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ORIGINAL ARTICLE

Studies on the Effect of *Asteracantha longifolia* Seed Powder on Cadmium Chloride Induced Testicular (Micrometry) Changes in Albino Rats

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Abstract

The present study was carried out to evaluate the effect of *Asteracantha longifolia* seed powder on cadmium chloride induced micrometry changes in testis of albino rats by means of an ocular micrometry. Thirty male albino rats were divided equally in to three groups; the experimental period was 60 days. Group I was fed on balanced diet of rat pellet, group II rats were given cadmium chloride in drinking water at a dose of 200 ppm daily and group III rats were fed on *A. longifolia* seed powder thoroughly mixed with rat feed at a dose of 500ppm level, simultaneously the rats were given $CdCl_2$ at a dose of 200ppm in drinking water throughout the experimental period. It is concluded that oral administration of *A. longifolia* seed powder significantly improved the micrometrical changes in testis and accessory sex organs, reducing the severity of $CdCl_2$ toxicity in male rats.

Keywords: *Asteracantha longifolia*, Cadmium chloride, Testis, Micrometry.

1. Introduction

Cadmium (Cd), a heavy metal, is toxic to both humans and animals. Cadmium in its elemental form occurs naturally in earth's crust and it is unusual to find in its pure form. It is commonly found in combination with other elements such as oxygen (cadmium oxide), sulfur (cadmium sulfate), chloride (cadmium chloride) and carbon (cadmium carbonate). The major source of cadmium to the environment include battery manufacturing (Adams,1992), plastic industries, smelting and refining of metals e.g. Zinc refining/ Cadmium smelting and production (Ellis *et al.*,1985), lead smelting and refining, iron and steel production and cadmium containing pigment production. Due to its favourable chemical properties, large scale use of cadmium started during 1940s (Muller *et al.*, 1994). Exposure to cadmium damages many organs including lungs, brain, liver, kidney and testis (Hardman *et al.*, 2001). Acute cadmium exposure induces circulatory defects in testis (Gunn *et al.*, 1975). The resultant hypoxia/ischemia causes a spectrum of secondary effects including seminiferous tubules necrosis and leydig cells damage within 24 hrs (Gunn *et al.*, 1975; Barlow *et al.*, 1982). Acute exposure to low doses of cadmium impacts mainly testis, even though liver, kidney, pancreas and spleen have higher affinities for cadmium (Lucis *et al*., 1972). The effects of cadmium on the testis appear to be manifested mainly in the sertoli cells, which showed more morphological changes under scanning electron microscopy. It also causes derangements in spermatogenesis and spermiogenesis (Boscolo *et al*., 1985).

Asteracantha longifolia (L.) Nees, Acanthaceae, is a source of the ayurvedic drug, 'Kokilaaksha' and the Unani drug, Talimakhana. *A. longifolia* belongs to the Ayurveda class of 'Vajikaran' for enhancement of sexual performance and also used as general tonic, sedative, antihistaminic, hepatostimulant and diuretic (Ahmed *et al.*, 2001; Sunitha *et al.*, 2008).

A. longifolia is known since the ancient ages in India for its medicinal values. The roots of *A. longifolia* Nees used as tonic, hypnotic, antidiarrhoeic, antidysentric, aphrodisiac, hepatoprotective and antianaemic (Kirtikar and Basu, 1935). The seeds of *A. longifolia* were used in impotency and seminal or other debilities as an aphrodisiac (Nadkarni, 1954). The seeds extract of *A. longifolia* in rats showed androgenic as well as aphrodisiac activity in dose dependent manner (Chauhan *et al.*, 2011; Sahu *et al.*, 2010). The ethanolic extract showed androgenic activity by increasing testosterone level and marked improvement in histoarchitecture of testis and sperm count in epididymus (Chauhan *et al.*, 2009; 2010). The *A. longifolia* Nees has shown to possess hypoglycemic activity in human subjects (Fernando *et al*., 1989), hepatoprotective activity against paracetamol and thioacetamide intoxification in rats (Singh and Handa, 1999) and carbon tetrachloride induced liver dysfunctions (Shailajan *et al.,* 2005), antitumour (Mazumdar *et al.,* 1997), anabolic and androgenic activities (Jayatilak *et al.*, 1976). The antinociceptive effect of *A. longifolia* Nees, has been proved using chemical and thermal method of nociception in mice (Shanmugasundaram and Venkataraman, 2005). Protection of betulin, an aliphatic ester present in *A. longifolia* on cadmium chloride induced cytotoxicity in HepG2 cells appeared to be related to the inhibition of apoptosis, as determined by PI staining and DNA fragmentation analysis (Oh *et al.*, 2006).

Hence this study was aimed to understand the effects of simultaneous administration of Cadmium chloride and *A. longifolia* seed powder on testicular micrometrical values, it was hypothesized that *A. longifolia* will have protective effects against cadmium induced toxicity in testis.

2. Materials and Methods

2.1 Chemicals and Plant Materials

Cadmium chloride ($CdCl_2$, Qualigens, Mumbai) was used to induce cadmium toxicity in male wistar rats. The seeds of *A. longifolia* Nees were procured from local market and identified by Department of Botany, Marathwada Agricultural University, Parbhani, Maharashtra (MS). The seeds were crushed in to coarse powder, packed and sealed in airtight container and used as per requirement for experimental studies.

2.2 Experimental Animals

The investigation was carried out on thirty male wistar rats (120-180 gms), procured from Raj Biotech India Pvt Ltd., Wing, Satara, Maharashtra, India and maintained at Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Maharashtra Animal Fisheries Sciences University, Parbhani, Maharashtra, India. Rat chow and water were given ad libitum. The rats were housed under temperature controlled (22-25^{o}C) conditions with a 12:12 light: dark cycle. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC, Project No: 04/Pharmacology/2007, Dt 5/2/2007).

Rats were randomly selected divided into 3 groups of 10 rats. The study duration was sixty consecutive days. Group I was fed on balanced diet of rat pellets, group II rats were given freshly prepared cadmium chloride solution in the deionized drinking water at a dose of 200ppm daily for 60 days. The rats in group III were fed with *A. longifolia* plant powder thoroughly mixed in rat feed at the concentration of 500 ppm level. Simultaneously these rats were administered cadmium chloride at a dose of 200ppm in deionized drinking water throughout the experimental period.

At the end of experimental period all the experimental animals were sacrificed by use of gaseous inhalation (chloroform), animal dissected, organs are collected and fascia is removed for further examination. The histological sections of testis and accessory sex organs were subjected to micrometry to study the size of important structures by means of an ocular micrometry. The micrometry included the following aspects *viz.* thickness of the capsules of testis, diameter of seminiferous tubules, thickness of interstitial space, height of sertoli cells, diameter of various stages of germ cells, diameter of lumen of seminal vesicles, height of secretary epithelial cells of seminal vesicles and diameter of lumen of prostate gland. The micrometry was carried out by examining the maximum possible histostructures in many slides of each tissue. All the measurements were expressed as Mean ± Standard Error.

3. Results and Discussion

The results of the study on cadmium chloride treatment and cadmium chloride with *A. longifolia* seed powder on micrometry of testis, accessory sex organs, liver and kidney were depicted. There was no treatment related adverse reaction in rats and mortality, the animals were apparently healthy throughout experimental period. Table 1 summarizes the mean micrometry values of different anatomical structure of testis. There was no significant difference in thickness of capsule of testis between the three groups. The diameter of seminiferous tubules and height of sertoli cells were significantly lowered. Thickness of the interstitial space was increased upon administration of $CdCl_2$ (Group-II) as compared to control (Group-I). Concurrent administration of $CdCl_2$ with *A. longifolia* seed powder significantly improved the diameter of seminiferous tubules, increased height of sertoli cells and reduced the increased thickness of interstitial space caused by the $CdCl_2$.

Table 2 summarizes the mean micrometry values of diameter of different stages of germ cells. All the stages of germ cell diameter were significantly decreased ($P<0.01$) when treated with $CdCl_2$ alone (Group-II) as compared to control (Group-I). However, $CdCl_2$ with *A. longifolia* seed powder (Group-III) significantly increased the micrometric measurements of spermatogonia, primary spermatocytes, secondary

Table 1: Effect of $CdCl_2$ alone and $CdCl_2$ with *A.longifolia* seed powder on micrometry of testis of rats

Group No	Treatment	Thickness of capsule (µ) Mean±SE	Diameter of seminiferous tubules (µ) Mean±SE	Thickness of interstitial space (µ) Mean±SE	Height of sertoli cells (µ) Mean±SE
I	Control	59.64*±5.38 (32.24-80.06)	292.75*±2.0 (279.87-302.12)	44.03*±1.37 (37.92-49.9)	61.6*±2.02 (52.7-70)
II	$CdCl_2$ alone	51.58**±2.14 (48.30-64.48)	186.5**±9.0 (107.34-205.72)	51.58**±4.01 (32.24-64.48)	33.95**±1.80 (28-45.5)
III	$CdCl_2$ with *A. longifolia* seed powder	54.80**±2.62 (48.36-64.48)	266.71***±1.75 (252.74-274.33)	36.47***±0.78 (32.89-40.11)	46.6**±2.8 (35-60.5)
	CD	10.7	16	7.2	6.5

$^{*}P < 0.001$; $^{**}P < 0.01$; $^{***}P < 0.05$; Number of rats =10 in each group.

Table 2: Effect of $CdCl_2$ alone and $CdCl_2$ with *A.longifolia* seed powder on micrometry of germ cells of rats

Group No.	Treatment	Spermatogonia (µ) Mean±SE	Primary spermatocytes (µ) Mean±SE	Secondary spermatocytes (µ) Mean±SE	Spermatids(µ) Mean±SE
I	Control	4.28*±0.05 (4.05-4.5)	12.25*±0.6 (10.5-14)	5.23±0.6 (4.2-10.5)	5.17*±0.2 (4.5-5.25)
II	$CdCl_2$ alone	3.93**±0.05 (3.85-4.2)	8.4**±0.6 (7-10.5)	3.61±0.07 (3.5-4.2)	3.7**±0.13 (3.5-4.5)
III	$CdCl_2$ with *A. longifolia* seed powder	3.99**±0.08 (3.85-4.55)	8.75**±0.6 (7-10.5)	4.62±0.5 (3.5-7)	3.78**±0.10 (3.5-4.2)
	CD	0.8	1.7	1.4	0.48

$^{*}P < 0.001$; $^{**}P < 0.01$; $^{***}P < 0.05$; Number of rats =10 in each group.

Table 3: Effect of $CdCl_2$ alone and $CdCl_2$ with *A. longifolia* seed powder on seminal vesicles and prostate gland of rats

Group No.	Treatment	Diameter of lumen of seminal vesicles (µ) Mean±SE	Height of secretary epithelial cells (µ) Mean±SE	Diameter of lumen of prostate gland(µ) Mean±SE
I	Control	317.59*±9.0 (270.99-354.55)	16.1*±0.6 (14-17.5)	286.82*±7.2 (260.79-336.02)
II	$CdCl_2$	187.04**±8.0 (148.97-221.02)	6.36**±0.9 (3.5-10.5)	119.85**±5.07 (99.97-142.02)
III	$CdCl_2$ with *A. longifolia* seed powder	278.80***±9.0 (228.71-315.35)	11.55**±0.5 (10.5-14)	190.21***±7.7 (120.97-224.04)
	CD	26.3	2	24.63

$^{*}P < 0.001$; $^{**}P < 0.01$; $^{***}P < 0.05$; Number of rats =10 in each group.

spermatocytes and spermatids. Micrometric changes in testis induced by cadmium chloride in rats were drastically improved by concurrent administration of the *A. longifolia*, which could have exerted androgen like activity necessary for spermatogenesis in rats (Steinberger and Duckett, 1965). It is well known fact that cadmium reduces testosterone production and disrupt in regulatory mechanism of hypothalamic-pituitory-gonadal axis (Lafuente *et al.,* 2001; Pillai *et al.,* 2002).

Table 3 summarizes the micrometric measurement of seminal vesicles and prostate gland of rats. There was significant decrease ($P<0.01$) in diameter of lumen of seminal vesicles, height of secretary epithelial cells and diameter of lumen of prostate gland in $CdCl_2$ treated rats (Group-II) as compared to control (Group-I). Further, there was an increase in the above parameters among the $CdCl_2$ with *A. longifolia* seed powder treated rats (Group-III) compared to $CdCl_2$ treated rats (Group-II). Similar observations were made regarding the micrometric changes for seminal vesicles and prostate gland in *A. longifolia* treated rats by earlier studies (Wale, 2004).

Conclusion

It is concluded from the above study that, oral administration of *A. longifolia* seed powder for 60 days at a dose of 500ppm significantly improved the micrometric changes of testis and accessory sex organs reducing the severity of cadmium chloride toxicity in male wistar rats.

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