

Full Length Research Paper

Effects of extensive consumption of hot red pepper fruit on liver of rabbit

Mohamed A. Dkhil^{1,2,*} and Saleh Al-Quraishy²

¹Department of Zoology and Entomology, Faculty of Science, Helwan University, Egypt.

²Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia.

Accepted 1 May, 2022

In the present study, we examined the effects of hot red pepper on rabbit's hepatic tissue. Rabbits were orally ingested 2 g/Kg hot red pepper every day for 10 days. Hot red pepper induced a significant increase in temperature of rabbits after oral ingestion of each dose. Hepatic tissue damage was recorded through examination of the stained paraffin embedded sections. Inflammatory cellular infiltration and hepatocytic vacuolation were marked in rabbits ingested with the hot red pepper. Histochemical studies reveal a decrease in both of carbohydrates and protein contents in the liver. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, triglycerides and glucose were decreased in rabbit due to oral ingestion of hot red pepper. These scientific evidences show that hot red pepper possesses some chemical and pharmacological properties which are capable of inducing liver damage. Therefore, based on the findings of this study, the excessive consumption of red pepper is capable of inducing liver damage and so should be avoided.

Key words: Red pepper, rabbit liver, histology, biochemistry.

INTRODUCTION

Red pepper (*Capsicum annum*) is widely used as a spice and exhibits a wide range of physiological and pharmacological properties (Srinivasan and Sambaiah, 1991; Srinivasan and Chandrasekhara, 1992; Choi and Suh, 2004; Srinivasan, 2005). Red pepper is the mostly used spicy for food throughout the world, especially in Central America, Latin America, Africa and Asia (Nopaintaya and Nye, 1974; Chukwu, 2006). Red pepper is known to be harmful if consumed in excess (Nwaopara et al., 2004; Nwaopara et al., 2007). Red pepper possesses some chemical and pharmacological properties similar to the classes of drugs that are capable of inducing tissue damage (Govindarajan and Sathyanarayana, 1991).

In the past three decades, it has been experimentally documented that several common spices can also exert health beneficial physiological effects (Srinivasan, 2005).

These physiological effects of spices in most instances have been attributed to the main spice active principles present in them. Among these physiological influences spices are documented to exhibit, their hypolipidemic and antioxidant properties have far-reaching health implication (Manjunatha and Srinivasan, 2008).

For more than a century, capsaicin (8-methyl-N-vanillyl-6-noneamide), the active ingredient of pepper has captured the attention of many investigators, because its biting and burning properties suggest it could have physiological and pharmacological effect (Govindarajan and Sathyanarayana, 1991).

Capsaicinoids are mainly ingested as naturally occurring component of capsicums (chili, cayenne pepper, and red pepper). Their levels range from 0.1 to 2.5 mg/g (Parrish, 1996). The consumption of capsicum spices was reported to be 2.5 to 20 g/person/day in India, Thailand and Mexico (Monsereenusorn, 1983; Lopez-Carrillo et al., 1994). The maximum daily intake of capsaicin in USA and Europe from mild chili and paprika was roughly estimated to be 0.025 mg/Kg bw/day (Council of Europe, 2001).

*Corresponding author. E-mail: mohameddkhil@yahoo.com.
Tel: 00966-14675754. Fax: 00966-14678514.

Early studies demonstrated that peripheral administration of capsaicin produced a hypothermic effect, which failed to produce detectable changes in the metabolic rate. However, results from later investigations indicate that the ingestion of red pepper decreases appetite and subsequent protein and fat intake in Japanese females and energy intake in Caucasian males (Yoshioka et al., 1999).

Limited research has been reported into the effect of hot red pepper on the histopathology and biochemistry of liver. So it makes interesting to study the effect of extensive consumption of hot red pepper on the histopathology and biochemistry of rabbit's liver.

MATERIALS AND METHODS

Experimental animals

Ten adult male New Zealand rabbits (2000 to 2250 g) from the animal house of the faculty of pharmacy, King Saud University, Saudi Arabia were used. Rabbits were bred under specified pathogen-free conditions and fed a standard diet and water ad libitum. Animals were divided into two groups. The first groups were considered as the control group (6 non-treated rabbits). They received only normal feed with carrot every day for 10 days. The second group was orally ingested 2 g/Kg of red pepper fruit with carrot every day for 10 days. Rectal temperature was measured by an electric thermometer one min before and after eating of the hot red pepper. The experiments were approved by State authorities and followed Saudi Arabian rules for animal protection.

Histological and histochemical preparations

Animals from control and treated groups were slaughtered; dissected and small pieces of the liver were quickly removed, then fixed in Carnoy's fixative fluid. Following fixation, specimens were dehydrated, embedded, and then sectioned to five microns thickness. For histological examinations, sections were stained with Ehrlich Haematoxylin and Eosin (Drury and Wallington, 1980). Modified quantitative Ishak scoring system (Ishak et al., 1995) were used; scores of 1 to 3 were assigned to cases of minimal liver damage, scores of 4 to 8 to mild, scores of 9 to 12 to moderate and scores of 13 to 18 to severe cases. For the histochemical study, sections were stained with periodic acid-Schiff's method to demonstrate total carbohydrates (Hotchkiss, 1948), with bromophenol blue method to demonstrate total proteins (Maize et al., 1953). Many slides have been carefully examined for each animal (each group contained 6 animals and for each animal at least 3 slides from different areas of the organ were examined).

Biochemical and haematological studies

The blood was collected and allowed to clot for 30 min at 4 °C, and centrifuged at 3000g for 3 min. Sera were collected and stored at -20°C. Total cholesterol and triglyceride were measured in an auto-analyzer (Hitachi autoanalyzer, Hitachi Co., Tokyo) using special kits (DiaSys, Germany). Serum aspartate aminotransferase (AST), aniline aminotransferase (ALT) and glucose (Biosystems, Spain) levels were determined using commercial kits on a Shimadzu-UV 1230 model spectrophotometer. Some blood was collected into tubes with ethylene diamintetra acetic acid for the determination of some hematological parameters (total erythrocytes count, total

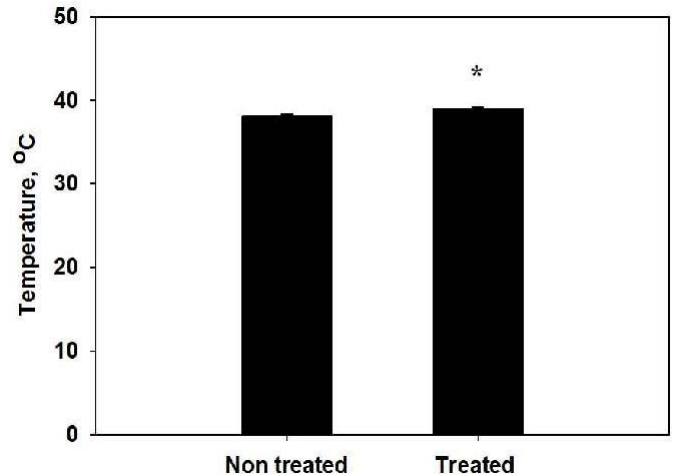


Figure 1. Effect of hot red pepper administration on body temperature of rabbit. Data presented as means \pm S.D. *Student's t-test was performed and a value of $P < 0.05$ were considered statistically significant

Leucocytic count, hemoglobin contents and hematocrit) using an automatic counter (VET-530 CA Medonic; Medonic, Stockholm, Sweden).

Statistical analysis

Statistical analyses were performed using an unpaired Student's t-test.

RESULTS

Our results showed that hot red pepper induced a significant increase ($P < 0.05$) in body temperature after eating (Figure 1). Hepatic tissue was affected and became darker in color. Hepatic interlobular areas of all examined sections were rich in inflammatory cellular infiltration (Figure 2B), in comparison to the control rabbits (Figure 2A). The lobular inflammation is characterized by predominant infiltrations of lymphocytes, plasma cells, and histiocytes, which are localized in perivascular and parenchymal areas. Apoptotic bodies were rare. All these alterations are considered in the histological liver activity index according to Ishak, which can be categorized as 9 to 11 for the liver of the treated rabbits in comparison to 1 and 3 for non-treated controls (Table 1). Hot red pepper treatment induced a hepatocytic vacuolation in most of the hepatocytes (Figure 2B). Sinusoids became moderately dilated containing more kupffer cells on its periphery.

Treated animals suffered impaired liver function. Both levels of ALT and AST enzymes were increased about 45%, when compared to the control rabbits (Table 2). Serum blood levels of cholesterol and triglycerides in rabbits receiving diet containing 4 gm hot red pepper for 10 days were significantly reduced reaching 31.2 ± 8.2

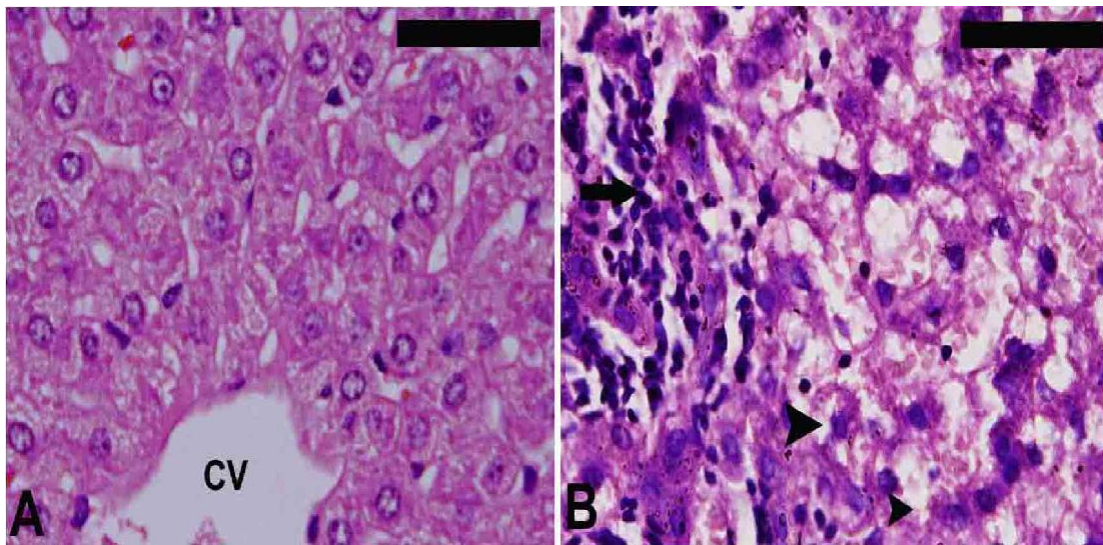


Figure 2. Histological changes in liver of rabbit. A, Control liver with central vein (CV) and surrounding hepatocytes, sinusoids lined with Kupffer cells. B, Liver sections of rabbits administered red pepper with inflammatory cellular infiltrations (arrow), hepatocytic vacuolations (arrow head) and prominent Kupffer cells. Sections were stained with hematoxylin-eosin. Scale bar = 50 μ m.

Table 1. Histopathological scores of inflammation after treatment of rabbit with hot red pepper.

Liver parameter	Control non-treated rabbits	Treated rabbits
Histological activity index ^a	1-3	9-11
Sinusoid dilatation	+	++
Cytoplasmic vacuolization	+	+++
Binucleated cells	+	++
Cell swelling	NO	++
Hyperplasia of Kupffer cells	+	++

^a Modified according to Ishak et al. (1995). Score: 1 - 3, minimal; 4 - 8, mild; 9 - 12, moderate; 13 - 18, severe.

Table 2. Effect of hot red pepper supplementation on some biochemical and hematological parameters.

Variable	Control \pm SD	Treated \pm SD	P-value
ALT (IU/L) *	38.6 \pm 8.7	56.0 \pm 4.3	< 0.05
AST (IU/L) *	41.2 \pm 5.6	59.0 \pm 8.6	< 0.05
Cholesterol (mg/dl)*	37.1 \pm 7.3	31.2 \pm 8.2	< 0.01
Triglycerides (mg/dl)*	154.6 \pm 9.7	87.2 \pm 7.3	< 0.01
Glucose (mg/dl) *	94.3 \pm 8.3	82.7 \pm 6.4	< 0.05
Erythrocytes $\times 10^6/\text{mm}^3$	5.7 \pm 0.9	6.4 \pm 0.6	NS
Leucocytes $\times 10^3/\text{mm}^3$	9.7 \pm 3.2	8.9 \pm 2.7	NS
Hemoglobin g/dl	12.2 \pm 2.2	13.8 \pm 0.9	NS
Hematocrit %	41.5 \pm 5.9	45.0 \pm 3.1	NS

Data presented as means \pm S.D. * Student's *t*-test was performed and a value of *P* < 0.01 or *P* < 0.05 were considered statistically significant. NS, not significant. AST, aspartate aminotransferase; ALT, aniline aminotransferase.

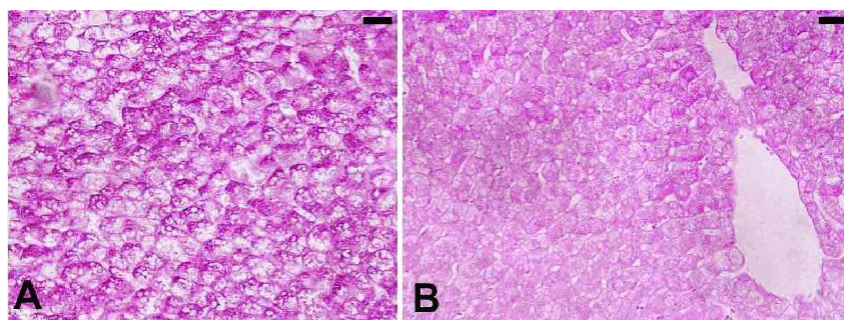


Figure 3. Total carbohydrates in liver sections. A, Control liver, carbohydrates in hepatocytes with intense red color, nuclei have no stains. B, Liver sections of rabbit administered red pepper with a slight decrease in carbohydrate content. Sections were stained with periodic Schiff's method. Scale bar=100 μ m.

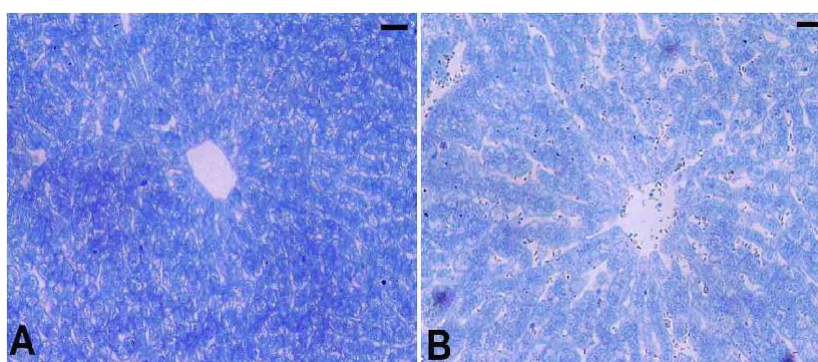


Figure 4. Protein content in liver sections. A, Control liver with a considerable amount of protein elements in the cytoplasm of hepatocytes. B, Treated liver section with a weak response towards bromophenol blue reaction. Sections were stained with bromophenol blue method. Scale bar = 100 μ m.

and 87.2 ± 7.3 mg/dl, respectively (Table 2). Hot red pepper was able to significantly decrease the level of serum blood glucose to 82.7 ± 6.4 mg/dl, compared to 94.3 ± 8.3 mg/dl, in control rabbits (Table 2). Administration of hot red pepper has no significant effect on both of erythrocyte and leucocyte counts. In addition, the hemoglobin and hematocrite values were not significantly affected (Table 2).

Control liver sections stained with PAS method is shown in Figure (3A). The second group administered hot red pepper had a slight decrease in glycogen in some hepatocytes. Glycogen appeared around the cell membrane (Figure 3B). Examination of liver sections from the control group, stained by bromophenol blue method, showed normal protein content (Figure 4A). Protein content was moderately decreased in the hepatocytes of rabbits inoculated with hot red pepper (Figure 4B).

DISCUSSION

Here, we show that hot red pepper exhibits a thermogenic activity, evidenced as a significant increase

in rabbit's body temperature after eating. This thermogenic activity was reported by Yoshioka et al. (2001, 2004). Hot red pepper consumption causes a red face, increase in whole body temperature, and much sweating. This shows the increase in metabolic rate, which mean that capsaicin is a thermogenic substance, help to burn energy from food, especially food with high fat content. For this reason, most diet products contain red pepper as an ingredient (Kawada et al., 1988; Yoshioka et al., 2001).

These results indicate that extensive consumption of hot red pepper reduce adiposity, a phenomenon which can be explained partly by the enhancing effects of capsaicin on energy and lipid metabolism via catecholamine secretion from the adrenal medulla (Watanabe et al., 1987a,b; Kawada et al., 1988). Since an increase in sympathetic nervous system (SNS) activity affects food intake behaviour (Russek et al., 1987; Bray, 1991; Raben et al., 1996), we hypothesized that the usage of red pepper (capsaicin) to the diet can decrease food intake and that this is associated with an increase in SNS activity. These facts were found in our results demonstrating the significant decrease in both of blood serum

cholesterol and triglycerides after administration of hot red pepper for 10 days.

Results also showed a remarkable cellular infiltration in the hepatic tissue. This supports Nwaopara et al. (2007) whose studies suggested that abundance of leucocytes, in general, and lymphocytes, in particular, are a prominent response of body tissues facing any injurious impacts. Leukocyte elevations and adherence to the vascular endothelium have been suggested by Miura et al. (1991) and McCafferty et al. (1995) to play an important role in the pathogenesis of drugs-associated injury.

Zhang and Wang (1984) suggested that the cytoplasmic vacuolation is mainly a consequence of considerable disturbance in lipid inclusions and fat metabolism occurring during pathological changes. Also, vacuolar degeneration has been regarded by Durham et al. (1990) to be an alteration produced to collect the injurious substances in the cells. In this study, the vacuolation of the cytoplasm of the liver cells appeared at first in the hepatocytes of the peripheral zone of the hepatic lobules, extending gradually toward the center. This may be due to the direction of the lobular blood supply. Vacuolation and damage of liver cells were noted by other investigators following treatment with different agents (Yabe, 2000; Samaranayake et al., 2000).

The induced hepatocellular damage was demonstrated by the increased activities of ALS and AST after hot red pepper administration. Decrease in blood serum glucose due to red pepper administration or due to its active compound capsaicin was reported by Monsereenusorn (1983) and Chaiyasit et al. (2009). The metabolic disorder in the rabbit's hepatic tissue leads to a disturbance in both of carbohydrate and protein contents in liver as demonstrated histochemically.

These scientific evidences show that hot red pepper under study possesses some chemical and pharmacological properties similar to the classes of drugs that are capable of inducing liver damage and thus, explains its capability to affect the histological changes observed. Therefore, based on the findings of this study, the excessive consumption of red pepper is capable of inducing liver damage and so should be avoided.

ACKNOWLEDGMENTS

This work was gratefully supported by the centre of Excellence for Biodiversity Research, College of Science, King Saud University, Riyadh, Saudi Arabia.

REFERENCES

Bray G (1991). A disorder of nutrient partitioning: MONA LISA hypothesis. *J. Nutr.*, 121:1146–1162.
Chaiyasit K, Khovidhunkit W, Wittayalertpanya S (2009). Pharmacokinetic and the effect of capsaicin in *Capsicum frutescens* on decreasing plasma glucose level. *J. Med. Assoc. Thai.*, 92:108-113.

Choi YM, Suh HJ (2004). Pharmacological effects of fermented red pepper. *Phytother. Res.* 18: 884-888.
Chukwu LO (2006). Histophysiological and basal metabolic responses of albino rat, *Rattus norvegicus* (L) exposed to aqueous pepper extracts. *A. J. Biotechnol.* 5: 1279-1283.
Council of Europe (2001). Committee of experts on flavouring substances. Datasheet on capsaicin.
Drury RA, Wallington EA (1980). Carleton's histological technique, 5th edn. Oxford University Press, Oxford New York Toronto, pp. 188–189, 237–240, 290–291.
Durham SK, Brouwer A, Barelds RJ (1990). Comparative endotoxin-induced hepatic injury in young and aged rats. *J. Pathol.* 162: 341-349.
Govindarajan VS, Sathyanarayana MN (1991). Capsicum production, echnology, Chemistry and quality. Part V. Impact on physiology, pharmacology, nutrition and metabolism: structure, pungency, pain and desensitization sequences. *Crit. Rev. Food Sci. Nutr.* 29: 435–474.
Hotchkiss RD (1948). A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch. Biochem.* 16: 131.
Ishak K, Baptista A, Bianchi L, Callea F, Degroote J, Gudat F, Denk H, Desmet V, Korb G, Macsween RNM, Phillips MJ, Portmann BG, Poulsen H, Scheuer PJ, Schmid M, Thaler H (1995). Histological grading and staging of chronic hepatitis. *J. Hepatol.* 22: 696–699.
Kawada T, Sakabe S, Watanabe T, Yamamoto M, Iwai K (1988). Some pungent principles of spices cause the adrenal medulla to secrete catecholamine in anesthetized rats. *Proc. Soc. Exp. Biol. Med.* 188: 229–233.
Lopez-Carrillo L, Avila HM, Dubrow R (1994). Chili pepper consumption and gastric cancer in Mexico: A case-control study. *Am. J. Epidemiol.* 139: 263-271.
Maize D, Brewer PA, Affert M (1953). The cytochemical staining and measurements of protein with mercuric bromophenol blue. *Bid Bull.* 104: 57-67.
Manjunatha H, Srinivasan K (2008). Hypolipidemic and antioxidant potency of heat processed turmeric and red pepper in experimental Rats. *Afr. J. Food Sci.* 2: 1-6.
McCafferty D, Granger DN, Wallace JL (1995). Indomethacin-induced gastric injury and leukocyte adherence in arthritic versus healthy rats. *Gastroenterol.* 109:1173-1180.
Miura S, Suematsu M, Tanaka S, Nagata H, Houzawa SI, Suzuki M, Kurose I, Serizawa H, Tsuchiya M (1991). Microcirculatory disturbance in indomethacin-induced intestinal ulcer. *Am. J. Physiol.*, 24:213-309.
Monsereenusorn Y (1983). Subchronic toxicity studies of capsaicin and capsaicin in rats. *Res. Commun. Chem. Pathol. Pharmacol.*, 41: 95-110.
Nopaintaya W, Nye SW (1974). Duodenal mucosal response to the pungent principle of Hot pepper (capsaicin) in rat: light and Electron microscopic study. *Toxicology and applied pharmacology*, 30: 149-161.
Nwaopara AO, Anyanwu LC, Oyinbo CA, Anaikot IC (2004). The histological changes In pancreas of Wister rats fed with diets containing Yaji (Local meat sauce). *J. Expt. Clin. Anat.*, 3: 44-47.
Nwaopara AO, Odike MAC, Inegbenebor U, Adoye MI (2007). The combined effects of excessive consumption of ginger, clove, red pepper and black pepper on the histology of the liver. *Pakistan. J. Nutr.*, 6: 524-527.
Nwaopara AQ, Odike MAC, Inegbenebor U, Adoye MI (2007). The combined effects of excessive consumption of ginger, clove, red pepper and black pepper on the histology of the liver. *Pak. J. Nutr.*, 6: 524-527.
Parrish M (1996). Liquid chromatographic method of determining capsaicinoids in capsicums and their extractives: Collaborative study. *J. Assoc. Off. Anal. Chem. Int.*, 79: 738-745.
Raben A, Holst JJ, Christensen NJ, Astrup A (1996). Determinants of postprandial appetite sensations: macronutrient intake and glucose metabolism. *Int. J. Obes.*, 20: 161–169.
Russek M, Vega C, Berrera J, Soto-Mora LM, Langazorta A, Racotta R (1987). Anorexia elicited by different catecholamines in rats. *Appetite.* 9: 119–126.

- Samaranayake MD, Wickramasinghe SM, Angunawela P, Jayasekera S, Iwai S, Fukushima S (2000). Inhibition of chemically induced liver carcinogenesis in Wistar rats by garlic (*Allium sativum*). *Phytother Res.*, 14: 564-567.
- Srinivasan K, Sambaiiah K. (1991). The effect of spices on cholesterol 7 alpha-hydroxylase activity and on serum and hepatic cholesterol levels in the rat. *Int. J. Vitam. Nutr. Res.*, 61:364-369.
- Srinivasan K. (2005). Role of spices beyond food flavouring: Nutraceuticals with multiple health effects. *Food Reviews Int.*, 21: 167-188.
- Srinivasan MR, Chandrasekhara N (1992). Comparative influence of vanillin and capsaicin on liver and blood lipids in the rat. *Ind. J. Med. Res.*, 96: 133-135.
- Watanabe T, Kawada T, Iwai K (1987a). Enhancement by capsaicin of energy metabolism in rats through secretion of catecholamine from adrenal medulla. *Agric. Biol. Chem.*, 51, 75-79.
- Watanabe T, Kawada T, Yamamoto M, Iwai K (1987b). Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the adrenal medulla of anesthetized rats. *Biochem. Biophys. Res. Commun.*, 142. 259-264.
- Yabe N, Matsui H (2000). *Ampelopsis brevipedunculata* (Vitaceae) extract inhibits a progression of carbon tetrachloride-induced hepatic injury in the mice. *Phytomedicine*, 7: 493-498.
- Yoshioka M, St-Pierre S, Drapeau V, Dionne I, Doucet E, Suzuki M, Angelo Tremblay A. (1999). Effects of red pepper on appetite and energy intake. *Br. J. Nutr.*, 82: 115-123.
- Yoshioka M, Doucet E, Drapeau V, Dionne I, Tremblay A (2001). Combined effects of red pepper and caffeine consumption on 24 h energy balance in subjects given free access to foods. *Br. J. Nutr.*, 85: 203-211.
- Yoshioka M, Imanaga M, Ueyama H, Yamane M, Kubo Y, Boivin A, St-Amand J, Tanaka H, Kiyonaga A (2004). Maximum tolerable dose of red pepper decreases fat intake independently of spicy sensation in the mouth. *Br. J. Nutr.*, 9: 991-5.
- Zhang LY, Wang CX (1984). Histopathological and histochemical studies on toxic effect of brodifacoum in mouse liver. *Acta. Acad. Med. Sci.*, 6: 386-388.