VETERINARSKI ARHIV 79 (5), 509-516, 2009

The histological investigation on the testes of mice after an application of capsaicin

Hatice Erdost*, Cansel G. Özgüden Akkoç, Nesrin Özfiliz, Tuncay İlhan, and Şerife Tütüncü

Department of Histology and Embryology, Faculty of Veterinary Medicine, Uludağ University, Bursa, Turkey

ERDOST, H., C. G. Ö. AKKOÇ, N. ÖZFILIZ, T. İLHAN, Ş. TÜTÜNCÜ: The histological investigation on the testes of mice after an application of capsaicin. Vet. arhiv 79, 509-516, 2009.

ABSTRACT

In the present study we examined the effects of capsaicin, a pungent principle of red hot pepper, on mice testes. Capsaicin was injected subcutaneously at a dose of 1 mg/kg body means everyday for a week each time on 21, 35 and 50-day-olds respectively. The body and testes means of all mice comprising the experiment groups were higher than the control groups. The intertubular compartment of the testes in both group mice showed ++ positive reaction with Oil Red O staining. Spermatogenic cell serial formation took place earlier compared to the control group. Our study results indicate that capsaicin is relatively non toxic at this dose (1 mg/kg body mean) tested in male mice, no mortality was observed during the study, the experiment groups were hyperactive and spermatogenic series develop earlier.

Key words: capsaicin, testes, mice, light microscopy

Introduction

Red hot chili peppers have come into their own recently, both as a culinary spice and as a hot new medical remedy. Long used as a food spice and an aid to digestion, red chilies or cayenne peppers were once thought to aggravate stomach ulcers (MAKARA et al., 1965). Although capsaicin may cause neurogenic inflammation per se under certain physiologic conditions, it also has analgesic and anti-inflammatory activities and is used currently in topical creams and gels to mitigate neurogenic pain (SZALLASI, 1995; CATERINA et al., 1997; TOMINAGA et al., 1998; GUO et al., 1999; MICHAEL and PRIESTLEY, 1999).

*Corresponding author:

Prof. Dr. Hatice Erdost, Uludağ Üniversitesi, Veteriner Fakültesi, Histoloji-Embriyoloji Anabilim Dalı, 16059 Görükle/ Bursa, Turkey, Phone: +90 224 2941262; Fax: +90 224 2941202; E-mail: edost@uludag.edu.tr

ISSN 0372-5480
Printed in Croatia

Capsaicin is the pungent ingredient in a wide variety of red peppers of the genus Capsicum. A large number of studies have established the molecule of capsaicin as an important probe for sensory neuron mechanism, but the mechanism underlying the neuronal effects of capsaicin is not completely clear (HOLZER, 1991; GOVINDARAJAN and SATHYANARAYANA, 1991; NEWSON et al., 2005). Now its consumption is expanding worldwide owing to various functional properties such as anti-carcinogenic and anti-oxidative activities (MODLY et al., 1986; MATERSKA and PERUCKA, 2005).

TRAURIG et al. (1984) found a reduction in the oestrus cycles of female rats treated with capsaicin; the proportion of pregnancy declined; and there was a decrease in decidua formed in the uterus; in males there was a decrease in the proportion of rats found to impregnate. Red hot pepper added to the diet of cocks in low doses during the developing period decreased body weight gain, but it stimulated the development of the reproductive organs (ÖZER et al., 2006). The information about the effects of capsaicin on the testes is limited.

The present study aimed at examining whether capsaicin would cause any developmental differences in the prepubertal (day 35th), pubertal (day 50th) and adult (day 75th) periods of mice testes on histological examinations. Data obtained from the present study will be used either in human medicine or veterinary medicine to provide evidence for the possible utility of capsaicin in reproductive systems.

Materials and methods

Animals and protocols. In the present study, 60 Swiss albino male mice were used at 21 days of age. Water and food were provided ad libitum. They were on a standard light cycle and housed at a temperature of 23 ± 1 °C.

Animals were divided into two groups each containing 30 mice. The first group was the control group. The experiment group was given capsaicin (98% pure, purchased from Sigma Chemical Company, St. Louis, MO.). Capsaicin was dissolved in a solution comprising 90% phosphate buffer saline and 10% Tween 20 and then injected subcutaneously at a dose of 1 mg/kg body mass everyday for a week starting from the 21th, 35th and 50th days of age. In the control group, mice were injected subcutaneously with phosphate buffer saline and Tween 20 as in the experiment group.

At the end of the 35th, 50th and 75th days, animals were sacrificed by cervical dislocation under diethyl ether inhalation anesthesia. Both control and experiment group mice were weighed at the same age. In this study, the experimental protocols were approved by the Experimental Animal Research Committee of Uludag University (Protocol number: 1.06.2004/1).

Light microscopy. Testes were removed carefully, trimmed of extraneous tissue, and then weighed. For light microscopic examination, samples were fixed in Bouine

and Ca-formalin solutions. Five µm sections obtained from the parafin embedded tissue pieces. The slides for each stage were stained with triple stain Crossmonn's modification (CROSSMONN, 1937) and Oil Red O method (GRIMSTONE and SKAER, 1972). The gradation of Oil Red O positive reaction was assessed as follows, weak (+), normal (++), strong (+++). The slides were viewed with a Nikon ecilipse 80i Microscope-Ds Camera Control Unit DS-L1, and then figures were taken.

Statistical analysis. Body and testes weights of the three groups were compared using the Univariate Analysis of Variance (one-way ANOVA test) with post hoc test Bonferroni. All statistical analyses were performed using SPSS statistical program (Edition 13.0, SPSS Inc., Chicago, IL, USA).

Results

No mortality was observed during the experiment period and the appearance of all animals was indicative of good health. Mice in the experiment group, especially on the 50^{th} and 75^{th} days, were hyperactive compared with mice in the control group, but no other specific physical signs related with the treatment were observed.

Body and testes weight data for mice are shown in Table 1. Total body weights were less in all mice in the control group and significant differences ($P<0.05$) were observed in favor of the experiment group. There were no significant differences between the testes weights of the control and experiment groups.

Table 1. Morphologic values from control and experiment groups (Mean ± SE)

Parameters	n	35 day-old		50 day-old		75 day-old	
		Control	Experiment	Control	Experiment	Control	Experiment
Body means (g)	10	20.60 ± 0.83	27.70 ± 0.83*	27.10 ± 0.83	32.80 ± 0.83*	28.60 ± 0.83	32.90 ± 0.83*
Testes means (g)	10	0.05 ± 0.003	0.05 ± 0.003	0.07 ± 0.003	0.09 ± 0.003	0.09 ± 0.003	0.08 ± 0.003

*Differences between control and experiment groups are statistically significant ($P<0.05$)

Standard protocol for parafin-embedded tissues allows us to distinguish the nuclei of cells based upon their staining affinity for the basic stain triple. Nuclear characteristics within each cell association are sufficiently different so that, with experience, we can identify the cell type.

In the 35 day-olds, the wall of the seminiferous tubules contains spermatogenic cell lines. In the first line, there were spermatogonia and Sertoli cells. Then spermatocytes and round spermatids were seen within the epithelium. Formation of spermatogenic cell lines

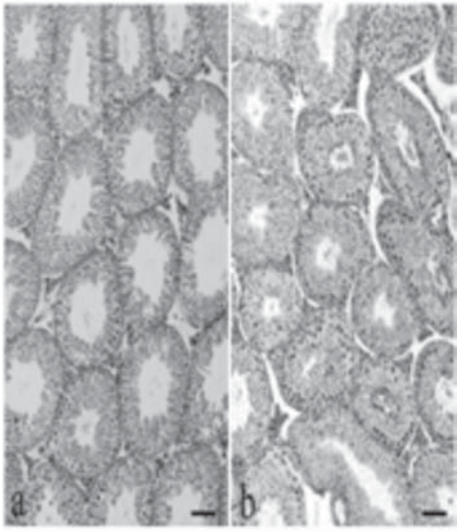

Fig. 1. Histological findings in 35 day old mice, the control group testes (a) and experimental group testes (b). Triple stain. Scale bar = 50 μm.

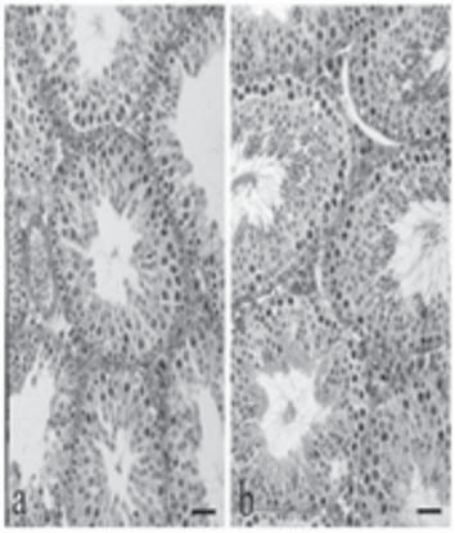

Fig. 2. Histological findings in 50 day old mice, the control group testes (a) and experimental group testes (b). Triple stain. Scale bar = 25 μm.

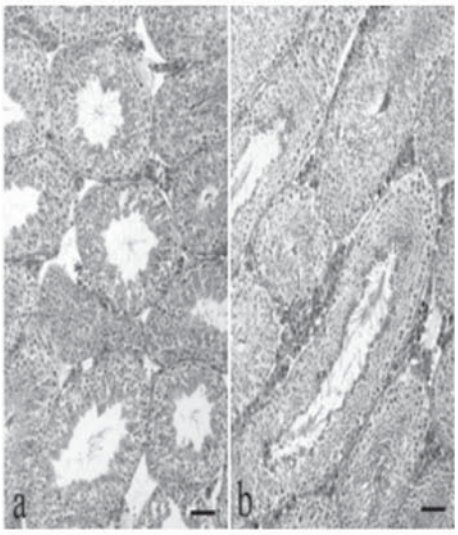

Fig. 3. Histological findings in 75 day old mice, the control group testes (a) and experimental group testes (b). Triple stain. Scale bar = 50 μm.

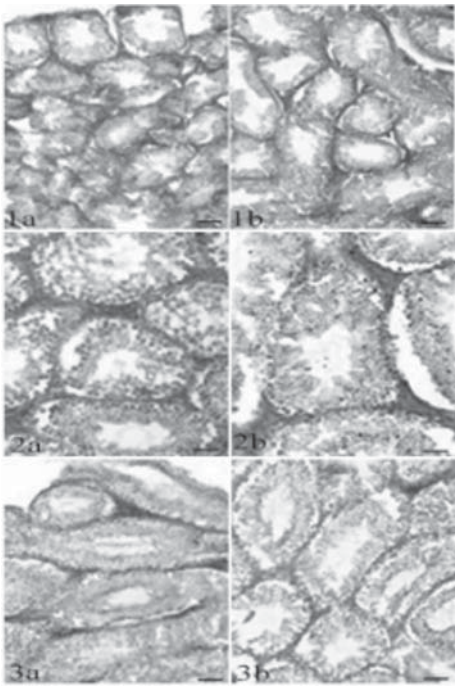

Fig. 4. Positive Oil Red O staining in Leydig cells surrounding the seminiferous tubules in both control and experiment groups testes: (1a) 35th day old control group testes, (1b) 35 day old experiment group testes, scale bar = 50 μm, (2a) 50 day old control group testes, (2b) 50 day old experiment group testes, scale bar = 25 μm, (3a) 75 day old control group testes, (3b) 75 day old experiment group testes, scale bar = 50 μm.

showed numerous spermatocytes in the experiment group compared to the control group, as shown in Fig. 1.

In the 50 day-olds of the experiment group, the seminiferous epithelium were observed to be thick and secondary spermatocytes and spermatids formed 4 or 5 cell lines near the seminiferous tubules in the experiment group. Spermatocytogenic cell lines were arranged in a cell stage composed of spermatogonia, spermatocytes, and two generations of spermatids (elongate and round). Spermatozoa in the seminiferous tubule lumen of the experiment group were seen to be more abundant than in the control group, as seen in Fig. 2.

In the 75 day-olds of the experiment group spermatocytes and spermatids were more dominant than in the control group, as seen in Fig. 3.

The data obtained by Oil Red O staining were similar at the end of the 35th, 50th and 75th days in both the control and experiment groups' testes. A positive reaction was observed in the Leydig cells surrounding the seminiferous tubules in the intertubular compartments showing ++ positive reaction in both the control and experiment groups, as shown in Fig. 4.

Discussion

NEWSON et al. (2005), reported capsaicin-treated rats were hyperactive compared with controls and we also observed hyperactivity in the experiment group, especially at the 50th and 75th days, compared to the control groups. The long-term biological effects of the application of chilli extract on the tongue of Balb/c mice have been studied. It resulted in high mortality and weight loss in mice (AGRAWAL and BHIDE, 1987). Our study results indicate that capsaicin is relatively non toxic at this dose; during the study there was no mortality in either the control or treated groups.

We observed Oil Red O stained slides giving ++ positive reactions in all groups in the intertubular compartment. A similar positive reaction has been described in the intersititial cells, the oocyte cytoplasm and the theca interna of the ovaries of both red hot pepper treated and control groups (ÖZER et al., 2005). During every phase of this study (ÖZER et al., 2005) it was observed that the fat content in the abdominal cavity of the control group was greater in quantity than in the experiment group. In cocks fed with a diet containing 1% red hot pepper (10 g/kg diet), their body weight gain decreased, whereas the testes' weight, length, width and wall thickness of tubules seminiferous contortus increased, and the completion of spermatogenic cell serial formation took place earlier when compared to control group (ÖZER et al., 2006). ÖZER et al. (2006) pointed out that red hot pepper added to the cock diet in low doses during the developing period affected lipid metabolism negatively and slowed down body growth. Our findings showed that the subcutaneous injection of capsaicin increased body weight gain and testes weight. Significant total body weight differences ($P<0.05$) were observed in favor of the experiment group but there was no significant difference between the testes weight of the control and experiment groups. ERDOST et al. (2006) pointed out that the number of FSH and LH immunoreactive cells increased in the unit area of the hypophysis when red hot pepper was added to chicken diets. In the present study, spermatogenic cell serial formation took place earlier compared to the control group.

The beneficial effects are particularly associated with long usage by some ethnic groups and its safe consumption levels, with a critical review of the studies on the reproductive system. Our study results indicate that capsaicin is relatively non toxic at this dose (1 mg/kg body weight) tested in male mice, no mortality was observed during the study, experimental groups were hyperactive and spermatogenic cell series developed

earlier. These findings suggest that capsaicin may have a stimulating function in the testes. Furthermore, this study would be a preliminary step for more studies in the future.

Acknowledgements

This work was supported by funds of University of Uludag (Project number: 2003-82).

References

AGRAWAL, R. C., S. V BHIDE (1987): Biological studies on carcinogenicity of chillies in Balb/c mice. Indian J. Med. Res. 86, 391-396.

CATERINA, M. J., M. A. SCHUMACHER, M. TOMINAGA, T. A. ROSEN, J. D. LEVINE, D. JULIUS (1997): The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816-824.

CROSSMONN, G. (1937): A modification of Mallory's connective tissue stain with a discussion of the principles involved. Anat. Rec. 69, 33-38.

ERDOST, H., A. ÖZER, M. YAKIŞIK, N. ÖZFILIZ, B. ZIK (2006): FSH and LH cells in the laying hens and cocks, fed with a diet containing red hot pepper. J. Food Agricult. Environ. 4, 119-123.

GRIMSTONE, A. V., R. J. SKAER (1972): A guide book to microscopical methods, Cambridge University Press, London, pp. 53-54.

GOVINDARAJAN, V. S., M. N. SATHYANARAYANA (1991): Capsicum-production, technology, chemistry, and quality. Part V. Impact on physiology, pharmacology, nutrition, and metabolism; structure, pungency, pain, and desensitization sequences. Crit. Rev. Food Sci. Nutr. 29, 435-471.

GUO, A., L. VULCHANOVA, J. WANG, X. LI, R. ELDE (1999): Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. Eur. J. Neurosci. 11**,** 946-958.

HOLZER, P. (1991): Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol. Rev. 43, 143-201.

MAKARA, G. B., L. CSALAY, R. FRENKL, Z. SOMFAI, K. SZEPESHÁZI (1965): Effect of capsaicin on experimental ulcer in the rat. Acta Med. Acad. Sci. Hung. 21, 213-216.

MATERSKA, M., I. PERUCKA (2005): Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L). J. Agric. Food Chem. 53, 1750-1756.

MICHAEL, G. J., J. V. PRIESTLEY (1999): Differential expression of the mRNA for the vanilloid receptor subtype 1 in cells of the adult rat dorsal root and nodose ganglia and its downregulation by axotomy. J. Neurosci. 19, 1844-1854.

MODLY, C. E., M. DAS, P. S. DON, C. L. MARCELO, H. MUKHTAR, D. R. BICKERS (1986): Capsaicin as an *in vitro* inhibitor of benzo(a)pyrene metabolism and its DNA binding in human and murine keratinocytes. Drug Metab. Dispos. 14, 413-416.

NEWSON, P., A. LYNCH-FRAME, R. ROACH, S. BENNETT, V. CARR, L. A CHAHL (2005): Intrinsic sensory deprivation induced by neonatal capsaicin treatment induces changes in rat brain and behaviour of possible relevance to schizophrenia. Br. J. Pharmacol. 146, 408-418.

ÖZER, A., H. ERDOST, B. ZIK (2005): Histological investigations on the effects of feeding a diet containing red hot pepper on the reproductive organs of the chicken. Phytother. Res. 19, 501-505.

ÖZER, A., B. ZIK, H. ERDOST, N. ÖZFILIZ (2006): Histological investigations on the effects of feeding a diet containing red hot pepper on the reproductive organs of the cock. Türk J. Vet. Anim. Sci. 30, 7-15.

SZALLASI, A. (1995): Autoradiographic visualization and pharmacological characterization of vanilloid (capsaicin) receptors in several species, including man. Acta Physiol. Scand. (Suppl.) 629, 1-68.

TRAURIG, H., A. SARIA, F. LEMBECK (1984): The effect of neonatal capsaicin treatment on growth and subsequent reproductive function in the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 327, 254-259.

TOMINAGA, M., M. J. CATERINA, A. B. MALBERG, T. A. ROSEN, H. GILBERT, K. SKINNER, B. E. RAUMANN, A. I. BASBAUM, D. JULIUS (1998): The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron. 21, 531-543.

Received: 14 April 2008
Accepted: 2 September 2009

ERDOST, H., C. G. Ö. AKKOÇ, N. ÖZFILIZ, T. İLHAN, Ş. TÜTÜNCÜ: Histološka istraživanja sjemenika miša nakon primjene kapsaicina. Vet. arhiv 79, 509-516, 2009.

SAŽETAK

Istraženi su učinci kapsaicina, jetkog i glavnog sastojka crvenoga papra, na sjemenike miševa. Kapsaicin je bio primijenjen miševima u dobi od 21, 35 i 50 dana supkutano u dozi od 1 mg/kg tjelesne mase svakoga dana u tjednu. Prosječna tjelesna masa i masa sjemenika u miševa pokusnih skupina bila je veća nego u kontrolnih skupina. Intertubularni odjeljci sjemenika obiju skupina miševa pokazivali su ++ pozitivnu reakciju bojenjem uljnim crvenilom. Spermatogene stanice oblikovale su se ranije u pokusnoj skupini u odnosu na kontrolnu. Rezultati pokazuju da je kapsaicin relativno netoksičan u miševa u dozi od 1 mg/kg tjelesne mase. Tijekom istraživanja uginuća nisu bila zabilježena. Životinje pokusne skupine bile su hiperaktivne s ranije razvijenim spermatogenim stanicama.

Ključne riječi: kapsaicin, testes, miševi