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RESEARCH ARTICLE

ASSESSMENT OF PROTECTIVE EFFECT OF CURCUMIN ON CADMIUM CHLORIDE INDUCED TESTICULAR TOXICITY IN MALE SWISS ALBINO MICE (MUS MUSCULUS).

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Abstract

It is a well established fact that cadmium is a known clastogen, mutagen and a carcinogen. Its ingestion results in long term multifaceted alterations and impaired biological and physiological functions. It is also known that curcumin a mandatory dietary condiment has potent healing and curative properties. Hence the aim of the present study was to investigate the protective effects of curcumin against cadmium chloride induced testicular toxicity in male Swiss albino mice (*Mus musculus*). Male Swiss albino mice were divided into four groups, each comprising of six mice. (a) Control group administered only vehicle; (b) experimental group administered only curcumin (10mg/animal/day); (c) experimental group administered a single oral dose of cadmium chloride (50mg/kg/animal/day) and (d) experimental group administered curcumin (10mg/animal/day) for 15 days and on the 16th day cadmium chloride (50mg/kg/animal/day). After completion of dose administration mice were sacrificed and testes were excised. Sections of testes were processed for histopathological, histochemical and biochemical studies. Histopathological examination showed cadmium induced toxicity as manifested by damaged disorganized cellular layers of seminiferous tubules, leydig cells, apoptotic cells, and loss of interstitial tissue ameliorated by the administration of Curcumin. Histochemical staining resulted in altered distribution pattern of lactate dehydrogenase (LDH) enzyme on cadmium administration. The curcumin pre treated group showed an enzymatic distribution profile similar to that of control group. Biochemical test result in a marked decrease in the activity levels of the lactate dehydrogenase (LDH) significantly ($P < 0.05$) in the cadmium treated mice. The degree of decrease of these enzymes was significantly less ($P < 0.05$) when mice were pre treated with curcumin. Curcumin appear to possess an ameliorative potential against cadmium-induced testicular toxicity.

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Introduction:-

The turmeric (*Curcuma longa*) plant is a perennial herb belonging to the Zingiberaceae family, cultivated extensively in south and southeast tropical Asia. The most active component of turmeric is curcumin, which makes up 2 to 5% of the spice (Aggarwal et al. 2006). Curcumin (diferuloylmethane) derived from the rhizomes of turmeric (*Curcuma longa* L.), is the yellow biologically active, non – toxic chemical and has been used in Indian and Chinese traditional medicines for hundreds of years (Zhang et al. 2013). Extensive clinical trials over the past quarter century have addressed the pharmacokinetics, safety, and efficacy of this nutraceutical against numerous diseases in humans. Curcumin possess more than 25 beneficial biological activities viz anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, antifertility, antidiabetic, anticoagulant, and hypocholesteremic etc. and it is a potent inhibitor of various reactive oxygen-generating enzymes (Ruby et al. 1995; Sugiyama et al. 1996;

Chainani 2003; Pandey et al. 2010 and Naksuriya et al. 2014). In Indian subcontinent turmeric is mandatory dietary condiment, and from birth to death it is consumed daily as a food constituent. It can be postulated that the consumption of these condiments and spices may protect individuals from many opportunistic disabilities caused by harmful environmental contaminants. Cadmium is an omnipresent substance present in all strata's of environment viz. air, water and soil. It is a known mutagen, carcinogen and clastogen (Ohsawa and Kawai 1981; Mukherjee, Sharma and Talukder 1988b; Nodberg et al. 2007; ATSDR 2012). It gains entry in humans mainly via oral route (food and water) and inhalation route (dust, fumes and cigarette smoking) (Jurasovic 2004; Mortensen et al. 2011; Neill et al. 2012 and Satarug et al. 2013). In humans it serves no beneficial purpose and once absorbed it remains resident for many years. Various modes and methodologies are being developed to flush out such toxicants from the biological systems (Czekaj et al. 2002; Li et al. 2008; Kundu et al. 2009; Sokkary and Awadalla 2011; Ivanova et al. 2013 and Lu et al. 2013). Hence in the present experiment an effort has been made to assess whether curcumin had the ameliorative potential to prevent cadmium induced testicular toxicity.

Materials and methods:-

Chemicals:-

Cadmium chloride (Molecular weight = 201.32) was obtained from Glaxo company (India). Curcumin was purchased from Loba Chem. Pvt. Ltd. All the other chemicals and solvents used were of analytical grade.

Animals and treatment:-

The study was conducted on adult male Swiss albino mice 32-50 days old and weighing around to 30-40g. These were maintained in plastic cages under controlled lighting conditions (12:12 light: dark cycle) relative humidity (50 \pm 5%) and temperature (37 \pm 2°C), fed with mice feed and had ad libitum access to water.

Experimental protocol:-

The experimental protocol constituted of four groups consisting of six mice per experiment. The doses of cadmium chloride were prepared fresh in distilled water and (0.2ml) was administered by gastric gavages route., Curcumin was administered along with food pellets

Group 1:- Mice were administered only the vehicle (distilled water). The group served as control.

Group 2:- Mice were administered with curcumin (10mg/animal/day).

Group 3:- Mice were administered cadmium chloride at a dose 50mg/kg/animal/day for a day.

Group 4:- Mice were administered curcumin (10mg/animal/day) for 15 days and on the 16th day cadmium chloride (50mg/kg/animal/day) was administered.

24 hrs after administration of last dose, the control and the experimental animals were sacrificed by cervical dislocation and both the testes were dissected out.

Histological preparation:-

Dissected testes were fixed in Bouins solution for 24 hours and subsequently processed for paraffin wax block preparation as per the technique of Drury and Wallington (1967). Routine 5-6 μ thick sections were cut with a rotary microtome. The deparaffinized sections were stained with haematoxylin and eosin stains. Appropriate sections were observed under the microscope and photographed.

Histochemical method:-

Testes were fixed in chilled calcium formol (4°C) and kept in a refrigerator, for 18-20 h and were further processed for localization of LDH as per the technique of Hess, Scarpelli and Pearse (1958).

Biochemical estimation:-

A 10 % (w/v) homogenate of testis was prepared in ice-cold normal saline using a chilled glass-teflon porter tissue grinder tube, and then centrifuged at 3000 rpm for 15 min. The supernatant was used for estimation of LDH. LDH levels were estimated by using kits supplied by Span Diagnostics.

Statistical Analysis: The values were represented as mean \pm SEM at n=6 experiments. The values were taken as significant at P < 0.05 ANOVA (analysis of variance).

Results:-

Histopathological profile of testes:- Histoarchitecture of control and Curcumin treated groups displayed normal morphological profile round seminiferous tubules, compact interstitial connective tissue and germ cells (FIG. 1). In cadmium treated mice (50mg/kg) there was no significant alteration in the oval shape of testis. However, in certain areas induction of hemorrhage in the testicular vasculature was observed. The significant alterations in testicular histoarchitecture, where there was a distinctive variation in the shape of seminiferous tubules which changed from typical round to irregular and testis of Cadmium treated groups showing damaged seminiferous tubules and Leydig cells. Loss of interstitial connective tissue was observed (FIG.1 and FIG.2). In cadmium treated animal due to loss of germinal cells there was a significant expansion in the width of seminiferous tubular lumen which appeared to be time dependent (FIG.3).

As compared to only cadmium treated group a decline in the area of hemorrhage in the testicular vasculature was observed in pre treated group. The loss of cell contact and initiation of exfoliation appeared to be checked and the comparative damage was significantly less. There was no total disruption of cellular association were observed (FIG.4).

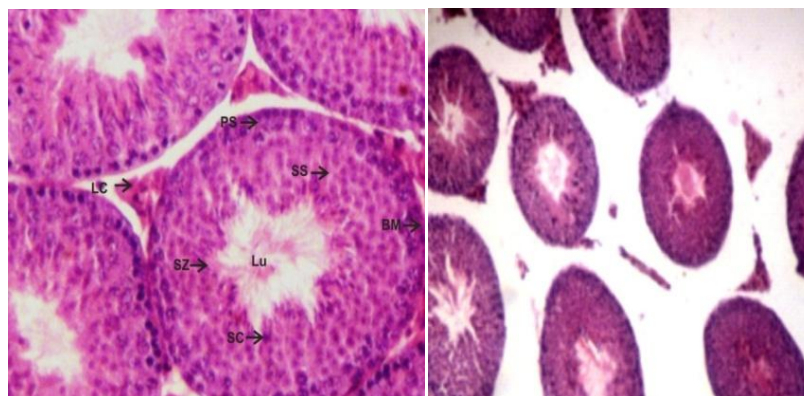


Fig.1

fig.2

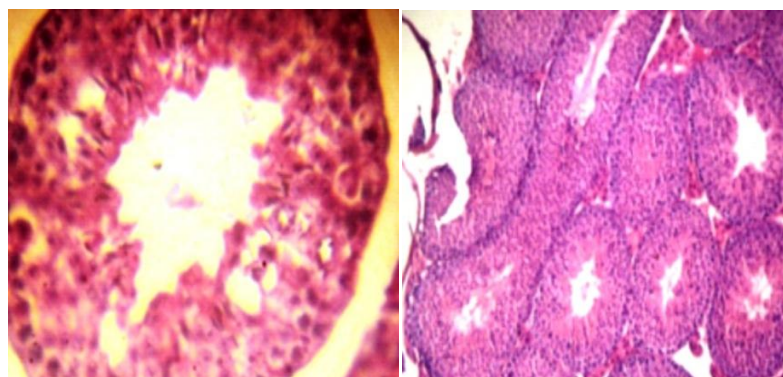


Fig.3

fig.4

Fig.1:- Photomicrograph of testes of control group administered only vehicle. Primary spermatocytes (PS), secondary spermatocytes (SS), spermatids and spermatozoa (SZ) in lumen(LU),basement membrane(BM), Leydig cells (LC), Sertoli cells (SC) are seen.(20x).

Fig.2:- Photomicrograph of testes of CdCl_2 treated group mice. Damaged seminiferous tubules and leydig cells and loss of interstitial tissue are clearly visible. (10X)

Fig.3:- Photomicrograph of testes of CdCl_2 treated group mice. Apototic cells, disorganized cellular layers of seminiferous tubules, loss of cell contact resulting in empty space are visible. (40X)

Fig.4:- Photomicrograph of testes Curcumin pretreated group mice. Packed seminiferous tubules, the loss of cell contact and initiation of exfoliation checked are seen.(10X)

Histochemical profile of ldh in testes:-

The apparent localization of LDH is shown in FIG. 5 and TABLE I. Strong staining is evident in control and Curcumin treated groups, spermatogonia mother cells displayed light to moderate enzyme reactions. As these grow and enter premeiotic prophase, the LDH staining was elevated. The spermatogonia and spermatids in various phases of differentiation exhibited intense enzyme activity. The Sertoli cells also showed moderate LDH activity. Except for Leydig cells which showed intense LDH reaction, the other interstitial cells and tunica did not show any reaction (FIG.5 (a)).

Cadmium chloride (50 mg/kg/ animal for a day) treated group showed differential LDH reaction in various cells of the testes as compared to control. Spermatogonia mother cells and spermatogonia did not show any reaction whereas moderate reaction was seen in spermatids. Light reaction was evinced in Sertoli cells and Leydig cells whereas in other cells of interstitium and tunica negligible reaction was showed (FIG.5 (b)).

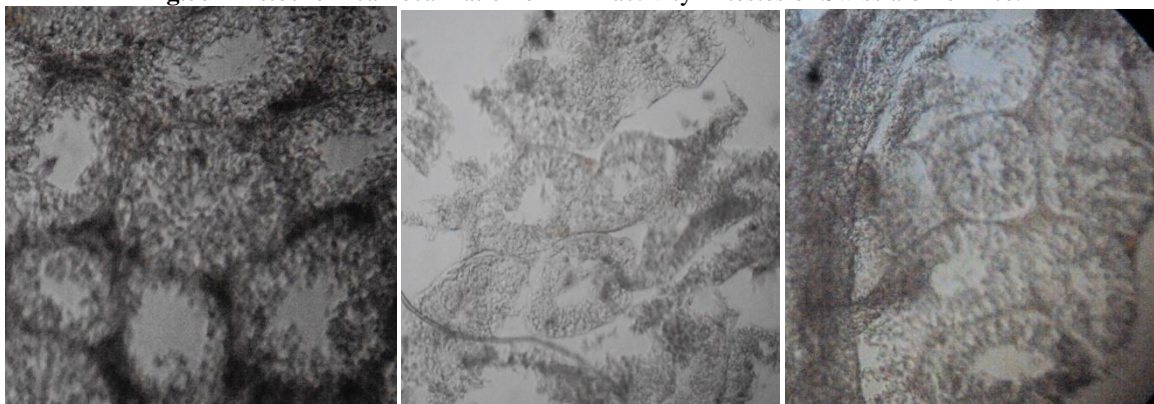
Curcumin pre treated group displayed light reactions in spermatogonia mother cells. The LDH staining was elevated in spermatogonia. The spermatids in various phases of differentiation exhibited moderate enzyme activity. The Sertoli cells and Leydig cells also showed light LDH activity, the other interstitial cells and tunica showed moderate reaction (FIG.5 (c)).

Table I:- Protective effect of curcumin against cadmium chloride induced decline in LDH Activity.

Dose	Spermatogonia Mother Cell	Spermatocytes	Spermatids	Sertoli Cells	Leydig Cells	Tunica
Control	++	++	+	++	++++	-
CUR	++	++	+	++	++++	-
CdCl ₂	-	-	++	+	+	-
CUR+ CdCl ₂	+	+	++	+	++	++

Histochemical index taken for enzymatic activity was as follows: + + + + (very strong) > + + + (strong) > + + (moderate) > + (light) > - (no activity)

Fig.5:- Histochemical localization of LDH activity in testes of Swiss albino mice.



(a) T.S. of control group testes (10X); (b) T.S. of CdCl₂ treated group testes (10X); (c) T.S. of Curcumin pretreated group testes (10X)

Biochemical profile of ldh in testis:-

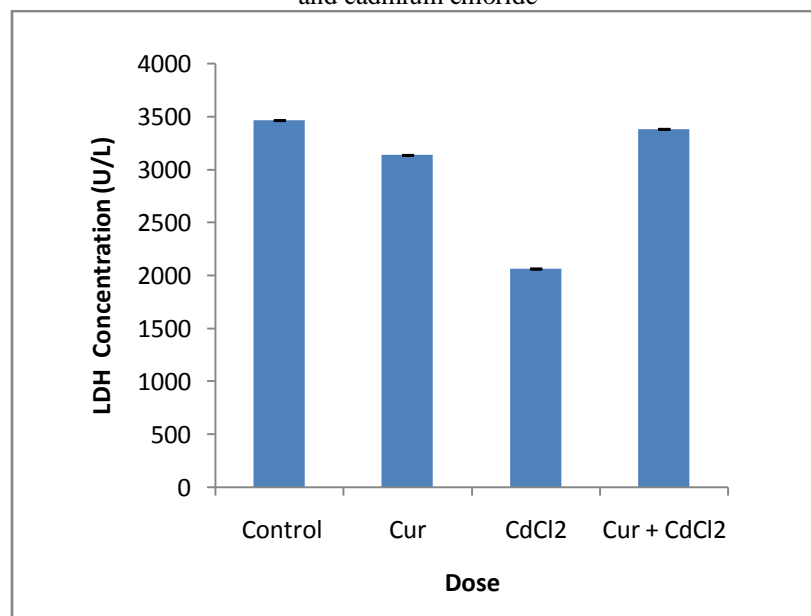
The biochemical results of LDH activity in testes are presented in Table II. The estimates of LDH activity in testes calculate according to the following formula: LDH(U/L) = (Δ Abs/min) x factor, $\epsilon_{\text{NAD/NADH}} = 6230 \text{ M}^{-1} \text{ cm}^{-1}$

LDH activity in cadmium treated group was significantly lower than the activity in the control group testes ($p < 0.05$). However a significant increased in LDH activity in pre treated with curcumin groups when compared with control and cadmium treated groups (FIG.6, table II).

Table II:- Quantitative estimation of LDH (U/L) in testes of male Swiss Albino mice administered cadmium chloride and cadmium chloride.

S.No.	Dose protocol	LDH estimates (Mean \pm SEM)
1	Control	3461.167 \pm 0.989
2	Curcumin(10mg/animal/day)	3133.833 \pm 1.626
3	CdCl ₂	2060.667 \pm 1.223*
4	Cur cumin + CdCl ₂	3379.587 \pm 1.115*,**

Values are Means \pm SEM of 6 animals for each group. Values bearing superscript are significantly different by ANOVA at $p \leq 0.05$. *: when compared different groups with control group.; **: when compared (cur+CdCl₂) with cadmium chloride group.

Fig.6:- Quantitative estimation of LDH (U/L) in testes of male Swiss Albino mice administered cadmium chloride and cadmium chloride

CdCl₂=Cadmium chloride, Cur = Curcumin

Discussion:-

The process of spermatogenesis is a highly sensitive phenomenon depending upon an interplay of a number of enzymes and hormones. Any disturbance in the micro and macro environment of the testis results in the perturbation in sperm production resulting in infertility problems. The management of infertility problems has become an increasingly important part of health services during the past decades. The importance of drugs from plant origin, as fertility regulating agents for the males has long been recognized. Medicinal plants present a repertoire capable of providing varied constituents which could be helpful in infertility management. Curcumin, a potent antioxidant compound found in turmeric, has been used for centuries as a natural dye, seasoning and medicine. In Ayurveda, a 5000 year old system of medicine originating in India, curcumin in turmeric has been used to treat a number of common conditions. Hence, in the present experiment an effort has been made to observe ameliorative effects of curcumin on testicular damage induced by cadmium chloride.

Moreover, cadmium being a metal has been recognized as a reproductive toxicant and has been reported to reduce male fertility and altered sexual behaviour in both humans and rodents (Thomas and Brogan, 1983). Lohiya and Dixit (1976) observed the effect of cadmium chloride on testis fructose contents of shrew (*Suncus murinus* anderson), gerbil (*Meriones hurrianus* jerdon) and hedgehog and reported that a single low dose of subcutaneous injection of cadmium chloride (0.04 m mol/kg body weight) did not cause any change in the macroscopic and microscopic features of testes of *Suncus*. However the testes of gerbil under comparable condition showed significant weight loss and shrinkage in tubule diameter. Destruction of seminiferous epithelium was observed. A

single intratesticular injection of CdCl₂ (0.5 mg/kg body weight) in hedgehog resulted in testicular necrosis. These observations are similar to the present results where it was observed that cadmium treatment resulted in degeneration of seminiferous tubular epithelium. It has been reported that as low as 1-2 mg Cd/kg body wt. can cause testicular damage without pathological changes to other organs (Prozialeck et al., 2006). Acute doses of cadmium are known to have a destructive action on testicular tissue (Parizek, 1960; Gunn and Gould, 1970; Friberg et al., 1974). Necrosis, hemorrhage in testicular vasculature and testicular oedema as observed in the present study which has been also reported in number of earlier studies (Madlafousek, 1971; Choudhury, 2009; Honda et al., 2010 and Tonglian et al., 2011, Singh et al., 2011). Present result shows that cadmium chloride induce perturbations in the normal histology of testis that are ameliorated on pre treatment with curcumin as curcumin thwart per oxidative changes in the testis.

Lactate dehydrogenase form an important class of oxido-reductase which are intimately linked with the events of spermatogenesis and androgenesis. This enzyme has been found mainly in the spermatogonia and spermatocytes, with less activity in the interstitial tissue. Xu Lichun et al. (2000) observed the biochemical toxicity effects of cadmium on testis, different doses of cadmium chloride (0.2, 0.4, 0.8 mg Cd/kg body weight) administered ip to the adult male Sprague Dawley rats. They observed the activities of lactate dehydrogenase isozyme X (LDH X) decreased in the middle and high dose groups significantly. These suggest cadmium toxicity on male reproductive system is associated to activities of testicular enzymes.

Jianguo et al. (1996) observed LDH-X activity of seminiferous tubules of mouse testis by histochemical method after cadmium and cadmium plus zinc administration. They showed that LDH-X activity had no difference between the control group and the group of cadmium administration for 1 hour, but the activity reduced significantly after cadmium administration for 3 hours ($P < 0.05$), and highly significantly reduced for 6, 12 hours ($P < 0.01$). The results suggest that LDH-X activity of seminiferous tubules of mouse testes reduces in acute cadmium poisoning.

Donatus et al. (1990) reported the cytoprotective effect of curcumin in rat hepatocytes. At low concentrations curcumin was found to protect significantly against paracetamol-induced lipid peroxidation, without protection against paracetamol-induced LDH-leakage. At a 100 times higher concentration of curcumin the observed protective effect on LDH-leakage.

Padmaja and Raju (2005) reported that selenium administration resulted in a marked decrease in the activity levels of the liver lactate dehydrogenase in the Wistar rat. The degree of decrease of these enzymes was significantly less ($P < 0.001$) when rats were treated with curcumin, a natural constituent of *Curcuma longa*. Curcumin seems to prevent oxidative damage mediated through selenium and protect the dehydrogenases possibly through its anti-oxidative property.

Sadik (2008) reported that harmful effect of cadmium on testis manifested by germ cell degeneration and impairment of testicular enzymes. Male adult Wistar rats treated with cadmium (2.5 mg/kg body wt, five times a week for 4 weeks) showed decreased testicular enzymes, such as lactate dehydrogenase (LDH) after cadmium exposure.

Zoheb et al. (2014) assessed the toxicity of mixture of six metals including lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), iron (Fe), and copper (Cu) at environmentally realistic concentrations and to determine whether curcumin was able to prevent testicular injury in rats. Administered curcumin (100 mg/kg body wt.), 10 x mixtures, 100 x mixtures, 10x plus curcumin, and 100x plus curcumin. Testes were collected for the analysis of oxidative stress markers, testicular enzymes, and histopathology. Exposure of 10x and 100x mixture significant decrease in LDH activity at 100x exposure level were observed. Lactate dehydrogenase activity was comparable amongst all groups.

El-Fattah et al. (2015) reported the protective role of resveratrol and curcumin on oxidative testicular damage induced by di-(2-ethylhexyl) phthalate (DEHP). Male Wistar rats were given either resveratrol (80 mg/kg BW) or curcumin (200 mg/kg BW) orally for 30 days before and 45 days after DEHP administration. Oxidative damage was observed lactate dehydrogenase (LDH) showed severe declines. Histopathological observations provided evidence for the biochemical and molecular analysis. These DEHP-induced pathological alterations were attenuated by pretreatment with resveratrol and curcumin.

Lonare et al. (2015) reported the toxic effects of imidacloprid (IM) on male reproductive system and ameliorative effect of curcumin (CMN) in male Wistar rats. IM (45 and 90 mg/kg, body weight) and CMN (100 mg/kg, body weight) were administered orally to the rats either alone or in combinations for a period of 28 days. IM treatments resulted in significant decrease ($p < 0.05$) lactate dehydrogenase-x. However, the reproductive toxicity parameters, oxidative stress indicators, and histopathological changes were minimized and functional restorations were noticed by co-administration of CMN in IM-treated rats. Thus, on the basis of above findings it can be concluded that cadmium chloride induce perturbations in the normal histology of testis that are ameliorated on pre treatment with curcumin as curcumin thwart per oxidative changes in the testis. Moreover, it can also be suggested that everyone who is supplementation of curcumin led to the improvement in altered structure of testis due to cadmium chloride intoxication. This mitigating effect can be attributed to the potential of curcumin in inducing antioxidant defense mechanisms reported in earlier studies (El-Bahr and Salama 2007; El-Wakf et al., 2011). These observations are similar to the research findings of several researchers who have reported that curcumin has the innate potential to counterbalance reproductive damage induced by heavy metals viz. chromium (Chandra et al., 2007) and arsenic (Demerdash et al., 2009).

Conclusion:-

In present study, concluded that where testes is greatly targeted to damage by cadmium intoxication. Along with evidence derived from present study, where exposure to cadmium constitutes a great threat being associated with reproductive injurious effects. Hence, concern should be directed to limit the inadvertent incorporation of cadmium in human body. The curcumin has the ameliorative potential to prevent cadmium induced testicular toxicity.

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