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Status of Oxidative Stress in Cerebral Cortex and Testes, Acetylcholinesterase Activity in Cerebral Cortex and Sperm Parameters in Cadmium-Exposed Rats

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ABSTRACT

The present study was carried out to evaluate oxidative stress mediated pathophysiological alterations in brain cerebral cortex and testes of rats exposed to cadmium chloride at 15, 50 and 100 ppm in drinking water for 28 days. The activity of SOD in brain of rats of all toxicity groups was non- significantly decreased. The SOD activity in testes was significantly decreased in animals exposed to 50 and 100 ppm level of cadmium. The catalase activity in brain cerebral cortex and testes was significantly decreased in dose dependent manner. The GSH levels in brain and testes tissue were increased at all tested levels of exposure of cadmium. The acetylcholinesterase activity in brain of rats exposed all levels of Cd were significantly decreased. Cadmium exposure at 100 ppm level significantly reduced the total epididymal sperm count. However, the epididymal sperm motility was significantly reduced in rats exposed to all tested levels of cadmium. The different levels of cadmium exposure produced pathological lesions in brain cerebral cortex and testes which were remarkable at 100 ppm level of exposure as compared to other levels of exposure in rats. In conclusion, cadmium exposure at 100 ppm for 28 days in rats produced marked alterations in both brain and testes through oxidative insult.

Key words: Brain, Cadmium, Oxidative stress markers, Rats, Subacute toxicity, Testes.

INTRODUCTION

Cadmium (Cd) is considered as a hazardous environmental pollutant. Major occupational exposures to Cd occur in non-ferrous metal smelters, production and processing of Cd alloys (WHO, 1992; Fay and Mumtaz, 1996). Cigarette smoke is also the source of Cd exposure to humans (Zalups and Ahmad, 2003). It is well known that long-term exposure to Cd causes various toxic effects in organs such as heart, kidneys, liver, brain, lung, bones, haemopoietic organs, endocrine and reproductive organs (Cuypers *et al*., 2010, Gomathy and Sabarinathan, 2010; Jiraungkoorskul and Jiraungkoorskul 2016; Wang *et al.,* 2019).

The brain is very sensitive to oxidative stress as it has low-to-moderate activity with respect to enzymatic defense systems compared to other organs (Cooper, 1997). Cadmium is able to induce neurotoxicity with a wide spectrum of clinical entities including neurological disturbances and changes in the normal neurochemistry of the brain (Viaene *et al*., 2000). Acetylcholinesterase (AChE) is a very important enzyme for cholinergic neurotrans mission. Areas of higher AChE expression generally correlate with brain regions that degenerate early in Alzheimer's disease.

Male reproductive toxicity caused by Cd involves various mechanisms such as direct effect on the testicular tissue, altered accessory sex gland secretions, resulting in decreased semen quality and indirect endocrine effects (Benoff *et al*., 2009; Pandya *et al*., 2012). Cadmium affects multiple cellular processes, including cell proliferation, differentiation, and apoptosis (Waisberg *et al.,* 2013). In the testes, disruption of intracellular junctions due to Cd toxicity on vascular system results in haemorrhage, oedema, necrosis and germ cells damage at high level of exposure (Siu *et al*., 2009). Another factor that may cause disruption of spermatogenesis in the testes is oxidative stress. The excessive production of reactive oxygen species (ROS) leads to altered morphology, concentration and motility of sperms (Meeker *et al.,* 2008).

Various studies have demonstrated specific organ toxicity due to exposure to particular level of Cd in rodent. However, the effects of Cd exposure at different levels on brain and testes have not been studied with special reference to oxidative damage. Thus, the present study was carried out to evaluate the subacute toxicity of Cd at low (15 ppm), medium (50 ppm) and high (100 ppm) level of exposure with special attention to oxidative stress mediated changes in brain cerebral cortex and testes.

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MATERIALS AND METHODS

Chemicals

Cadmium chloride (Lot No: 0000298204) was purchased from Himedia, Mumbai. The chemicals like KH_2PO_4 (Lot No: I12A/3212/0907/53) and $Na_2HPO_4.2H_2O$ (Lot No: K14A/0514/2104/31) were purchased from S.D. fine Chemicals, Mumbai. Other chemicals like RBC lysis buffer (Lot No: RNBG5300), Pyrogallol (Lot No: 1002139642), dTNB (Lot No: SHBG1688V), acetylthiocholine iodide (Lot No: SHBG1688V) and Bradford reagent (Lot No: SLBV5669) of analytical grade were purchased from Sigma Aldrich, USA. EDTA (Lot No: 61803701001730) and H_2O_2 (Lot No: CE6C660325) were purchased from Merck Ltd., Mumbai. All chemicals used in the study were of analytical grade.

Experimental animals and design

The study was conducted on 24 albino rats (270-340 g weight, 8-9 weeks of age). The rats were acquired from registered breeder and were maintained as per the national guideline (CPCSEA, 2003). The experimental protocol (No. JAU/JVC/IAEC/SA/32/18) was approved by the Institutional Animal Ethics Committee (IAEC). The experiment was conducted during July to August, 2018. The rats were maintained in standard polypropylene cages with stainless steel top grill and Corn Cobb was used as bedding material. During whole study period, feed (VRK Nutritional Solutions, Maharashtra) and water were supplied *ad libitum.* Rats were accommodated in cool environment (25 ± 2°C) with relative humidity ranged between 42 to 55% along with 12 hours light dark cycle.

The rats were randomly divided in to four groups (six rats in each group). The group I received the *ad libitum* drinking RO water for a period of 28 days and it served as a control. The rats of group II, III and IV were exposed to cadmium chloride at 15, 50 and 100 ppm, respectively through drinking water for a period of 28 days.

Collection of samples

Male rats were anesthetised by injecting thiopental sodium (40 mg/kg, I.P.) for collection of epididymis to evaluate the sperm parameters. The cauda epididymis was excised and then placed in watch glass containing 1 mL of DPBS medium [(Dulbecco's PBS with 1% BSA and 0.1% glucose (dextrose)] (Slott *et al.,* 1991) for evaluation of epididymal sperm count and motility. All rats were humanely sacrificed at the end of study to observe gross pathological changes in brain and testes. The tissue samples of brain and testes were collected and homogenized in ice cold 10% phosphate buffer (7.5 pH; PBS: KH_2PO_4 and $Na_2HPO_4 \times 2H_2O$) and centrifuged at 12000g for 10 minutes and resulted supernatant was used for estimation of various antioxidant enzymes except for SOD in which Tris-EDTA buffer (8.5 pH) was used and centrifuged at 12000g for 40 minutes. Protein estimation in tissues of brain and testes was carried out using the standard method (Bradford, 1976). These data were used to calculate catalase activity in brain and testes. Cortex part of brain was also collected in phosphate buffer (PB: KH_2PO_4 and $Na_2HPO_4 \times 2H_2O$ pH 8.0, 0.01M) for estimation of acetylcholinesterase activity. The tissues of brain and testes of all animals were collected in 10% formalin for histopathological examination.

Evaluation of Oxidative parameters in brain and testes

Superoxide dismutase (SOD) activity in collected tissue was performed according to the method of Marklund and Marklund (1974). Catalase activity in tissue sample was determined according to the method of Aebi, (1974). The levels GSH in blood and tissue were estimated according to standard method (Ellman, 1959).

Evaluation of acetylcholinesterase activity in brain cerebral cortex

A photometric method was used for determining acetylcholinesterase activity in tissue. The enzyme activity is measured with the production of yellow color from thiocholine when it reacts with dithiobisnitrobenzoate ion (Ellman *et al.,* 1960).

Evaluation of sperm parameters

Each cauda epididymis was minced and allowed the sperm to swim out in media at 37°C for 5 minutes. The sperm suspension was used for the evolution of sperm parameters for further experimentation. Different sperm parameters were evaluated using a Nikon Eclips Ci microscope with DS 4L camera control unit (Nikon Corporation, Japan). Epididymal sperm counting was carried out as per the procedure suggested by Khan and Sinha (1996). Epididymal sperm motility was carried out as per the procedure suggested by Ciftci *et al*. (2012).

Histopathology

The formalin-fixed tissues were embedded in paraffin and processed as per standard procedures. These tissue samples were sectioned at 5 - 6 micrometer thickness with an automatic section cutting machine semi-automated rotary microtome (Leica Biosystems, Germany) and were stained with hematoxylin and eosin (H & E) stain (Luna, 1968). The H & E stained slides were observed under the microscope and pathological lesions were recorded.

Statistical analysis

Numerical data obtained from this study have been expressed as mean ± standard error (SE). Data were analyzed statistically by ANOVA and mean of different treatment group's means were compared by Duncan's multiple range tests (DMRT) to observe difference among the treatments (Snedecor and Cochran, 1980**)**.

RESULTS AND DISCUSSION

SOD activity, catalase activity and GSH level in cerebral cortex and testes of rats of different groups are shown in Table 1, 2 and 3 respectively. Mean values of SOD activity in brain and testes of normal control rats were 0.63 ± 0.13 and 0.45 ± 0.06 (U/mg of tissue), respectively. The activity of SOD in brain of rats of all toxicity groups were slightly decreased (non-significant, P>0.05) as compared to that of

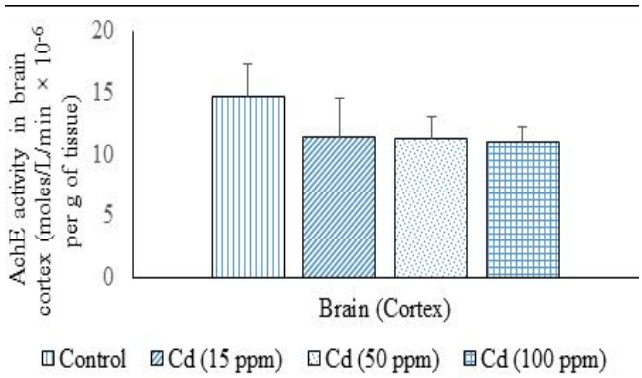


Fig 1: AChE activity in brain cortex of animals exposed to various levels of cadmium.

Table 1: SOD activity (U/mg of tissue) in brain cortex and testes of rats of different groups.

Organ	Treatment groups			
	Control (C1)	15 ppm (T1)	50 ppm (T2)	100 ppm (T3)
Brain	0.63 ± 0.13^{a}	0.43 ± 0.09^{a}	0.48 ± 0.09^{a}	0.50 ± 0.05^{a}
Testes	0.45 ± 0.06^{b}	0.42 ± 0.03^{b}	0.27 ± 0.05^{a}	0.38 ± 0.02^{a}

Value with different superscripts in a row were differ significantly (P<0.05).

Table 2: Catalase activity (U/mg protein) in brain and testes of rats of different groups.

Organ	Treatment groups			
	Control (C1)	15 ppm (T1)	50 ppm (T2)	100 ppm (T3)
Brain	5.05 ± 2.1^{b}	2.34 ± 0.88^{ab}	1.55 ± 0.36^{a}	1.31 ± 0.30^{a}
Testes	6.45 ± 2.25^{a}	3.70 ± 1.82^{ab}	1.35 ± 0.12^{b}	1.70 ± 0.40^{b}

Value with different superscripts in a row were differ significantly (P<0.05).

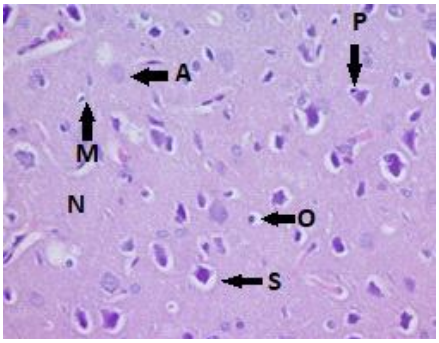
Table 3: GSH levels (μg/mg of tissue) in brain and testes of rats of different groups.

Organ	Treatment groups			
	Control (C1)	15 ppm (T1)	50 ppm (T2)	100 ppm (T3)
Brain	0.02 ± 0.006^{a}	0.02 ± 0.008^{a}	0.03 ± 0.004^{a}	0.03 ± 0.003^{a}
Testes	0.01 ± 0.004^{a}	0.02 ± 0.007^{a}	0.04 ± 0.12^{b}	0.04 ± 0.007^{b}

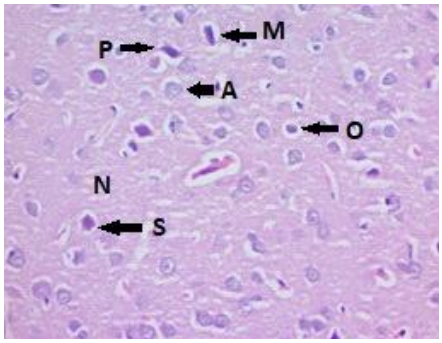
Value with different superscripts in a row were differ significantly (P<0.05).

control group. The SOD activity in testes of rats exposed to 50 and 100 ppm levels of Cd was significantly decreased as compared to that of control animals and animals exposed to 15 ppm Cd level. Mean values of catalase activity in brain and testes of normal control rats were 5.05 ± 2.1 and 6.45 ± 2.25 (U/mg protein), respectively. Catalase activity in cerebral cortex was non-significantly (P>0.05) decrease at 15 ppm level of Cd exposure, whereas, rats exposed to 50 and 100 ppm showed significant (P<0.05) decreased catalase activity in cerebral cortex as compared to that of control group. Similarly, catalase activity in testes was also non-significantly (P>0.05) decrease at 15 ppm level of Cd exposure, whereas, rats exposed to 50 and 100 ppm showed significant (P<0.05) decrease in catalase activity in testes as compared to that of control group. However, the levels of GSH in cerebral cortex was not significantly affected following exposure to all tested level of Cd in rats, Whereas level of GSH in testes was significantly increased as compared to control group. The acetylcholinesterase activity (moles/L/min × 10^{-6} per g of tissue) in cerebral cortex of rats exposed to 15, 50 and 100 ppm of Cd level were 11.38 ± 3.23, 11.30 ± 1.81 and 10.96 ± 1.23, respectively which were significantly lower (P<0.05) as compared to the value of 14.64 ± 2.61 in control animals (Fig 1). No dose dependent effect of Cd was observed in activity of AchE in toxicity groups.

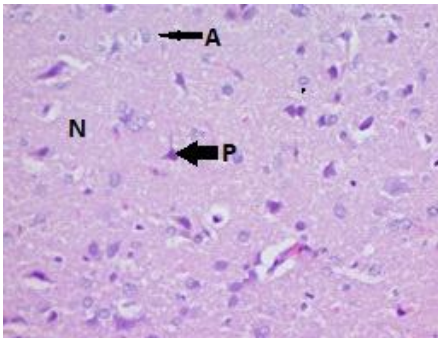
Total epididymal sperm count (x 10^{6}/cauda epididymis) in rats of control group was 21.00 ± 0.35. The total epididymal sperm count was not altered in rats exposed to Cd at 15 and 50 ppm levels. However, the Cd exposure at 100 ppm level significantly (P<0.05) reduced the total sperm count as compared to that observed in control animals. The epididymal sperm motility in rats exposed to all tested levels of Cd was significantly lower as compared to that observed in control animals.



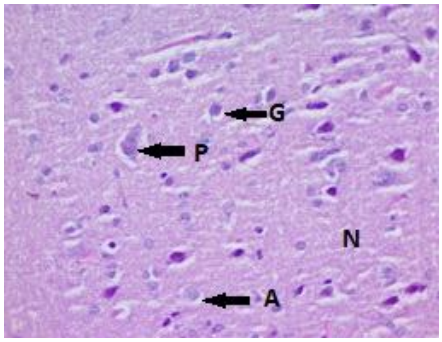
Microscopic view of brain cortex of group C1 showed normal histological architecture with astrocytes (A), pyramidal cell (P), stellate cells (S), oligodendrocytes (O), microglia (M), neuropil (N) (H & E ×400)



Microscopic view of brain cortex of group T1 showed mild degenerated neuropil (N), swollen astrocytes with increase surrounding space (A), irregular shape of pyramidal cell (P) and stellate cells (S) (H & E ×400)



Microscopic view of brain cortex of group T2 showed slight more degeneration of neuropil (N) and pyramidal cells (P), decrease in size of astrocytes (A) with irregular shape as compared to T1 group (H & E × 400).



Microscopic view of brain cortex of group T3 showed more degenerated neuropil (N), irregular shape of pyramidal cells (P) with increase surrounding space and decrease in size of astrocytes (A) with irregular shape, pyknotic glial cells (G) as compared to other toxicity groups (H & E × 400)

Fig 2: Microscopic lesions in brain cortex of rats exposed to various levels of cadmium chloride**.**

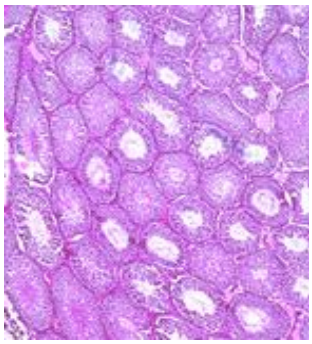
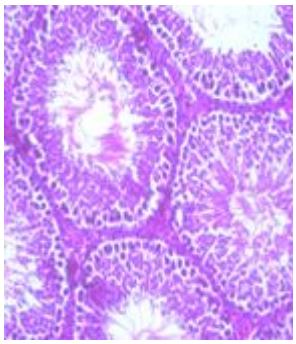
Table 4: Reproductive parameters in rats of different groups.

Parameters	Treatment groups			
	Control (C1)	15 ppm (T1)	50 ppm (T2)	100 ppm (T3)
Total sperm count (x10^6/ cauda epididymis)	21.00 ± 0.35^a	21.33 ± 0.51^a	21.16 ± 0.38^a	17.66 ± 0.98^b
Epididymal sperm motility (%)	80.83 ± 0.31^b	64.44 ± 0.39^a	62.50 ± 0.92^a	65.44 ± 0.31^a

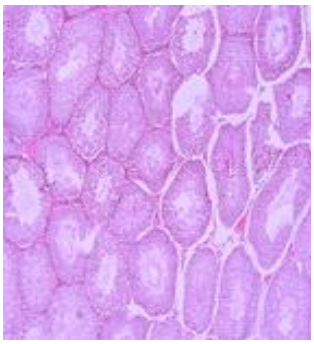
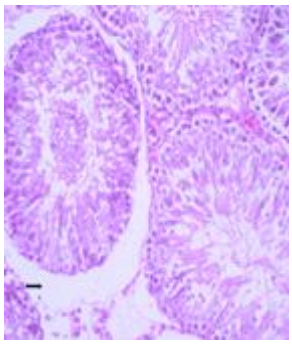
Value with different superscripts in a row were differ significantly (P<0.05).

Upon histopathological evaluation of cerebral cortex of control rats, normal histological architecture with astrocytes, pyramidal cell, stellate cells, oligodendrocytes, microglia, neuropil was observed. Cerebral cortex of rats exposed to 15 ppm Cd for 28 days showed degenerated neuropil, swollen astrocytes with increase surrounding space, irregular shape of pyramidal cell and stellate cells. Cerebral cortex of rats exposed to 50 ppm Cd for 28 days showed mild degeneration of neuropil and pyramidal cells, decrease in size of astrocyte with irregular shape. Cerebral cortex of rats exposed to 100 ppm Cd for 28 days showed severe degenerated neuropil, irregular shape of pyramidal cells with increase surrounding space and decrease in size of astrocytes with irregular shape, pyknotic glial cells (Fig 2). The histopathological changes were more pronounce in cerebral cortex of rats exposed to Cd at 50 and 100 ppm levels as compared to those observed at 15 ppm level.

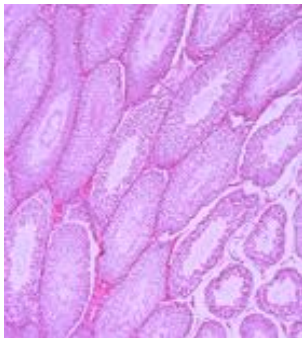
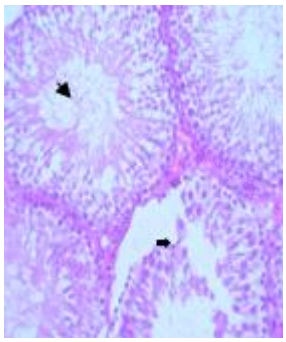
Upon histopathological evaluation of testes of normal control rats, normal structure of seminiferous tubules with normal spermatogenic cells layers and spermatozoa, lumen of seminiferous tubules filled with mature spermatozoa were observed. Testes of rats exposed to 15 ppm level of Cd

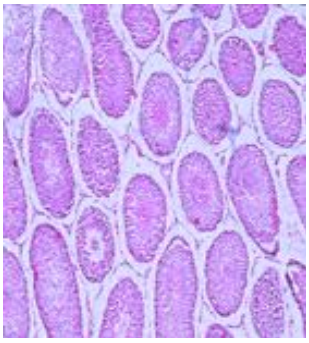
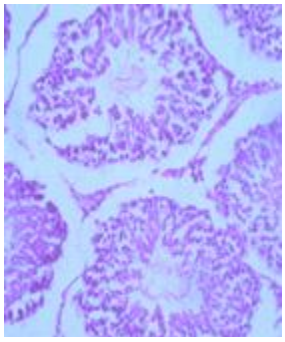
Microscopic view of testis of group C1 showed normal structure of seminiferous tubules with normal spermatogenic cells layers and spermatozoa, lumen of seminiferous tubules filled with mature spermatozoa (SG: spermatogonia, ST: spermatid, SP: spermatozoa, IT: interstitial tissue (H & E, x 100 & ×400)

Microscopic view of testis of group T1 showed disorganized seminiferous tubules with sloughing of layer of spermatogonia, increase in interstitial space, lumen of seminiferous tubules filled with mature spermatozoa (H & E, x 100 & ×400)

Microscopic view of testis of group T2 showed slight more shrunken and disorganized seminiferous tubules with sloughing of layer of spermatogonia and slight increase in interstitial space, degeneration of germinal layer with decrease thickness as compared to T1 group (H & E, x 100 & ×400)

Microscopic view of testis of group T3 showed more shrunken and disorganized seminiferous tubules with sloughing of layer of spermatogonia and increase in interstitial space, degeneration of germinal layer with decrease thickness, decrease number of spermatogonia as compared to other toxicity groups (H & E, x 100 & ×400)

Fig 3: Microscopic changes in testes of rats of exposed to various levels of cadmium chloride.

showed disorganized seminiferous tubules with sloughing of layer of spermatogonia, increase in interstitial space, lumen of seminiferous tubules filled with mature spermatozoa. Testes of rats exposed to 50 ppm level of Cd showed slight shrunken and disorganized seminiferous tubules with sloughing of layer of spermatogonia and increase in interstitial space, degeneration of germinal layer with decrease thickness. However, more shrunken and disorganized seminiferous tubules with almost sloughing of layer of spermatogonia and increase in interstitial space, degeneration of germinal layer with decrease thickness, decrease number of spermatogonia were observed in testes of rats exposed to 100 ppm level of Cd (Fig 3). The histopathological changes were more pronounce in testes of rats exposed to Cd at 50 and 100 ppm levels as compared to low level exposure.

Cadmium is one of the most toxic substances and it produces deleterious effect on health of human and animals. It is a heavy metal that has greater potential to induce oxidative stress by enhancing the production of reactive oxygen species (ROS). Super oxide dismutase is the first line of antioxidant defense, catalysing the conversion of $O2^{\bullet -}$ to the less toxic H_2O_2. With proper activity of catalase or glutathione peroxidase, H_2O_2 is neutralized with the formation of a water molecule (Harris, 1992). It has been reported that the potential replacement of Zn by Cd, which subsequently lead to the reduction in SOD activity (Chronology *et al.,* 2002; Hussain *et al.,* 1987). Additionally, Cd and enzyme interaction may hinder the functioning the enzyme due to disruption of the topography of the channel localized in SOD (Okabe, 2000). The brain is a vital part of the body which has role in regulation of function of other parts of the body and damage or form of stress in this region may have severe deleterious effect on entire body. Ogunrinola *et al*., (2016) observed significantly decreased SOD activity in erythrocytes, plasma, whole brain and liver of rats in a dose-dependent manner following 6 weeks of exposure to Cd at 100, 200 and 300 ppm levels which were as a result of an imbalance between oxidants and antioxidants level caused oxidative stress. They did not observed the effect of the cadmium on oxidative stress markers in testes at lower or at 100 ppm exposure level. In

the present study, exposure to cadmium for 4 weeks moderately altered the SOD activity in cerebral cortex and testes which could explain the possibility of stimulation of adaptive mechanism. A mechanism has been suggested to be involved in the interaction between Cd and the catalytic subunit of CAT leading to the reduction of CAT activity (Cuypers *et al.,* 2010). The increase in GSH level is clear indication of defensive mechanism against the Cd toxicity in brain and testes. Increased activity of brain microvessel SOD, glutathione peroxidase and CAT have also been reported in rats at 30 days of exposure to 10 ppm level of cadmium. However, the continuation of the Cd treatment for 90 days reported to decrease the levels of superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase, vitamin E, glutathione and ascorbic acid in the microvessal preparation compared to controls (Shukla *et al.,* 1996). Lipid peroxidation along with the depletion of microvessel antioxidant substances may be related to Cd-induced blood-brain barrier dysfunction (Shukla *et al.,* 1996). Intraperitioneal administration of 0.4 mg/kg Cd daily for 45 days was found to inhibit the activity of glutathione peroxidase and catalase in liver, kidney, testes and various brain regions at different time intervals (Shukla *et al.,* 1989).

Various mechanisms were proposed for the Cd toxicity. One of these mechanisms includes Cd binding to –SH groups from cell membrane proteins, cytoplasmic proteins, and enzymes. Kikelomo *et al.* (2008) observed that Cd (1.5 mg/kg P.O. for 3 weeks) decreased SOD activity, CAT activity and alkaline phosphatase (ALP) levels in rats. Cadmium produces toxicity by altering the thiol status and cellular defense mechanism. In view of the fact that metallothionein (MT) is cysteine-rich and Cd has a high affinity for thiols, MTs are known to sequester Cd. Therefore, cadmium is stored as a Cd-Mt complex in the liver. Lysosomes have role in metabolism of Cd-MT and librates cadmium ions. Librated Cd ions again make a complex with newly synthesized MT. If demand of MT in Cd toxicity is not fulfilled by the body, free Cd crushes the SOD defense systems. The observed decrease SOD level in the cerebral cortex and testes in the present study indicates that cadmium toxicity causes oxidative stress by challenging the thiol status of cells. Activation of mitogen, activated protein kinase; c-Jun N-terminal kinase and other signalling pathways might be responsible for Cd-induced overwhelmed cellular defence mechanism. As a consequence, down regulation of genes coding for molecules involved in the biological defence and cellular repair, including antioxidants-SOD occurs (Hussain *et al.,* 1987; Tobwala *et al.,* 2014). Catalase can be a quite efficient inhibitor of lipid peroxidation when hydrogen peroxide accumulates in a cell containing free ferrous ions. The importance of catalase becomes even more apparent particularly in the situations like Cd toxicity where glutathione peroxidase activity is inhibited and its reaction participants are short supplied. In the present study, increased GSH level with significant decreased CAT activity clearly demonstrated the capacity of cadmium to cause the alteration in oxidative defence system.

The activity of AchE in tissue of cerebral cortex collected from the each animal under study was also evaluated in the present study. The activity of AChE in different treatment groups was decreased as compared to that of normal control animals. In relation to the study of brain enzyme activity, the AChE activity is a key enzyme in detecting the neurotoxic effect of certain heavy metals. However, numerous studies have suggested that the free radicals production could at least in partly associated with the decreased activity of brain AChE activity (Tsakiris *et al*., 2000). It has been reported that decreased activity AChE leads to the accumulation of acetylcholine, which causes cholinergic hyperactivity, convulsion and status epilepticus (Olney *et al*., 1986). Alterations in the mechanisms of neurotransmitters release have also been implicated in Cd neurotoxicity and Cd may block the influx of Ca^{+2} through membrane channels into the nerve terminal following the action potential. These decreases in calcium influx caused by Cd would be associated with an altered transmitter release (Antonio *et al*., 2002). However, the symptoms associated with the cholinergic stimulation were not observed in the present study which might be due to comparatively less alteration caused by Cd to AChE. Similar to our observation, Cd administration for 3 weeks in rats (3 mg/kg body weight through intraperitoneal injection) has been reported to decrease the AChE, Mg^{2+}-ATPase and Na^{+}/K^{+}-ATPase activity and ACh content (Devi and Konduru, 2018). Cadmium induced reactive oxygen species may play a significant role in the mechanism of down-regulation of nAChRs and as such, a decrease in AChE activity, a key enzyme of cholinergic central and peripheral nervous system. The observations of decreased AChE activity in the brain of rodents exposed to lead and fluoride provide support to our present findings on Cd induced decrease in AChE activity (Bhatnagar *et al*., 2006; Reddy *et al*., 2007; Bouaziz *et al*., 2010; Devi and Konduru, 2018).

Findings in present study related to toxic effect of varying level of Cd exposure on testes were in agreement with previous findings (Acharya *et al*., 2008; Ola-Mudathir *et al*., 2008; Yari *et al*., 2010) that Cd toxicity led to alteration in sperm parameters with necrotic degenerative changes in the testes (El-Shahat *et al.,* 2009; de Souza Predes *et al*., 2010), reduced testicular weight and decreased sperm count and motility (WHO, 1992; Wang *et al*., 2006). Cadmium treatment to rats at high dose (5 mg/kg b.wt. intraperitoneally) has also been reported to cause a significant ($p>0.01$) reduction in spermatozoa count, spermatozoa motility, percentage number of morphologically normal spermatozoa and a significant increase in the percentage number of morphologically abnormal spermatozoa (Akunna *et al*., 2017). In the present study, subacute exposure at 100 ppm to rats showed alterations in sperm parameters and testicular toxicity which clearly demonstrate the toxicity potential of cadmium upon repeated exposure at 100 ppm.

In the present study, microscopic view of cerebral cortex of rats exposed to 100 ppm level of Cd showed more

degenerated neuropil, irregular shape of pyramidal cells with increase surrounding space and decrease in size of astrocytes with irregular shape, pyknotic glial cells. Cd intoxicated rats (5 mg/kg body, P.O., 4 weeks) in previous report also exhibited marked gliosis, nuclear pyknosis spongiform necrosis and lymphocytic inflammatory infiltrates in brain (Shagirtha *et al.*, 2011). Khan and parvez, (2015) reported Cd-induced (3 mg/kg, S.C. for 21 days) abnormal structural changes in the brain tissue including degenerated neurons along with vacuolated spaces and apoptosis. The vacuolation in the surrounding neuropil might be attributed to the shrinkage of cells and withdrawal of their processes secondary to cytoskeletal affection leaving pericellular spaces. The variation in histopathological changes have been noticed which might be due to difference in dose, route and duration of exposure.

In the present study, microscopic view of testes of rats exposed to 100 ppm level of Cd showed more shrunken and disorganized seminiferous tubules with sloughing of layer of spermatogonia and increase in interstitial space, degeneration of germinal layer with decrease thickness, decrease number of spermatogonia as compared to other toxicity groups. Aruldhas *et al*. (2005) also reported that Cd-induced oxidative stress by generating free radicals led to infertility in non-human primates. Santos *et al.* (2004) and Blanco *et al.* (2007) reported extensive widening of interstitial spaces due to diffuse eosinophilic, edematous vacuolated fluids infiltration, necrosis, congestion and hemorrhage in testes due to Cd exposure to mice. Mohamed *et al*. (2014) examined the histopathological changes in testes following Cd chloride exposure to rats (20 mg/kg/day) and they observed seminiferous tubules with variable morphological changes in the form of multiple distortions, wide interstitial spaces, severe damage, necrosis and interstitial tissue degeneration with reduction of size of some seminiferous tubules. Accumulated degenerated germ cells in the seminiferous tubules depicted in the previously reported work may be attributed to the failure of Sertoli cells to perform their function (O'Donnell *et al*., 2011).

CONCLUSION

The cadmium exposure to rats at 50 and 100 ppm level through oral route for 28 days could be able to produce oxidative insult by altering SOD and catalase activity. The histopathological changes in cerebral cortex and testes were more remarkable in rats exposed to 100 ppm level of cadmium. Thus, cadmium exposure at 100 ppm can be used to underline the mechanism of cadmium toxicity and related studies in rodent model of heavy metal induced oxidative stress.

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Conflict of Interest

Authors declare no conflict of interest.

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