



## COMPARATIVE BENEFICIAL EFFECT OF N-ACETYLCYSTEINE AND GALLIC ACID ON ANTIOXIDANT AND ACETYL CHOLINE STATUS OF MERCURY INTOXICATED RATS

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### ABSTRACT

Mercuric Chloride ( $\text{HgCl}_2$ ) is one of the most toxic heavy metal which induces oxidative stress in the body. And also it acts as a neurotoxin. In the present experimental study, the antioxidant and acetyl choline property of mercury intoxicated brain tissue of rat was investigated. And withdrawal effect of mercury toxicity in the brain tissue with the help of N-acetylcysteine (NAC) and Gallic acid (GA) treatments were also investigated. NAC and GA, at a concentration of 30mg/kg body weight, accelerate the oxidation of neurotoxicity induced by  $\text{HgCl}_2$  (1.29mg/kg Body weight of the animal). The aim of this study was to investigate the protective potential of N-acetylcysteine and Gallic acid against  $\text{HgCl}_2$  induced brain damage. The antioxidant activities of NAC and GA concentration were mainly due to the scavenging of lipid peroxide in this system. The oxidant mechanism for NAC and GA is most likely due to the strong reducing power and weak metal Chelating ability. During the  $\text{HgCl}_2$  – treatment, the mercury toxicants mainly induced the toxicity in the brain tissues of rats to exhibit elevated level of lipid peroxidation (LPO). At the same time the level of enzymic antioxidants (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)), AchE activity and non-enzymic antioxidants (reduced glutathione (GSH)) were significantly decreased in brain tissues. But during the recovery period (N-Acetylcysteine (NAC) and Gallic acid (GA) treatment on mercury intoxicated animal), both compounds are enhances the not only the GSH levels, and also promote the AChE activities. It leads to protects cell damage against neurotoxicity induced by mercuric chloride. Histological and histopathological observations are also supporting these findings by the way of restoration of brain histoarchitecture. Oral administration of NAC and GA for 21 days improved the status of above parameters towards normal level or near normal level. Thus, results of the present study NAC and GA exhibited potent antioxidant, neuroprotective activities on  $\text{HgCl}_2$  induced neurotoxicity in rats.

**Key words:** Mercuric chloride, Neurotoxicity, N-Acetylcysteine, Gallic acid, Antioxidant.

### INTRODUCTION

Mercury is one of the wide spread environmental and industrial pollutant, which cause severe physiological and bioenzymological alteration in the tissues of both animal and men (Lund *et al.*, 1993). It is known that mercury promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxide. In organic

mercury present in the environment is a well-established toxicant to human health (Clarkson *et al.*, 1988).  $\text{HgCl}_2$  causes oxidative damage (Ricardo Brandao *et al.*, 2011) in normal cells. Normally  $\text{HgCl}_2$  has been widely used to study the hemodynamics changes, functional alteration and tissue damages in animals (Flmenbaum *et al.*, 1974).  $\text{HgCl}_2$ -induced oxidative damage is generally attributed to the formation of highly reactive hydroxyl radical (OH), the stimulator of lipid peroxidation and the sources of destruction of damage to the cell membrane (Bharathi and Jagadeesan, 2012; Kavitha and Jagadeesan, 2006).  $\text{HgCl}_2$  highly affected the cells of brain tissue in animals to

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promote the neurotoxicity (Jagadeesan and Shankersami P, 2007).  $\text{HgCl}_2$  is one of the most toxic forms of mercury because it easily forms organomercury complexes with proteins (Wargovich *et al.*, 2001), leading to functional and structural alterations in many organs, such as central nervous system (Mahboob *et al.*, 2001). The most serious effect of heavy metal poisoning is damage the central nervous system.

N-Acetylcysteine (NAC, N-Acetyl-L-Cysteine) is a thiol compound, a mucolytic agent and a precursor of L-cysteine and reduced glutathione. NAC is a source of sulfhydryl containing antioxidant that has been used to mitigate various conditions of oxidative stress. Its antioxidant action is believed to originate from its ability to stimulate GSH synthesizing level and scavenging reactive oxygen species (ROS) in animals (Ottenwalder *et al.*, 1987; Gurer *et al.*, 1998). NAC is also known to have metal chelating properties to scavenging the unwanted metal from the animal body. NAC is used as a mucolytic drug, because of its antimutagenic and anticarcinogenic qualities making it a potential chemopreventive agent (DeVries and DeFlora, 1993). Number of experimental research work proves that NAC potentiates the toxicity induced by heavy metals in animals. But none of them proves that NAC has been used to reduce the neurotoxicity in mercury intoxicated rat.

Gallic acid (GA, 3,4,5-trihydroxybenzoic acid), a naturally occurring plant product, which is available in various plants. And it also found to be a strong natural antioxidant to scavenging the reactive oxygen species (Madsen & Bertelsen, 1995). Recently, GA was described as having a cytotoxic effect on isolated hepatocytes (Nakagawa & Tayama, 1995). GA is a strong natural antioxidant and is pharmacologically active as an anti-allergic, antimutagenic, anti-inflammatory and anticarcinogenic agent. Its inhibits melanogenesis which may be related to GA is antioxidant activity in scavenging reactive oxygen species (Inoue, 1995).

The present study was planned to evaluate the therapeutic potential of two differently acting antioxidant, NAC and GA treatments on  $\text{HgCl}_2$  intoxicated brain tissue of rat. We assessed the markers of oxidative stress and oxidant injury in brain tissue, and also we examined the potential beneficial effect of N-acetylcysteine and Gallic acid on mercury intoxicated brain tissue of rats through bio-enzymological and histological observations.

## MATERIALS AND METHODS

### Chemicals

Mercuric chloride ( $\text{HgCl}_2$ ), N-Acetylcysteine, Gallic acid and all other necessary reagents of analytical grade were bought from Hi-Media laboratories Ltd, Mumbai, India.

### Animals

Healthy adult male albino Wistar rats (180-200 g), were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained in an air-conditioned room ( $25 \pm 3^\circ\text{C}$ ) with a 12 h light/12 h dark cycle. The animals were allowed free access to water and standard pellet diet (Amrut Laboratory Animal feed Pranav agro-Industries Ltd. Bangalore, India). All experimental studies were conducted in the Department of Zoology, Faculty of Science, Annamalai University, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 1985). The experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital (Reg No.160/1999/CPCSEA, Pro. No. 822), Annamalai nagar.

### Experimental design

The animals were randomly divided in to six groups of six rats each. Physiological saline (0.9%) was used as vehicle solution to the control as well as for the administration of  $\text{HgCl}_2$ , NAC and GA and fed by gastric intubation.

- Group I** : Control rats
- Group II** :  $\text{HgCl}_2$  control (1.29 mg/kg BW)
- Group III** :  $\text{HgCl}_2$  + NAC treatment
- Group IV** :  $\text{HgCl}_2$  + GA treatment
- Group V** : Control + NAC (30 mg/kg BW)
- Group VI** : Control + GA (30 mg/kg BW)

Total weight of the diet was kept constant throughout the experimental period. The total experimental duration was 21 days. On 22<sup>nd</sup> day the rats were sacrificed by cervical dislocation. The whole brain tissue was isolated immediately from the animals in the cold room and then used for estimation of lipid peroxidation (LPO) by adopting the method of Nichals and Samuelson (1968), reduced glutathione (GSH) by the method of Beutler and Kelley (1963), glutathione peroxidase (GPx) by the method of Rotruck *et al.*, (1973), catalase (CAT) by the method of Sinha (1972), superoxide dismutase (SOD) by the method of Kakkar *et al.*, (1984). And it was also used for histological and histopathological observations by adopting the method of Gurr (1959)

### Statistical analysis

Statistical analysis was performed by one way analysis of variance (ANOVA) and the groups were compared by Duncan's Multiple Range Test (DMRT) using SPSS Software Package, version 17.0. Results were expressed as means  $\pm$  standard deviation for six rats in each group. A value of  $P \leq 0.05$  was considered to be statistically significant.

## RESULTS

### Histological and histopathological observations in the rat brain tissue

In the present experimental study, the untreated control brain tissue shows the normal histoarchitecture of neuroglial cells, pia matter, blood space, pyramidal cell and granular cells. At sublethal dose of mercuric chloride treatment, the brain tissue shows the damaged neuroglial cells in some area. In most of the area, the irregular size and shape of the neuroglial cells was noticed. Vacuoles and damaged blood vessels and granular cells also noticed. During the recovery period, NAC administrated on mercury intoxicated brain tissue shows remarkable recovery of its histoarchitecture is mainly due to the withdrawal effect of mercury from the brain tissue. GA administrated on mercury intoxicated brain tissue also shows the restoration of histoarchitecture of the brain tissue is not only due to the elimination of mercury toxicity from the brain tissue and also promote the regeneration of brain cells.

### Level of LPO contents in the rat brains tissue

Table 1 shows the levels of LPO content in the brain tissue of rat when treated with HgCl<sub>2</sub>-and followed by N-Acetylcysteine and Gallic acid, N-Acetylcysteine alone treated, Gallic acid treated alone rats. Our results indicate that the administration of NAC and GA on mercury intoxicated animals were promote the healthy condition. During the mercuric chloride treatment, the level of LPO content was significantly elevated. During the recovery period, administration of NAC and GA on mercury intoxicated brain tissue shows decreased level of LPO content. Treatment with NAC and GA significantly decreased the levels of LPO.

### Level of non-enzymatic antioxidants in the brain tissue of rats

Table 1 shows the levels of reduced glutathione (GSH) content in the brain tissue of rat when treated with

HgCl<sub>2</sub>-and followed by N-Acetylcysteine and Gallic acid, N-Acetylcysteine alone treated, Gallic acid treated alone rats. At sub-lethal dose of mercuric chloride treatment, the level of GSH content in the brain tissue was significantly decreased. During the recovery period, administration of NAC and GA on mercury intoxicated brain tissue shows that the decreased level of GSH content was increased. This result suggests that the animal get recovery from the toxicity effect of mercury with the help of NAC and GA administration. Treatment with NAC and GA alone on control rat also shows significantly elevated levels of GSH content in the brain tissue.

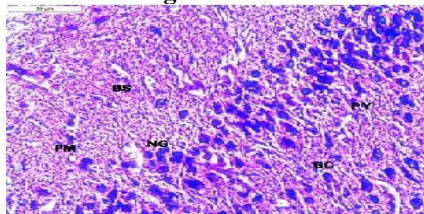
### Level of enzymatic antioxidants in the brain tissue of rats

Table 1 and 2 show the levels of enzymatic antioxidants, the activities of SOD, CAT and GPx in the tissues are given in respectively. At sub-lethal dose of mercuric chloride treated brain tissue shows the lowered the levels of SOD, CAT and GPx activities. During the recovery period, administration of NAC and GA on mercury intoxicated brain tissue shows that the decreased level of SOD, CAT and GPx activities. This result also suggests that the animal get recovery from the toxicity effect of mercury with the help of NAC and GA administration. Treatment with NAC and GA alone on control rat also shows significantly elevated levels of SOD, CAT and GPx activities in the brain tissue.

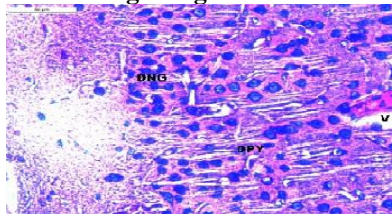
### Level of AChE activity in the brain tissue of rats

Table 3 shows the levels of AChE activities in the brain tissue of control and treated rats. In the present experiments, the level of AChE activity was significantly decreased in the brain tissue of rat when treated with HgCl<sub>2</sub>. During the recovery period, the administration of NAC and GA on HgCl<sub>2</sub> intoxicated rats brain tissue shows the remarkable increase in the levels of AChE activity.

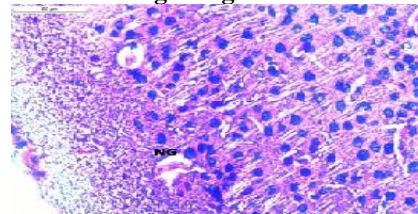
**Fig 1. Control**



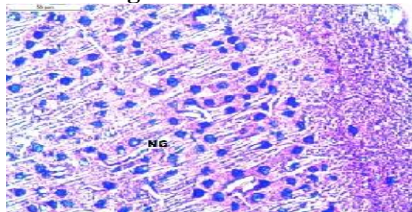
**Fig 2. Hg Treated**



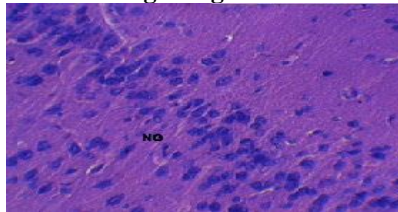
**Fig 3. Hg+NAC**



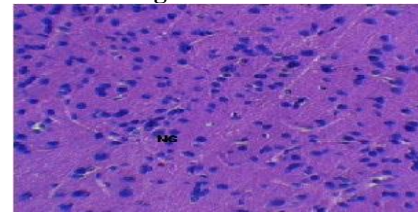
**Fig 4. NAC alone**



**Fig 5. Hg + GA**



**Fig 6. GA alone**



**Figure 1.** The brain tissue of untreated control rat showing the normal histoarchitecture of neuroglial cells (Ng), Pia matter (PM), blood space (BS), pyramidal cell (PY) and granular cells (GC).

**Figure 2.** At sub-lethal dose of mercury chloride treatment, the brain tissue shows irregular shape and size of neuroglial cells. And it also arranged in irregular manner. Numbers of vacuoles are also noticed in most of the area. The damaged blood vessels are also noticed.

**Figure 3.** Influence of NAC on mercury intoxicated brain tissue shows a remarkable recovery of neuroglial cells by the way of restoring its size and shape of the neuroglial cells, pyramidal cells and granular cells. Restoration blood space and reduced number of vacuoles are also noticed.

**Figure 4.** NAC alone treated brain tissue shows the complete normal histoarchitecture.

**Figure 5.** GA administrated on mercury intoxicated brain tissue shows a moderated recovery of its histoarchitecture.

**Figure 6.** GA alone brain tissue also shows the complete normal histoarchitecture.

**Table 1. Effect of NAC and GA on LPO, GSH and GPx in the brain tissue of HgCl<sub>2</sub>-Neurotoxicity and control rats**

Groups	LPO	GSH	GPx
Control	0.743 ± 0.02	23.28 ± 0.50	9.268 ± 0.66
HgCl <sub>2</sub> + control	1.537 ± 0.14	13.24 ± 0.89	5.345 ± 0.41
HgCl <sub>2</sub> + NAC	1.007 ± 0.17	20.74 ± 0.46	8.871 ± 0.29
HgCl <sub>2</sub> + GA	1.267 ± 0.06	19.46 ± 1.84	7.729 ± 0.39
Control + NAC	0.632 ± 0.02	26.75 ± 0.89	9.401 ± 0.50
Control + GA	0.724 ± 0.02	24.44 ± 0.64	9.285 ± 0.51

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at  $p < 0.05$ . (DMRT).

**Table 2. Effect of NAC and GA on SOD and CAT in the brain tissue of HgCl<sub>2</sub>-Neurotoxicity and control rats**

Groups	SOD	CAT
Control	6.180 ± 0.48	52.64 ± 0.82
HgCl <sub>2</sub> + control	3.882 ± 0.34	33.78 ± 1.28
HgCl <sub>2</sub> + NAC	5.380 ± 0.27	49.57 ± 1.41
HgCl <sub>2</sub> + GA	4.342 ± 0.37	45.18 ± 1.32
Control + NAC	6.606 ± 0.38	55.68 ± 1.10
Control + GA	6.333 ± 0.43	53.94 ± 0.86

Values are given as means ± SD for six rats in each group.

Values not sharing a common superscript differ significantly at  $p < 0.05$ . (DMRT).

**Table 3. Effect of NAC and GA on acetyl cholinesterase (AChE) in the brain tissue of HgCl<sub>2</sub>- Neurotoxicity and control rats**

Groups	AChE
Control	11.12 ± 0.21
HgCl <sub>2</sub> + control	6.13 ± 0.44
HgCl <sub>2</sub> + NAC	10.49 ± 0.48
HgCl <sub>2</sub> + GA	9.92 ± 0.43
Control + NAC	12.19 ± 0.41
Control + GA	11.96 ± 0.53

Values are given as means ± SD for six rats in each group

Values not sharing a common superscript differ significantly at  $p < 0.05$ . (DMRT).

## DISCUSSION

Mercury is one of the most potent thiol binding agents. It acts on reduced glutathione to decrease in its level. The most serious effects of mercury and its compounds poisoning are damage to the central nervous system. And they are known to accumulate in

various parts of brain and central nervous system in animals (Mary Chandravathy and Rajendrakum, 2000). The central and peripheral nerves were damaged directly by mercury and its compounds and the damaged function of motor nerves was imputed to osteoproliferation of vertebrae (Margarat *et al.*, 2001). The neurotoxic effect of

mercuric chloride on the brain may be exhibited by metabolic perturbations at the subcellular level. The brain is the target organ of mercuric chloride. Increased dose of inorganic mercury causes severe neurological damages in animal (Rao *et al.*, 1997).

In the present experimental study, the mercury treated brain histopathology shows the reduced number of neuroglial cells and pyramidal cells and these are arranged in irregular pattern and changed its size and shape. The damaged blood vessels also noticed in some area is mainly due to the mercury toxicity. These result suggests that the loss of active movement could be possibly due to the loss of co-coordinating movements caused by the destruction of neurological cells were observed in mercury intoxication in brain tissue. Long-term exposure of mercury and its compounds mainly promote the paralysis and death (Klassen *et al.*, 1980). Low level of mercury treatments promote the oxidant process in the cells and also decrease the antioxidant properties. In the present experimental work also support events by the way of enhanced level of LPO content and simultaneously decreased level of antioxidant profiles in the mercury intoxicated brain tissue. Treatment with NAC and GA on mercury intoxicated brain tissue shows an increased antioxidant activities (enzymatic and non-enzymatic system), indicating that NAC and GA *in vivo* have neuro-protective activity against brain injury induced by HgCl<sub>2</sub> (Flora *et al.*, 1999; Gali and Perchellet, 1991).

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 (Lund *et al.*, 1993). Free radical production and lipid peroxidation complex and natural deleterious process all are consider to dose related to toxicity of xenobiotics. (Harmann, 1981). Lipid peroxidation (LPO) is a chain reaction which is initiated molecular rearrangement and easily reacts with oxygen molecule to give a per-oxy radical (R-OO). Per-oxy radical could attach a hydrogen atom from another lipid molecule and continue the chain reaction finally yield the lipid hydro peroxide (R-OOH) and combine with the hydrogen atom that it abstracts to give lipid peroxide. (Halliwell and Gutteridge *et al.*, 1985). Lipid peroxidation is highly destructive process and induces a plethora of alteration in the structure and function of cellular membrane, which could lead to cell injury. In the present experimental study, an increased level of LPO was observed in the brain tissues of Wistar rats, when treated with sub-lethal dose of mercuric chloride. The significant increase in lipid peroxidation level in mercuric chloride intoxicated rat could lead to the damage of plasma membrane of the respective tissue which is causing oxidative stress induced by mercuric chloride (Bharathi and Jagadeesan, 2012; Rao and Gayatri, 2000).

Particularly an enormous amount of LPO production was noticed mercury intoxicated brain tissue because it possesses rich in lipids and biomembrane. The increase in the levels of LPO indicates enhanced lipid peroxidation leading to brain tissue injury and failure of the antioxidant defense mechanism to prevent the formation of excess free radicals.

During the recovery span, the level of LPO content in the brain tissue was found to be near normal level in NAC and GA treated on mercuric chloride intoxicated rats. Administration of NAC and GA treatment normally decreases the production of LPO in brain tissue of mercury intoxicated rat, which in turn might protect the system against the toxic manifestation of OH radical and H<sub>2</sub>O<sub>2</sub>. However, the increase was still significant when compared to the control. Girardi *et al.*, (1991) have also suggested that an enhanced level of LPO content was significantly decreased in mercury intoxicated brain mainly due to administration of NAC and it may exert a stabilizing action on brain cell membrane. (Gow-Chin Y *et al.*, 2002).

The reduced glutathione function as free radical scavenger and in the repair of radical caused biological damage (Valko *et al.*, 2007). The main function of glutathione is detoxication of endogenous metabolic peroxides through glutathione peroxidase (GPx) pathway and endogenous substances such as heavy metal like mercury. It binds to exogenous substances and their metabolites, which are ultimately, excreted resulting in decreased concentration of this tripeptide. It is a primary water-soluble antioxidant in the cell. It has a profound role in metal detoxification. Glutathione helps in removing toxic peroxides by a reaction catalyzed by glutathione peroxidase (Bharathi and Jagadeesan, 2012; Bose *et al.*, 1994). A significant recovery was evident following the administration of NAC and GA in the mercuric chloride intoxicated brain tissue. This recovery in GSH content may be due to free sulfhydryl group that NAC contains or may be due to its role as a precursor for cysteine which is used in GSH biosynthesis. Further, the restoration of GSH by NAC and GA protect the system against HgCl<sub>2</sub> induced oxidative stress by the way of promoting the histoarchitecture of brain tissue, but when compare to GA administration NAC treatment was shows fast restoration of GSH content and concomitant decrease in LPO content in mercury intoxicated brain tissue (Ricardo B *et al.*, 2011; Madlener and Illmer, 2007).

Glutathione peroxidase (GP<sub>x</sub>) is an antioxidant enzyme when it acts on production of oxidative stress which is promoted by toxicants in animals and it require glutathione as co-factor. Glutathione is present in both cytosol and in mitochondrial matrix in most of the cells. In the present experimental work, treatment of NAC and GA on mercury intoxicated brain tissue get an improved the level of GPx activity significantly to near normal level

(Banner, 1986; Gali, 1992). This result suggested that the intoxicated brain tissue slowly get recovery from the mercury toxicity with the help of NAC and GA.

In the present experimental study, an increased level of lipid peroxidation content and simultaneously decreased level of GPx, SOD and CAT activities were noticed in the mercury intoxicated brain tissue. During the recovery period, the level of GPx, SOD and CAT activities were slowly recovered from the decreased level in brain tissues of mercury intoxicated rat when dosed with NAC and GA. GPx helps in clearing the toxic intermediate hydrogen peroxide which is formed in the cells. Both GSH and GPx are worked together in the intoxicated cell to remove the toxicant and free radicals simultaneously. 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 (Das *et al.*, 1988). Other scavenging enzymes are Superoxide dismutase (SOD) and Catalase (CAT). SOD is a metallic enzymes (Salo *et al.*, 1988), which provides primary defense against free radical toxicity by catalyzing the dismutation of O<sub>2</sub> (Fridovich *et al.*, 1976). 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. 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. Catalase is an antioxidant enzyme which destroys H<sub>2</sub>O<sub>2</sub> that can form a highly reactive radical in the presence of iron as catalyst (Chatterjee and Bhattacharya shelly, 1984). CAT is involved in the detoxification of hydrogen peroxide in intoxicated cells (Baker *et al.*, 1980). In the present study, it was observed that the administration of NAC and GA on mercury intoxicated brain shows a significant increase in the level of SOD, GPx and CAT activities. 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 brain tissue (Nakatani, 1992; Seo SY, Sharma, 2003; Brando, 2006).

Acetylcholinesterase is an important regulatory enzyme that controls the transmission of nerve impulses across cholinergic synapses by hydrolyzing the excitatory transmitter acetylcholine (Mitrovic and Dettbarn, 1996). Mercury and its compounds are mainly affects the central nervous system in all animals. Due to the malfunctioning

of the central nervous system the animals were collapsed and died. The death of the animal may be due to the inhibition of AChE enzyme system by disrupting the nervous activity through the accumulation of acetylcholine at nerve endings in the central and peripheral nervous system during the toxicants exposure (Sankarsamipillai and Jagadeesan, 2006). The inhibitory effect on AChE activity indicates that toxicants might interfere in vital processes like energy metabolism of nerve cell (Nath and Kumar, 1999). AChE inhibitor not only induced the brain cell injury (Yang and Dettbarn, 1996) and also inhibit the AChE synthesis and also disrupts the smooth transmission of the nerve impulses across the synapses causing neurosis, tremor and depression in the respiratory centers (Murthy, 1980). The present investigation also confirms the brain damage which is caused by mercury through the observations of brain histoarchitecture. During the recovery period, the activity of AChE significantly increased to reach in near normal level. This result indicated that this might be due to physiological function of N-acetylcysteine and Gallic acid respectively. And it also suggested that the increased level of AChE is mainly due to the decrease in the mercury toxicity. In the present study, administration of N-acetylcysteine and Gallic acid on mercury intoxicated brain tissue plays a vital role to detoxify the mercury toxicity. It also promotes the membrane stabilization and proliferation in the brain tissue. The present studies suggest that the N-acetylcysteine has more efficacy than Gallic acid.

## CONCLUSION

In conclusion, mercuric chloride proved to be a neurotoxic effect and caused neuroglial cell damages and administration of NAC and GA can reduces the resulting damage probably due to its ability to neutralize or scavenge the free radicals that are generated by mercuric chloride. This study elucidated the protective role of NAC and GA against mercuric chloride toxicity, but the present result suggests that administration of NAC is comparatively better than GA administration.

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## REFERENCES

- Aroma OI, Halliwell B, Holly B and Butler J. The antioxidant action of N- acetylcysteine in reaction with hydrogen peroxide, hydroxy radical, superoxide and hypochlorous acid. *Free Rad. Biol. Med.*, 6, 1989, 593-597.
- Baker H, Frank O, DeAngelis B, Feingold S. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutr Res*, 21, 1980, 531-536.
- Banner W, Koch M, Capin DM, Hoft SB, Chang S and Tong TG. Experimental chelation therapy in chromium, lead and boron intoxication with N-acetylcysteine and other compounds. *Toxicol. Appl. Pharmacol.* 83, 1986, 142-147.

- Beutler E and Kelley BM. The effect of disodium nitrate on RBC glutathione. *Experientia*, 29, 1994, 96-97.
- Bharathi E, Jagadeesan G and Manivasagam T. Influence of s-allyl cysteine against mercuric chloride induced nephrotoxicity in albino rats. *J.Chem.Pharma. Res*, 4(3), 2012, 1470-1474
- Bose, Mukhopadhyaya SBS, Choudhry and Battacharya S. Correlation and metal distribution, reduced glutathione and metallo thionin levels in liver and kidney of rat. *Ind J. Exp. Biol.*, 32, 1994, 679-681.
- Brandao R, Santos FW, Zeni R, Nogueira CW. DMPS and N-acetylcysteine induced renal toxicity in mice exposed to mercury. *Biomaterials*, 19, 2006, 389-398.
- Chatterjee S and Bhattacharya shelly. *Toxicol. Lett.*, 22, 1984, 187.
- Clarkson TWL, Friberg JBH and Nylander M. The prediction of intake of mercury vapor from amalgams in T.W. clarkson, I. Friberg, Nordberg and PR Sager eds *Biological monitorin of toxic metals*, Plenum press New york, 1988, 247-264.
- Das M, Ghosh DC, Addya S and Chatterjee GC. Effect of actue oral administration of cadmium chloride on uptake of element and control lipoperoxidation process in hepatic and renal nuclear fractions of rats. *Indian J.Exp.Bio.*, 1988, 449-452.
- De Vries N, DeFlora S. N-acetylcysteine. *J. Cell. Biochem. (suppl.)*, 17 F, 1993, 270 – 277.
- Duncan BD. Duncan's multiple range tests for correlated and heteroscedastic mean. *Biometrics*, 13, 1957, 359-364.
- Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys*, 82, 1959, 70-77.
- Flmenbaum W, Huddleston ML, Neil JS and Hamburger RJ. Uranyl nitrate acute renal failure in the rat. Micro punture and renal hemodynamics studies. *Kidney Int*, 6, 1974, 408-418.
- Flora SJS. Arsenic induced oxidative stress and its reversibility following combined administration of N-acetylcysteine and meso 2,3-dimercaptosuccinic acid in rats. *Clin. Exp. Pharmacol. Physiol.*, 26, 1999, 865-869.
- Fridovich I. Superoxide dismutase. *Ann Res Biochem*, 44, 1976, 147-159.
- Gali HU, Perchellet EM, Perchellet JP. Inhibition of tumor promoter-induced ornithine decarboxylase activity by tannic acid and other polyphenols in mouse epidermis in vivo. *Cancer Res.*, 51, 1991, 2820-2825.
- Gali HU, Perchellet EM, Klish DS, Johnson JM, perchellet JP. Hydrolyzable tannins: potent inhibitors of hydroperoxide production and tumor promotion in mouse skin treated with 12-Otetradecanoylphorbol-13-acetate in vivo. *Int J Cancer*, 51, 1992, 425-432.
- Girardi G, Elias MM. Effectiveness of n-acetylcysteine in protcting against mercuric chloride-induced nephrotoxicity. *Toxicology*, 67, 1991, 155-164.
- Gow-Chin Y, Pin-Der D. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. *Food Chemistry*, 79, 2002, 307-313.
- Gurer H, Ozgunes H, Neal R, Spitz DR and Ercal N. Antioxidant effects of N- acetyl cysteine and succimer in red blood cell from lead exposed rats. *Toxicology*, 128, 1998, 181-189.
- Halliwell B and Gutteridge JMC. *Free radicals in biology and medicine*. Oxford University Press, New York, 1985, 139-145.
- Harmann D. Free radicals. *Proc. Natl. Acad. Sci., USA.*, 78, 1981, 7124.
- Inoue M, Suzuk R, Sakaguchi N, Li Z, Takeda T, Ogihara Y, Jiang BY, Chen Y, relative induction of cell death in cancer cells by gallic acid. *Boil pharm Bull*, 18, 1995, 1526 – 1530.
- Jagadeesan G and Sankarsami PG. Hepatoprotective effects of taurine against mercury induced toxicity in rats. *J. Env. Biol.*, 28(4), 2007, 753-756.
- Kakkar P, Das B, and Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys*, 21, 1984, 130-132
- Kavitha AV and Jagadeesan G. In vivo studies on the role of tribulus terrestris (Linn.) against mercuric chloride induced nephrotoxicity in mice, *Mus musculus* (Linn). *Journal of Environmental Biology*, 27(2), 2006, 397-400.
- Kroes BH, Van den Berg AJ, Qvarles van Ufford HC, Van Dijk H, Labadie RP. Anti – inflammatory activity of gallic acid. *Planta med*, 58, 1992, 499 -504.
- Lowry OH, Rosenbrough NJ, Farr A and Randell RJ. Profein measurement with folin phenal reagent. *J.Bio.Chem.*, 193, 1951, 265-273.
- Lund BO, Miller DM and Woods JS. Studies on Hg(11) induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. *Biochem. Pharmacol*, 45, 1993, 2017-2024.
- Madlener S, Illmer C and Horvath Z Gallic acid inhibits ribonucleotide reductase and cyclooxygenases in human HL-60 promyelocytic leukemia cells. *Cancer let*, 245, 2007, 156-162.
- Madsen HL and Bertelsen G. Spices as antioxidants. *Trends in food Science and Technology*, 6, 1995, 271-277.
- Mahboob M, Shireen KF, Atkinson A, Khan AT. Lipid peroxidation and antioxidant enzyme activity in different oranges of mice exposed to low level of mercury. *J. Environ. Sci. Health B*, 36, 2001, 687-697.
- Margarat,A., G.Jagadeesan and S.Sethupathy:Comparative effect of Penicillamine and taurine on mercury Poisoned mice, *Mus musculus*. *Pollut. Res.*, 20, 2001, 1-4.

- Mary CV, Rajendrakumar G and Pratap Reddy K. Lead induced oxidative stress and antioxidative enzymes responses in lungs and brain of mice. *Ind J. Environ. Toxicol.*, 10(2), 2002, 55-57.
- Mitratovic D and Dettbarn. Modification of acetyl cholinesterase during adaptation to chronic, subacute paraoxon application in rat. *Toxicol. Appl. Pharmacol.*, 136, 1996, 20-28.
- Murthy SD. Pesticide In: Cassarett and Doull's Toxicology. The basic science of poisons. (Eds) Douell CD, Klassen and Arnadur MO. Macmillan publication. New York, 1980, 357-405.
- Nakagawa Y and Tayama S. Cytotoxicity of propyl gallate and related compounds in rat hepatocytes. *Archives of Toxicology*, 69, 1995, 200-208.
- Nakatani, N. Natural antioxidants from spices. American Chemical Society, Washington, DC: ACS Symposium Series 507, 1992.
- Nichans WG and Samuelson B. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur J Biochem*, 6, 1968, 126- 130.
- Okiawa S, Yamada K, Yamashita N, Tada-Oikawa S, Kawanishi S. N-acetylcysteine, a cancer chemopreventive agent, causes oxidative damage to cellular and isolated DNA. *Carcinogenesis*, 20, 1999, 1485-1490.
- Ottenwalder H, Simon P. Differential effect of N-acetylcysteine on of the metals Hg, Cd, Pd and Au. *Arch. Toxicol.*, 60, 1987, 401-402.
- Rao, M.V, Gayatri R. and Patil. Mitigative role of ascorbic acid and glutathione combination in mercuric chloride toxicity in mice: a biochemical study. *Indian J. Environ. Toxicol.*, 10(1), 2000, 11-13.
- Rao, M.V. Mercury and its effects on mammalian system. A critical review. *Ind. J. Environ. Toxicol.*, 7, 1997, 3-11.
- Ricardo Brandao, Cristina W. Noguera. Inhibition of hepatic  $\delta$ -aminolevulinic acid dehydratase activity induced by mercuric chloride is potentiated by n-acetylcysteine in vitro. *Food and Chemical toxicology*, 49, 2011, 305-308.
- Rotruck JT, Pope AL, Ganther HE and Swason AB. Selenium: biochemical role as a component of glutathione peroxidase. *Science*, 179, 1973, 588-590.
- Salo D, Pacifini RE, Davier KJ. Superoxide dismutase is preferentially degraded by a proteolytic system from red blood cells following oxidative modification by hydrogen peroxide. *Free Radic. Biol.med.*, 5, 1988, 335-339.
- Seo SY, Sharma VK, and Sharma NJ. Mushroom tyrosinase: Recent prospects. *J Agric Food Chem*, 51, 2003, 2837-285.
- Sinha AK. Colorimetric assay of catalase. *Anal Biochem*, 47, 1972, 389-394. Superoxide dismutase activities in organs of rats with streptozotocin induced. *Diabetes*, 35, 1986, 503-7.
- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, and Telser J. Free radical and antioxidants in normal physiological functions and human disease. *Int J Biochem cell Biol*. 2007; 39:44-84.
- Wargovich MJ, Woods C, Hollis DM, Zander ME. Herbs, Cancer prevention and Health. *J. Nutr.*, 131, 2001, 3034S-3036.
- WHO. Inorganic mercury. *Environmental Health Criteria*, 118, 1991, 1-168.
- Yang ZP and Dettbarn WD. Disopropylphosphoro flouride-induced cholinergic hyperactivity and lipid peroxidation. *Toxicol. Appl. Pharmacol.*, 138, 1996, 48-53.
- Zhongbing L, Guangjun N, Peter S. Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. *Neurochemistry International*, 48, 2006, 263-274.