
- **Group II:** Mercury 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:** Mercury 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:** Mercury 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 kidney tissue was immediately segregated from the animal and placed in super cold saline and afterward utilized for the assessment of oxidant and antioxidant characters and furthermore for histological examination.

Determination of Lipid Peroxidation (TBARS): The LPO/TBARS quantity in the kidney tissue was determined by following the procedure of Nichans and Samuelsen [23]. A notable measure of entire kidney tissue homogenate was set up in 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) upto 15 min and cooled under running tape water. Subsequent to cooling, the mix was taken to study the chromophore absorption at 535 nm contra the reagent blank under 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 stated as n-moles of MDA delivered per 100 mg.

Determination Reduced Glutathione (GSH) Activity: The proportion of reduced glutathione in kidney tissue was estimated through adopting Beutler and Kelley 24 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 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 kidney tissue was analyzed by using the procedure of Kakkar method [25]. The known amount of kidney 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 and 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 min and centrifuged for 5 min at 3000 rpm, and the n-butanol layer was is 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 kidney tissue was assayed with the help of Sinha 26 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 test. 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 kidney tissue was estimated by adopting the procedure of Rotruck [27]. The weighed volume of kidney 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 H_2O_2 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.

Histology and Histopathological Examination: The subjective examination of tissue histo-architexture was examined by selected kidney tissue sample, fixed in 10% buffered formaldehyde upto 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 with the use of 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) [28]. 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: Table 1 evidenced that the impact of the mercuric chloride intoxication drastically increased the volume of lipid peroxidation and concurrently lowered the amount of total reduced glutathione (GSH) proportion in the kidney tissue of the rat when correlated with control animals. The protective potential of betulinic acid and rotundic acid restores LPO and GSH content closer to the normal (control) level in the kidney tissue.

TABLE 1: THE LIPID PEROXIDATION (LPO) AND REDUCED GLUTATHIONE (GSH) CONTENT OF KIDNEY TISSUE ON BETULINIC ACID AND ROTUNDIC ACID AGAINST MERCURIC CHLORIDE INTOXICATED RATS

Groups	LPO	GSH
Control	1.608 ± 0.02^{a}	52.38 ± 2.98^{a}
$HgCl_2$	3.364 ± 0.03^{b}	23.76 ± 3.12^{b}
$HgCl_2$+Betulinic acid	2.568 ± 0.01^{c}	43.45 ± 3.96^{c}
$HgCl_2$+Rotundic acid	2.659 ± 0.04^{c}	41.67 ± 3.68^{d}
Betulinic acid	1.568 ± 0.02^{a}	54.19 ± 4.44^{e}
Rotundic acid	1.575 ± 0.01^{a}	53.66 ± 2.74^{a}

Values are expressed as mean ± SD; values are taken as a mean of six individual experiments values not sharing a common superscript letter or differ significantly (DMRT)

Analysis of Superoxide Dismutase (SOD), catalase (CAT) and Glutathione Peroxidase (GPx) Activities: Table 2 indicated that the antioxidant enzymes (SOD, CAT, GPx) activities were decreased significantly ($p < 0.05$) in mercuric chloride intoxicated rats kidney tissues when correlated with the control animals. In the course of the recovery period, the post-treatment of betulinic acid and rotundic acid enhanced all these antioxidant enzyme activities towards ($p < 0.05$) nearly to the control.

TABLE 2: THE LEVEL OF SUPEROXIDE DISMUTASE (SOD), CATALASE (CAT), GLUTATHIONE PEROXIDASE (GPX) ACTIVITY OF BETULINIC ACID AND ROTUNDIC ACID ON MERCURIC CHLORIDE INTOXICATED RAT KIDNEY TISSUE

Groups	SOD	CAT	GPx
Control	3.954±0.38[a]	37.86±1.67[a]	6.982±0.28[a]
$HgCl_2$	2.112±0.29[b]	25.58±2.01[b]	3.983±0.36[b]
$HgCl_2$+Betulinic acid	3.559±0.36[c]	34.24±1.98[c]	5.677±0.40[c]
$HgCl_2$+Rotundic acid	3.436±0.42[c]	31.45±2.54[c]	5.616±0.38[c]
Betulinic acid	4.245±0.44[a]	39.06±1.85[a]	6.763±0.42[a]
Rotundic acid	4.166±0.46[a]	37.98±1.96[a]	6.745±0.42[a]

Values are expressed as mean ± SD, values are taken as a mean of six individual experiments values not sharing a common superscript letter or differ significantly (DMRT)

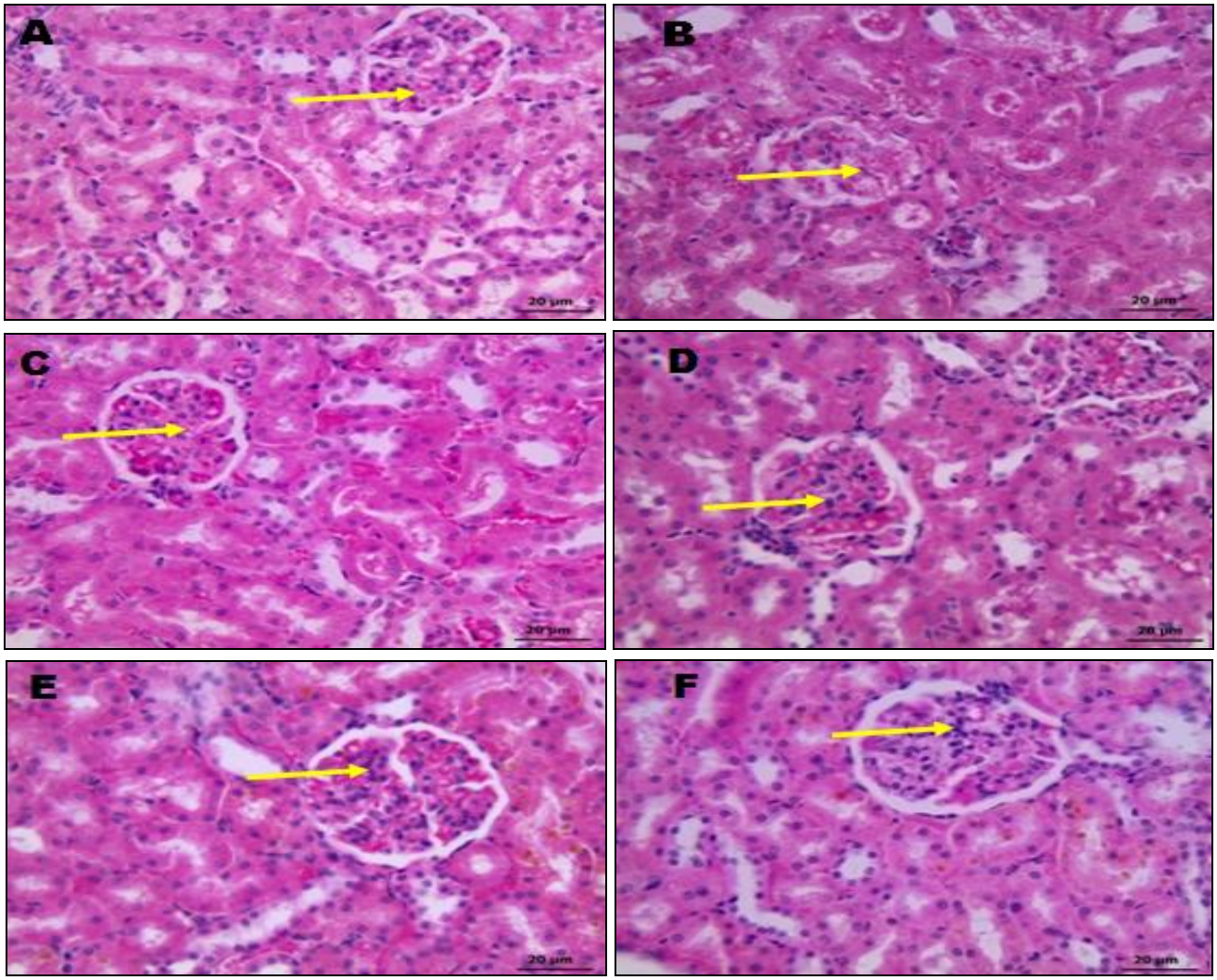

FIG. 1: HISTOLOGY OF EXPERIMENTAL RAT KIDNEY TISSUE BY LIGHT MICROSCOPE WITH H&E STAINING AT 40× MAGNIFICATION. A. CONTROL RAT KIDNEY SECTION SHOWS NORMAL ARRANGEMENT OF HISTOARCHITECTURE OF RENAL TUBULES, DISTAL AND PROXIMAL CONVOLUTED TUBULES. B. MERCURIC CHLORIDE TREATED RAT KIDNEY TISSUE SHOWS DEGENERATED RENAL TUBULES AND GLOMERULUS. C. MERCURIC CHLORIDE FOLLOWED BY BETULINIC ACID-TREATED RAT KIDNEY TISSUE WAS LIKELY TO BE CONTROL. D. MERCURIC CHLORIDE FOLLOWED BY ROTUNDIC ACID-TREATED RAT KIDNEY TISSUE SHOWS REGENERATING RENAL TUBULES AND GLOMERULUS. E AND F, BETULINIC ACID, AND ROTUNDIC ACID-TREATED RAT KIDNEY TISSUE

Histological and Histopathological Study: Kidney of the control group exhibits the normal absolute histroarchitecutre of the renal tubules, proximal and distal convoluted tubules **Fig. 1A**. But in the mercury intoxicated group, the renal tubules of the kidney are drastically ruptured. These renal tubules are damaged, crumbled, and disintegrated in many regions. Vacuolization and

moderate necrosis at some areas are known to be the major histopathological changes. Damaged cell boundaties and small lesions are found in the renal tubules. In some region tubular necrosis are observed and glomerular hyperemia, deterioration of glomerulus accompanied by tubular necrosis are noticeable. Haemorrhage in Bowman's capsule and tubular deterioration are also evident **Fig. 1B**.

At the course of the post-dosage of betulinic acid, the kidney tissue of rat intoxicated mercury exhibits no evidence of degeneration in the renal tubules, and they are regenerated by cells that originated recently. The absence of fragmentation and vacuoles in the kidney tubules are observed. Tubular necrosis and deterioration of glomerulus are absent. Haemorrhage in the Bowman's capsule is not noticeable **Fig. 1C**.

When the $HgCl_2$ intoxicated rat post-treated with rotundic acid reveals partial rehabilitation of renal tubules. Moderate vacuolization and non-extreme necrosis were also observed in certain regions. Newly regenerating cells replace the vacuolated spaces at certain locations. Damaged kidney tubules, partly regenerated cell margins, and hemorrhage in the Bowman's capsule are evident in certain areas **Fig. 1D**.

DISCUSSION: Mercury is considered a highly dangerous metal among the heavy metals that prompt toxicity in the lung, liver, brain, kidney [7, 29], cardiorespiratory system [30], and genital system [31]. Mercury chloride is the main toxic agent of mercury, causes oxidative stress through the development of ROS, prompt to the destabilization and disintegration on the membranes of the cells [32]. Oxidative stress caused through the formation of ROS like peroxides, anion radicals of superoxide, denaturation of protein, lipid peroxidation of the membrane, DNA damage, and cellular lesions [33, 34]. The exposure to mercury causes modifications in the inner membrane of mitochondria that induce the increase of H_2O_2 formation in the electron transport chain of mitochondria, and reduction of GSH levels in the mitochondria have been revealed [35]. Mercury has an ability to intercommunicate with the diverse eminent antioxidant thiol, GSH, that which leads to the development of an excretable GSH and mercury complex. Thus this kind of reaction lowered the volume of GSH, and therefore, the ratio of reduced glutathione gives rise to the existence of oxidative stress [36, 37].

The elevated level of LPO re-moulds the structure of the cell membrane and it is terribly distracting the free radical stimulating enzymes, namely CAT, SOD, and GPx, and it results in cellular injury [38, 39]. At the period of recovery, the treatment of betulinic acid and rotundic acid on the rats intoxicated with mercury incredibly decreased the level of lipid peroxidation in the kidney tissue. Thus the consequences recommended that betulinic acid and rotundic acid acquire antioxidant properties, and they also improve the antioxidant properties in the rats as they were treated [40, 41].

In the current investigation, the volume of reduced glutathione quantity was decreased drastically in the kidney tissue of rats intoxicated with mercury. Non-enzymic antioxidants, such as GSH is the major thiol, that which tries to electrophilic sub-atomic species and forms intermediates for free radical. It performs an important function in the antioxidant defense system, metabolism, and detoxification of endogenous and exogenous materials [42, 43]. Mercury is a transition metal that has a greater affinity towards endogenous thiol molecules as GSH, and the toxicity of mercury ions binds with up to two GSH tripeptides irreversibly [44]. The formation of metal GSH composite action results in the elimination of the toxic metal via the kidney tissues. These actions exhaust the GSH from the cells, and they lowered the antioxidants potentiality [45, 46]. GSH is a primary cellular defending agent against the compounds of Hg. An excessive proportion of Hg ions deposited in the tissues of the kidney disorganizing the metabolism of GSH and causes damage to the cells of the kidney [47, 48]. Therefore, the administration of betulinic acid and rotundic acid therapy followed by mercury chloride elevated the volume of GSH in the kidney tissues. The enrichment of GSH might be because of facilitating GSH biosynthesis, which is accommodated by betulinic acid and rotundic acid correspondingly.

Lipid peroxidation is the process of molecular mechanisms of cell damage in acute poisoning of mercury and is incorporated with the reduction in cellular antioxidants, namely superoxide dismutase (SOD) and catalase (CAT) [48, 49, 50].

The reduction in the action of antioxidant enzymes (SOD, CAT, GPx) in the mercuric chloride intoxicated kidney tissues of rats might be because of the emergence of H2O2 and nitric oxide (NO) [51, 52]. NO and H_2O_2 convoluted with mercury prompted acute renal damage. The comparable type of results in the tissues of rats kidney was also reported by Bharathi and Jagadeesan [8], Joshi *et al.,* [32] and Caglayan *et al.,* [53] in previous experiments at the point when induced with a sub-lethal dose of mercuric chloride. They are revealed that mercury engenders extreme reduction in the non-enzymatic activities of the glutathione in the metabolic pathway GSH in the tissues of the rat.

The enhanced amount of antioxidant diversion by the therapy of betulinic acid and rotundic acid while analogize to the intoxication of mercuric chloride might be accelerated the composite reaction in metabolism of xenobiotics and they might promotes the occurrence of un-indebted nucleophile for inactivating of electrophiles and consequently they plays an important role in metallo conservation [46, 55]. It was scrutinized that betulinic acid and rotundic acid while dosed to rats intoxicated with mercury exhibits significant increase in the amount of GSH, SOD, CAT, and GPx reactions through their antioxidant potentiality and thereby decreases the volume of lipid peroxidation sequentially ameliorates the toxicity of mercury [40, 41, 54].

The free radicals might also perform a prominent role through the deterioration of unsaturated fatty acids and further potential susceptible materials [56]. The present investigation results are also authenticating that the noticed lipid peroxidation alongside histopathological disfigurement with modification in SOD, CAT, and GPx reaction in the tissues of the kidney. These constituents additionally mean that free radicals conjured by mercuric chloride changed the endogenous antioxidant action and effectuated oxidative stress in tissues [57, 58].

Dismutation of superoxide anion radical catalyzed through SOD as hydrogen peroxide. Thus, despite the of underlying mechanism, SOD hindrance might be accorded to the upgraded oxidation notified in rats exposed to mercury [51, 58, 59]. The radical of superoxide was recognized to be vastly malignant to the cellular constituents as a progenitor of the additional reactive oxygen species, promotes to tissue injury and several diseases. In a physiological system, the elimination of toxicity might be through the superoxide dismutase [8]. A potential clarification of our identification could be rendered to the excellent antioxidant potential of betulinic acid and rotundic acid through scavenging a numerous number of free radicals and protecting the cell membrane of lipid from oxidation, reduced lipid peroxidation, and enhancing the quantity of antioxidant enzymes [40, 41, 54].

CONCLUSION: The present study suggests that betulinic acid and rotundic acid has the potentiality to safeguards the kidneys from mercury chloride induced damage. This safeguarding reaction against mercury chloride-induced nephrotoxicity may be described for the anti-oxidant, anti-inflammatory, anti-apoptotic properties of betulinic acid and rotundic acid. As a whole, our results suggest that betulinic acid and rotundic acid might be a promising compounds for the treatment of kidney toxicity induced by mercury chloride.

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