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·*Original Article*·

The inhibitory effects on adult male reproductive functions of crude garlic (*Allium sativum*) feeding

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Abstract

Aim: To investigate the effects of crude garlic on adult male rat reproductive functions. **Methods:** Thirty male rats were divided into five groups: group 1 (untreated) and groups 2, 3, 4 and 5 were fed for 30 days with 5%, 10%, 15% and 30% crude garlic, respectively. Testes and accessory organs were weighed and some markers were assessed. Light and electron microscopy observations were also performed. **Results:** A significant decrease was observed in the body weight of groups 4 (14%; $P < 0.01$) and 5 (20%; $P < 0.01$); of the prostate weight in group 5 (29.1%; $P < 0.05$) and of seminal vesicle weight in groups 3 (14.4%; $P < 0.01$), 4 (18.3%; $P < 0.01$) and 5 (27.3%; $P < 0.01$). In contrast, testis and epididymis weights were unchanged. In epididymis tissue, the alpha glucosidase activity and the spermatozoa density were unchanged. The treatment resulted in a significant decrease in testosterone serum levels in groups 3 (77.3%; $P < 0.01$), 4 (77.3%; $P < 0.01$) and 5 (90.9%; $P < 0.01$), associated with a significant increase in LH serum levels ($P < 0.01$). Testicular histology showed a dose-dependent increase in the percentage of empty seminiferous tubules. Moreover, testicular function was affected; a significant decrease in phosphatase acid activity ($P < 0.01$) and testosterone ($P < 0.05$) contents were observed. **Conclusion:** Crude garlic consumption during 1 month reduced testosterone secretion and altered spermatogenesis at 10%, 15% and 30% doses. *(Asian J Androl 2008 Jul; 10: 593–601)*

Keywords: crude garlic; spermatogenesis; testosterone; luteinizing hormone; testis; sexual accessory organs; Sertoli cell; Leydig cell; germ cells

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1 Introduction

Alternative medicines, automedication and phytotherapy are part of the way of life of most populations, particularly in Africa. Side effects of many medicinal

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plants on fertility are unknown. Some of these plants contain estrogenic substances and, therefore, might alter gametogenesis production [1]. Some plants like *Morinda lucida*, *Ricinus communis* and *Yohimbe* are known to cause reductions in sperm density [2], to alter androgenic secretion [3] and to reduce mobility and density of mice spermatozoa [4].

Allium sativum (As) is a frequently used plant in Mediterranean cooking. In Tunisia, it is regularly consumed at various doses both crude and cooked. Therapeutic virtues of this plant are numerous [5]; however, its impacts on the male reproductive system have been not clearly defined. Some studies have reported that garlic impairs testicular function [6] and has spermicidal effects on spermatozoa [7, 8], but others demonstrate its beneficial effects on recovery of testicular functions [9]. These discrepancies could be related to the type of preparations, doses and way of administration.

In our study, we investigated the effects of chronic consumption of crude garlic, as is largely used in Mediterranean cooking, on the following variables of male rats' reproductive functions: testicular and plasma testosterone, luteinizing hormone (LH) levels, prostate and seminal vesicle markers, sperm density and testicular integrity on histological sections.

2 Materials and methods

2.1 Plant and preparation

The type of As used in the present study was "spring garlic". This variety has pink bulbs and is planted between December and March (according to the weather) in Tunisia and collected in July. This type of garlic contains 2.1% proteins, 30% carbohydrates, 1.5% fibre, 0.2% fat, 0.015% vitamins and 0.7% minerals. The plant (As) used in this study was grown in Tunisia and purchased at a local market. Every day the garlic pellets were made by mixing peeled cloves of garlic with powdered standard rat pellet diet (Industrial society of food, Sfax, Tunisia) at four doses: 5%, 10%, 15% and 30%. For example, the 30% pellets for one rat were prepared by mixing 9 g of crude garlic with 21 g of powdered standard diet in 5 mL of water. Cloves were crushed in distilled water to minimize volatile compound loss. A similar volume of water was added to the other doses.

2.2 Animals and treatment

A number of 30 adult male Wistar rats (Pasteur Institute of Tunis, Tunisia), whose average weight ranged between 200 g and 250 g, were used for the study. The animals were housed with proper aeration at 25 ± 2°C, and were given tap water *ad libitum*. The rats were allowed to acclimatize in the laboratory for a period of 1 week before the beginning of the study. The rats were randomly assigned into the different groups (of six animals each) using a hazard permutation table. Control animals received a standard pellet diet (group 1). The other groups received a diet supplemented with 5%, 10%, 15% and 30% of As (for groups 2, 3, 4 and 5, respectively). Every day, 30 g of food (garlic mixed with standard diet) was given to each rat. The animals consumed 30 g of food daily, as no pellet was observed the following day. All rats were weighed daily. After 30 days of treatment rats were killed and a cardiac blood sample was taken from each rat and then put into a sterile tube. Blood was allowed to clot at room temperature. When the clot was retracted, the sample was centrifuged at 3 000 × *g* for 15 min and the serum was transferred to a new tube. The serum samples were stored frozen at –20ºC until use. All the rats were killed by decapitation the same day between 09:00 and 11:00 o'clock. Reproductive organs were dissected out and weighed.

All studies on animals were conducted in accordance with current regulations and standards approved by the Faculty of Medicine of Tunis animal care committee.

2.3 Hormonal analysis

The same radioimmunoassay (RIA) system was used to measure testosterone contents in both testicular tissue and serum samples. The RIA kit was obtained from Biosource (Nivelles, Belgium). The intra-assay and interassay coefficients of variations (CV) were 4.6% and 6.2%, respectively. The detection limit of the testosterone assay was 0.05 ng/mL. LH (Biocode-Hycel, Liège, Belgium) concentrations were determined according to the manufacturer's recommendations. The detection limit of the LH assay was 0.05 ng/mL. The intra-assay and interassay CV were 8.2% and 6.8%, respectively.

2.4 Tissue biochemistry

2.4.1 Testis

Testosterone and cholesterol contents were determined in testicular tissue. One testis was crushed into

2 mL of 0.9% NaCl in distilled water. The homogenate was centrifuged at 13 000 × *g* for 10 min. The supernatant was removed and used for determination of testosterone and cholesterol contents with the same assay as for blood samples. The results were expressed as mg/g or ng/g of testicular tissue for cholesterol and testosterone, respectively. Cholesterol levels (Diagnostics Elitech, Sees, France) were assayed with a colorimetric method [10]. The intra-assay and interassay CV were 1.7% and 3.8%, respectively. The detection limit was 0.05 g/L.

Acid phosphatase activity was determined using a colorimetric assay (Diagnostics Elitech, Sees, France) according to the manufacturer's recommendations. The intra-assay and interassay CV were 1.6% and 2.3%, respectively. The detection limit of acid phosphatase activity assay was 0.5 μmol/min/L. 0.5 g of testicular tissue were homogenized in 2 mL of citric acid buffer (0.1 mol/L citric acid, 0.2 mol/L Na_2HPO_4, pH 6.2, supplemented with 0.4% Triton X-100 solution) and centrifuged at 80 000 × *g* at 4ºC for 30 min. The reaction medium containing 0.1 mL supernatant, 0.05 mL 4-paranitrophenol (PNP, 23 mmol/L) and 0.5 mL buffer (0.1 mol/L citric acid, 0.2 mol/L Na_2HPO_4, pH 5.0) was incubated at 37ºC for 30 min. Then, 2.5 mL of NaOH (0.2 mol/L) was added to stop the reaction, and the absorbance (Metertek SP-850, Metertech, Taipei, Taiwan) was recorded at 405 nm. A standard PNP curve was obtained using the same method. Acid phosphatase activity was expressed as μmol/min/g of tissue.

2.4.2 Epididymis

One caudal epididymis of each rat was cut, homogenized in citric acid buffer (0.1 mol/L citric acid, 0.2 mol/L Na_2HPO_4, pH 6.2, supplemented with 0.4% Triton X-100 solution) and centrifuged at 80 000 × *g* at 4ºC for 30 min. The alpha-glucosidase activity was measured using the colorimetric method [11]. The reaction system contained 1.2 mL buffer (69 mmol/L citric acid, pH 6.8), 0.2 mL paranitrophosphateglycerol (PNPG, 23 mmol/L) and 0.2 mL supernatant. The reaction medium was incubated at 37ºC for 4 h and 0.25 mL Na_2CO_3 (0.1 mol/L) was added to stop the reaction. The absorbance was measured at 400 nm with a Metertek SP-850 (Metertech, Taipei, Taiwan) spectrophotometer and PNPG content was estimated to a standard curve. The alpha-glucosidase activity was expressed as μmol/min/g of tissue. The detection limit of alpha-glucosidase activity assay was 0.5 μmol/min/L. The intra-assay and interassay CV were 2.1% and 2.6%, respectively.

2.4.3 Prostate and seminal vesicle

Extraction procedures were similar for prostate and seminal vesicle. 0.2 g of tissues were homogenized in 2 mL of 0.33% perchloric acid at 4ºC and centrifuged at 2 500 × *g* for 10 min. Then, 1 mL of the supernatant was added to 0.5 mL K_2CO_3 (0.75 mol/L). The reaction medium was centrifuged at 2 500 × *g* for 10 min and supernatants were used for determination of prostate citric acid (r-Biopharm, Darmstadt, Germany) and seminal vesicle fructose (r-Biopharm, Darmstadt, Germany) using an ultra violet method according to the manufacturer's recommendations. The detection limits were 0.5 mg/L and 0.4 mg/L, respectively. The interassay CV were 4.2% and 1.8% for prostate citric acid and seminal vesicle fructose, respectively. The intra-assay CV were 1.3% for prostate citric acid and 1.8% for seminal vesicle fructose.

2.5 Sperm density

The caudal epididymis was removed, and cut in small pieces into 1 mL of 0.9% NaCl. The NaCl solution was transferred into a new tube. The epididymis tissue was rinsed with 0.5 mL of NaCl that was added to the previous tube. The NaCl solution containing spermatozoa was incubated for 30 min at room temperature. Then, to 50 μL of the spermatozoa suspension was added 200 μL of formaldehyde 1%. The number of spermatozoa was determined using a Thomas' cytometer cell. The results were expressed as the number of spermatozoa (10^6/mL).

2.6 Histopathological studies

Testes were fixed in a 10% formaldehyde solution, passed through ascending series of ethanol baths, cleared in toluene and embedded in paraffin. Tissues were sectioned at 4 μm and stained with haematoxylin and eosin. For the determination of the number of empty seminiferous tubules, a slide from each animal was used. All the seminiferous tubules were counted and the results were presented as a percentage of empty seminiferous tubules. For the determination of the seminiferous tubule area, only round and almost round (oval-shaped) tubules were analyzed. To calculate the area, the diameter was measured (with a micrometer objective) for round seminiferous tubules and the small and large diameters were

measured for oval-shaped tubules. Some fragments of testis were processed for electron microscopy. They were fixed in 4% glutaraldehyde, postfixed in a 1% osmium tetroxide solution, and embedded in Epon 812. Ultra-thin sections were observed on a JEOL1010 transmission electron microscope after lead citrate and uranyl acetate contrast.

2.7 Statistical analysis

All data are presented as mean ± SD and median. Statistical analyses were performed using SPSS 10.0 for Windows (SPSS, Chicago, IL, USA). To determine whether there were differences between all groups the Kruskall-Wallis test was performed and this was followed by the Mann-Whitney *U*-test to determine the significance ($P < 0.05$) of the differences between the pair of groups.

3 Results

3.1 Body and organ weights

Compared to the control group, rats in groups 4 and 5 showed significant decreases in body weight, 14% ($P < 0.01$) and 20% ($P < 0.01$), respectively (Table 1). Concerning the weight of the reproductive organs, crude garlic treatment significantly decreased seminal vesicle weight in group 3 (14.4%; $P < 0.01$), 4 (18.3%; $P < 0.01$) and 5 (27.3%; $P < 0.01$). The prostate weight (Table 1) was significantly decreased (by 29.1%; $P < 0.05$) only in group 5. In contrast, no significant modification of testis and epididymis weights was observed after crude garlic treatment.

Table 1. Effects of the 30-day *Allium sativum* (As) treatment on rat body and reproductive organ weights. All data are presented as mean ± SD and median. $^bP < 0.05$, $^cP < 0.01$, compared to controls.

Group	Body weight	Reproductive organ weight (mg/100 g body weight)			
($n = 6$)	(g)	Testis	Epididymis	Seminal	Prostate
Group 1	287.7 ± 20.2	983.2 ± 61.1	446.3 ± 27.8	254.4 ± 12.0	132.5 ± 9.0
(Control)	292.0	995.5	453.9	251.1	135.9
Group 2	280.2 ± 19.0	982.2 ± 57.2	451.3 ± 30.6	249.0 ± 7.1	126.6 ± 11.6
(5% of As)	273.0	958.7	446.9	251.3	132.3
Group 3	275.8 ± 14.6	975.3 ± 25.6	446.6 ± 30.6	213.3 ± 21.3^c	119.9 ± 13.6
(10% of As)	273.5	976.9	456.0	215.0	120.5
Group 4	253.2 ± 12.7^c	968.8 ± 30.5	423.9 ± 41.2	206.3 ± 16.2^c	120.6 ± 13.1
(15% of As)	251.0	963.9	419.3	205.1	125.7
Group 5	238.4 ± 18.0^c	929.1 ± 33.9	422.4 ± 25.3	184.5 ± 16.8^c	95.1 ± 5.7^b
(30% of As)	233.7	943.2	429.6	182.5	96.3

Table 2. Effects of the 30-day *Allium sativum* (As) treatment on rat serum hormone levels. The serum was obtained from cardiac blood samples. All data are presented as mean ± SD and median. $^bP < 0.05$, $^cP < 0.01$, compared to controls.

Group ($n = 6$)	Testosterone (ng/mL)	Luteinizing hormone (ng/mL)
Group 1	2.00 ± 0.30	0.30 ± 0.10
(Control)	2.20	0.30
Group 2	1.80 ± 0.06	0.40 ± 0.15
(5% of As)	1.80	0.40
Group 3	0.50 ± 0.03^c	0.60 ± 0.18^c
(10% of As)	0.50	0.60
Group 4	0.50 ± 0.02^c	0.70 ± 0.07^c
(15% of As)	0.50	0.70
Group 5	0.20 ± 0.01^c	1.10 ± 0.18^c
(30% of As)	0.200	1.10

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3.2 Hormonal measurement

A significant decrease in serum testosterone levels was observed in groups 3 (77.3%; $P < 0.01$), 4 (77.3%; $P < 0.01$) and 5 (90.9%; $P < 0.01$), accompanied by significant increases in LH concentration ($P < 0.01$) at these doses (Table 2).

3.3 Accessory gland functions

The treated rats showed no significant reductions in alpha-glucosidase activity in caudal epididymis and no significant reductions in spermatozoa density in caudal epididymis (Table 3).

However, prostate citric acid content was significantly decreased (19.4%; $P < 0.05$) in group 5. There were 32.7%, 63.8% and 75.1% reduction in seminal vesicle fructose at 10%, 15% and 30% doses of As, respectively ($P < 0.01$) in comparison to the untreated rats (Table 3).

3.4 Testis

3.4.1 Testicular morphology and ultrastructure

Morphological alterations of seminiferous tubules were observed in group 5 (30% of As) (Figure 1C and 1D) when compared to the control testis (Figure 1A and

Table 3. Effects of the 30-day *Allium sativum* (As) treatment on several rat accessory gland variables. The alpha-glucosidase activity, the citric acid and fructose contents were obtained on epididymis, prostate and seminal vesicles tissues, respectively. Sperm density was determined from epididymis tissue. All data are presented as mean ± SD and median. $^{b}P < 0.05$, $^{c}P < 0.01$, compared to controls.

Group ($n = 6$)	Epididymis		Prostate	Seminal vesicles
	Alpha-Glucosidase (μmol/min/g)	Sperm density (10^6/mL)	citric acid (mg/g)	fructose (mg/g)
Group 1	176.4 ± 54.5	25.7 ± 3.8	381.1 ± 43.3	204.8 ± 15.9
(Control)	192.8	24.7	365.4	200.7
Group 2	186.9 ± 45.2	25.0 ± 4.5	362.6 ± 55.6	180.5 ± 15.6
(5% of As)	172.0	26.2	358.7	176.0
Group 3	172.9 ± 82.3	22.5 ± 4.2	320.8 ± 55.7	134.3 ± 11.1^{c}
(10% of As)	127.0	21.7	321.5	135.0
Group 4	168.6 ± 25.9	22.1 ± 2.6	382.2 ± 35.3	75.9 ± 8.8^{c}
(15% of As)	180.0	22.5	388.6	72.6
Group 5	166.5 ± 43.9	22.1 ± 3.0	290.1 ± 60.4^{b}	49.0 ± 6.2^{c}
(30% of As)	142.9	23.4	294.3	49.9

Table 4. Effects of the 30-day *Allium sativum* (As) treatment on several testicular variables. Cholesterol, testicular contents and acid phosphatase activity were determined on testicular tissue. The percentage of empty seminiferous tubules was determined on testicular sections. All data are presented as mean ± SD and median. $^{b}P < 0.05$, $^{c}P < 0.01$, compared to controls.

Group ($n = 6$)	Cholesterol (mg/g)	Acid phosphatase activity	Testosterone (ng/g)	Percentage of empty seminiferous tubules
Group 1	12.5 ± 2.1	130.3 ± 7.4	1.50 ± 0.02	11.50 ± 2.90
(Control)	13.5	130.5	1.50	11.50
Group 2	13.1 ± 2.7	69.0 ± 9.4^{c}	1.00 ± 0.02^{b}	11.16 ± 4.30
(5% of As)	12.0	67.4	1.00	9.50
Group 3	12.0 ± 1.7	72.1 ± 11.6^{c}	0.40 ± 0.01^{b}	25.70 ± 6.70^{c}
(10% of As)	12.5	73.6	0.40	23.50
Group 4	13.2 ± 2.5	78.4 ± 13.7^{c}	0.30 ± 0.02^{b}	34.30 ± 1.70^{c}
(15% of As)	13.5	87.1	0.30	35.00
Group 5	12.7 ± 2.2	70.2 ± 9.4^{c}	0.15 ± 0.01^{b}	37.80 ± 4.10^{c}
(30% of As)	13.5	67.7	0.16	39.00

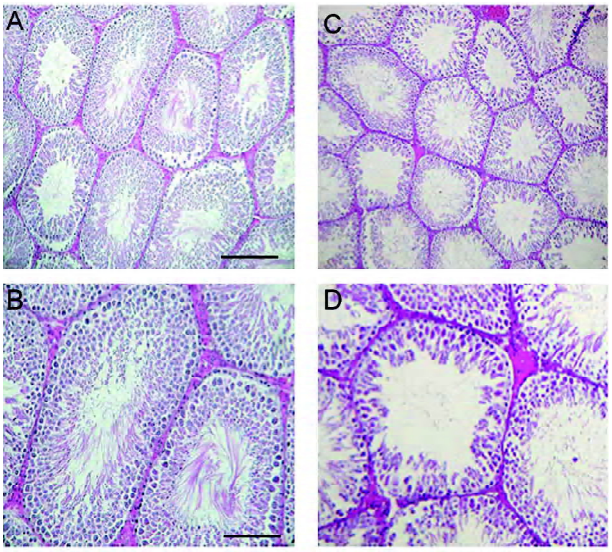

Figure 1. Histology of the rat testis (H&E): (A) and (B): Testis of control rat depicting normal stages of spermatogenesis. (C and D): Testis of rat treated with 30% *Allium sativum* (As). (A) and (C): Bar = 200 μm. (B) and (D): Bar = 50 μm.

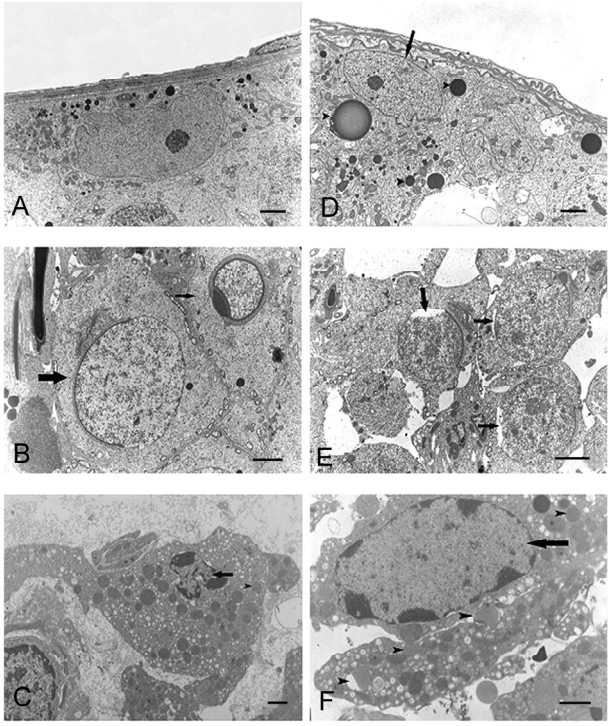

Figure 2. Ultrastructure of rat testis: (A, B, C) control rat and (D, E, F) rat treated with 30% *Allium sativum* (As). (A): Sertoli cells showing normal cytoplasm characteristics. The nucleus shows irregular pattern with deep indentation and contains a prominent nucleolus and abundant euchromatin. Cytoplasm shows numerous mitochondria and few lipid droplets. Bar = 2 μm. (B): Spermatids showing two different stages: a round spermatid with acrosome in formation (➞); a spermatid at "cap phase" with well-defined nucleus and acrosome membranes (➞). Bar = 2 μm. (C): Leydig cell showing prominent nucleus (➞) with slightly tortuous nuclear membrane and patchy chromatin material. Some lipid droplets are present in cytoplasm (➤). Bar = 2 μm. (D): Sertoli cells showing numerous and voluminous lipid droplets (➤). Note the reduction of nucleus volume (➞) and the presence of more condensed chromatin. Bar = 2 μm. (E): Spermatids: note the presence of vacuolisations in the nucleus (➞) and interruptions of the nuclear envelope. Bar = 1 μm. (F): Leydig cell showing a normal structure except for the abundance of lipid droplets (➤). Bar = 1 μm.

1B). A significant and dose-dependent increase in the percentage of empty seminiferous tubules was observed after treatment with 10% ($P = 0.002$), 15% ($P = 0.002$) and 30% ($P = 0.004$) of crude garlic (Table 4). An approximate threefold increase was observed in the percentage of empty seminiferous tubules in the group fed 30% garlic as compared to the control group. In contrast, the area of the seminiferous tubules was unchanged by As feeding (63.43 ± 3.05 μm^2 for 30% As *vs.* 63.08 ± 2.66 μm^2 for the control group). The testicular ultrastructure of rats treated during for 30 days with 30% of As displayed cellular alterations (Figure 2). Sertoli cells had a reduced volume and presented vacuolization, sparse organelles and a few scattered mitochondria in their cytoplasm (Figure 2D) compared to untreated rats (Figure 2A). Nuclear degeneration was evident in the primary spermatocytes and spermatids: nuclear envelopes were frequently interrupted (Figure 2E). Leydig cells displayed more lipid droplets (Figure 2F) than untreated ones (Figure 2C).

3.4.2 Testicular functions

In the testicular tissue, the acid phosphatase activity was significantly decreased in groups 2 (48.3%; $P < 0.01$), 3 (47.4%; $P < 0.01$), 4 (33.2%; $P < 0.01$) and 5 (48.1%; $P < 0.01$). Similarly, a significant decrease in intra-testicular testosterone concentration was observed in groups 2 (33.3% decrease; $P < 0.05$), 3 (73.3% decrease; $P < 0.05$), 4 (80% decrease; $P < 0.05$) and 5 (89.3%; $P < 0.05$). In contrast, no significant change in intra-testicular cholesterol concentration was detected com-

pared to the values observed in the control (Table 4).

4 Discussion

In the present study, rats fed a diet consisting of 15% or 30% crude garlic had significantly reduced body weights compared to rats who did not consume garlic. Our results are in accordance with the study by Dixit and Joshi [6] reporting a decrease in body weight after treatment with a powder garlic preparation by daily gavage. Concerning the accessory gland functions, the present study showed no significant difference between treated and untreated groups in epididymis α-glucosidase activity or in sperm density in caudal epididymis. However, we detected significant increase in the number of empty seminiferous tubules in the testes from rats fed 10%, 15% or 30% As. One possible explanation for the absence of significant modifications in sperm density observed in caudal epididymis in the present study might be that the treatment was conducted during 30 days, whereas the rat seminal cycle lasts 53 days. Al-Bekairi *et al*. [12] reported an increase in epididymis spermatozoa after feeding rats with garlic water extract over 3 months. The reasons for this discrepancy in the studies could be linked to the difference in the garlic preparation (crude garlic versus water extract).

We showed here that a reduction in prostate weight was associated with a decrease in citric acid content when rats were fed 30% garlic. These results suggest a dysfunction of the prostate gland, which might be a result of low testosterone levels, because the secretion of citric acid is regulated by androgens [13]. Moreover, a low fructose concentration and a reduction in seminal vesicle weight were observed in rats treated with high doses of garlic. These results could also be attributed to decreased testosterone levels. Fructose provides energy for sperm motility [14]: an interesting question to address would be whether sperm motility is modified in rats fed crude garlic.

In the testis, acid phosphatase is widely distributed in lysosomes of Sertoli cells, spermatogonia and late spermatids [15]. Activities of free lysosomal enzymes have been shown to rise when testicular steroidogenesis is increased [16]. In the present study, the decrease in acid phosphatase activity might reflect decreased testicular function in the treated rats and might be associated with the reduced secretion of testosterone. However, the possibility exists that the effects observed here on male reproductive functions were linked to the body weight loss detected at doses of 15% and 30% As. However, hallmarks of the negative effects of As on male reproductive functions (such as decreased seminal vesicle weight and plasma and testicular tissue testosterone contents) were also observed at doses of 5% and 10% As that did not induce a body weight loss. Moreover, by using a protocol of daily gavage administration, that reduced the possibility of adulteration of rats pellets, Dixit and Joshi [6] showed that As induced a reduction in accessory gland weight and hypospermatogenesis.

Administration of crude garlic resulted in decreased serum and testicular testosterone levels, suggesting that crude garlic has an inhibitory effect on testosterone production. Interestingly, this effect is dose-dependent (10%, 15% and 30%). The reduction in circulating and intra-testicular testosterone levels was associated with elevated LH levels in rats treated with 10%, 15% and 30% crude garlic. These results suggest a diminished responsiveness of Leydig cells to LH and/or a direct inhibition of testicular steroidogenesis and as such a testicular alteration in the gonadotropin-testosterone axis. Previous data from Yuriko *et al*. [17] indicated that increased testicular testosterone concentrations after treatment with 8 g of garlic powder was associated with an increase in LH plasma levels. The discrepancies observed in testosterone levels between our present study and the Yuriko's study could be attributed to the different types of garlic preparations used. Indeed, it is possible that crude garlic (present study) and garlic powder [17] do not contain the same active compounds. However, As is most frequently used in its crude form in cooking.

Because a decrease in testosterone levels was observed after crude garlic feeding, it was of interest to examine if the substrate of androgen was affected by the treatment. Indeed, cholesterol is involved in testicular steroidogenesis and is the most important precursor in the synthesis of steroid hormones. In our study, the cholesterol content in testicular tissue remained unchanged. These results suggest that crude garlic might inhibits steroidogenesis by an other way than a decrease in its substrate income. Therefore, one may hypothesize that As inhibits steroidogenesis in three different ways: (i) it might affect free cholesterol mobilization towards Leydig cell mitochondria; (ii) it might disrupt cholesterol mitochondrial translocation, which is an important step of steroidogenesis with the STAR protein as an effector;

and (iii) it might prevent cholesterol conversion into testosterone by impairing activities of key regulatory enzymes of steroidogenesis. These hypotheses are currently being investigated.

Testosterone has been shown to be essential for spermatogenesis completion, because it stimulates the conversion of round spermatids into elongated spermatids between stage VII and stage VIII of the spermatogenetic cycle. Androgen deficiency disturbs the spermiation process [18] by altering spermatid-Sertoli cell junctions, which results in premature detachment of round spermatids from Sertoli cells and seminal epithelium [19], along with apoptosis and activation of caspases [20]. In this context, the decrease in plasma and testicular testosterone production observed in the present study might explain the increased percentage of empty seminiferous tubules in As-fed rats. Moreover, decreased testosterone levels have been previously associated with histological alterations in Sertoli and Leydig (androgen target) cells [21]. In this context, the possibility exists that the ultrastructural alterations of Sertoli and Leydig cells observed here were related to the decreased testosterone levels. Therefore, our results are in accordance with the study of Dixit and Joshi [6] who reported a spermatogenesis arrest at the primary spermatocyte stage with 50 mg of garlic powder oral administration for 70 days. We showed here that raw crude garlic feeding impaired male reproductive function and spermatogenesis in male rats. Other data obtained with different garlic preparations has shown that garlic is effective in assisting the recovery of testicular function after experimental testicular hypogonadism [9]. These discrepancies could be related to the type of preparations used (e.g. garlic powder [6, 17], water extract [12], aged garlic, raw garlic juice and heated garlic juice [9]) or the doses and the method of administration (gavage, i.p. injection, *ad libitum*). The active principle in garlic supporting the inhibitory effect remains to be identified. One molecule, attridium (diallyl trisulfide), is a good candidate because it is known for its spermicidal activity *in vitro* [7, 8].

In summary, we showed that crude garlic feeding altered the reproductive function in adult male rats in accessory glands (prostate, epididymis and seminal vesicle) and testis (spermatogenesis). This action is probably related to an effect of garlic on the Leydig cells, and perhaps also on the Sertoli cells, with a decrease in serum and testicular testosterone levels and a disruption of normal spermatogenesis.

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