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In-vivo Ameliorative Potential Effect of N-Acetylcysteine and Gallic Acid against Hepatotoxicity Induced by Mercuric Chloride in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Mercury is a highly toxic metal induces oxidative stress in the body and results in a variety of adverse health effects including liver damage. In the present experimental study was to investigate the hepatoprotective effect of some phytochemical on Mercury intoxicated rats. During treatment periods, a sub-lethal dose of mercuric chloride (1.29 mg/kg body weight) treated rat liver tissue shows hepatic injury is mainly associated with distortion of the metabolic function of the liver in rats, it is Hepatic damage can be evaluated by biochemical analysis of the serum tests, includes levels of serum Alanine and Aspartate aminotransferases, alkaline phosphatase, lactic dehydrogenase of

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liver marker enzymes. In the present experimental study, drastically altered in the level of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Lactic dehyrogenase (LDH) and Bilirubin levels were observed in the blood serum of mercuric chloride intoxicated Wistar rats. The activity of liver marker enzymes such as ALT AST, ALP and LDH were significantly increased. In addition, lipid peroxidation (LPO) were significantly increased while the activities of the levels of non enzymatic antioxidants (reduced glutathione (GSH)) and enzymatic antioxidants (glutathione peroxidase (GPx) superoxide dismutase (SOD) and catalase (CAT)) were significantly decreased in liver tissues and the toxicity with mercury is associated with oxidative stress in which mercury induces the formation of free radicals including ROS. It alters the antioxidant capacity of the cells to promote cellular damages. During the recovery period, the hepatic marker enzymes, enzymatic antioxidant and non-enzymatic antioxidant are restored to near normal level in liver tissues. Our experimental studies indicate that treatment for N-Acetylcysteine and Gallic acid exhibited the strong hepatoprotective activity against mercuric chloride induced liver damage in Wistar rat.

Keywords: Mercuric chloride; N-Acetylcysteine; gallic acid; ALT; AST; ALP; LDH; LPO; SOD; CAT; GPx.

1. INTRODUCTION

"Heavy metals are one of the most hazardous environmental pollutants and the toxic to many living organisms. It is the most common hazards found in today's environment are the toxic metals and metalloids. It is the toxic metals shown to affect almost every organ system of the body often due to their persistence and the Mercury is one of the heavy metal responsible for environmental pollution" [1]. "Mercury exposure of its forms in different ways such as water, air, soil, and food poses serious threats to our health and the environment, it is can cause damage to tissues and through diverse mechanisms such as interrupting intracellular calcium homeostasis, disrupting membrane potential, altering protein synthesis, and interrupting excitatory amino acid pathways in the central nervous system. Mitochondrial damage, lipid peroxidation, microtubule destruction and the neurotoxic accumulation of serotonin, aspartate, and glutamate cause hepatootoxicity" [2]. "Mercury has high affinity to lipid allowing movement across cell membranes and can interfere with cell metabolism" [3]. "Mercury (II) is a potential hepatotoxic agent and oxidative stress is its major cause. The toxic effects of mercury involve interactions with a large number of cellular processes, including the formation of complexes with free thiols and protein thiol groups, which may lead to oxidative damage" [4]. "Once absorbed in the cell, $HgCl_2$ forms covalent bonds with glutathione and cysteine residues of proteins glutathione the primary intracellular antioxidant and the conjugating agent, was shown to be depleted and impaired function in mercury toxicity. A single mercury ion can bind to and cause irreversible excretion of upto two glutathione molecules. Released mercury ions form complexes with glutathione and cysteine results in greater activity of free mercury ions disturbing glutathione metabolism and damaging cells" [5]. "Liver is the central organ to carry out the metabolic activities and detoxification in the rats and also carried out a different type of functions which is associated with metabolic activities and detoxification process of toxic substances" [6]. "Mercuric chloride induced oxidative damage is generally attributed to the free radical formation and subsequent lipid peroxidation has been reported to be a cause of cell death in mercuric chloride induced hepatotoxicity" [7].

"N-acetylcysteine (NAC) is an excellent source of sulfhydryl (SH) groups, and it is converted in the body into metabolites capable of stimulating glutathione synthesis, and it is the promoting detoxification, and acting directly as free radical scavengers" [8]. "Administration of NAC has historically been as a mucolytic agent in a variety of respiratory illnesses; however, it appears to also have beneficial effects in conditions characterized by decreased GSH or oxidative stress [9], N-acetylcysteine also appears to have some clinical usefulness as a chelating agent in the treatment of acute heavy metal poisoning [10], both as an agent capable of protecting the liver from damage and as an intervention to enhance elimination of the metals" [11,12].

"Gallic acid (GA) is a polyhydroxyphenolic compounds to play an important antioxidant role as dietary antioxidants for the prevention of oxidative damage in living organisms. It is can act

as an effective antioxidant and the free hydroxyl or carboxyl groups in gallic acid molecule contribute to its oxidation potential effect. GA is a strong natural antioxidant and is pharmacologically active as an anti allergic, antimutagenic, anti-inflammatory and anticarcinogenic agent and decreases the peroxidation of liver tissues, and it is was described as an excellent free radical scavenger" [13].

In the present study, N-acetylcysteine (NAC) and Gallic acid [GA] may have protective effect on the tissue damage that results from oxygen free radicals in Mercuric chloride induced hepatotoxicity in rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Mercuric chloride and N-acetylcysteine (NAC), Gallic acid (GA) and all other necessary reagents of analytical grade were bought from Hi-Media laboratories, Mumbai, India.

2.2 Experimental Animals

"Healthy male albino Wistar rats (180-200 g), were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained in an air-conditioned room (25 ± 3 ºC) with a 12 h light/12 h dark cycle. Feed and water were provided ad libitum to all the animals" [14].

2.3 Experimental Induction of Hepatotoxicity

Hepatotoxicity was induced in animals using an oral dose of $HgCl_2$ (1.29 mg/kg body wt) freshly prepared in physiological saline.

2.4 Experimental Design

"The animals were randomized and divided into six groups, each group containing six rats. The toxic dosage of mercuric chloride has been determined from our previous study as sufficient to elicit mild or moderate oxidative stress for mercuric chloride" [14].

- Group I: only vehicle (0.9% Nacl) was given to these animals (Control).
- Group II: the animals were administered $HgCl_2$ 1.29 mg/kg body weight in 0.9 intraperitoneally for of 21 days.
- Group III: the animals were administered orally N-Acetylcysteine (NAC) (30 mg/kg body weight) alone for 21 days.
- Group IV: the animal were administered N-Acetylcysteine (NAC) after the intoxication of mercuric chloride administration.
- Group V: the animals were administered orally Gallic acid (GA) (30 mg/kg body weight) alone for 21 days.
- Group IV: the animal were administered Gallic acid (GA) after the intoxication of mercuric chloride administration.

2.5 Sample Preparation

"At the end of the experimental duration, rats were fasted overnight and anaesthetized with intramuscular injection of ketamine hydrochloride (24 mg/kg body weight) and sacrificed by cervical dislocation. Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 40 min. Serum was separated by centrifugation at 2000rpm for 10 min. Plasma was separated by collecting blood in tubes containing heparin and centrifuged at 2000rpm for 10 min. The serum samples were used for hepatic marker assay ALT AST, ALP, LDH and bilirubin" [14]. Liver tissues (250 mg) were sliced into pieces and homogenized in appropriate buffer under cold condition (pH 7.0) to give 20% homogenate (w/v). The homogenate was centrifuged at 1000 rpm for 10 min at 0 °C in cold centrifuge. The supernatant was used for biochemical estimations.

2.6 Biochemical Analysis

The activity of AST and ALT was determined by adopting the method of King [15]. The alkaline phosphate was estimated by King and Armstrong method [16]. The activity of lactate dehydrogenase was assayed by the method of King [17]. The level of serum Bilirubin was estimated by the method of Mallay and Evelyn [18]. The level of lipid peroxidase was estimated with the method of Nichans and Samuelson [19]. The glutathione (reduced) was determined according to the method of Beutler and Kelley [20]. The activity of glutathione peroxidase was assayed using the method of Rotruck [21]. SOD activity was assayed according to the method of Kakkar [22]. Catalase activity was determined according to calorimetrically method of Sinha [23].

2.7 Statistical Analysis

"Values are given as mean ± S.D. for six rats in each group. The data for various biochemical parameters were analyzed using analysis of t-test and the group means was compared by Duncan's multiple range test (DMRT)" [24]. Values were considered statistically significant when $P < 0.05$ and the values sharing a common superscript did not differ significantly.

3. RESULTS

Table 1 shows that the level of ALT AST, ALP, LDH and bilirubin was significantly increased in mercury intoxicated rat blood serum compared to control. On administration of N-acetylcysteine and Gallic acid to animals, the level of ALT AST, ALP, LDH and bilirubin was significantly decreased near to normal level compared to mercuric chloride induced rat.

Table 2 shows that the level of LPO was significantly increased in mercury intoxicated rat liver tissues compared to control. On administration of N-acetylcysteine and Gallic acid to animals, the level of LPO was significantly decreased near to normal level compared to mercuric chloride induced rat.

The levels of non-enzymatic antioxidants reduced glutathione activity is a significant decreased in mercuric chloride intoxication rats. Treatment of N-acetylcysteine and Gallic acid orally administrated the rats the reduced glutathione activity was significantly increased near to normal level compared to mercuric chloride intoxicated rat liver tissues, the level of enzymatic antioxidant GPx, SOD and CAT activity is a significant decreased in mercuric chloride intoxication rats. Treatment of N-acetylcysteine and Gallic acid orally administrated the rats the GPx, SOD and CAT activity was significantly increased near to normal level compared to mercuric chloride intoxicated rat liver tissues.

4. DISCUSSION

"Mercury is one of the wide spread environmental pollutant; it is cause severe physiological and biochemical alteration in the tissues of animals and high levels of mercury are associated with hepatotoxicity, nephrotoxicity, and neurological damage in different tissues of animals, it is Toxicity with mercury is associated with oxidative stress in which mercury induces the formation of free radicals including ROS and RNS, and alters the antioxidant capacity of the cells" [25].

Table 1. The effect of hepatic metabolic enzymes of ALT, AST, ALP, LDH and Bilirubin activity on N-Acetylcysteine and Gallic acid with Mercuric chloride induced rat blood serum

Group	ALT	AST	ALP	LDH	Bilirubin
Control	23.82 ± 4.54	52.08 ± 4.69	92.27 ± 1.48	108.24 ± 2.87	0.530 ± 0.04
HgCl2	48.39 ± 3.78	82.41 ± 5.78	197.25 ± 2.63	175.52 ± 2.45	0.993 ± 0.09
HgCl2 + N-Acetylcysteine	28.92 ± 1.58	59.1 ± 4.69	108.15 ± 2.71	113.67 ± 4.30	0.644 ± 0.05
HgCl2 + Gallic acid	30.33 ± 2.20	65.42± 3.79	112.36 ± 1.42	119.38 ±1.92	0.659 ± 0.02
N-Acetylcysteine	19.45 ± 2.64	48.69 ± 2.27	90.78 ± 3.48	109.70 ± 2.42	0.510 ± 0.04
Gallic acid	20.3 ± 1.54	50.21 ± 2.68	91.55 ± 2.57	110.61 ± 1.61	0.522 ± 0.06

Values are expressed as mean±S.D. Values are taken as a mean of six individual experiments values not sharing a common superscript letter of differ significantly (DMRT)

Table 2. The effect of N-Acetylcysteine and Gallic acid on LPO, SOD, CAT, GPx and GSH in the liver tissue of Mercuric chloride-Hepatotoxicity and control rats

Group	LPO	SOD	CAT	GPx	GSH
Control	0.827 ± 0.01	9.331 ± 0.42	80.37 ± 0.55	12.942 ± 0.27	29.75 ± 0.37
HgCl2	1.917 ± 0.10	5.278 ± 0.50	51.18 ± 1.26	7.331 ± 0.38	15.82 ± 0.73
HgCl2 + N-Acetylcysteine	1.145 ± 0.05	8.530 ± 0.39	76.39 ± 0.72	11.817 ± 0.46	25.68 ± 0.48
HgCl2 + Gallic acid	1.441 ± 0.02	7.031 + 0.19	71.53 ± 1.25	11.023 ± 0.29	21.06 ± 1.33
N-Acetylcysteine	0.721 ± 0.03	9.773 ± 0.15	83.51 ± 0.82	13.224 ± 0.10	32.27 ± 0.65
Gallic acid	0.815 ± 0.02	9.525 ± 0.28	82.44 ± 1.31	13.025 ± 0.30	30.85 ± 0.99

Values are expressed as mean±S.D. Values are taken as a mean of six individual experiments values not sharing a common superscript letter of differ significantly (DMRT)

"It is development of oxidative stress causes cell damages and the deleterious effects of mercury on hepatocyte cells were clearly reflected in increased levels of serum enzymes taken as indices for liver functions. Like increased serum of AST, ALT and ALP activities demonstrated the severity of mercuric chloride induced tissue damage of animal. When the cell membrane of hepatocytes is damaged a several of enzymes such as ALT, AST, and ALP are released into the blood stream from the cytosol of animal" [26].

In the present experimental study, sub-lethal dose of mercuric chloride administration on rat serum shows significantly increase in the level of liver bio-markers such as AST, ALT, ALP and LDH activities. An enhanced level of these bio-marker enzyme activities indicates the damage and destruction of liver tissue. In the present study, liver damage induced by $HgCl_2$ was indicated from biochemical and bio-enzymological examinations. Significant increases in the activities of AST, ALT, ALP and LDH were observed in $HgCl_2$-treated rats, normally Alanine transaminase (ALT) activity was higher in the liver tissue of an animal. During the mercury intoxication, mercury toxicity promotes injury to the liver results in release of ALT substance into the blood stream. An enhanced level of ALT activity in the serum of mercury intoxicated rat confirmed the result and also damages occurred in the liver tissue. An increased level of ALT often means that liver damage is present. And as the ALT is an indicator of overall health of an animal. Heavy metal accumulates mainly in liver and kidney tissues in an intoxicated animal. Because, as these organs are play a vital role in the process of detoxification and excretion of foreign materials. Long term exposure of heavy metals leads to intoxication of these organs. "Liver damage mainly induced by $HgCl_2$ generally reflects disturbances of liver cell metabolism, which leads to characteristic changes in the serum enzyme activities" [27]. "The increased levels of AST and ALT may be the liver cell destruction or changes in the membrane permeability. The deleterious effects of mercury on hepatocytes were clearly reflected in elevated levels of serum enzymes taken as indices for liver functions" [28]. "The role LDH is to utilize the glucose molecule for energy production. So, the increased level of LDH in the bloodstream could indicate the cellular damage. LDH isoenzyme plays a key role to involve in the energy metabolism in tissues, facilitating the production of ATP through glycolysis process during the oxygen demand or energy demand" [29]. "During the energy demand leads to stimulated the secretion of LDH as an alternate anaerobic pathway to increase ATP production hence high levels of LDH available in bloodstream of mercury intoxicated rat" [30]. "Another reason for the enhancement of LDH levels may be increased may be due to the formation of cell necrosis caused by mercury treatment" [31]. In the present experimental study also confirms that an enhanced level of serum LDH was occurred in rats when treated with mercuric chloride. Treatment with mercury significantly increased serum ALT, AST, ALP and LDH activities.

During the recovery period ($HgCl_2$ followed by NAC and $HgCl_2$ followed by GA) shows, the activity of AST, ALT, ALP and LDH significantly decreased to reach in near normal level. These observations confirm improved liver functions in mercury intoxicated rat when dosed with NAC and GA and the animals are also showed drastic recovery from the mercury toxicity. NAC and GA is being used against various diseases for a long time for the strengthening of the body's resistance, restoring normal function of the body to consolidate the constitution and promoting blood circulation [32]. The above results suggest that NAC and GA have some protective value against mercury–induced oxidative stress, but these effects are independent of mercury depletion from these organs. N-acetylcysteine also appears to have some clinical usefulness as a chelating agent in the treatment of acute heavy metal poisoning, both as an agent capable of protecting the liver from damage and as an intervention to enhance elimination of the metals, decreased levels of circulatory AST, ALT, ALP and LDH in Gallic acid treated rats may be due to the free radical scavenging property of Gallic acid. GA significantly decreased liver enzymes indicating recovery of liver cells from $HgCl_2$ effect [33]. Treatment with NAC and GA attenuated these increased enzyme activities, indicating that both NAC and GA have hepatoprotective activity against liver injury induced by $HgCl_2$.

Bilirubin is formed from haemoglobin in the reticuloendothelial system such as spleen, bone marrow, kupffer cells in liver and then circulates, it is a product of haemoglobin degradation and its accumulation is a measure of the hepatocyte formation and the rate of erythrocyte degradation [34]. In the present work, sub lethal dose of mercuric chloride treated rats showed in significant increased of serum bilirubin compared

to untreated control rats, the increased of bilirubin in rat mainly suggested that cellular damage occurred in the liver tissue leading to the failure of excretion of bilirubin into the bile [35]. "The increase of bilirubin formation due to activation of HO_{-2} and protects against hydrogen-peroxide-induced hepatotoxicity. It has been also demonstrated that intracellular bilirubin concentrations can be locally and temporarily increased by induction of HO_{-1} or rapid activation of HO_{-2}, so as to resist short- and long-lasting oxidative stress. Bilirubun levels may rise in disease of hepatocytes is necrosis, of excretion is duct obstruction, defects in conjugating enzymes and in hemolysis. There may be a reduction in the number of functioning liver cells as in chronic hepatitis" [36]. "So, that all liver functions are impaired. Bilirubin estimation is reliable sensitive in the diagnosis of hepatic diseases. Elevated levels of serum enzymes are the indication of cellular leakage and loss of functional integrity of the cell membrane in liver" [37]. During the recovery period, N-acetylcysteine and Gallic acid on mercuric chloride intoxicated rats, showed decreased level of bilirubin in the mercuric chloride intoxicated rats. "Decrease in serum bilirubin level after treatment with the NAC and GA indicates the effectiveness of the NAC and GA in normal function status of liver. It is helps in the remove of free radicals, which was produced the hemolysis. This might be the possible reason for reduced bilirubin level. These observations also suggested the protective action of NAC and GA against the damage of the liver" [38].

"Lipid peroxidation refers to the oxidative degradation of lipids. Free radicals are the "steal" electrons of the lipids in cell membranes when the membranes are damaged. This process proceeds by a free radical chain reaction mechanism. These free radicals mainly affect polyunsaturated fatty acids, because they contain multiple double bonds in between reactive hydrogens" [39]. Lipid peroxidation may be defined as the oxidative deterioration of polyunsaturated fatty acids (PUFA) to form free radicals intermediate and peroxides, which damage cellular constituents [40]. In the present experimental study, an increased level of LPO was observed in the liver tissues of Wistar rats, when treated with sub-lethal dose of mercuric chloride. A rise in the reactive oxygen species (ROS) stimulation by the influence of $HgCl_2$ also promotes biochemical changes in the membranes, functional alterations and also causes actuated cell damages in liver. When the physiological condition of a system was normal, there will be a low accumulation of lipid peroxide was established in the tissues [41]. Free radicals derived from oxygen in excessive amounts are responsible to deferent stimuli may inhibits cytotoxicity of the cells. The greater part of the tissue injury is viewed as intervened through those free radicals with assaulting peroxidations of polyunsaturated fatty acids of the cell membranes [42]. These lipid peroxides may cause the cell membrane damage and destroy the cell to disturb the normal function of the cell [43].

"Animal cells have a much more limited production of antioxidants. Oxidative damage can therefore more easily accumulate in animal cells. Antioxidants are substances that delay or inhibit oxidative damage to the target molecule. These include enzymatic and non-enzymatic antioxidants that keep in check ROS/RNS level and repair oxidative cellular damage. A series of enzymes act as free radical-scavenging systems and break down hydrogen peroxide and superoxide anion" [44]. These enzymes are located near the site of generation of these oxidants and include the following reactions carried out [45]. SOD, CAT and GPx are the three primary antioxidant enzymes located in the liver, with different of action in hepatocytes [49]. All these act by one or more of the mechanisms like reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen [46].

"Superoxide dismutase (SOD) can catalyze dismutation of O_2•- into H_2O_2, which it is then deactivated to H_2O by catalase. A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion (O_2•-) and hydrogen peroxide (H_2O_2), which, in turn, generate hydroxyl radicals (•OH), resulting in initiation and propagation of lipid peroxidation" [47]. Superoxide dismutase has been detected in a large number of tissues and organisms, it is present to protect the cell from damage caused by free radicals. Several investigators also indicated that the production of free radicals induced by mercuric chloride intoxication, which could be through inhibition of superoxide dismutase (SOD) enzyme leading to alterations in cells intrinsic antioxidant defense system and resulting in oxidative stress promoted by mercury toxicity in rats [48]. In the present study the level of SOD activity was drastically decreased liver tissues of mercury intoxicated rats were observed. This result indicated that the

decreased level of SOD enzyme in the tissues mainly prevent the formation of excessive oxidative stress [49].

"Reduced glutathione (GSH) is one of the most important endogenous antioxidants; it plays the role of a sulfhydryl group provider for direct scavenging reaction. GSH provides major protection in oxidative injury by involving in the cellular defense system against oxidative damage" [50]. Glutathione is a tripeptide metabolic regulator and a putative indicator of health. It is tripeptide is present in high amount in the liver. GSH scavengers O_2-and protects protein thiol groups from oxidation, ts main role is detoxification of endogenous metabolic peroxides through glutathione peroxidase pathway and also the exogenous substances mercury [51]. "It is GSH conjugation process is desirable in that it result in the excretion of the toxic substances into the bile. As a result of the binding of mercury to glutathione and subsequent elimination of intracellular glutathione, levels of reduced glutathione are lowered in the cell and thus decrease the antioxidant potential of the cell" [52]. In the present investigation it was observed that Hg intoxication significantly depletes the content in the liver and thus reducing the antioxidant potential and accelerating the lipid peroxidation, resulting in hepatocytes damage.

Glutathione peroxidase is the most abundant enzyme and is a very efficient scavenger of hydrogen peroxide (H_2O_2). The detoxifying action of GPx against H_2O_2 protects cell membrane against oxidative damage. In the present study, the GSH level was found to decline and the GPx activity was significantly enhanced, which may be due to the binding of metals with GSH and the process of elimination of free radicals (H_2O_2) promoted by mercury [53]. The treatment of metals like mercuric chloride causes depletion of liver glutathione, among this maximum depletion was observed on mercuric chloride treatment, its produced liver damage.

Catalase (CAT) functions are catalyzing the decomposition of hydrogen peroxide to water and oxygen, it is found to act faster than peroxidase and localized mainly in mitochondria and in sub-cellular respiratory organelles [54]. In the present experimental study, the level of catalase activity was decreased in the mercury intoxicated tissues. During the mercury intoxication the formation of free radicals may induce the inactivation and consumption of CAT activity in the respective tissues [55]. High level of peroxides may cause the inhibition of catalase activity in liver tissue. In this study a marked decreased in liver CAT activities were observed. Mercury leads to increased lipid peroxidation oxidative stress and promote hepatotoxicity in the animal mainly due to reduced its antioxidant system [56].

"During the recovery period ($HgCl_2$ followed by NAC and $HgCl_2$ followed by GA) shows, the level of GPx, SOD CAT and GSH activities were slowly recovered from the decreased level in liver tissues of mercury intoxicated rat when dosed again with NAC and GA respectively. GPx helps in clearing the toxic intermediate hydrogen peroxide which is formed in the cells" [56]. "Both GSH and GPx are worked together in the intoxicated cell to remove the toxicant and free radicals simultaneously" [57]. "Glutathione peroxides alone are not enough to remove the free radicals from the living system with the help of other scavenging enzymes it completes the process" [58]. "Other scavenging enzymes are Superoxide dismutase (SOD) and Catalase (CAT). SOD is a metallic enzyme, which provides primary defense against free radical toxicity by catalyzing the dismutation of O_2. It is a principle chain breaking antioxidant in the living organism, SOD is considered to be a stress protein, which is synthesized in response to oxidative stress promoted by toxicants" [59]. "Superoxide dismutase has been detected in a large number of tissues and organisms, and is thought that it is present to protect the cell from the damage caused by free radicals" [60]. "Catalase is an antioxidant enzyme which destroyes H_2O_2 that can form a highly reactive radical in the presence of iron as catalyst" [61,62]. "CAT is involved in the detoxification of hydrogen peroxide in intoxicated cells. In the present study, it was observed that the administration of NAC and GA on mercury intoxicated liver shows a significant increase in the level of SOD, GPx and CAT activities" [63]. "This result suggests that the NAC and GA can reduce the reactive oxygen free radicals and also improve the anti-oxidant enzymes activities in the mercury intoxicated liver tissue" [64]. "The animals orally treated with NAC and GA significantly enhanced the activities of SOD, CAT and GPx. They have anti-oxidative and free radical scavenger activities with various degrees" [65,66].

During the recovery span, N-Acetylcysteine and Gallic acid when administrated to orally with mercury intoxicated rat serum and tissues shows

a decreased trend. An elevated level of both biochemical and bioenzymological functions were reached to near normal level in the mercury intoxicated rats when administrated with N-Acetylcysteine and Gallic acid respectively. The present experimental study suggests that the rats given N-Acetylcysteine and Gallic acid followed with mercuric chloride showed significant improvement of elevated serum and liver tissues biochemical and bioenzymological parameters and enzymatic and nonenzymatic antioxidant activities are indicative of liver function as compared with mercuric chloride treated group. N-Acetylcysteine and Gallic acid followed by mercuric chloride significantly promoted the health status of the animals and also rectify the tissue damages induced by toxicant.

5. CONCLUSION

In conclusion, the current investigation revealed that, mercuric chloride produced oxidative stress and this was associated with impairment in hepatic functions. The use of naturally occurring antioxidants like N-Acetylcysteine and Gallic acid is potentiated with their synergetic combination in ameliorating the hazardous effects of mercuric chloride. This combination may have therapeutic effects in inflammatory liver diseases. The present studies suggest that N-Acetylcysteine is having more potent than Gallic acid.

ETHICAL APPROVAL

The study protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No: 160/1999/CPCSEA, Proposal Number: 822), Annamalai University, Annamalainagar.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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