



## Study on Neuroendocrine Disrupting Potential of Cadmium in Rats and Evaluation of Role of Green Tea

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### ABSTRACT

The protective role of green tea extract was studied in neuroendocrine disrupting actions of cadmium. Twenty four weaned *Sprague dawley* male rats were randomly divided into 4 groups of 6 rats in each. Group 1 served as Sham control, Group 2 was treated with CdCl<sub>2</sub> @5mg/kg b.wt. per orally for 3 months, Group 3 was treated with Green tea extract(1.5%) and Group 4 with CdCl<sub>2</sub> + green tea extract. The serum testosterone, Tri-iodothyronine (T<sub>3</sub>) and Thyroxine (T<sub>4</sub>) hormones were monitored at monthly interval. At the end of 3 months rats were sacrificed and testes were collected for estimation of thio barbituric acid reacting substance (TBARS), reduced GSH, protein carbonyls and sperm count. Before sacrifice, rats were subjected to elevated plus maze (EPM) and morris water maze (MWM). Administration of CdCl<sub>2</sub> resulted in decrease of serum testosterone, T<sub>4</sub> hormones, testicular GSH, sperm count and increase in serum T<sub>3</sub> and testicular TBARS and protein carbonyls while, number of entries and time spent in open arm of EPM increased and the total distance travelled in MWM increased. Treatment with green tea significantly ameliorated (p<0.05) toxic effects of CdCl<sub>2</sub> by restoring biochemical and hormonal profile to normal. It is concluded that green tea extract exhibits neuro-endocrine protective property in CdCl<sub>2</sub> induced neuroendocrine disruption.

**Keywords:** Cadmium, green tea, neuroendocrine disruption

An environmental endocrine or hormone disruptor may be defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour (Lintelmann *et al.*, 2003). Effects of endocrine disruptors on human and animals include reproductive impairment and abnormalities, reduced immune function, reduced or abnormal growth, decreased cognitive abilities, increased susceptibility to disease and increased mortality (Clotfelter *et al.*, 2004).

Cadmium is an inorganic toxicant, occurring naturally in ores containing copper, zinc or iron. It is distributed widely in environment and workplaces and is of great concern as an environmental and occupational toxicant, especially with increasing industrialization (Jarup, 2003). Cd<sup>2+</sup> has a long biological half-life of 15–30 years (Henson and

Anderson, 2000), mainly due to its low rate of excretion from the body and accumulates over time in blood, kidney and liver (Varga *et al.*, 1993; Bhattacharyya *et al.*, 2000) as well as in the reproductive organs, including the placenta, testis and ovaries (Zadorozhnaja *et al.*, 2000; Fiala *et al.*, 2001; Piasek *et al.*, 2001). Cd and Pb damage the structure and function of the thyroid gland. Probable mode of action or mechanism of Cd and Pb was by interference in the synthesis and/or secretion of T<sub>4</sub> by the damage of thyroid follicular cells, decrease transformation rate of the T<sub>4</sub> to T<sub>3</sub> in peripheral tissue by inhibiting the activity of (5'-D) and interference with pituitary gland or hypothalamus gland (Ashraf and Asma, 2009). Cadmium interferes with reproduction of both males and females by interfering in the steroidogenesis and exhibit estrogen-like and androgen-like activities both *in vitro* and *in vivo* through direct binding to ER  $\alpha$  and androgen receptors (AR) (Masufumi and Shinichi, 2006).

The World Health Organization (WHO) has shown that, over 80% of population in traditional medicinal system depends on medicinal plants (Barua *et al.*, 2012). Tea, a product made up from leaf and bud of the plant *Camellia sinensis*, is the second most consumed beverage in the world. Green tea is mainly produced from *Camellia sinensis* var *sinensis*. Polyphenols constitute the most interesting group of green tea leaf components, and in consequence, green tea can be considered an important dietary source of polyphenols, particularly flavonoids. Consumption of green tea provides a protection against stroke (Weisburger, 1996), liver disease (Jonathan, 2008), bacterial infection (Horiba *et al.*, 1991; Terada *et al.*, 1993), cancer (Yang and Wang, 1993), viral infection (Nakayama, 1990) and lowers the risk of osteoporosis (Adak and Gabar, 2011). Green tea has also been shown to be effective against the immune suppression caused by ultraviolet radiation (Katiyar *et al.*, 1995).

In addition, green tea polyphenols have shown protection against cytokines induced by tumors. A variety of epidemiologic studies showed the preventive effect of green tea consumption against atherosclerosis and coronary heart disease (Thelle, 1995). Polyphenols found in green tea have a greater antioxidant activity than do either vitamins C or E and are believed to be suitable for protection against reactive oxygen species (ROS) and their associated pathologies (Mitscher *et al.*, 1997). Green tea polyphenols (GTPs) and especially the gallic acid moiety are known to scavenge  $O_2^-$ ,  $HO^-$  and  $ROO^-$  (Guo *et al.*, 1999; Lin *et al.*, 2000).

## MATERIALS AND METHODS

Twenty four male weaned (21 days) Sprague-dawley rats were obtained from NCLAS (National Centre for Laboratory Animal Sciences), National Institute of Nutrition, Hyderabad. Animals were reared in animal house attached to the Department of Veterinary Pharmacology & Toxicology with 12 h – 12 h dark and light cycle. Before conducting the experiment, rats were kept for acclimatization for 1 week. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC). After acclimatization all the rats were divided into 4 groups of 6 each and treated as per the schedule for three months. Group 1 served as Sham control, Group 2 received  $CdCl_2$  @ 5mg/kg b.wt. by oral gavage daily, Group 3 Green tea extract (1.5%) treatment

as sole source of drinking water while Group 4 received  $CdCl_2$  + green tea extract.

All the chemicals (for preparation of reagents and buffers) were procured from Qualigens Pvt. Ltd., Mumbai and SRL Pvt. Ltd., Mumbai.  $T_3$ ,  $T_4$ , and Testosterone ELISA kits were obtained from Omega Diagnostics Ltd., Scotland, United Kingdom. Green Tea was procured from Nilgiri Tea Emporium, Hyderabad.

Blood was collected from all the groups at monthly intervals for analyzing serum hormone profile ( $T_3$ ,  $T_4$  and Testosterone). At the end of 3 months rats were subjected to neuro-behavioural studies (Elevated plus maze and Morris water maze). At the end of 3 months, rats were sacrificed to collect testes and subjected them to biochemical studies. The tissues were stored at  $-20^\circ C$  for further estimation of GSH, TBARS and protein carbonyls in testis homogenates. Epididymus was collected for total sperm count.

The data was subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 15.0. Differences between means were tested using Duncan's multiple comparison test and significance level was set at 0.05.

## RESULTS AND DISCUSSION

The serum  $T_3$  concentrations at different time intervals showed a decrease in  $T_3$  concentration in groups 2, 3 and 4. Follicular cells of the thyroid are designed for hormone synthesis and secretion.  $T_3$  and  $T_4$  are the predominant circulating thyroid hormones synthesized and secreted by follicular cells in vertebrates.  $T_3$  is considered a biologically active thyroid hormone and most of the circulating  $T_3$  is generated by extra-thyroidal deiodination of  $T_4$ , taking place mainly in the liver. Thyroid hormones are metabolized in peripheral tissues (by deiodination, conjugation, deamination and decarboxylation) and alterations in their metabolism might significantly influence the function of thyroid hormone metabolites at the cellular level (Barbara Piłat *et al.*, 2003). The thyroidal Cd accumulation in the Cd-exposed rats was very low, when compared to its accumulation in other organs of these animals, especially in the liver and kidney. In spite of low Cd retention in the thyroid, less or more serious damage to the thyroid follicular cells was observed in the rats chronically

exposed to this heavy metal. It is suggested that Cd might have interfered with the thyroid function at the glandular level as well as at the peripheral level by inhibiting the conversion of  $T_4$  to  $T_3$  (Gupta and Kar, 1999). Since the thyroid gland is the only organ involved in  $T_4$  synthesis, the decrease in the serum level of this hormone in the Cd-exposed rats, together with decreased concentration of  $T_3$ , might suggest that Cd would have influenced the production and/or secretion of  $T_4$  by follicular cells and inhibited the enzyme 5'-D activity through binding to sulfhydryl groups of this enzyme. Yoshizuka *et al.* (1991) speculated that the metal accumulated in the mitochondria of thyroid follicular epithelial cells can inhibit the synthesis and release of thyroid hormones influencing the oxidative phosphorylation of these organelles. Reduced  $T_4$  levels in group 3 might be due to the inhibition of TPO-catalyzed reactions resulting in decrease in serum levels of thyroid hormones. Available literature showed that dietary flavonoids had an antithyroidal effect and found to be goitrogenic (Amar *et al.*, 2010). Although flavonoids are reported to exert antithyroid effects through a variety of mechanisms, reports on the antithyroidal effects of catechins of green tea are limited (Table. 1&2).

**Table 1:** Serum  $T_3$  (ng/ml) in different groups of rats (Parent stock)

Group	1 <sup>st</sup> Month	2 <sup>nd</sup> Month	3 <sup>rd</sup> Month
Group 1	0.518±0.01 <sup>b</sup>	0.543±0.02 <sup>b</sup>	0.578±0.02 <sup>b</sup>
Group 2	0.513±0.01 <sup>ab</sup>	0.532±0.02 <sup>a</sup>	0.572±0.02 <sup>b</sup>
Group 3	0.506±0.01 <sup>a</sup>	0.530±0.01 <sup>a</sup>	0.564±0.02 <sup>a</sup>
Group 4	0.497±0.01 <sup>a</sup>	0.528±0.03 <sup>a</sup>	0.562±0.02 <sup>a</sup>

Values are mean ± standard error (n=6)

Means with different alphabets as superscripts differ significantly (p<0.05) with in the column

**Table 2:** Serum  $T_4$  (ng/ml) in different groups of rats

Group	1 <sup>st</sup> Month	2 <sup>nd</sup> Month	3 <sup>rd</sup> Month
Group 1	97.48±4.36 <sup>b</sup>	102.44±5.92 <sup>c</sup>	108.46±4.91 <sup>c</sup>
Group 2	94.27±3.52 <sup>a</sup>	91.64±4.36 <sup>b</sup>	91.22±3.89 <sup>b</sup>
Group 3	95.64±5.01 <sup>b</sup>	93.74±4.93 <sup>b</sup>	92.65±5.06 <sup>b</sup>
Group 4	88.27±4.07 <sup>a</sup>	86.64±4.09 <sup>a</sup>	81.27±4.67 <sup>a</sup>

Values are mean ± standard error (n=6)

Means with different alphabets as superscripts differ significantly (p<0.05) with in the column

There was significant increase in TBARS and protein carbonyls, and decreased GSH, serum testosterone and sperm count in group 2. Most of the toxic effects of Cd are related to its ability to generate free radicals at a rate high enough to overwhelm the natural antioxidant defence system of the body (Rajender *et al.*, 2011). The decreased serum testosterone in group 2 may be attributed to testicular damage caused by CdCl<sub>2</sub>, which competes with zinc in zinc-containing enzymes and decreases activity of testis-specific enzymes, metal accumulates in this tissue, a disruption of the regulatory mechanism of the hypothalamic- pituitary axis and Cd up-regulates testicular PGF2 $\alpha$ , which causes negative feedback of testosterone production. The results are in agreement with the findings of Ekhoeye *et al.* (2013). The improvement in the study parameters in group 4 may be due to high concentrations of polyphenols, which have strong antioxidant properties. Antioxidants have been shown to reduce free radical oxidative damage (Fabricia *et al.*, 2010; Jalil *et al.*, 2011) as evident from the results of this study (Table 3, 4, 5).

**Table 3:** Oxidative stress parameters in testis of different groups of rats

Group	TBARS concentration (n moles of MDA released/ mg protein)	GSH concentration (n moles/mg protein)	Protein carbonyls concentration (n moles/mg protein)
Group 1	3.65±0.14 <sup>a</sup>	3.96±0.17 <sup>c</sup>	0.58±0.01 <sup>a</sup>
Group 2	6.37±0.24 <sup>c</sup>	0.83±0.04 <sup>a</sup>	3.61±0.12 <sup>c</sup>
Group 3	3.02±0.12 <sup>a</sup>	4.28±0.23 <sup>c</sup>	0.55±0.01 <sup>a</sup>
Group 4	4.14±0.31 <sup>b</sup>	1.72±0.12 <sup>b</sup>	2.04±0.06 <sup>b</sup>

Values are mean ± standard error (n=6)

Means with different alphabets as superscripts differ significantly (p<0.05) with in the column

**Table 4:** Epididymal sperm count (million/ml) in different groups of rats

Group	3 <sup>rd</sup> Month
Group 1	80.34±4.36 <sup>c</sup>
Group 2	56.76±2.91 <sup>a</sup>
Group 3	81.28±3.99 <sup>c</sup>
Group 4	62.73±4.01 <sup>b</sup>

Values are mean ± standard error (n=6)

Means with different alphabets as superscripts differ significantly (p<0.05) with in the column

**Table 5:** Serum Testosterone (ng/ml) in different groups of rats

Group	1 <sup>st</sup> Month	2 <sup>nd</sup> Month	3 <sup>rd</sup> Month
Group 1	4.92±0.13 <sup>b</sup>	5.74±0.54 <sup>b</sup>	6.63±0.26 <sup>b</sup>
Group 2	4.71±0.16 <sup>a</sup>	4.18±0.16 <sup>a</sup>	3.88±0.19 <sup>a</sup>
Group 3	5.04±0.15 <sup>b</sup>	6.05±0.17 <sup>c</sup>	6.86±0.19 <sup>b</sup>
Group 4	4.83±0.16 <sup>ab</sup>	4.09±0.13 <sup>a</sup>	4.03±0.13 <sup>a</sup>

Values are mean ± standard error (n=6)

Means with different alphabets as superscripts differ significantly (p<0.05) with in the column

Elevated plus maze (EPM) determines the animal's unconditioned response to a potentially dangerous environment. This is an effective and most simple test for evaluating memory and learning in rats. A normal rodent avoids open arm for fear or anxiety and likes to spend most of the time in closed arms. Such behaviour is called thigmotaxis, the tendency to remain close to the walls. After the animal is accustomed, it may explore the open arm environment. Cadmium treated rats have spent more time in the open arms of elevated plus maze and made more number of visits into open arms (Table 6). Cadmium has a long-term effect in the synapses. Cadmium inhibits the voltage dependent calcium channels *in vitro* and hence might affect the release of neurotransmitters from neuron terminals. Sub-chronic oral exposure to cadmium can cause anxiety and alterations in the biochemical activity.

Long-term changes in anxiety-like behaviour can be related to dopaminergic and serotonergic alterations detected in hippocampus (Bull, 2010), where serotonin system is important in the pathophysiology of psychiatric disorders including mood and anxiety. The oxidative damage mechanism caused by Cd intoxication might be related to its displacement to iron (Fe<sup>+2</sup>) and copper (Cu<sup>+2</sup>) from cytoplasmic and cell membrane proteins with consequent elevation in their ions inside the cell leading to free radical generation and binding of cadmium with -SH groups of various essential enzymes (Hussein *et al.*, 2010). Morris water maze is the gold standard for determining cognitive skills in animals. Changes in the central cholinergic system can be detected (Fibiger, 1991) and cued version is capable of revealing deficits in sensory, motor or motivational processes. Repeated acquisition (learning) and performance (memory) were assessed in all the groups. Animals in all the groups learned to locate the platform with continued training. Rats in group 2 took more time than other groups to locate the platform in

the probe trial. A detailed analysis of swimming patterns revealed thigmotaxis in group 2 i.e., higher percentage of time in outer zone and lesser time in the middle zone, the zone of platform location (Table 7).

**Table 6:** Oxidative stress parameters in testis of different groups of rats

Group	TBARS concentration (n moles of MDA released/mg protein)	GSH concentration (n moles/mg protein)	Protein carbonyls concentration (n moles/mg protein)
Group 1	3.65±0.14 <sup>a</sup>	3.96±0.17 <sup>c</sup>	0.58±0.01 <sup>a</sup>
Group 2	6.37±0.24 <sup>c</sup>	0.83±0.04 <sup>a</sup>	3.61±0.12 <sup>c</sup>
Group 3	3.02±0.12 <sup>a</sup>	4.28±0.23 <sup>c</sup>	0.55±0.01 <sup>a</sup>
Group 4	4.14±0.31 <sup>b</sup>	1.72±0.12 <sup>b</sup>	2.04±0.06 <sup>b</sup>

Values are mean ± standard error (n=6)

Means with different alphabets as superscripts differ significantly (p<0.05) with in the column

**Table 7:** Epididymal sperm count (million/ml) in different groups of rats

Group	3 <sup>rd</sup> Month
Group 1	80.34±4.36 <sup>c</sup>
Group 2	56.76±2.91 <sup>a</sup>
Group 3	81.28±3.99 <sup>c</sup>
Group 4	62.73±4.01 <sup>b</sup>

Values are mean ± standard error (n=6)

Means with different alphabets as superscripts differ significantly (p<0.05) with in the column

**Table 8:** Serum Testosterone (ng/ml) in different groups of rats

Group	1 <sup>st</sup> Month	2 <sup>nd</sup> Month	3 <sup>rd</sup> Month
Group 1	4.92±0.13 <sup>b</sup>	5.74±0.54 <sup>b</sup>	6.63±0.26 <sup>b</sup>
Group 2	4.71±0.16 <sup>a</sup>	4.18±0.16 <sup>a</sup>	3.88±0.19 <sup>a</sup>
Group 3	5.04±0.15 <sup>b</sup>	6.05±0.17 <sup>c</sup>	6.86±0.19 <sup>b</sup>
Group 4	4.83±0.16 <sup>ab</sup>	4.09±0.13 <sup>a</sup>	4.03±0.13 <sup>a</sup>

Values are mean ± standard error (n=6)

Means with different alphabets as superscripts differ significantly (p<0.05) with in the column

The improvement in green tea-treated group 4 may be attributed to 1) inhibition of the redox-sensitive transcription factors; 2) inhibition of 'pro-oxidant'

**Table 9:** Performance of rats on Elevated Plus Maze

Group	No. of entries			Duration of time spent(sec)	
	Closed arm	Open arm	Centre of the maze	Closed arm	Open arm
Group 1	9.01±1.73 <sup>b</sup>	4.04±0.64 <sup>a</sup>	53.64±6.93 <sup>b</sup>	137.28±15.32 <sup>c</sup>	93.63±10.68 <sup>a</sup>
Group 2	2.02±0.25 <sup>a</sup>	8.51±0.98 <sup>c</sup>	58.29±9.37 <sup>b</sup>	42.19±5.82 <sup>a</sup>	178.27±16.93 <sup>b</sup>
Group 3	10.07±1.38 <sup>c</sup>	3.86±0.53 <sup>a</sup>	51.04±5.18 <sup>b</sup>	146.97±20.38 <sup>c</sup>	86.41±7.37 <sup>a</sup>
Group 4	10.07±1.38 <sup>c</sup>	6.04±1.06 <sup>b</sup>	42.37±7.09 <sup>a</sup>	107.26±14.27 <sup>b</sup>	137.22±14.34 <sup>c</sup>

Values are Mean +SE (n=6); ONE WAY ANOVA (SPSS);

Means with different alphabets as superscripts differ significantly (P< 0.05) with in the column

**Table 10:** Performance of rats on Morris Water Maze

Groups	Escape Latency (in seconds)			Probe trial (sec)	Total Distance travelled (cm)
	EL1	EL2	EL3		
Group 1	46.24±7.31 <sup>a</sup>	38.27±5.13 <sup>a</sup>	37.28±4.91 <sup>a</sup>	35.23±2.90 <sup>a</sup>	378.29±26.26 <sup>a</sup>
Group 2	56.22±5.71 <sup>b</sup>	47.91±4.85 <sup>b</sup>	45.26±3.87 <sup>c</sup>	44.08±3.06 <sup>c</sup>	672.17±31.03 <sup>c</sup>
Group 3	48.14±4.62 <sup>a</sup>	35.26±3.15 <sup>a</sup>	35.09±4.04 <sup>a</sup>	35.01±3.07 <sup>a</sup>	353.04±15.06 <sup>a</sup>
Group 4	55.23±4.28 <sup>b</sup>	46.23±5.09 <sup>b</sup>	41.27±3.05 <sup>bc</sup>	39.36±4.06 <sup>bc</sup>	479.31±21.15 <sup>b</sup>

Values are Mean +SE (n=6); ONE WAY ANOVA (SPSS)

Means with different alphabets as superscripts differ significantly (P< 0.05) with in the column

enzymes, such as inducible nitric oxide synthase, lipooxygenases, cyclooxygenases and xanthine oxidase; and 3) induction of antioxidant enzymes, such as glutathione-S-transferases and superoxide dismutases. Cabrera *et al.* (2006) reported protective role of green tea polyphenols against Parkinson's and Alzheimer's diseases and other neurodegenerative diseases.

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