Original Paper

Toxicological Evaluation of Ingestion of Raw Bamboo (*Bambusa arundinacea*) Shoots on Male Reproductive System of Rat

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ABSTRACT

Bamboo shoots (*Bambusa arundinacea*) are well known as highly nutritious food used in chutney and pickles in Indian cuisine. But they contain a potent antithyroid cyanogenic glycoside to the extent of 800-1000mg/kg. Abnormal thyroid function can influence normal reproductive function.

The current study was designed to evaluate the morphological and functional status of testes after ingestion of raw *Bambusa arundinacea* shoots for two consecutive reproductive cycles. The experimental animals were divided into two groups: six in each division. Control group rats were maintained with normal diet, while the treatment group received a diet with 1/3 amount supplemented by raw *Bambusa arundinacea* shoots.

The results show that raw *Bambusa arundinacea* shoots intake could lower bodyweight as well as testicular weight; testosterone level and sperm count could also undergo decrease, with inhibition of Δ5-3β HSD and 17β HSD.

Key Words: *Bambusa arundinacea*; Male reproductive toxicology; Testicular function; Food toxicity

Introduction

Young shoots of bamboo plant (*Bambusa arundinacea*) usually harvested before they are two weeks old are regarded as highly nutritious, low in fat and calories, high in potassium and dietary fibre, all of which help in maintaining normal blood pressure, steady heart rate, low cholesterol level and prevention of colon cancer.[1] Bamboo shoots however contain a potent antithyroid agent cyanogenic glycoside to the extent of 800–1000 mg/kg. This cyanogenic glycoside is hydrolyzed in the gut resulting in the production of glucose and hydroxybenzaldehyde cyanohydrin. The latter then decomposes to hydroxyl benzaldehyde and hydrogen cyanide which are then converted to thiocyanate.[2] Toxicity of cyanogenic plants depend primarily on the potential concentration of hydrogen cyanide.[3,4] The lethal dose of HCN has been reported to be 10–15 mg/kg body weight for various species of albino rat, whereas 3.7 mg/kg body weight has been reported for mouse.[5]

Thiocyanate is an anion that blocks iodine channel and causes goitre. It is well known that abnormal thyroid function can influence normal reproductive function. But there is very little information about the antifertility effect of *Bambusa arundinacea* shoot extracts in male rats, and animal studies are not sufficient enough to establish the effect of ingestion of raw shoots of this plant on the male reproductive system.

In the current study, raw *Bambusa arundinacea* shoots were selected to evaluate the morphological and functional status of testes by measuring the body weight, testicular weight, sperm count, assay of serum testosterone level, and Δ5-3β HSD and 17β HSD activity.

Materials and Methods

Maintenance of experimental animals: In the present study, 12 male mature albino rats of Wistar strain weighing about 198 ± 8.27 grams were selected. The rats were divided into two equal groups of "control" and "treatment"

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each consisting of 6 rats. All animal were caged in unheated, well ventilated (25° ± 2°C with plenty of light and air) stainless steel cages and acclimatized to the conditions for at least one week prior to the actual experimental under 12:12 h L:D cycle. The rats were maintained on standard diet ad libitum: wheat (60%), Bengal gram (30%), salt mixture (3%), milk powder (5%), groundnut oil (1%), vitamins (1%), and water.[7] Approval for the animal study was provided by the Institutional Ethical Committee, Department of Physiology, University of Calcutta.

Animal treatment: The rats of control group were maintained under normal diet (10 g of food per 100 g of body weight). The rats of treatment group received 1/3 amount supplement in the form of raw *Bambusa arundinacea* shoots for 28 days. On the 29th day, all the animals were weighed and then sacrificed. Testes were excised and immediately weighed on electronic balance.

Assay of testicular enzyme: Testicular enzyme Δ5-3β HSD and 17β HSD activity of 6 rats from each group were measured separately. Testicular tissues were homogenized with homogenizing fluid containing 20% spectroscopic grade glycerol, 5 mM potassium phosphate and 1mM EDTA at a tissue concentration of 105 homogenizing mixture. Then it was centrifuged at 10,000 rpm for 30 minutes at a constant temperature (4°C); the supernatant was then used for assay procedure. The activity was determined by optical measurement of the rate of reduction on NAD.[8]

Enzyme linked immunosorbent assay of testosterone: Circulating testosterone level was determined by using Clonital Inc [kit no. code no. ST020]. In this method, 25 microlitres of serum testosterone and 25 microlitres of standard were taken into a microplate well. Then 100 microlitres of diluted marker (testosterone-HRP conjugate) was added to all wells. The microplate was gently swirled for mixing and it was then covered. It was then incubated at 37°C for 1 hour. The contents of microplate were discarded by decantation and 300 µl of distilled water was added for proper washing. Then 100 µl of chromogen were added to all wells and the plate was incubated at room temperature for 15 minutes in the dark. 100 microlitres of sulphuric acid, 2 mol/L, as stop solution was added to each well and mixed by rotation. The absorbance in each well was then read at 450 nm by ELISA reader.[9]

Sperm count: Sperm samples were collected from cauda epididymis after sacrifice of the rat. Sperm count was determined by haemocytometer. Some amount of cauda epidydimis free from fat was placed in one vial containing 5 ml 1% (w/v) sucrose in phosphate buffer saline (pH 7.4). The cauda epidydimis was punctured with fine scissors and spermatozoa were washed with diluents. A drop of suspension was placed on the haemocytometer and covered with cover glass. The number of spermatozoa in fine small squares was counted.[10]

Statistical analysis: All data were statistically analyzed by using a suitable statistical software package and presented as Mean ± Standard Deviation (SD). Significance of difference of control and treated groups was estimated using Student's "t" test. The level of significance was expressed as $p<0.05$.

Results

Body weight and testicular weight: In the *Bambusa arundinacea*-treated group, the body weight as well as testicular weight decreased significantly in comparison to the control group when these are expressed in gm and gm% of body weight respectively (**Table 1**).

Assay of testicular enzyme Δ5-3β HSD and 17β HSD activity: The activity of regulatory enzymes of stereodogenic pathway, 5-3 HSD and 17 HSD were decreased significantly in the *Bambusa arundinacea*-treated group when these are expressed in OD/min/mgpr (**Table 2**).

Serum testosterone level and epididymal sperm count: Serum testosterone level and epididymal sperm count reduced significantly in the *Bambusa arundinacea*-treated group. Testosterone level was expressed in ng/ml and epididymal sperm count was expressed in million cells/cauda epididymis (**Table 3**).

Discussion

From this study it is clear that *Bambusa arundinacea* treatment during two consecutive reproductive cycles of adult male albino rats lowered the body weight and testis weight significantly in comparison with the control group. For the development of reproductive organs, thyroid hormone plays an important role, and thus the decreased testis weight is due to less thyroid hormone production under the influence of bamboo shoot feeding. Δ5-3β HSD and 17βHSD are microsomal enzymes that are essential for the synthesis of testosterone from its precursor's

molecule. Any alteration of action of these enzyme leads to the impairment of testosterone production. Δ5-3β HSD convert pregnenolone to progesterone and dehydroprogesterone to andosterone in testosterone biosynthesis. In this study, *Bambusa arundinacea* intake in male rats significantly reduced Δ5-3β HSD activity in comparison to control group. Therefore it can be assumed that HCN directly or indirectly inhibits enzyme activity.[2] 17β HSD is the regulatory enzyme in testosterone biosynthesis. This specific enzyme converts andosterone to testosterone. *Bambusa arundinacea* intake in male albino rats had also inhibited 17β HSD activity in comparison to the control group.

The testosterone level decreased significantly when compared to the control group. For testicular hormogenesis, there must be presence of optimum amount of cholesterol in blood because cholesterol is the precursor of all classes of steroid hormones. *Bambusa arundinacea* being a cardioprotective substance, its fibre content keeps the blood LDL level low.[1] Therefore in this investigation, decreased Δ5-3β HSD and 17β HSD, and low level of cholesterol and blood LDL had decreased testosterone production. *Bambusa arundinacea* intake in rats has shown significantly lower sperm count in comparison to control rats. Testosterone is necessary for sperm maturation and normal spermatogenesis. Testosterone level falls due to reduced activity of Δ5-3β HSD and 17β HSD, and thus the production and sperm maturation were decreased.

Acknowledgement

I acknowledge the University of Calcutta, Dept of Physiology for research support and equipment, and Prof (Dr) Amar K Chandra and Mrs Shyamosree Roy Choudhury of Dept of Physiology, University of Calcutta for their constant support and supervision.

Table 1 *Bambusa arundinacea* intake: Alteration in body weight & testicular weight in male albino rat [Values are mean ± SD of 6 observations in each group]

Group	Body weight (gm)	Testicular weight (gm% of body weight)
Control	198 ± 8.270	1.182 ± 0.726
Treatment	134 ± 5.099	1.040 ± 0.534
Two tail t-tests were performed. A significant difference was found between control and treated group at $p<0.05$		

Table 2 B*ambusa arundinacea* intake: Alteration in testicular enzyme Δ5-3β HSD & 17ß HSD activity in male albino rat [Values are mean ± SD of 6 observations in each group]

Group	Δ5-3β HSD activity (OD/min/mgpr)	17β HSD activity (OD/min/mgpr)
Control	0.344 ± 0.051	0.0065 ± 0.0003
Treatment	0.075 ± 0.005	0.0057 ± 0.0002
Two tail t-tests were performed. A significant difference was found between control and treated group at $p<0.05$		

Table 3 *Bambusa arundinacea* intake: Alteration in serum testosterone level and epididymal sperm count in male albino rat [Values are mean ± SD of 6 observations in each group]

Group	Serum testosterone level (ng/ml)	Epididymal sperm count (million cells/cauda epididymis)
Control	3.2 ± 0.34	88.83 ± 8.084
Treatment	1.39 ± 3.74	46.82 ± 3.957
Two tail t-tests were performed. A significant difference was found between control and treated group at $p<0.05$		

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Corrigendum

Under the article titled **"**Awareness of Protective Measures and Qualitative Analysis of Blood in Farmers with Chronic Exposure to Pesticides: A Cross Sectional Study" by Pujar SS, Pushpa MG, Kadagoudar S (JIST, Vol 8, No 2, 2012; 11-15), the conclusion part featured some information pertaining to another un-related study, due to a formatting error that occurred at the time of printing. The error is regretted.