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·Original Article·

Effect of piperine on the epididymis of adult male rats

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Abstract

Aim: To study the effect of piperine on the epididymal antioxidant system of adult male rats. **Methods:** Adult male rats were orally administered piperine at doses of 1 mg/kg, 10 mg/kg and 100 mg/kg body weight each day for 30 consecutive days. Twenty-four hours after the last treatment, the rats were weighed and killed with ether and the epididymis was dissected from the bodies. Sperm collected from the cauda region of the epididymis was used for the assessment of its count, motility and viability. Caput, corpus and cauda regions of the epididymis were separated and homogenized separately to obtain 10 % homogenates. The supernatants were used for the assays of sialic acid, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, lipid peroxidation and hydrogen peroxide generation. **Results:** Body weight of the piperine-treated rats remained unchanged. The weights of the caput, corpus and cauda regions of the epididymis significantly decreased at dose of 100 mg/kg. Epididymal sperm count and motility decreased at 10 mg/kg and 100 mg/kg, and sperm viability decreased significantly at 100 mg/kg. Sialic acid levels in the epididymis decreased significantly at 100 mg/kg while significant decrease in the cauda region alone was observed at 10 mg/kg. A significant decline in the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, along with an increase in hydrogen peroxide generation and lipid peroxidation were observed at 10 mg/kg and 100 mg/kg. **Conclusion:** Piperine caused a decrease in the activity of antioxidant enzymes and sialic acid levels in the epididymis and thereby increased reactive oxygen species levels that could damage the epididymal environment and sperm function. *(Asian J Androl 2005 Dec; 7: 363–368)*

Keywords: piperine; epididymis; reactive oxygen species; antioxidant enzymes; sialic acid

1 Introduction

Piperine (1-piperoylpiperidine) is an alkaloid present in the fruits of black pepper (*Piper nigrum*), long pepper (*Piper longum*) and other piper species (family: Piperaceae). Piperine is the major pungent substance present in these plants and is commonly used as a spice all over the world for seasoning and flavoring food. Piperine is known to possess pharmacological properties, such as antipyretic, analgesic and anti-inflammatory activities [1], and has been used in Ayurvedic medicine (a traditional Indian system of medicine) for the treatment of various diseases for thousands of years.

For many years it has been known that piperine can interfere with the reproductive process. Piper from different species has been used in indigenous drug preparations for inducing menstruation and terminating of early

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pregnancy [2]. Black pepper has been reported to be used by Malay women as an abortifacient. An Ayurvedic preparation consisting of a mixture of *Piper longum*, *Embelia ribes* and borax, used to prevent pregnancy, has also been shown to induce sterility in male mice [3]. In female mice, it has been shown that piperine effectively inhibits implantation, produces abortion and delays labor when administered during gestation and it has also been proposed that it disturbs the estrogen–progesterone balance essential to maintain pregnancy [4]. Piperine was shown to impair fertilization *in vitro* by inhibiting calcium influx to sperm which is necessary for sperm capacitation and acrosome reaction [5]. On the other hand, one study has also shown that piperine enhances fertilization *in vivo* by relaxing the smooth muscles of the isthmus, allowing a higher number of spermatozoa to enter the fertilization site in the female oviduct [6].

Very few studies have demonstrated the potential antifertility effects of piperine on male reproduction *in vivo*. In male albino rats, piperine has been reported to elevate serum gonadotropin levels by impairing the feedback signal to the pituitary and decreasing intratesticular testosterone concentrations. The desquamation of germ cell types and shrinkage of seminiferous tubules, along with the disruption of spermatogenesis has been reported [7].

Piperine is known to possess protective antioxidant properties and a dose of 100 mg/kg of piperine has been shown to augment the antioxidant defense system against benzo[a]pyrene-induced carcinogenesis in lungs [8]. Experimental evidence has indicated that piperine inhibits lipid peroxidation and prevented reduced glutathione depletion against carbon tetrachloride-induced cytotoxicity in the liver [9]; it has also been shown that piperine protects intestinal lumen from carcinogen-induced lipid peroxidation. On the contrary, piperine has initiated lipid peroxidation and free radical generation in rat intestinal epithelial cells *in vivo* [10]. Piperine has also been reported to potentiate carbon tetrachloride-induced hepatotoxicity *in vivo* at a 100-mg dosage by increasing lipid peroxidation [11]. The present study was undertaken to evaluate whether piperine administration could decrease the activity of antioxidant enzymes in the epididymis and affect epididymal function. The experiments done during the study comply with the current laws of India.

2 Materials and methods

2.1 Chemicals

Piperine of 97 % purity was obtained from Sigma-Aldrich (St. Louis, MO, USA). Nicotinamide adenine dinucleotide phosphate (NADPH reduced) and glutathione (oxidized) were obtained from SISCO Research Laboratories (Mumbai, India). Bovine serum albumin, horseradish peroxidase, thiobarbituric acid and pyrogallol were obtained from Himedia Laboratories (Mumbai, India). All other chemicals used for various assays were of analytical grade and were obtained from local commercial sources.

2.2 Animals

Male Wistar rats (90 days old) were housed in plastic cages under standard conditions of light and dark (12 h : 12 h) with an ambient temperature of 24 °C ± 2 °C. They were fed with standard laboratory chow and tap water *ad libitum*.

2.3 Treatment

Animals were divided into four groups of four rats each. Piperine was dissolved in vehicle (10 % Dimethyl Sulphoxide [DMSO] in ethanol and groundnut oil [ratio of 1:1]) and was administered orally each day for 30 consecutive days using a micropipette (Accupipet, Tarson Products Pvt. Ltd., Kolkata, India). Groups I, II and III received piperine at doses of 1 mg/kg, 10 mg/kg and 100 mg/kg, respectively. A corresponding group of control animals were administered with vehicle alone. After 24 h of the last treatment, the rats were weighed and killed using an overdosage of anesthetic ether. The epididymis of each animal was dissected, cleared of the adhering tissues and weighed. Epididymal sperm count, motility and viability were assessed immediately thereafter.

2.4 Epididymal sperm motility, viability and count

Epididymal sperm was collected by cutting the cauda region of the epididymis into small pieces in 5 mL of Ringer's medium at 32 °C. A sperm viability test was done by the method described by World Health Organization (WHO) [12]. Epididymal sperm count and motility were evaluated and the methods used have been detailed elsewhere [14].

2.5 Biochemical parameters

The caput, corpus and cauda regions of the epididymis were separated and homogenized separately in cold normal saline with the help of a glass-teflon homogenizer (Remi RQ-127A, Remi Motors, Mumbai, India). Superna-

tants were collected and assays of superoxide dismutase, catalase, hydrogen peroxide generation, lipid peroxidation, glutathione peroxidase and glutathione reductase were done. Protein was estimated by the method of Lowry *et al*. [13]. The methods have been standardized in our laboratory and have been detailed elsewhere [14]. Sialic acid was estimated using the method of Aminoff [15].

2.6 Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was performed using Duncan's Multiple Range test. Differences were considered to be significant at $P < 0.05$.

3 Results

3.1 Body and organ weight

The administration of piperine at any dose did not alter the body weight of the animals. The weights of the caput, corpus and cauda epididymis in rats after 10 mg/kg (group II) and 100 mg/kg (group III) of piperine administration were found to be significantly decreased, compared with those in the corresponding control groups (Table 1). The weights, expressed relative to the body weight, were also found to be significantly decreased after the 100-mg/kg dose piperine was administered (data not shown).

3.2 Epididymal sperm count, motility and viability

The cauda epididymal sperm count and motility decreased significantly after 10 mg/kg (group II) and 100 mg/kg (group III) of piperine were administered; whereas sperm viability was found to decrease only after the 100 mg/kg dose of piperine. Piperine did not cause any significant change in sperm count, motility or viability when 1 mg/kg (group I) was administered (Figures 1, 2).

3.3 Sialic acid levels in the epididymis

Sialic acid levels in the caput, corpus and cauda regions decreased significantly in the 100-mg/kg piperine-treatment group (group III); while in the 10-mg/kg piperine-treatment group (group II), sialic acid levels de-

Table 1. Effect of piperine at three doses on body weight and weights of different regions of the epididymis of male adult rats. Data were expressed as mean ± SD, n=4; $^bP < 0.05$, compared with the control group.

		piperine (mg/kg body weight)		
Parameters	Control	1	10	100
Body weight (g)	151.25 ± 4.79	149.25 ± 2.75	147.00 ± 4.76	146.00 ± 1.41
Caput weight (mg)	185.00 ± 2.50	181.75 ± 2.22	172.00 ± 2.50^b	141.00 ± 2.50^b
Corpus weight (mg)	81.00 ± 1.83	77.75 ± 1.71	67.25 ± 2.63^b	35.50 ± 2.08^b
Cauda weight (mg)	177.25 ± 1.71	172.75 ± 1.71	164.00 ± 2.94^b	138.25 ± 2.98^b

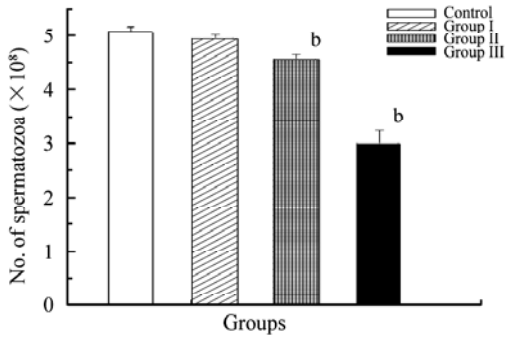

Figure 1. The effect of piperine at three doses on the epididymal sperm count of male adult rats ($^bP < 0.05$, compared with the control group). The data were expressed as $\times 10^8$ spermatozoa (mean ± SD). Group I: 1 mg/kg; Group II: 10 mg/kg; Group III: 100 mg/kg.

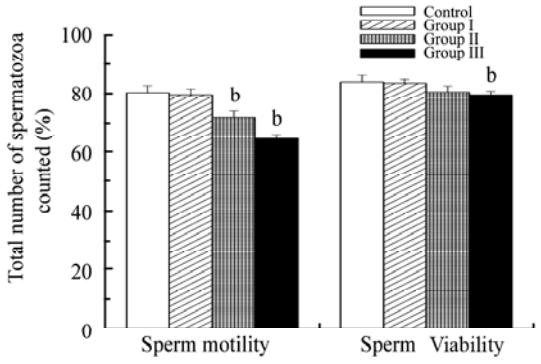

Figure 2. The effect of piperine at three doses on epididymal sperm motility and viability of male adult rats ($^bP < 0.05$, compared with the control group). The data were expressed as percentages of the total spermatozoa counted (mean ± SD). Group I: 1 mg/kg; Group II: 10 mg/kg; Group III: 100 mg/kg.

creased only in the cauda region. No significant changes in the levels of sialic acid were observed in any regions of the epididymis in the rats treated with 1 mg/kg dose of piperine (group I) (Figure 3).

3.4 Antioxidant enzymes and lipid peroxidation activities in the epididymis

The activities of antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase were found to be significantly decreased in the caput, corpus and cauda regions of the epididymis at 10 mg/kg (group II) and 100 mg/kg (group III), while no changes were observed at 1 mg/kg compared with that in the control. A dose-dependent increase in hydrogen peroxide generation and lipid peroxidation were also observed with significant increase at dose of 100 mg/kg (group III) (Table 2).

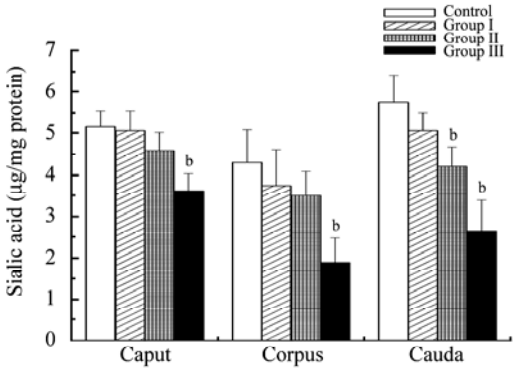

Figure 3. The effect of piperine at three doses on sialic acid levels in the caput, corpus and cauda regions of the epididymis ($^bP < 0.05$, compared with the control group). The data were expressed as μg/mg protein (mean ± SD). Group I: 1 mg/kg; Group II: 10 mg/kg; Group III: 100 mg/kg.

Table 2. Effect of piperine at three doses on the antioxidant system of various regions of the epididymis of male adult rats. ^{m}μmol pyrogallol oxidized/min/mg protein at 32 °C; ^{n}μmol H_2O_2 consumed/min/mg protein at 32 °C; ^{o}μmol malondialdehyde produced/15 min/mg protein at 32 °C; ^{p}μmol H_2O_2 generated/30 min/mg protein at 32 °C; ^{q} nmol Nicotinamide adenine dinucleotide phosphate (NADPH)(reduced) oxidized/min/mg protein at 32 °C. Data were expressed as mean ± SD, $n = 4$. $^bP < 0.05$, compared with the control group.

Parameters	Control	Piperine (mg/kg body weight)		
		1	10	100
		CAPUT		
Superoxide dismutase^m	72.67 ± 11.03	66.31 ± 15.26	52.56 ± 14.69	43.26 ± 6.54^b
Catalase^n	1.59 ± 0.12	1.47 ± 0.22	1.34 ± 0.18	1.20 ± 0.12^b
Lipid peroxidation^o	60.31 ± 6.03	66.90 ± 11.58	109.31 ± 11.44	165.15 ± 30.12
Hydrogen peroxide^p	13.71 ± 3.47	16.75 ± 6.73	23.32 ± 10.24	41.56 ± 8.99^b
Glutathione peroxidase^q	147.04 ± 9.31	144.55 ± 21.18	124.39 ± 11.28	108.50 ± 15.71^b
Glutathione Reductase^q	136.03 ± 18.71	130.24 ± 11.78	114.75 ± 4.98	84.16 ± 10.44^b
		CORPUS		
Superoxide dismutase^m	52.91 ± 10.12	40.43 ± 10.64	36.52 ± 3.81^b	34.36 ± 2.96^b
Catalase^n	1.22 ± 0.06	0.98 ± 0.19	0.52 ± 0.12^b	0.46 ± 0.02^b
Lipid peroxidation^o	43.36 ± 9.33	63.52 ± 7.65	81.46 ± 11.38^b	151.77 ± 23.24^b
Hydrogen peroxide^p	28.35 ± 4.35	35.37 ± 13.37	51.31 ± 9.47^b	88.21 ± 7.72^b
Glutathione peroxidase^q	133.09 ± 8.80	125.53 ± 15.16	119.59 ± 8.48	69.49 ± 2.46^b
Glutathione reductase^q	131.79 ± 7.78	117.25 ± 18.79	112.33 ± 13.63	99.52 ± 12.89^b
		CAUDA		
Superoxide dismutase^m	84.72 ± 3.34	80.68 ± 6.44	74.46 ± 3.48^b	52.45 ± 1.35^b
Catalase^n	1.91 ± 0.23	1.75 ± 0.44	1.59 ± 0.21	1.31 ± 0.11^b
Lipid peroxidation^o	53.01 ± 6.58	67.43 ± 16.30	74.56 ± 12.09	91.01 ± 7.39^b
Hydrogen peroxide^p	33.70 ± 10.42	44.23 ± 10.12	57.22 ± 5.56^b	108.51 ± 15.63^b
Glutathione peroxidase^q	123.69 ± 7.12	115.52 ± 19.42	78.15 ± 8.69^b	66.23 ± 6.74^b
Glutathione reductase^q	145.55 ± 9.10	129.51 ± 13.30	88.21 ± 3.10^b	48.35 ± 9.05^b

4 Discussion

Piperine has been reported to induce sterility in laboratory male mice [3] and to disrupt spermatogenesis by impairing the pituitary-testicular negative feedback system and leading to impaired fertility. In the present study, the body weight of the piperine-treated animals remained unchanged, which showed that the doses selected were not toxic and the metabolic processes of the treated animals were normal. The administration of 10 mg/kg and 100 mg/kg of piperine decreased the weights of various regions of the epididymis, the epididymal sperm count, motility, viability and the sialic acid levels in the epididymis. The weights of the seminal vesicle and ventral prostate also decreased (data not shown). Piperine has been shown to decrease intratesticular testosterone concentrations at dose of 10 mg/kg by inhibiting cytochrome P_{450}, which is involved in the steroidogenic pathway [7]. In the present study, depletion of sialic acid levels along with the decreased weights of accessory sex organs are indicative of the decreased bioavailability of testosterone [16]. Testosterone withdrawal has been shown to cause DNA fragmentation by stimulating caspase activation in Sertoli cells *in vitro*, which indicated that decreased testosterone levels can stimulate apoptotic pathways [17]. It has been reported that orchidectomy induced a wave of apoptotic cell death in the epididymis along with histochemical changes, such as a reduction in the levels of lipids, polysaccharide complexes, glycogen and an 80 % loss of epididymal tissue weight. Androgen replacement therapy after orchidectomy could completely prevent apoptosis in the caput, corpus and cauda regions of the epididymis, thereby proving the androgen dependency of the epididymis [18]. As the epididymis and the fertilizing potential of its contained spermatozoa are dependent on testicular androgens, the observed decrease in sperm motility, count and viability shown in the present study could be due to the reduced bioavailability of testosterone. Principal cells of the epididymal epithelium secrete sialic acid and their reduced levels in the epididymis can be caused by the impaired secretory functions of the epididymis [19]. It has been shown that androgen withdrawal induced apoptosis mainly in the epididymal epithelium and was localized specifically to principal cells of the epididymis [18]. Decreased levels of androgen along with apoptotic damage to principal cells can be the causes of an observed reduction in sialic acid secretion. Sperm absorbs sialic acid from epididymal luminal fluid which facilitated their downward movement without friction [19] and also helps in the acquisition of motility and viability [20] and hence the decreased sialic acid levels in the epididymis can also be a causative factor for impaired sperm functions. Decrease in the specific activities of antioxidant enzymes in the caput, corpus and cauda regions of the epididymis were observed following piperine-treatment. Increase in hydrogen peroxide generation and lipid peroxidation were also observed, which indicated that piperine induced stress in the epididymis by decreasing the activity of antioxidant enzymes thereby leading to an excessive generation of reactive oxygen species (ROS). Superoxide dismutase is involved in the conversion of superoxide anion radical to hydrogen peroxide, which in turn is degraded by catalase and glutathione peroxidase/reductase system. Sialic acid has been reported to act as a potent hydrogen peroxide scavenger by imparting a defense against oxidative damage [21]. Reduction in catalase, glutathione peroxidase and sialic acid levels can be the cause of increased hydrogen peroxide levels in the epididymis as these antioxidants are unable to scavenge hydrogen peroxide generated in the epididymis as a result of excessive ROS formation. Hydrogen peroxide, through the production of hydroxyl radicals, can initiate lipid peroxidation, which can cause structural damage to the epididymal cell membrane [22]. Sperm cytoplasm contained very low concentrations of scavenging enzymes and they were well protected by the antioxidant system of the epididymis; decrease in antioxidant enzyme levels can cause damage to spermatozoa. The mechanism by which piperine induced stress and brought about an imbalance in prooxidant/antioxidant levels in the epididymis was not clear.

In conclusion, piperine decreases the activity of the antioxidant enzymes and sialic acid and hampers the epididymal environment where sperm maturation takes place. Considering the above results, we propose that the inhibition of antioxidant enzyme activities along with a decrease in sialic acid levels that could generate ROS in the epididymis, is the reason for the potential antifertility effects of piperine.

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