P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-5* ISSUE-10* January- 2021
E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Testicular Toxicity Following Cadmium Exposure in Rats: Ameliorative Potential of Camelliasinensis

Paper Submission: 15/01/2021, Date of Acceptance: 26/01/2021, Date of Publication: 27/01/2021

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Abstract

In the present study, the effect of green tea extract (GTE) was studied on cadmium induced toxicity in testes of male albino rats (Wistar strain). The effect was studied on total proteins, sugar, lipids, enzymes - alkaline phosphatase (ALP), acid phosphatase (ACP), alanine transaminase (ALT), and aspartate transaminase (AST) in the testes of treated rats. The results show that aqueous extract of green tea restores the normal activity of these enzymes. GTE appears to be beneficial in inhibiting and restoring the testicular injuries induced by $CdCl_2$ intoxication in mammals.

Keywords: GTE, Testicular, Cadmium, Acid Phosphatase, Alkaline Phosphatase, Alanine Transaminase, Aspartate Transaminase.

Introduction

Pollution has become one of the major problems of modern world and heavy metals are also contributors of pollutions. Man is always exposed to toxic metals because they are present in environment including food and drinking water. Cereals which are consumed worldwide, in particular, are the source of this contamination of toxic metals (EFSA 2012 a, b). There is no natural way of reducing the concentration of these metals in the body. Research is being done since long to find out how their absorption can be reduced in the tissues, and thus reducing their toxic effect.

Review of Literature

Cd is able to accumulate in tissues, and even its minute quantity has deleterious effect as it has long half- life, (Winiarska –Mieczan 2014). Toxic action of cadmiumis firstly due to its affinity for thiol, histidine and carboxyl groups of proteins, and attaches itself to active sites of enzymes, (Rubino, 2015). Secondly, calcium in bones and iron in erythrocytes which are necessary for their functioning are replaced by metals, such as cadmium leading to damage and changing their structure, (Puertyo-Parejo et al 201, Jai Shankar et al 2017,). Thirdly reactive form of oxygen (ROS) is increased in the body due to Cd, changing the antioxidant system of the body. (Mao et al, 2018).

From nutritional point of view, food products having antioxidant compounds must be used in daily diet, so that the dangerous effect of this heavy metal can be reduced,Camellia sinensis (Green tea) is one such plant, supposed to possess anti-oxidant properties which is easily available and consumed by masses.

Chemical Constituents of Green Tea

The chemical composition of green tea includes polyphenols (catechins and flavonoids) which are primarily responsible for its remedial quality, the content varies from 30% to 40%. Polyphenols have active hydroxyl hydrogen that can end the chain reaction of excessive free radicals that otherwise result in pathological changes of body. The polyphenols increase the scavenging rate by enhancing the activity of glutathione peroxidase and superoxide dismutase. The major therapeutic benefit of green tea is due to presence of epicatechin, epigallocatechin (ECG), epigallocatechingallate (EGCG)(Butia et al 2015).

The present study is an attempt to study the protective effect of GTE on Cd induced toxicity on testes of albino rats.

Materials and Methods

Preparation of Green Tea Extract

Green tea was procured from Tea State of Tata Group of Company, TALAT, Assam. The green tea leaves were powdered, sieved with a sieve of 0.3 mm aperture size. 100 g of this powder was steeped in 600 ml of distilled water and heated in water bath for 3 hours at 90 ° C.The mixture was cooled and filtrate was used. (Dahiru et. al.)

Aim of this Study

Is to show the reduction of pernicious effect of cadmium exposure hazards, by the antioxidant properties of C. sinensis.

Experimental Animals

Male albino rats, Wistar strain (7-8 weeks old) procured from Animal Division of IVRI, Izzatnagar, Bareilly were maintained in the animal facility of the Zoology Department of Meerut College, Meerut with standard food pellets and tap water ad libitum. Entire experiment was approved and all animals were cared for according to guidelines of the Institutional Animal Ethics Committee (IAEC).

Experimental Design

For two weeks animals were kept on normal diet and acclimatized for laboratory conditions. The duration of experiment was 45 days. Experimental animals were divided into three groups of 18 rats each.

Group I: Normal control group.

Group II: Cadmium control group received 2mg / 100gm body weight of cadmium chloride orally.

Group III: Animals were given a dose of 4.0 mg / 100gm. body weight aqueous extract of C. sinensisleaves orally along with 2 mg cadmium chloride.

Animals of each group were further divided into three sub-groups of six rats each. Animals of subgroup A were treated for 15 days, of subgroup B were treated for 30 days and of subgroup C were treated for 30 days then kept at normal diet for 15 days (total duration of experiments – 45 days) to study whether the effect C.sinenesis is permanent or temporary.

Biochemical Analysis

After completion of the experiment, experimental animalsof each subgroup were accordingly sacrificed under light anaesthesia (diethyl ether), one day after completion of treatment. One testis of each experimental animal was excised immediately for biochemical investigation, and other fixed for histopathological studies. One gm portion of testis was used for homogenate preparation, in ice cold KCl solution (1.15%w/v), using Teflon homogenizer. The homogenate was centrifuged at 4000 x g for 10 minutes to remove precipitated proteins. The supernatant was used for all biochemical estimations. (Dahiruet al 2007)

The parameters selected for biochemical analysis are sugar, total proteins, lipids, alkaline phosphatase (ALP), acidphosphatase (ACP), alaninetransaminase (ALT) and aspartate transaminase (AST)

Statistical Analysis

Results are expressed as Mean ±SD. For calculating level of significance,the Student's t-test was used for pair wise comparison of means. Statistical significance was accepted at $p < 0.05$.

Results

It has been suggested that reproductive system may be more susceptible to cadmium induced damage than other systems, since even low doses can produce haemorrhagic necrosis in the testes, despite the fact that relatively little cadmium can reach this organ (Norberg 1972, Laskey et al 1984). In cadmium control groups, sugar showed decrease after treatment with 2mg. $CdCl_2$ which was increased, by administration of 4 mg./100gm body wt. of GTE. Protein level was lowered by Cd treatment, but was restored by 4mg. GTE feeding. Increase in lipid level was very evident in testes after 2 mg. Cd administration, and 4mg. GTE was able to bring back the lipid level near to normalcy.

The reduced level of alkaline phosphatase was highly significant in testes in cadmium treatment group due to change in levels of protein, sugar and lipids. Treatment with GTE of 4mg. ALP also increased in significant amount. ACP was increased by 2mg. dose of Cd, and decreased in 4mg. C. sinensis dose group. Increase in Alanine transaminase (ALT) and aspartate transaminase (AST) was high in testes of Cd treated rats. The significant elevation in ALT and AST level after Cd treatment was decreased by GTE dose. The reversibility showed stability in the values of all parameters.The levels of all biochemical parameters studied are given in table below.

Parameters	Duration	Control	$CdCl_2$ group	$CdCl_2$ +GTE
		N=6	2mg/100gB.wt	4mg/100gB.wt
				N=6
			N=6	
Sugar(mg/dl)	15days	23.33±2.73	$15.83±2.86^{c}$	$22.17±2.86^{b}$
	30days	24.67±2.42	$14.83±3.19^{c}$	$23.17±3.60^{b}$
	45 days	23.50±1.38	$16.33±2.94^{c}$	$23.00±0.80^{c}$
Total Protein (gm/dl)	15 days	4.28±0.35	$2.47±0.64^{c}$	$3.70±0.28^{b}$
	30 days	4.20±0.18	$2.33±0.45^{c}$	$3.70±0.21^{c}$
	45 days	3.90±0.35	$2.30±0.49^{c}$	$3.67±0.41^{c}$
Lipid(mg/dl)	15 days	51.67±13.82	$77.83±7.70^{b}$	$55.17±5.31^{c}$
	30 days	52.00±3.58	$78.83±4.71^{c}$	$54.33±2.66^{c}$
	45 days	53.67±5.72	$74.83±0.85^{c}$	$55.33±3.27^{c}$
ALP(U/L)	15 days	26.33±5.72	$15.33±1.63^{b}$	$22.67±5.01^{a}$

	30 days	25.83±2.71	13.33±1.21[c]	24.67±2.73[c]
	45 days	24.67±4.50	14.00±2.19[c]	23.33±3.72[c]
ACP(K A unit)	15 days	1.33±0.27	1.80±0.42	1.43±0.23
	30 days	1.10±0.17	1.33±0.33[b]	1.02±0.22[b]
	45 days	1.27±0.16	1.90±0.30	1.33±0.16
ALT(U/L)	15 days	25.00±4.10	33.50±1.52[c]	26.00±3.58[c]
	30 days	24.67±3.27	32.83±3.82[a]	25.00±2.45[b]
	45 days	24.67±3.27	31.83±3.92[b]	25.00±2.45[b]
AST(U/L)	15 days	23.00±2.83	45.00±16.48[a]	28.17±4.02
	30 days	26.17±2.04	45.67±2.34[c]	27.00±4.52[c]
	45 days	24.50±1.97	44.33±2.94[c]	26.33±3.44[c]
Values are mean ± SD. Significance as per Student's "t" test. a=P<0.01, b=P<0.005, c=P<0.001				

Discussion

The testes are the important target organ of Cd toxicity. Although the testicular damage induced by Cd was recognized decades ago,the precise mechanisms underlying its toxicity to the testes remained unclear.Cd may also cause severe damage to the reproductive organs in adults including the ovary and testes, which are sensitive to Cd toxicity (Thompson and Bannigan, 2008).Cadmium has profound effect on sex organ weight, a primary indicator of possible alteration in androgen status (Biswas et al 2001, Laskey et al 1991). Several mechanisms of Cd-induced testicular toxicity have been proposed. In 2000, Lauenteet al., reported increased Cd accumulation in the hypothalamus, pituitary and testes and decreased plasma levels of follicle stimulating hormone in rats, suggesting a possible effect of Cd on the hypothalamic-pituitary-testicular axis.

Although the testes express several antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase to counteract the oxidative stress, their levels are greatly diminished upon Cd exposure (Sen Gupta et.al., 2004). Therefore, it is reasonable to assume that antioxidant agents (enzymatic and non-enzymatic) may prevent or at least reduce the Cd toxicity to the testes.

Basically, the biological activity of polyphenols present in C. sinensis is anchored on their remarkable antioxidant potential. There seem to be two generally accepted mechanisms of polyphenol antioxidant action; the first is the free radical-scavenging mechanism, whereby the polyphenols interfere to break any existing free radical chain reaction. The second being, suppression of free radical formation by chelating metal ions that are involved in free radical production or regulation of enzyme activity (Singh et al 2013). However, another possible antioxidant pathway for these compounds (polyphenols) has been proposed to be a possible interaction with other physiologic antioxidants (Perron et al 2009, Fraga 2010).

Catechin was effective in reducing the $CdCl_2$-induced augmentation of phase I (P450 and CYPB5) as well as phase II (DT-diaphorase and glutathione-S-transferase) enzymes in testes. Furthermore, $CdCl_2$ intoxication was found to attenuate the antioxidant potential of testes, which was however augmented when supplemented with green tea extract. Compared to $CdCl_2$-treated control mice, superoxide dismutase, glutathione peroxidase, glutathione, and catalase levels were significantly decreased in testes. Indeed, green tea catechin significantly increased testicular antioxidant enzymatic activities compared to those given $CdCl_2$ alone (Sharmaand Goel, 2015).

In testes sugar and protein levels were significantly decreased but lipid level was increased in Cd treated group, indicating that Cd damages testes also. Treatment with 4mg. of C. sinensis along with Cd for 15 and 30 days caused decrease in lipid level and increase in sugar and protein levels. Similarly, in the reversibility group, Cd treatment decreased sugar and protein level and increased lipid level, even after 15 days of discontinuation of the treatment. These observations are in accordance with the findings that Cd once accumulated in the tissue is not cleared easily due to long half-life of Cd in body tissues.

The level of protein is related with activities of cell division also. For actively dividing cells proteins are required for formation of new cells. The decreased level of protein is related with destruction of cellular components. Dixit (1978) administered M. conzatti flower extract for 25 days and reported reduced level of proteins in the testes showing mass atrophy of the spermatogenic element. Verma et al (1980) have also reported significant reduction in the level of proteins of gonadal glands after feeding high dose of alcoholic extract of M. conzatti. The difference in the results of the present study and findings of Dixit and Verma et al. may be due to the fact that M. conzatti.has antifertility activity and causes destruction of the testicular elements hence decreased level of proteins. Cadmium is also causing destruction of tissues, so level of proteins is decreased but with C. sinensis extract feeding, normal level of protein indicates no tissue damage, showing normal cell division is taking place in testes.

As far as activity of various enzymes is concerned, level of alkaline phosphatise (ALP) was decreased but level of acid phosphatase (ACP), aspartate transaminase (AST),alanine transaminase (ALT) wasincreased in the testes of Cd control group, as well as Cd control reversibility group.

Present results show that Cd inhibits alkaline phosphatase activity in testes as is indicated by decreased level of ALP in Cd control group. Co-treatment with antioxidant C. sinensis extracts restored activity of ALP in testes.ALP is known to play key role in transport of phosphates through cellular membranes. ALP being the key enzyme for metabolic pathway has to respond to greater number of different controlling sites in addition to the state of plasma membrane.

Acid phosphatase activity showed increase in Cd control group. But treatment with 4mg. dose of GTE showed decrease. Acid phosphatase (ACP) in tissues is associated with breakdown and catalytic activities. High level of ACP is observed in tissues with cell destruction and lytic activities. Increased level of acid phosphatase in testes showed its destructive activity.

ALT and AST activity is considered good indicator of any tissue function. Level of ALT and AST showed increase in Cd treated groups. In reversibility group, ALT remained elevated even after discontinuation of Cd feeding. A dose of 4 mg. of GTE treatment decreased the elevated level of ALT and AST.

Conclusion

As green tea contains numerous flavanols and polyphenols, it can be deducted from above study that presumably they negated the effect of free radical species generated by Cadmium. The protective effect of C. sinensismay have been fostered by altering the enzyme activity, thus the toxicity induced tissue damage was mitigated.

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