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SYSTEMIC AND LOCAL EFFECTS OF INTRAGASTRIC ADMINISTRATION OF THE HABANERO FRUIT (*CAPSICUM CHINENSE* JACQUIN C.V.) IN RATS

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The fruits of the habanero plant (*Capsicum chinense* Jacq.) are commonly used as spices. Their exceptionally hot flavour is the result of the substantial content of capsaicin that has among others the anticancer action. The experiments assess the impact of intragastric administration of a suspension of dried matter (dm) habanero fruit in peanut oil on the state of the digestive tract and parenchymal organs of rats. Habanero fruit with three different doses (0.08, 0.05 and 0.025 g of dry matter (d.m.) habanero fruit/kg b.w.) in 2 equal doses every 12 hours during 28 days was administered intragastrically in male rats. In day 8, 15 and 29 blood proofs were obtained to measure hematological parameters and alanine aminotransferase (ALT), aspartate aminotransferase (AST) activity, total bilirubin (BIL), total cholesterol (CHOL), glucose (GLUC), urea (U), and creatinine (CREA) content. Internal organs of rats were examined anatomopathologically. Between the study groups and control group there were no statistically significant differences in studied parameters. Post-mortem examinations as well as histological findings showed no pathological changes in the organs of rats. The study demonstrated a high level safety of the fruit habanero (*Capsicum chinense* Jacq.) administration in rats. There were no hematological, biochemical or post-mortem changes at doses that due to the amount of capsaicin can exhibit antitumor properties.

Key words: *capsaicin, habanero fruit, gastrointestinal tract, parenchymal organs, transaminases, urea, creatinine*

INTRODUCTION

The fruits of the habanero plant (*Capsicum chinense* Jacq.) are commonly used as spices and their exceptionally hot flavour is the result of the substantial content of capsaicin. When consumed in high doses they have beneficial effects in treating such diseases as cancer in humans and animals, however, the side-effects are no fully understood - information about the anticancer action of capsaicin originating in folk medicine have been confirmed in studies on cell cultures of prostate cancer, lung cancer, stomach, breast and pancreas cancer, and leukemia and glioma - actually it is known that the substance induces apoptosis of cancer cells (1-3). It was found that a dose of 10 mg/kg in mice, after implantation of human small-cell carcinoma, significantly reduced the size of the tumors without causing side effects (1). In other studies, after subcutaneous implantation of human colon adenocarcinoma cells into mice, an intraperitoneal dose of capsaicin (1–3 mg/kg body weight) was given every three days. In this 30-day experiment, an inhibition of tumor growth was observed and there were no side effects of the therapy (4). The anticancer effect of capsaicin is also subject to the inhibition of angiogenesis *in vitro* and *in vivo* by reducing the secretion of vascular endothelial growth factor (VEGF) (5). It was also found that capsaicin used at a dose of 1 mg/kg body weight (b.w.) reduced the levels of proinflammatory cytokines and stimulated the secretion of IL-10, demonstrating its anti-inflammatory action (6).

The average concentration of capsaicin originating from industrially produced foods consumed by people in Europe and the U.S. is 0.77 mg/person/day, the maximum level is 2.64 mg/person/day, and the recommended limit of capsaicin in ready-made meals is 5 mg/kg (7). However, in countries with culturally higher consumption of hot spices (Thailand, Mexico, India), the daily intake of capsaicinoids is 0.5–4 mg/kg b.w., which calculates to 25–200 mg/person/day, assuming that the average body weight is 50 kg (8). According to observations on the incidence of cancer in this group of people, too high an intake of capsaicin significantly increases the risk of gastric cancer, yet in an amount less than 30 mg/person/day (0.6 mg/kg b.w./day) the risk of the disease is greatly reduced (9). Nonetheless, this dose is still 24 times higher than the maximum consumed in Europe and the U.S.A.

Medical literature has a detailed description of the case of a 66-year-old man with prostate cancer which succumbed to the effects of the habanero fruit he consumed. Throughout the cancer, the important PSA (Prostate Specific Antigen) ratio always fell in the patient after eating sauce with habanero - up to 15 ml/day divided into 2 doses (10). The capsaicin content in this

sauce was set at 0.454 mg/ml, giving a dose of capsaicin 6.81 mg/person/day, which calculates to about 0.11 mg/kg b.w./day. Given the capsaicin content in dry matter (d.m.) of habanero fruit, the patient took about 1.58 g of d.m. habanero fruit per day, in two separate doses. This gives about 0.026 g of d.m. habanero fruit/kg b.w./day.

Capsaicin applied intragastrically in rats, even at a dose of 50 mg/kg b.w./day for 60 days showed a slight effect on the animals. The only side effect was the inhibition of growth (11). Typical symptoms of intoxication are seizures, convulsions, agitation, muscle spasms, yet these are only found after intraperitoneal administration of capsaicin at a dose of 9.5 mg/kg b.w. or orally at a dose of 154 mg/kg b.w. (LD_{50}) (12, 13). The safety of intragastric application of capsaicinoids is largely dependent on the concentration of substances in the applied mixture. The administration of pure capsaicin 0.014% solution in saline, in this manner, resulted in damage to the duodenal mucosa in the form of changes in the mitochondria, endoplasmic reticulum and ribosomes (14).

The aim of this study was to assess the impact of intragastric administration of a suspension of dried habanero fruit in peanut oil on the state of the digestive system mucosa and parenchymal organs of rats. The examined substance was administered in doses which can exert anti-cancer effect.

MATERIALS AND METHODS

Animals

The study was conducted on 72 male albino Wistar rats (120–125 g), originating from breeding laboratory animals. They were kept in an air-conditioned room at a relative humidity of 45–47% and a temperature of 22–23°C, with a light cycle of at 12 h light/12 h darkness. They were fed commercial feed for laboratory animals (LSM, Agropol Motycz, Poland) and were given tap water to drink *ad libitum*. The acclimation period was 16 days before the experiment. The animals were divided into 3 experimental groups (E1, E2 and E3) and 1 control group (C), numbering 18 individuals per group.

Experiments on animals were conducted with due approval of the procedures by the Local Animal Ethics Committee with regard to the care and use of animals.

Test substance

The test substance was ground dry ecologically grown habanero fruit from the Mexican states of Jukatan and Quintana Roo. In order to control the purity the content of the sum of aflatoxins (B1, B2, G1, G2) was determined with the immunoenzimatic method using the RIDASCREEN® Aflatoxin total test (R-Biopharm AG, Germany), and the ochratoxin A content of was determined with the RIDASCREEN® Ochratoxin A test (R-Biopharm AG, Germany). The obtained results were below the reference range (for the method). The capsaicinoids content in the substance measured with a specific HPLC was 7.64 mg/g d.m. (capsaicin and dihydrocapsaicin). The test substance after grinding was suspended in peanut oil and administered orally in the following daily doses: a low dose: 0.025 g d.m. habanero fruit/kg b.w. (E1 group), a medium dose: 0.05 g d.m. habanero fruit/kg b.w. (E2 group), and a high dose: 0.08 g d.m. habanero fruit/kg b.w. (group E3). The above mentioned doses were established on the basis of the capsaicinoids content in the test substance and the anti-cancer doses of capsaicin described in the literature. These daily doses were divided into 2 equal doses and given every 12 hours. The concentrations of the suspensions were prepared so that each animal received approximately 0.5 ml each. After immobilizing the animal, by gripping the nape of the neck, the suspension was applied using an atraumatic special plastic stomach tube for rodents: Instech-Solomon 15 ga. Control animals received approximately 0.5 ml of pure peanut oil using the same method.

Blood samples for testing

On the 8th, 15th and 29th day of the experiment, after a 12 h fast, six rats from each of the four groups (E1, E2, E3, C) were anesthetized using ketamine intramusculary, 80 mg/kg b.w. (Vetaketam; Vet-Agro, Poland). Under general anesthesia, blood was drawn by puncturing the right ventricle using a 0.8 mm needle. Blood samples were put into K_2EDTA test tubes in order to perform hematology tests, and into test tubes for coagulation in order to obtain serum. The animals were then euthanized with pentobarbital sodium (Morbital; Biowet Pulawy, Poland). Serum was obtained by centrifuging the tubes at 4°C for 30 minutes at 4000 rpm.

Hematological testing

The blood test was performed using a Scil Vet ABC Plus hematology analyzer (Horiba; Kyoto, Japan), obtaining the number of white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT) and the number of platelets (PLT). May-Grunwald staining was used for blood smears and viewed by light microscopy counting the percentage of individual leukocytes.

Blood serum biochemistry test

Determination of the activity of alanine transaminase (ALT), aspartate transaminase (AST) and the levels of glucose (GLU), cholesterol (CHOL), total bilirubin (BIL), urea (U) and creatinine (CREA) was performed by colorimetric method using a BS-130 biochemical analyser (Mindray; Shenzhen, China).

Morphological analysis

In necropsy testing performed immediately after euthanasia, biopsy samples from the stomach, duodenum, liver and kidneys were taken. The material was preserved in a 10% buffered formalin solution and submitted for standard histological processing. Sections 4 microns thick were stained with hematoxylin and eosin (HE) and inspected under a light microscope.

Statistical analysis

For all parameters, the mean and standard deviation (S.D.) were calculated. Statistical analysis was conducted by the Mann-Whitney U test at P-values of $P \leq 0.05$ (Statistica 10.0 software). For each parameter, statistically significant differences were calculated between control and experimental groups in day 8, 15 and 29.

RESULTS

Hematological and biochemical parameters

Hematology results are shown in *Table 1* and *2*. In group C, the WBC count during the whole experiment was relatively stable. In group E1, a slight decrease was observed on day 15. A similar observation was seen in groups E2 and E3 in day 29 of the experiment. There was a gradual slight increase in the number of RBCs in all test groups in proportion to the dose of the test

Table 1. Hematological parameters in experimental group E1 (0.025 g d.m./kg b.w.), E2 (0.05 g d.m./kg b.w.), E3 (0.08 g d.m./kg b.w.) and in control group C in day 8, 15 and 29.

			WBC			RBC			HGB			HCT			PLT		
Day			8	15	29	8	15	29	8	15	29	8	15	29	8	15	29
Group	C	X	5.98	5.68	5.03	7.27	7.97	8.39	15.32	16.12	15.63	42.25	45.60	46.00	628.33	581.50	626.00
		SD	0.92	0.84	2.27	0.33	0.58	0.50	0.40	0.81	0.73	2.45	3.36	2.24	61.02	198.31	85.89
	E1	X	5.38	4.53	5.22	7.51	8.20	8.42	14.90	15.90	15.82	43.42	46.85	47.13	633.67	488.83	632.33
		SD	2.73	2.12	1.57	0.31	0.38	0.67	0.63	0.87	0.73	2.31	2.82	4.13	76.13	90.35	38.76
	E2	X	5.07	5.45	4.37	7.26	8.46	9.05	15.72	16.13	16.27	41.83	48.07	50.23	662.17	576.00	613.00
		SD	1.64	1.92	0.32	0.73	0.66	0.45	3.26	1.16	1.88	5.36	3.52	2.42	169.98	86.48	82.82
	E3	X	5.02	5.02	4.40	7.84	8.52	8.36	14.78	16.25	14.18	45.68	48.48	46.13	635.83	530.83	538.00
		SD	0.67	0.59	1.05	1.05	0.36	0.87	0.39	1.16	0.82	6.72	3.74	3.98	186.51	38.44	76.31

There were no statistically significant differences between experimental and control groups.
WBC, white blood cells ($\times 10^3/mm^3$); RBC, red blood cells ($\times 10^6/mm^3$); HGB, hemoglobin (g/dL); HCT, hematocrit (%); PLT, platelets ($\times 10^3/mL$).

Table 2. Percentage of individual leukocytes in experimental group E1 (0.025 g d.m./kg b.w.), E2 (0.05 g d.m./kg b.w.), E3 (0.08 g d.m./kg b.w.) and in control group C in day 8, 15 and 29.

			BAND			SEG			EOS			BASO			LYM			MON		
Day			8	15	29	8	15	29	8	15	29	8	15	29	8	15	29	8	15	29
Group	C	X	0.00	0.00	0.00	16.33	10.33	10.83	0.00	0.00	0.67	0.00	0.00	0.00	83.67	89.67	88.50	0.00	0.00	0.00
		SD	0.00	0.00	0.00	7.74	3.98	4.07	0.00	0.00	1.03	0.00	0.00	0.00	7.74	3.98	4.51	0.00	0.00	0.00
	E1	X	0.00	0.00	0.00	10.17	16.33	9.00	0.00	0.00	1.33	0.00	0.00	0.00	89.83	83.67	89.67	0.00	0.00	0.00
		SD	0.00	0.00	0.00	6.01	7.99	3.35	0.00	0.00	1.21	0.00	0.00	0.00	6.01	7.99	3.14	0.00	0.00	0.00
	E2	X	0.00	0,00	0.00	17.33	13.33	8.50	0.00	0.00	0.50	0.00	0.00	0.00	82.67	86.67	91.00	0.00	0.00	0.00
		SD	0.00	0.00	0.00	6.68	6.15	4.23	0.00	0.00	0.84	0.00	0.00	0.00	6.68	6.15	4.98	0.00	0.00	0.00
	E3	X	0.00	0.00	0.00	10.50	18.50	13.33	0.00	0.00	2.00	0.00	0.00	0.00	89.50	81.50	84.67	0.00	0.00	0.00
		SD	0.00	0.00	0.00	5.75	6.89	8.64	0.00	0.00	2.00	0.00	0.00	0.00	5.75	6.89	9.48	0.00	0.00	0.00

There were no statistically significant differences between experimental and control groups.
BAND, bands; SEG, segmented neutrophils; EOS, eosinophils; BASO, basophils; LYM, lymphocytes; MON, monocytes.

Table 3. Biochemical parameters in experimental group E1 (0.025 g d.m./kg b.w.), E2 (0.05 g d.m./kg b.w.), E3 (0.08 g d.m./kg b.w.) and in control group C in day 8, 15 and 29.

			ALT			AST			BIL			CHOL			GLUC			U			CREA		
Day			8	15	29	8	15	29	8	15	29	8	15	29	8	15	29	8	15	29	8	15	29
Group	C	X	50.50	48.00	53.67	147.67	137.83	132.83	0.00	0.00	0.00	1.53	1.30	1.61	6.17	6.53	9.00	7.64	5.19	6.79	35.36	38.01	38.01
		SD	11.98	8.85	8.64	36.28	21.52	21.72	0.00	0.00	0.00	0.17	0.35	0.22	0.27	1.01	1.22	1.43	0.23	1.38	5.30	6.19	4.42
	E1	X	42.17	49.17	46.17	155.33	162.67	119.00	0.00	0.00	0.00	1.32	1.13	1.55	5.83	5.87	8.26	7.72	4.58	5.46	39.78	39.78	37.13
		SD	23.35	17.31	3.49	38.90	34.05	14.46	0.00	0.00	0.00	0.26	0.25	0.27	0.25	0.42	0.67	0.92	0.26	1.35	7.96	5.30	3.54
	E2	X	45.67	48.00	49.33	132.33	137.83	118.67	0.00	0.00	0.00	1.67	1.10	1.36	6.40	6.81	9.25	7.80	4.85	5.83	36.24	35.36	36.24
		SD	4.32	10.81	4.89	21.22	24.10	22.99	0.00	0.00	0.00	0.15	0.17	0.24	0.41	0.67	0.38	1.20	1.38	1.20	7.07	4.42	4.42
	E3	X	43.33	50.00	50.00	137.00	136.50	119.83	0.00	0.00	0.00	1.48	1.16	1.40	6.89	7.49	9.16	7.20	4.42	6.51	34.48	34.48	32.71
		SD	8.38	4.69	7.16	43.11	15.91	37.00	0.00	0.00	0.00	0.18	0.31	0.18	0.61	0.96	1.00	1.59	0.68	0.81	5.30	6.19	2.65

There were no statistically significant differences between experimental and control groups.
ALT, alanine aminotransferase (U/L); AST, aspartate aminotransferase (U/L); BIL, total bilirubin (μmol/L); CHOL, total cholesterol (mmol/L); GLUC, glucose (mmol/L); U, urea (mmol/L); CREA, creatinine (mmol/L).

substance on day 15 of the experiment. In the group receiving the highest dose of habanero (E3), the HGB content showed a slight decrease on day 29 of the study. HCT content in all groups was relatively stable. In group E1 on day 15, and in group E3 on day 29, a decrease in the number of PLTs was observed. Examination of blood smears showed no significant changes in the percentage of each leukocyte, however on the last day of the study all groups showed the appearance of eosinophils. Between the study groups and group C there were no statistically significant differences in any of the hematologic parameters studied, indicating a lack of effect of the test substance.

Results of the blood serum biochemistry test are shown in *Table 3*. Transaminases ALT and AST and CHOL, BIL, GLU and U and CREA concentration in the serum showed no statistically significant changes between the groups.

Post-mortem examinations

The bodies of all animals were tested immediately after euthanasia. Post-mortem examinations, performed in accordance with generally accepted practice, showed no pathological changes in the organs of rats.

Microscopic examination of the preparations obtained showed that the stomach and duodenum had normal histological structure, with the proper shaping of the individual layers of the stomach and distinct fundic and mucous glands (*Fig. 1*). Duodenal glands found in the submucosa were characterized by normal microscopic structure (*Fig. 2*). Microscopic images of the liver showed normal characteristics of the organ, with no pathological changes. Hepatocytes with eosinophilic cytoplasm were arranged in hepatic cords that arranged radially toward the central vein. The cross-sections of veins, arteries and bile ducts were well visible (*Fig. 3*). No differences were observed in the microscopic structure of rat kidney taken from either the experimental or control groups. They showed proper flesh structure in both the renal cortex and renal medulla regions. The proximal convoluted tubule, covered by simple epithelium were aligned regularly. The nucleus, surrounded by eosinophilic cytoplasm was clearly visible in the centre of the epithelial cells. The inner space of the tubules was star-shaped, concealed by the brush border membrane. Distal convoluted tubules were characterized by regular, round or oval inner space. Epithelial cell walls were somewhat indistinct (*Fig. 4*).

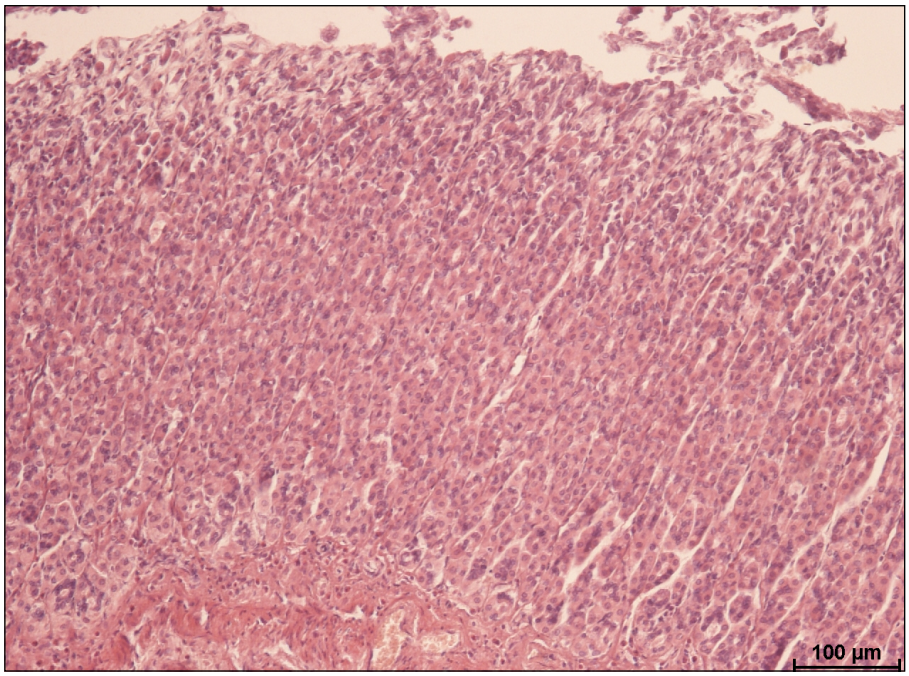

Fig. 1. Photomicrograph (H&E ×100) of stomach tissues from experimental animals treated with the high dose 0.08 g d.m. habanero fruit/kg b.w.

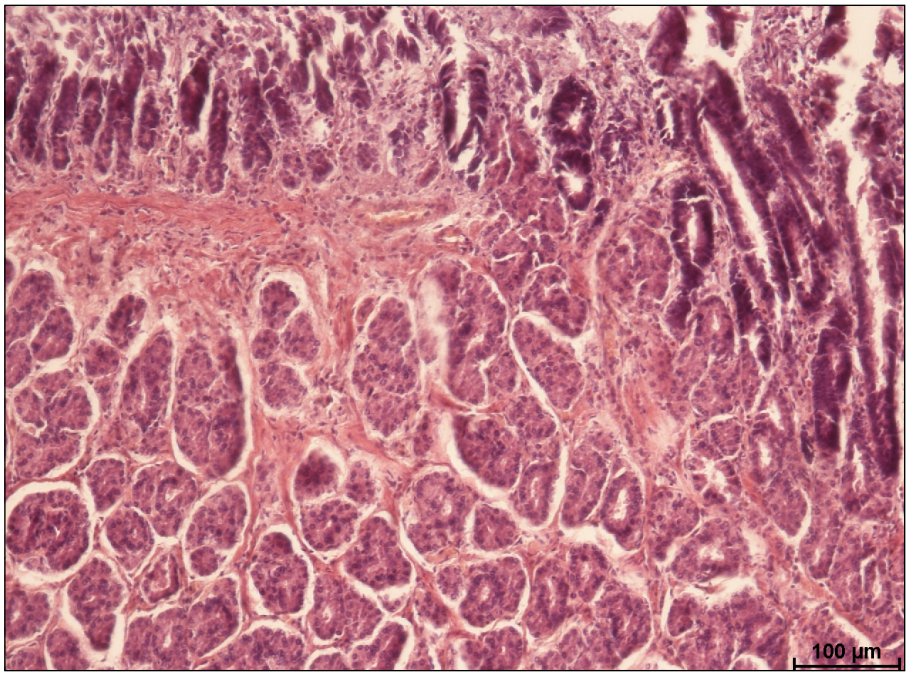

Fig. 2. Photomicrograph (H&E ×100) of duodenum tissues from experimental animals treated with the high dose 0.08 g d.m. habanero fruit/kg b.w.

DISCUSSION

Studies on the consumption of edible plants are commonly used to assess the risk in animals and humans, which is particularly important in the case of a pepper containing a significant amount of capsaicinoids (15). The sizes of the doses used in the experiment were based on the culturally conditioned high consumption of habanero by humans and data showing antitumor effect of capsaicin contained in the test substance (7-10). Studies have shown that the intragastric administration of the oil suspension of habanero fruit does not cause significant hematological, biochemical, anatomopathological or histopathological changes in rats.

Besides dihydrocapsaicin, nordihydrocapsaicin and homodihydrocapsaicin the most important ingredient of habanero in terms of toxicology is capsaicin. Suspended in peanut oil and administered intragastrically to rats it is rapidly absorbed, even up to 94%, with the maximum concentration achieved in blood after 1 hour (16, 17). It reaches the liver through the hepatic portal system, where it is partially metabolised by microsomes. The process is similar in rats, dogs and humans (18). In unchanged form, capsaicin is excreted in small amounts (0.095%) by the kidneys and in the feces (6.3%) (16, 19). With no data in the medical literature determining the toxicity in animals of the habanero fruit, it was necessary, therefore, to determine the effect of the test substance on parenchymal organs. Due to the different chemical composition of different peppers, it is difficult to interpret the results equitably. Hematology results obtained in all the groups studied showed no change, which is consistent with the results of

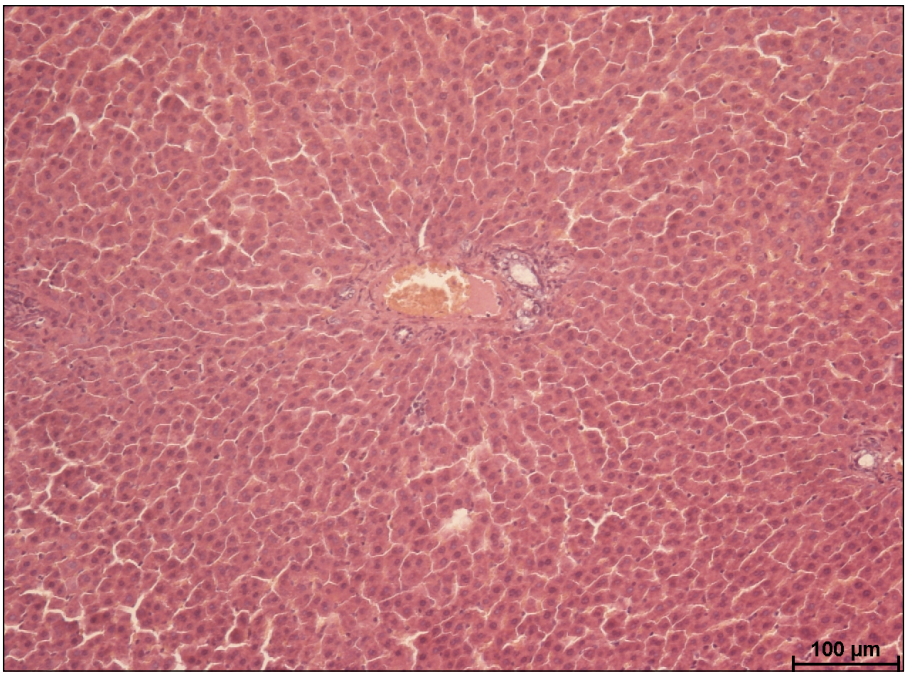

Fig. 3. Photomicrograph (H&E ×100) of liver tissues from experimental animals treated with the high dose 0.08 g d.m. habanero fruit/kg b.w.

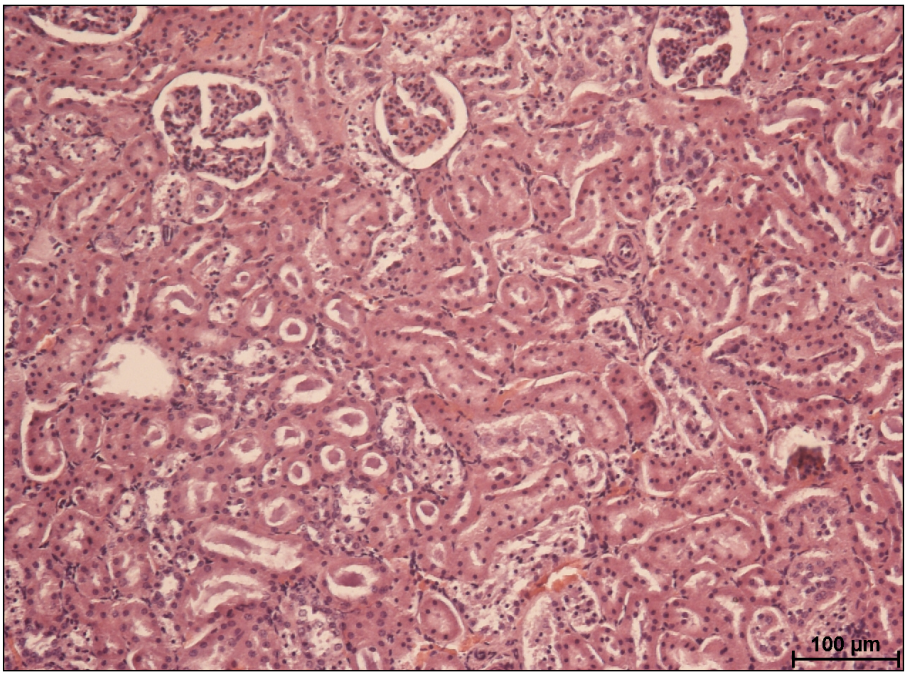

Fig. 4. Photomicrograph (H&E ×100) of kidney tissues from experimental animals treated with the high dose 0.08 g d.m. habanero fruit/kg b.w.

Zimmer *et al.* on the chronic use of the extract from the fruit of *Capsicum baccatum* on mice at a dose of 200 mg/kg b.w./day (19). This demonstrates the lack of significant effect of intragastrically administered habanero on the functional status of hematopoietic organs. The same authors also found no effect of the test substance on biochemical blood parameters (19). In day 29 of the examination an increase in the number of eosiniphils in blood was observed in both experimental and control groups, although the differences between the groups were not statistically significant. Moreover, eosinophilia was only diagnosed in some animals in all groups. The changes observed can be explained by a potential allergic reaction to peanut oil in which the test substance was suspended. Depending on sensory nerve modulation, capsaicin might also play a role in eosinoplilia (20). However, the addition of *Capsicum annum* to the diet may induce a slight dose-dependent increase of CHOL in the serum due to the high content of fatty acids in the fruit, yet there were no significant side effects at a concentration of up to 5% (21). However, intragastric administration *Capsicum baccatum* fruit extract at a dose of 0.5 g/kg/day causes a significant reduction in the level of blood urea nitrogen (BUN), GLU and CHOL (11) after 4 weeks. Similarly to Anthony *et al.* (22) in testing *Capsicum frutescens*, our study revealed no effect of habanero on the biochemical parameters of blood serum. This demonstrates the high safety of the test substance in the proposed doses. This is confirmed by the results of pathologic examination, which revealed no macroscopic changes within the internal organs. There were also no damage in the studied microscopic preparations. An addition of more than 10% ground fruit of

Capsicum frutescens to the diet of mice, however, results in glycogen depletion and anisocytosis of hepatocytes (23). The shedding of epithelial cells into the intestine and fatty vacuolation and necrosis of hepatocytes in the center of the lobules were observed in rats after 4 weeks of consuming the same amount, while a 2% addition to the diet did not result in any side effects (15, 21). The suspension of the test substance in peanut oil causes an additional intensification of the natural protective properties of peppers on gastrointestinal mucosa without reducing the bioavailability of the components (16, 24). Attention should be drawn to the gastroprotective role of capsaicin, which involves preventing gastric mucosa damage induced by indomethacin and ethanol (25, 26). It is even used as an orally administered gastroprotective drug for healthy human patients and patients with gastric mucosa damage (25). Capsaicin does not cause lasting tissue damage; it only stimulates neurons similarly to the way it happens in the case of mechanical damage or burns. Capsaicin-sensitive afferent neurons play an import ant role in sustaining the integrity of gastric mucosa - they exhibit a protective function through increasing blood flow in the mucous membrane as a result of the stimulation. A binding site for capsaicin, called transient receptor potential vanilloid 1 (TRPV-1), which is a non-selective cation channel, is located along gastric glands in the mucous membrane and submucosa, as well as around blood vessels and in the nerve plexus in muscle membrane and in the muscle layer (27-29). Capsaicin stimulates afferent neurons by activating (TRPV-1), which causes the release of calcitonin gene-related peptide (CGRP) and gastroprotection (30). A similar mechanism was also observed in the course of hyperosmolar detrusor overactivity, which in healthy rats leads to disorganisation of detrusor muscle contractility and difficulty in urination (31). Blocking TRPV-1 with capsazepine, similarly to blocking NOS, results in slower ulcer healing and decreases blood flow at the ulcer margin normally caused by the release of ghrelin, orexin A and nesfatin-1 (32). The presence of capsaicin in gastroprotection also depends on endogenous PGs and NO and by activating EP2 prostanoid receptor (33).

The study undertaken here demonstrated a high level of pharmacological safety of the fruit habanero (*Capsicum chinense* Jacq.) administration in rats. There were no hematological, biochemical or post-mortem changes at doses that due to the amount of capsaicin can exhibit antitumor properties. The use of capsaicin in cancer treatment and chemoprevention is widely debated, which has not prevented the introduction of capsaicin-containing dietary supplements to the market (34). The present study aimed at determining the safety of using habanero fruit with high capsaicin concentration may be a helpful for further consideration of the clinical application of this substance in humans and animals.

Conflict of interests: None declared.

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