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RESEARCH ARTICLE

Protective role of Bamboo (*Dendrocalamus strictus*) seed extract on endosulfan treated male rat testes and fertility

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ABSTRACT

In the present study, the effect of Bamboo seed extract was evaluated in endosulfan treated testes of male wistar rats. Effect of pesticide treatment and pesticide along with bamboo seed extract were studied through serum hormonal analysis (FSH, LH and Testosterone), testicular biochemistry (ascorbic acid), sperm function analysis. Administration of bamboo seed extract in endosulfan treated rat shows the revival of reproductive function by protecting the reproductive organ from endosulfan induced oxidative stress mediated testicular toxicity. The present study also reveals the bamboo seed extract significantly enhancing the male fertility by reversing the endosulfan induced adverse effect on rat testes.

INTRODUCTION

More than 90% of male infertility cases are due to low sperm counts, poor sperm quality or both (Stephen *et al.,* 1996). These manifestations resulted due to the endocrine disruptor present in the environment as a pollutant. Organochlorine pesticides are the major EDCs (Endocrine disrupting chemicals) which have widespread applications with regard to the field of agriculture (Simonich and Hite, 1995). Among the organochlorines pesticides (EDCs), endosulfan is the most commonly used pesticide; hence this study concentrates on the effect of endosulfan on reproductivity (Jayashree *et al*., 2006). It has been suggested that endosulfan exposure may delay sexual maturity and interfere with hormone synthesis in male children (Habibullah Saiyed *et al*., 2003). Delayed puberty was related to endosulfan as shown in human studies (referenced Den Hond, 2006). Hence this study concentrate on the effect of endosulfan on male rat reproductivity. The biosynthesis and secretion of testosterone, the vital hormone for male fertility are found to be affected by the endosulfan treatment at the varying doses (Marilyn 2000). Even the pesticides endosulfan has caused a number of adverse effects on most of the male reproductive parameters in rats, such as reduced fertility, degeneration of seminiferous tubule epithelium, reduced sperm count, altered spermatogenesis, increased abnormal sperm, testicular necrosis, and aspermatogenesis (Dalsenter *et al* 1999; ATSDR 2000; Sinha *et al.,* 2001). As an endocrine disruptor, it is oestrogenic and antiandrogenic in human cell and causes chronic depression of testosterone by interfering with male hormones (PANAP-2008). Both animal toxicology and human epidemiologic studies have revealed that pesticides may operate through hormonal or genotoxic pathways to affect spermatogenesis (Toppari *et al.*, 1996). Endosulfan was identified as a chemical inducing alteration in the activity of enzymes involved in oxidative stress and lipid peroxidation (Dorval *et al*., 2003; Frederick and Panemangalore, 2003). Several studies with liver, brain, and testis indicate that lindane (an organochlorine insecticide) causes oxidative stress (Sahoo *et al*., 2000; Junquira *et al*., 1988; Abdollahi *et al*., 2004). Environmental toxicity through pesticides is widespread, causing endocrine imbalance especially in male reproductive system. Therefore, there is a need to enhance reproductive efficiency using natural therapy of the effected organisms. It has been ethnically believed among tribes that bamboo seed consumption enhanced reproductive efficiency and increased the population of rat. It is also claimed that the bamboo seeds, which are favourites of the rodents, increase their fertility to such an extent that the number of their litter jumps from the normal 6-8 to 12-18 (Rokhuma, 1988). Pal (1993) stated that the sudden availability of nutritious food is likely to increase the prodigality of the local rodent population and then the population starts increasing. So the present study mainly focus on the role of bamboo seed extract on male reproductive status and its reproductive enhancing capability on endosulfan treated rats. It has been found that bamboo seeds components found to consist of carbohydrates, crude protein, calcium, phosphorous, riboflavin and high calories etc and bamboo seed are nutrient rich, having the overall nutritive quality slightly greater than rice and wheat (Lakshminarayana *et al*., 1955).

The kani tribes of Kanyakumari district Tamil Nadu not only use the seeds as a food, but also as commercial commodity to improve the economy and believed that the seed of *Bambusa arundinacea* enhance the fertility, and has a great demand of seeds of this species in pharmaceutical industry to manufacture drugs to improve fertility (Kiraba *et al.,* 2007). Dissection of adult females shows 35-42 little placental scars on the uterus which delivers even more convincing proof that a female black rats produced about as many as 40 pups during Mautam (bamboo flowering). Normally, a female black rat might produce only two litters in a lifetime; however, this appears to be doubled during bamboo flowering as they consumed the seed

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developing from the bamboo flower (Lalnunmawia, 2009). Bamboo plant grows particularly in Southeast Asia and represents a traditionally important commodity used for building materials, in traditional medicine, and as a source of food. Plant extracts, especially from bamboo leaves and bamboo roots, had been studied on their bioactivity (Chuyen *et al.* 1982, Nishina *et al.*1991). The Bamboo (*Phyllostachys edulis*) leaves have been also utilized clinically in the treatment of hypertension, arteriosclerosis, cardiovascular disease, and certain forms of cancer (Shibata *et al*. 1975). Bamboo seed is rich in phenolic components which possesses various bioactivities (Ikeshima 1999). Previous studies have reported the antioxidative activity of bamboo seed extract by in vitro assays like 1,1-diphenyl-2-picrylhydrazyl (DPPH assay), the inhibition of lipid peroxidation and reduction power (MuJun *et al.*,2004). It has been learnt from farmers that bamboo seed consumption enhances the reproductive efficiency and increases the population of rat in the field. Hence the present study has been decided to study the possible protective role of bamboo (*Dendrocalamus strictus)* seed extract in endosulfan induced reproductive toxicity of male rat testes.

MATERIAL AND METHODS

All the animal treatment and protocol employed in the study was approved by IAEC Institutional Animal Ethical Committee (Ref .No-BDU/IAE/2010/07). The male albino rats of Wister strain in adult stage weighing 160-180g were used in the study. The rats were maintained in normal day and night schedule (12 hour light: 12 hour dark). Each rat was given pellet foodstuff from Amrut laboratory animal feed, Pune, India for feeding and water ad libitum.

The animal were divided into four groups (n=6) after recording their initial body weight and they were treated orally for 10days.Group I(control) animals were given 0.5ml distilled water. Group II animals were given 0.5ml endosulfan (15mg/kg body weight). In group III the animals were treated with 0.5ml endosulfan (15mg/kg body weight) and bamboo (*Dendrocalamus strictus*) seed extract (90mg/kg body weight). Group IV animals were treated with 0.5ml bamboo seed extract (90mg/kg body weight) alone.

In the last day of experimental period before scarifying the animal the final body weight were collected and the entire animal groups were dispossessed of food overnight and then sacrificed by decapitation. Blood was collected by cardiac puncture in serum tubes and the serum separated was use for hormonal analysis. The weight of reproductive organ such as testes, epididymis and prostate gland were measured with the use of electronic balance. Then the tissues were processed for sperm functions analysis and testicular biochemistry. The hormonal assay for FSH, LH and testosterone were estimated by radio immunoassay kit. To check the sperm function, the sperm counts were made according to Gopalkrishnan *et al.,* (1987) and for sperm viability, it is carried out by the methods of Mortimer, D (1985, 1994). The ascorbic acid assay was estimated by the method of Omaye *et al.,* (1979) as a part of testicular biochemistry. Results were expressed as mean ± S.D of number of experiments. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 7.5 (SPSS, Cary, NC, USA) and DMRT was use to obtained individual comparison. A value of $P<0.5$ was considered to indicate a significant difference between groups.

RESULT AND DISCUSSION

The impact of endosulfan and bamboo (*Dendrocalamus strictus*) seed extract on adult male wistar rats were analysed in the present study. The endosulfan treated rats showed a significant decrease in body weight as compared with the control rats (Table 1). Interestingly it was significantly increased in endosulfan and bamboo seed extract treated group. The weight of testes showed significant difference between control and treated groups. There were no significant changes observed in the weight of epididymis and prostate gland. Though the post hoc analysis revealed the significant variation between control and treated groups. The combined treatment (endosulfan+bamboo seed extracts) had a stimulatory effect and increases the weight of testes. The testes weight of this group was significantly different from the control and endosulfan treated group. Among all the groups the absolute weight of testes was found to be high in seed extract alone treated group. The decrease in testicular weight of endosulfan treated rats may indicate impairment at testicular, pituitary or hypothalamic level.

Table 1. Effect of pesticides and bamboo seed extract on body weight and reproductive organs weight in control and experimental rats

Variables		Control	Endosulfan	Endosulfan + bamboo seed extract	Bamboo seed extract alone
Body weight	Initial (g)	170.0 ± 0.03^{b}	170.0 ± 0.01^{b}	160.0 ± 0.02^{a}	176.7 ± 5.16^{c}
	Final (g)	$181.7\pm7.53^{b,c}$	155.0 ± 7.74^{a}	179.0 ± 2.04^{b}	188.3 ± 2.58^{d}
Testes (g)		2.180 ± 0.33^{b}	2.071 ± 0.01^{a}	2.303 ± 0.14^{c}	2.378 ± 0.22^{d}
Epididymus (g)		0.621 ± 0.03^{a}	$0.636\pm0.04^{a,b}$	0.688 ± 0.06^{c}	0.690 ± 0.01^{c}
Prostate gland (g)		0.073 ± 0.01^{c}	0.059 ± 0.02^{a}	0.068 ± 0.01^{b}	0.080 ± 0.03^{d}

Values are articulated in mean ± SD for six rats in each group. Values with identical letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

Table 2. Effect of pesticides and bamboo seed extract on serum hormonal level and testicular biochemistry |(ascorbic acid estimation) in control and experimental rats

Variables	Control	Endosulfan	Endosulfan + bamboo seed extract	Bamboo seed extract alone
FSH(IU/ml)	0.059 ± 0.001^{c}	0.050 ± 0.001^{a}	0.057 ± 0.001^{b}	0.060 ± 0.003^{d}
LH(IU/ml)	0.073 ± 0.002^{c}	0.069 ± 0.001^{a}	0.071 ± 0.003^{b}	0.079 ± 0.001^{d}
Testosterone (ng/ml)	3.97 ± 0.003^{b}	2.79 ± 0.002^{a}	9.87 ± 0.001^{d}	4.37 ± 0.001^{c}
Ascorbic acid	1.185 ± 0.001^{b}	0.037 ± 0.003^{a}	1.410 ± 0.002^{c}	2.548 ± 0.007^{d}

Values are articulated in mean ± SD for six rats in each group. Values with identical letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

Table 3. Effect of pesticides and bamboo seed extract on sperm function analysis in control and experimental rats

Variables	Control	Endosulfan	Endosulfan + bamboo seed extract	Bamboo seed extract alone
Sperm count (millions/ml)	31.7 ± 0.16^{c}	8.04 ± 0.01^{a}	28.1 ± 0.06^{b}	42.3 ± 0.12^{d}
Sperm viability	76.26 ± 0.01^{c}	16.34 ± 0.01^{a}	65.18 ± 0.02^{b}	78.09 ± 0.09^{d}
Sperm non-viability	23.74 ± 0.02^{b}	83.65 ± 0.02^{d}	25.11 ± 0.01^{c}	21.91 ± 0.01^{a}

Values are articulated in mean ± SD for six rats in each group. Values with identical letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

The androgen dependent organs such as epididymis, seminal vesicles and prostate gland are all relying on testosterone for their growth and function (Klinefelter *et al.,* 1988). A reduction in their weight may reflect a decreased bioavailability and production of androgens (Mathur *et al.,* 1982). The FSH level and LH level was significantly changed in all the groups as noted by the post hoc analysis observation (Table-2). The level of testosterone was significantly varied among the groups. The post hoc analysis revealed a significantly higher level of testosterone in combined treatment (endosulfan+bamboo seed extracts) than that of the control, endosulfan treated and seed extract alone treated group. Testosterone is known to be critically involved in the development of sperm cells and deranged results widely in leydig cell dysfunction and testicular steriodogenic disorder (Zhang *et al.,* 2001). Testosterone is a male hormone that has a significant impact on spermatogenesis (Lee *et al.,* 2001) where endosulfan has been reported to interfere in the spermatogenic (Sinha *et al.,* 1995) and steriodogenic cycle in adult (Singh and Pandey 1989, 1990). Endosulfan has been shown to reduce plasma FSH, LH and testosterone in rats and cause testicular damage (Singh *et al.,* 1996; Srivastava *et al.,* 1980). In this current study also the significant lower level of testosterone was noted in the endosulfan treated group whereas increased level of testosterone was observed in combined treatment. Even the seed extract treated alone showed higher level of testosterone than control group. Hence, the bamboo seed extract has enhanced the testosterone secretion that can increase the fertility of sperm. The data on testicular biochemistry, that is the ascorbic acid estimation in testes showed a statistical significant variation (Table 2). The ascorbic acid concentration was comparatively low in endosulfan treated group while there was a higher concentration or notable increased in ascorbic acid concentration on combined treatment (endosulfan+bamboo seed extracts) than that of the control and endosulfan treated group. The seed extract alone treated group showed significantly higher level of ascorbic acid when compared to control, endosulfan treated group and combined treatment (endosulfan+bamboo seed extracts)group.

A decrease in the concentration of ascorbic acid in the testes may reflect a decrease in testicular steriodogenesis (Dabrowski *et al.,* 1996). The seed extract administration to endosulfan induced rat restored the concentration of ascorbic acid thereby preventing endosulfan induced oxidative stress mediated testicular damage and it has been strongly supported with the result of seed extract alone treatment group. Ascorbic acid in semen has been shown to play an important role in preventing oxidative damage to spermatozoa (Latchoumycandane *et al.,* 1999). The evaluation in a study revealed that the toxicity of endosulfan on reproductivity organs rather than reproductive function showed evidence of the ability of endosulfan to adversely affect male reproductive organs (Gupta and Gupta 1977a; Khan and Sinha 1996; NCI 1978; Singh and Pandey 1989; Singh and Pandey 1990; Sinha *et al.,* 1997). Administration of endosulfan caused a significant effect on sperm with notable reduction in both the sperm count and sperm viability. Similar result was observed in male mice showing a significant reduction in sperm counts after 35 days of treatment with 3mg/kg/day (Khan and Sinha 1996) and a related effect were seen in adult male rats treated with 5mg/kg/day for 70 days endosulfan (Sinha *et al.,* 1995). Comparatively the oral administration of seed extract to endosulfan treated rat showed significant restoration of sperm count and sperm viability (Table-3). The highest sperm count and sperm viability was observed in seed extract alone treated group when compared to control, endosulfan treated group and combined treatment (endosulfan+bamboo seed extracts) group. The same result was also revealed in post hoc analysis showing, the revival of male rat fertility by the administration of bamboo seed extract. From this investigation it has been concluded that the treatment of bamboo seed extract would have adverse effect on reviving the reproductive toxicity induced by the endosulfan in male rat testes. Additionally bamboo seed extract similarly act as an antioxidant as it restored the level of ascorbic acid in high level in male rat testes.

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