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Clinical assessment of the anti-cancer activity of the capsaicin-containing habanero pepper extract in dogs: a preliminary study

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Summary

The aim of the study was a preliminary assessment of the anti-cancer efficacy of the capsaicin-containing habanero pepper extract in dogs. The study was conducted on a group of 50 dogs (33 females, and 17 males aged 6-18 years) diagnosed with different tumours, and 20 dogs (12 females and 8 males, aged 2-12 years) forming a control group. All animals were administered with a diet supplement based on habanero pepper extract containing capsaicin. Observations were conducted for a period of 6 months, during which time the general condition of the animals administered with the extract was monitored, and haematological as well as biochemical examinations were conducted at 2-week intervals in order to assess the tolerance of the animals to the extract. In the animals of the test group, tumour sizes were measured at monthly intervals. After the end of observations, the tumours were removed and subjected to histopathological tests. As a result of habanero pepper extract administration, the tumour size decreased by 5-50% in 15 dogs, the tumours size remained unchanged in 29 dogs, whereas tumour size increased by 10-30% in 5 animals despite the administration of the extract. The extract was well tolerated by the animals. Temporary undesirable symptoms in the form of vomiting or diarrhoea and licking of the anal region, which could stem from its administration, were observed in only nine dogs of the test group and 5 of the control group. An increase in asparagine aminotransferase (AST) activity was observed in 13 dogs of the test group, alanine aminotransferase (ALT) activity was elevated in 11 dogs, whereas alkaline phosphatase (ALP) increased its activity in 18 dogs. Increases in total bilirubin, urea and creatinine concentration were noted in the serum of 10, 9 and 9 dogs respectively. In the control group, the AST activity increased in 7 dogs, ALT in 5, ALP in 5, and total bilirubin concentration in 6. The preliminary clinical observations indicate that the capsaicin-containing habanero pepper extract exhibits favourable effects on different tumours in dogs and is well tolerated by the animals, thus the obtained results are a good sign for future studies on alternative medications used in dog oncology.

Keywords: capsaicin, neoplasms, dogs

Cancer pharmacotherapy is a dynamically developing branch of human medicine as well as in veterinary medicine. Currently, the directions in which oncological treatment is heading include making cancer curable, and to transform it from a lethal disease into a long-term chronic one. It is hardly surprising then, that in order to achieve these oncological goals, new substances are with increasing frequency tested that, apart from their effectiveness, should also be inexpensive in production. Lately, with regard to this significant attention has been given to capsaicin. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is an organic compound belonging to the alkaloids, obtained from plants of the *Capsicum* family, with the following chemical formula: $C_{18}H_{27}NO_3$ and a molecular mass of 305.41 g/mol. Relatively large amounts of it are

present in the fruits of various pepper variants, and the compound itself is responsible for the spicy taste of this plant. Capsaicin bonds with TRPV1 (transient receptor potential vanilloid subtype 1) (2), which is present in large amounts in the hypothalamus, on the endings of the sensory neurons, in the dorsal root ganglia as well as in the trigeminal nerve. This receptor is also present in the kidneys, liver, bladder, and pancreas (12). After bonding with the TRPV1 receptor capsaicin causes the cation channel to open, resulting in a flow of cations to the inside of the cell and its depolarisation. The resulting action potential is passed on to the spinal cord and is responsible for the feeling of warmth and pain (17).

It has been proven that capsaicin modulates the metabolism of carcinogenic and mutagenic compounds, making this alkaloid an object of study for oncologists, who have begun to consider it a substance that could be used in cancer prevention and therapy (15). In human medicine it has been proven that capsaicin is effective in cancer therapy, including for breast, pancreas, bladder, and prostate tumours (3, 4). In veterinary medicine, current research on the habanero pepper extract and capsaicin itself has been limited mainly to the safety of its application in laboratory animals (6, 13).

The aim of the study was a preliminary assessment of the anti-cancer efficacy of the capsaicin-containing habanero pepper extract in dogs.

Material and methods

Animals. The study was conducted on a group of 50 dogs of different breeds, sexes (33 females and 17 males) and ages (6-18 years), which were diagnosed with tumours (mammary gland cancer was diagnosed in 29 females, 7 dogs had a chest wall tumours, 6 dogs had a tumour in the anal area, 5 had testicular tumours, 2 had tumours on the neck/head, and 1 had a tumour on its thigh) as well as 20 healthy dogs (12 females and 8 males, aged 2-12 years) as a control group. All the animals were provided with a diet supplement based on the habanero pepper extract containing capsaicin (at a dose of 74 mg of pure capsaicin per kg of animal body weight per specimen). Permission of the owners of each of the animal was obtained for the application of the diet supplement. For the tests, animals of both the study and control groups were selected where the haematological and biochemical tests did not reveal any deviations from the physiological norm. Observations were conducted for a period of 6 months, during which time the general condition of the animals receiving habanero extract containing capsaicin was monitored, with haematological and biochemical tests being carried out at two-week intervals in order to assess the tolerance to the extract in animals. The test group animals were examined for the tumour size at monthly intervals, during the entire observation period. After the end of the observations, the tumours were removed and subjected to histopathological tests.

General condition assessment. The general condition assessment of the animals used in the study was performed on the basis of clinical observations and data obtained during interviews. Special attention was paid to the possible occurrence of undesired effects in both dog groups, which could be linked to the application of habanero pepper extract, such as: vomiting, diarrhoea, or licking of the anal region.

Haematological and biochemical examination. The haematological examination was performed using an Exigo Boule analyser (Sweden), whereas the biochemical examination was conducted using a Mindray BS-300 analyser (Poland).

Tumour size measurement. Tumour diameter was determined by measurements with a ruler. The first measurement was performed prior to commencing the pepper extract administration, and subsequent measurements were made at monthly intervals.

Histopathological examination. Tumour fragments taken as samples for the histhopathological examination were fixed in 10% buffered formalin with pH = 7.2 and subsequently immersed in alcohol solutions of increasing concentrations, acetone and xylene, and moved to paraffin blocks in a tissue processor (Leica TP-20). Tissue sections with a thickness of 4 μm, performed on a microtome (Leica SR-200), were transferred onto slides. Preparations for the histopathological examination were stained with hematoxylin and eosin (HE) and assessed under an optical microscope (Nikon Eclipse E-600) following the WHO histological classification for tumours (7, 8, 10).

Classification of malignant mammary gland tumours of epithelial origin (G1-low grade of malignancy, G2 – intermediate grade of malignancy, and G3 – high grade of malignancy) was performed according to the guidelines presented by Goldschmidt et al. (5). Criteria for the malignant histologic grade were: tubule formation, nuclear pleomorphism, and mitoses per 10 HPF (high power field).

Statistical analysis. For easier statistical analysis the dogs were divided into 2 groups: 1 – a group with malignant tumours (37 animals), and 2 – a group with benign tumours (13 dogs). The Mann-Whitney rank test was used to demonstrate the differences between size of tumours before and after application of habanero pepper extract. Changes were considered statistically significant at $p < 0.05$. The Statistica 10.0 PL software was used for the calculations.

Results and discussion

Clinical examination (haematological and biochemical testing). In nine dogs of the test group and 5 of the control group, undesired effects of the administration of the habanero pepper extract were observed in the form of vomiting or diarrhoea and licking of the anal region (Tab. 1). Such disorders were mostly of a temporary character and were resolved spontaneously within 2-3 days without the need to discontinue the diet supplement. Only in one dog of the control group did diarrhoea persist for 7 days, after which time the owner did not consent to continue the administration of the extract to the animal, which was withdrawn from the study. During the observation, one of the dogs from the test group with testicular cancer died, thus the final number of tested animals was 49. In the dead dog, a post-mortem histopathological examination determined its tumour to be a Sertoli cell tumour (sertolioma). Haematological and biochemical examinations of the serum provided the following results. In the test group, a decrease in

Tab. 1. Description of the studied dogs

No.	Breed	Age	Sex	Change type	Effects of 6-month administration of habanero pepper extract containing capsaicin	Histhopathological examination	Sides effects after pepper extract administration
1	Mix	10	Female	Mammary tumour	10% decrease in tumour size	Complex carcinoma G1	–
2	Dalmatian	8	Female	Mammary tumour	15% decrease in tumour size	Complex carcinoma	vomiting
3	Mixed breed	10	Male	Tumour on chest wall	50% decrease in tumour size	Lipoma	–
4	Mixed breed	8	Female	Tumour over spatula	30% decrease in tumour size	Lipoma	–
5	Mixed breed	10	Female	Mammary tumour	10% decrease in tumour size	Complex carcinoma G1	–
6	Mixed breed	8	Male	Tumour on the neck	No change in tumour size	Histiocytic sarcoma	–
7	Mixed breed	7	Male	Tumour on thigh	No change in tumour size	Hemangiopericytoma	vomiting
8	Mixed breed	7	Female	Mammary tumour	No change in tumour size	Complex carcinoma G1	diarrhoea/anal region licking
9	Mixed breed	8	Female	Mammary tumour	No change in tumour size	Complex carcinoma G1	–
10	Boxer	8	Male	Tumour on chest wall	No change in tumour size	Mastocytoma	–
11	Mixed breed	3	Female	Mammary tumour	No change in tumour size	Tubulo-papillare carcinoma G1	vomiting
12	German Shepherd	8	Female	Mammary tumour	20% decrease in tumour size	Complex carcinoma G2	–
13	Mixed breed	12	Female	Tumour on forechest	No change in tumour size	Cystadenomatosis	–
14	Mixed breed	8	Female	Mammary tumour	No change in tumour size	Complex carcinoma G1	–
15	Mixed breed	7	Female	Tumour on forechest	10% increase in tumour size	Hemangiopericytoma	–
16	American Staffordshire Terrier	8	Female	Mammary tumour	No change in tumour size	Complex carcinoma G2	–
17	Mixed breed	7	Female	Mammary tumour	50% decrease in tumour size	Complex carcinoma G1	–
18	German Shepherd	8	Female	Mammary tumour	No change in tumour size	Complex carcinoma G1	–
19	Mixed breed	8	Female	Mammary tumour	No change in tumour size	Complex carcinoma G1	–
20	German Shepherd	11	Female	Mammary tumour	30% increase in tumour size	Complex carcinoma G2	–
21	American Staffordshire Terrier	10	Male	Tumour in anal area	No change in tumour size	Adenocarcinoma	–
22	Mixed breed	13	Female	Mammary tumour	10% increase in tumour size	Complex carcinoma G2	–
23	Mixed breed	18	Female	Mammary tumour	No change in tumour size	Adenoma	–
24	Mixed breed	10	Female	Mammary tumour	No change in tumour size	Tubulo-papillare carcinoma G1	diarrhoea/anal region licking
25	Mixed breed	6	Female	Mammary tumour	No change in tumour size	Adenocarcinoma	–

erythrocyte numbers was observed in 7 dogs, whereas a haemoglobin concentration below the lower normal limit was observed in 9 dogs. Similarly, leucocytosis was observed in 9 dogs, and thrombocytopenia occurred in 12 of the test group. The elevated activity of asparagine aminotransferase (AST) was observed in 13 dogs of the test group, alanine aminotransferase (ALT) activity increased in 11 dogs, whereas alkaline phosphatase (ALP) activity increased in 18. An increase of total bilirubin, urea and creatinine concentration was noted in the serum of 10, 9 and 9 dogs, respectively (Tab. 2).

In dogs of the control group, no disorders in the haematological examinations were detected during the observation period. A biochemical assay determined an increase in AST activity in 7 dogs, ALT in 5, ALP in 5 and increase in total bilirubin concentration in 6 (Tab. 3).

Haematological disorders in the test group persisted during the entire experiment, whereas elevated AST, ALT and ALP activities persisted during the entire study period in 7 dogs of the test group and 4 of the control group. In the remaining cases, the abnormal results of the biochemical examination returned to normal after 6-8 weeks of observation, both in dogs of the control group and of the test group.

Tumour size assessment after administration of habanero pepper extract. As a result of administration of the habanero pepper extract to those animals with tumours, a decrease in tumour sizes by 5-50% was observed in 15 dogs; no change in size during the entire observation period in 29, whereas an increase in size by 10-30% occurred in 5 despite the administration of the extract.

Histopathological examination. The most numerous group of tumours was represented by mammary gland tumours. 13 females were diagnosed with a complex low-grade G1 carcinoma. On the other hand, a complex carcinoma with a higher G2 malignancy grade was found in 5 females, and simple G1 adenocarcinomas

No.	Breed	Age	Sex	Change type	Effects of 6-month administration of habanero pepper extract containing capsaicin	Histhopathological examination	Sides effects after pepper extract administration
26	German Shepherd	12	Female	Mammary tumour	No change in tumour size	Adenoma	–
27	Dachshund	5	Female	Mammary tumour	No change in tumour size	Complex carcinoma G1	–
28	Mixed breed	10	Female	Mammary tumour	No change in tumour size	Adenoma	–
29	Mixed breed	12	Male	Tumour in facial part of the head	20% decrease in tumour size	Basal cell carcinoma	–
30	Dachshund	7	Female	Tumour in anal area	No change in tumour size	Adenocarcinoma	–
31	Mixed breed	8	Male	Testicular tumour	10% decrease in tumour size	Seminoma	–
32	Mixed breed	7	Female	Mammary tumour	No change in tumour size	Complex carcinoma G1	–
33	Mixed breed	8	Male	Tumour on chest wall	25% decrease in tumour size	Lipoma	diarrhoea/anal region licking
34	Mixed breed	10	Male	Testicular tumour	No change in tumour size	Sertolioma	–
35	Mixed breed	12	Male	Tumour in anal area	No change in tumour size	Adenocarcinoma	–
36	Golden Retriever	14	Female	Mammary tumour	No change in tumour size	Adenoma	–
37	German Shepherd	10	Male	Tumour in anal area	No change in tumour size	Adenocarcinoma	–
38	German Shepherd	8	Male	Tumour in anal area	10% increase in tumour size	Adenocarcinoma	–
39	Mixed breed	6	Male	Testicular tumour	No change in tumour size	Sertolioma	–
40	Beagle	8	Female	Mammary tumour	20% decrease in tumour size	Complex carcinoma G1	–
41	Mixed breed	9	Female	Mammary tumour	No change in tumour size	Adenoma	–
42	Mixed breed	12	Female	Mammary tumour	15% decrease in tumour size	Complex carcinoma G1	–
43	German Shepherd	7	Male	Tumour on chest wall	40% decrease in tumour size	Lipoma	diarrhoea/anal region licking
44	Boxer	9	Female	Mammary tumour	No change in tumour size	Adenoma	diarrhoea/anal region licking
45	German Shepherd	10	Male	Tumour in anal area	5% decrease in tumour size	Adenoma	–
46	Schnauzer	8	Female	Mammary tumour	10% increase in tumour size	Complex carcinoma G2	–
47	Irish Setter	15	Male	Testicular tumour	No change in tumour size	Sertolioma	–
48	Great Dane	8	Male	Testicular tumour	Dog died during observation	Sertolioma	–
49	Mixed breed	9	Female	Mammary tumour	No change in tumour size	Tubulo-papillare carcinoma G1	diarrhoea/anal region licking
50	Mixed breed	9	Female	Mammary tumour	20% decrease in tumour size	Adenoma	–

were diagnosed in only 1 case. Another numerous group of malignant tumours of epithelial origin was represented by adenocarcinoma, located primarily in the anal region. Testicular tumours occurred in 5 males, where sertolioma was diagnosed in four specimens. Of tumours of vascular origin, 2 cases of hemangiopericytoma were found. Only singular cases of mastocytoma with malignancy grade I, basal cell carcinoma and histiocytic sarcoma and seminoma were determined. Lipomas were dominant among the benign changes (Tab. 1).

Demonstration of the relationship between tumour histopathological classification and change in size after the administration of capsaicin-containing habanero pepper extract to dogs. The habanero pepper extract exhibited the greatest efficacy in relation to mammary tumours, where a complex carcinoma with a low G1 malignancy grade was histologically determined. In the case of 6 dogs, the tumour size decreased, while in a further 7 the tumour size remained unchanged. Further tumours where the size changed as a result of administration of the tested diet supplement were lipomas (in 4 dogs), adenomas (in 2 dogs), G2 complex mammary tumour (in 1 dog), basal cell carcinoma (in 1 dog) and testicular seminoma (in 1 dog). Among the 29 tumours whose size remained unchanged during the entire observation period, a histological examination revealed the above-mentioned G1 complex carcinoma (in 7 dogs), adenoma (in 6 dogs), adenocarcinoma (in 5 dogs), tubulo-papillare carcinoma (in 3 dogs), sertolioma (in 2 dogs), grade I mastocytoma (in 2 dogs), hemangiopericytoma (in 1 dog), histiocytic sarcoma (in 1 dog), and cystic hyperplasia of the sweat glands (1 dog). Despite the use of the extract, G2 complex carcinomas (3 dogs), hemangiopericytoma (1 dog) and adenocarcinoma (1 dog) increased in size.

Statistical analysis of the tumour size before and after administration of habanero pepper extract showed statistically significant differences in tumour size reduc-

Tab. 2. Results of haematological and biochemical tests performed in dogs of the study group

No of the dog	RBC (10^{12}/l)			Hb (g/dl)			WBC (10^9/l)			PLT (10^9/l)			AST (U/l)		
	D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180
1	6.7	7.1	7.3	14.2	13.3	14.5	12.4	15.1	14.3	305	176	122	14	28	33
2	5.8	6.0	5.7	15.8	16.8	15.0	13.8	12.6	15.7	444	236	246	28	77	69
3	5.6	6.2	5.7	14.4	17.1	15.2	9.6	6.2	14.2	428	324	290	15	28	24
4	6.0	7.1	6.8	15.1	15.5	14.7	8.7	10.2	11.5	390	388	222	19	17	32
5	7.7	8.6	6.9	14.2	13.6	14.9	15.2	12.1	11.8	450	298	300	9	35	29
6	5.9	6.3	6.0	12.6	11.1	9.8	14.8	12.4	10.9	410	402	322	16	33	35
7	6.6	6.2	5.9	16.1	14.3	12.6	13.0	10.8	13.3	299	333	314	36	64	35
8	5.5	3.8	4.1	12.9	7.6	7.5	14.2	12.0	14.6	398	455	405	12	59	33
9	6.0	5.8	6.4	14.6	15.2	13.7	9.9	16.0	15.3	284	126	111	21	32	27
10	7.1	6.0	6.5	17.2	12.7	13.9	10.5	8.9	11.0	487	289	305	30	28	19
11	6.2	5.6	5.7	15.0	14.9	16.2	12.5	14.6	15.1	290	254	259	33	88	92
12	5.5	4.4	5.0	13.3	8.9	10.5	12.8	13.4	13.2	436	188	117	14	28	25
13	6.3	6.0	5.9	13.2	13.0	14.1	10.3	14.7	11.7	377	278	255	27	28	30
14	6.4	5.7	5.9	16.7	17.1	15.3	13.1	11.4	15.7	412	277	306	18	28	33
15	7.0	6.3	6.6	13.9	12.6	14.0	14.8	16.3	15.4	481	402	306	22	30	37
16	7.1	6.1	6.7	15.3	17.1	15.2	15.5	18.2	19.6	379	360	372	34	81	85
17	8.0	7.5	7.5	14.7	13.5	12.8	13.3	15.6	16.1	322	205	245	30	28	21
18	5.7	5.5	6.4	14.1	13.7	13.3	14.6	12.3	13.5	413	407	264	7	26	29
19	8.1	8.4	7.3	12.8	14.5	14.3	15.8	14.9	15.2	308	379	299	25	31	35
20	5.5	4.2	3.6	12.4	11.0	10.6	14.6	23.0	27.4	306	183	153	19	12	29
21	7.1	6.6	5.6	17.1	15.2	13.9	8.2	9.1	13.8	283	164	130	17	19	24
22	5.5	4.8	4.8	13.0	8.9	8.2	9.7	14.1	14.8	215	95	88	8	15	16
23	8.2	7.4	7.1	15.2	14.8	14.0	13.3	10.0	12.7	306	402	354	30	29	27
24	6.0	5.8	5.4	15.9	16.5	15.1	7.2	8.0	9.9	375	455	440	15	53	27
25	5.6	5.6	6.0	16.3	17.1	16.2	8.0	8.5	10.3	442	162	94	13	34	30
26	5.6	5.7	6.3	15.7	14.8	14.3	6.8	10.2	11.8	256	289	218	5	32	35
27	7.9	7.3	6.7	17.2	14.4	13.5	14.0	24.5	28.3	415	207	222	16	52	24
28	5.5	3.9	4.3	12.4	8.5	7.4	11.5	13.3	15.9	406	144	66	34	23	20
29	6.0	5.9	5.6	16.3	17.5	14.9	11.9	10.2	15.6	305	495	402	16	28	32
30	7.5	7.2	6.9	16.0	16.8	14.0	12.6	11.7	13.6	266	434	332	8	23	28
31	5.9	6.2	6.4	13.3	12.2	12.8	14.3	9.2	12.8	322	451	430	19	14	24
32	7.5	7.3	6.9	16.4	15.6	13.4	9.0	7.2	14.5	318	399	390	5	17	30
33	5.9	5.5	6.0	16.0	13.9	13.7	14.1	10.3	13.3	254	266	414	23	69	33
34	6.6	6.9	7.1	15.8	13.6	12.6	12.8	19.8	22.1	388	216	320	19	32	29
35	5.6	4.3	4.6	12.4	10.3	9.9	13.1	16.0	15.6	328	122	147	25	70	72
36	5.9	5.8	6.4	12.7	14.7	14.0	7.4	9.1	15.2	299	333	285	14	36	33
37	6.0	5.7	6.6	14.3	9.1	8.7	10.6	19.8	25.7	355	381	304	11	35	29
38	8.2	9.2	7.4	15.6	17.9	15.4	9.9	8.4	11.1	433	221	216	33	30	26
39	5.7	5.8	6.5	14.0	12.8	12.0	11.3	13.4	15.2	416	157	123	28	23	35
40	7.2	7.8	5.9	13.5	15.0	15.6	12.7	9.4	12.6	375	288	212	15	26	33
41	7.4	9.6	6.9	13.2	13.9	14.6	13.2	14.4	14.5	416	451	386	16	33	35
42	6.9	6.6	5.6	14.8	16.2	14.7	15.3	13.8	16.1	322	463	257	35	17	21
43	8.1	9.8	8.1	12.3	14.5	12.8	11.1	12.0	14.6	305	182	98	11	90	88
44	7.6	8.7	7.9	14.7	17.0	15.5	16.0	15.7	14.0	406	228	255	25	63	61
45	6.9	7.2	8.2	16.6	15.5	15.0	12.3	8.9	10.6	266	290	215	19	30	27
46	5.5	5.1	4.9	12.7	9.2	8.8	11.6	15.3	15.0	399	318	414	10	50	33
47	8.2	7.6	7.9	13.2	12.9	12.3	15.7	22.4	27.4	355	407	298	28	36	33
48	Dog died														
49	6.8	7.1	6.8	17.2	16.5	15.5	16.1	28.3	26.6	376	118	143	23	49	55
50	7.0	6.8	7.0	14.0	12.4	12.2	12.6	11.6	9.8	288	226	312	31	28	29
Range	5.5-8.5			12-18			6-16.5			200-500			1-37		

Explanation: D 0 = day 0, before application of the habanero pepper extract, D 28 = 28th day of the observation; D 180 = 180th day

ALT (U/l)			ALP (U/l)			Urea (mg/dl)			Creatynine (mg/dl)			Total bilirubine (mg/dl)		
D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180
8	24	37	56	121	113	21.6	20.2	29.2	1.27	1.10	1.34	0.29	0.18	0.22
32	82	96	131	222	199	28.4	34.6	28.7	1.16	0.98	1.39	0.40	0.22	0.27
19	36	30	115	88	133	31.3	37.1	35.8	1.23	1.11	1.65	0.37	0.53	0.31
33	21	28	98	200	142	33.0	21.4	40.2	1.43	1.61	1.21	0.29	0.56	0.36
40	17	29	150	114	122	39.8	36.6	44.0	1.60	1.96	1.48	0.26	0.48	0.34
14	51	44	78	108	68	23.6	26.9	39.3	1.00	1.50	1.13	0.55	0.94	0.51
25	101	78	122	165	94	27.9	50.2	42.2	1.39	1.82	1.65	0.18	0.42	0.18
50	75	50	108	159	87	42.2	72.4	36.8	1.52	2.00	1.50	0.49	1.21	0.38
29	44	32	44	132	105	38.7	41.5	33.1	1.11	1.04	1.52	0.44	0.33	0.12
16	32	19	72	99	127	32.9	43.6	29.7	1.42	1.34	1.44	0.36	0.51	0.22
48	84	71	69	190	231	35.5	47.1	40.7	1.10	0.99	1.42	0.33	0.19	0.34
33	45	39	65	85	62	40.6	62.4	41.4	1.44	1.73	1.47	0.30	0.82	0.56
19	26	19	99	114	119	29.6	37.9	30.3	1.10	1.04	1.33	0.39	0.57	0.26
22	35	28	73	166	144	28.0	24.2	36.6	1.30	1.34	1.28	0.12	0.31	0.55
28	44	33	29	151	86	33.3	35.6	29.1	1.12	0.99	1.16	0.27	0.20	0.41
25	22	40	57	201	115	40.2	38.0	26.8	1.50	0.76	1.63	0.35	0.41	0.29
37	31	25	88	122	81	33.6	38.5	30.7	1.33	1.20	1.42	0.33	0.47	0.32
39	32	37	104	99	64	29.4	37.6	44.1	1.18	1.80	1.50	0.29	0.56	0.18
26	51	43	112	133	142	34.7	29.6	39.5	1.23	1.40	1.26	0.50	0.52	0.38
7	21	29	76	69	99	31.1	39.8	27.8	1.00	1.04	1.39	0.56	0.72	0.50
15	19	13	99	85	76	40.2	32.2	23.4	1.18	1.34	1.56	0.47	0.33	0.27
44	32	28	127	144	141	44.6	46.4	42.1	1.66	1.99	1.55	0.39	1.61	0.39
13	44	25	118	115	140	27.7	30.2	26.5	1.42	1.61	1.52	0.22	0.39	0.22
45	92	50	132	176	115	21.8	37.7	29.9	1.33	1.20	1.38	0.21	0.48	0.33
18	31	30	119	151	99	33.1	24.8	33.9	1.38	1.80	1.26	0.44	0.22	0.52
26	32	48	82	77	95	29.9	35.4	28.3	1.53	1.40	1.55	0.37	0.36	0.56
33	51	29	56	157	110	22.2	28.1	25.5	1.15	1.04	1.40	0.43	0.72	0.42
30	21	37	94	120	125	27.6	38.0	35.1	1.24	1.34	1.32	0.55	1.16	0.40
42	19	28	99	69	73	38.0	37.3	30.5	1.36	0.99	1.28	0.48	0.15	0.16
48	42	42	104	114	150	33.2	29.6	26.4	1.50	1.62	1.49	0.29	0.54	0.24
18	33	25	130	214	143	40.5	39.1	28.2	0.99	1.23	1.52	0.38	0.39	0.37
10	29	19	88	151	139	28.5	32.2	30.6	1.33	1.28	1.45	0.29	0.48	0.33
39	73	33	143	182	107	30.3	36.0	34.1	1.26	1.42	1.27	0.36	0.28	0.40
25	50	45	29	88	119	35.8	30.2	37.3	1.15	1.04	1.35	0.24	0.13	0.19
28	76	68	65	166	185	29.6	80.5	33.2	1.00	1.87	1.59	0.33	1.00	0.55
46	41	39	70	123	130	35.8	34.2	28.5	1.28	1.38	1.23	0.25	0.42	0.36
30	27	41	77	144	116	29.6	42.6	44.4	1.44	1.40	1.62	0.29	0.60	0.29
17	44	28	133	185	122	34.4	38.9	40.1	1.19	1.58	1.67	0.33	0.58	0.36
25	31	37	116	69	88	30.8	34.0	39.6	1.26	1.59	1.37	0.29	0.14	0.19
33	24	29	145	238	148	39.2	40.7	28.6	1.25	1.51	1.25	0.34	0.33	0.52
17	34	46	53	88	81	44.4	43.2	37.7	1.61	1.13	1.33	0.40	0.11	0.50
27	50	39	61	82	108	39.2	35.4	44.0	1.22	0.96	1.24	0.22	0.55	0.45
50	88	75	114	242	212	31.8	38.4	26.4	1.10	0.98	1.19	0.18	0.43	0.39
36	80	91	129	193	233	27.6	55.8	37.8	1.26	1.75	1.47	0.43	0.29	0.32
40	38	47	106	123	101	38.1	42.1	35.2	1.15	1.00	1.34	0.11	0.16	0.44
18	24	22	133	114	99	31.5	62.3	41.3	1.45	1.90	1.53	0.51	0.80	0.48
25	82	41	99	205	262	34.7	36.0	38.2	1.23	1.33	1.46	0.44	0.25	0.22
Dog died														
20	78	72	148	222	199	42.1	59.0	39.1	1.63	1.82	1.45	0.52	0.80	0.37
31	45	36	73	150	128	26.3	20.0	35.4	1.12	1.45	1.28	0.16	0.52	0.41
3-51			20-155			20-45			1.0-1.7			≤ 0.6		

of the observation.

Tab. 3. Results of haematological and biochemical tests performed in control dogs

No of the dog	RBC (10^{12}/l)			Hb (g/dl)			WBC (10^9/l)			PLT (10^9/l)			AST (U/l)		
	D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180
1	5.6	6.0	5.8	15.3	14.9	15.0	6.8	8.7	15.2	233	288	255	12	22	31
2	5.9	6.7	6.3	15.6	15.2	16.2	10.4	9.5	13.8	312	321	290	19	77	44
3	6.2	5.9	5.8	16.2	16.6	14.8	7.2	8.8	10.9	344	401	315	33	35	26
4	7.6	7.0	8.0	15.9	14.9	15.6	7.7	12.6	10.6	288	412	377	14	17	28
5	8.3	7.5	7.9	17.0	14.0	13.7	12.6	13.2	15.1	290	324	403	18	52	33
6	6.5	7.0	6.1	16.3	14.8	15.2	15.1	13.0	11.3	386	333	328	21	66	56
7	5.7	6.8	7.1	14.1	13.3	12.9	14.2	16.1	14.7	366	367	296	25	29	35
8	6.1	6.9	7.4	13.0	12.7	13.0	9.7	13.2	10.5	202	224	299	30	18	27
9	7.3	7.0	8.1	13.9	12.5	14.5	6.5	10.8	12.6	447	407	344	32	44	33
10	7.9	7.1	5.9	15.0	17.4	14.9	11.3	13.2	16.1	305	365	311	6	33	19
11	8.1	8.3	5.6	15.6	15.3	15.3	11.0	15.7	14.8	318	382	368	18	25	22
12	8.0	5.9	6.4	15.8	15.0	17.0	13.4	14.9	14.6	410	412	299	7	19	30
13	5.6	7.1	6.9	14.8	16.1	13.8	9.9	10.6	12.5	266	399	314	12	14	26
14	7.3	7.3	8.1	17.5	13.8	12.9	12.6	15.4	14.6	280	355	406	15	28	16
15	7.8	8.1	8.4	17.1	15.5	14.1	14.1	13.4	15.3	344	312	395	33	40	55
16	7.8	6.5	6.2	16.5	15.8	13.6	11.8	14.2	13.4	391	405	455	30	35	18
17	8.0	6.1	6.7	15.2	14.7	16.3	15.7	12.5	13.8	331	453	410	21	83	88
18	5.5	5.8	5.9	12.6	14.0	14.9	14.4	14.0	15.5	425	393	423	13	29	17
19	5.9	7.0	7.7	13.4	15.3	15.7	16.0	14.9	12.3	414	410	389	17	39	20
20	6.4	6.4	7.0	12.9	16.6	14.5	10.3	13.6	12.8	356	444	322	22	28	15
Range	5.5-8.5			12-18			6-16.5			200-500			1-37		

Explanation: as in Tab. 2.

tion in both groups – benign (p = 0.036) and malignant (p = 0.047) tumours. Differences in p value show that habanero pepper extract is more effective against benign tumours.

Preliminary clinical observations by the authors indicate that the capsaicin-containing habanero pepper extract exhibits a favourable effect on different tumour types in dogs, and it is relatively well tolerated by the animals. Among the 49 dogs with tumours that were administered a diet supplement based on the pepper extract, in 15 (30.61%) the tumour size was observed to decrease, in 29 (59.18%) the tumour size did not decrease or increase during the entire observation period, whereas only in 5 dogs (10.2%) did the tumour size increase during the experiment. This demonstrates that the habanero pepper extract may inhibit tumour growth, and even induce its resorption. This effect may be linked to the fact that the capsaicin contained in the extract induces cell apoptosis as a result of inhibition of the final stage of cellular respiration performed in mitochondria. Cancer cell apoptosis is a consequence of the inhibition of electron transport from NADH to the ubiquinone by capsaicin, or its direct bonding with coenzyme Q, which causes the change of electron flow direction and creation of reactive oxygen forms in excess. The consequence of this is the dispersion of the transmembrane potential in mitochondria (14), which is crucial for the functioning of these organelles. Apoptosis starts from the transmembrane potential breakdown, while active oxygen forms may cause damage to the structure and impairment of the mitochondrial function and, consequently, the death of the cancer cell.

Capsaicin-induced apoptosis of cancer cells is related to this alkaloid stimulating the transcription of, e.g., the p53 gene. Such a mechanism, accompanied by DNA fragmentation in cancer cells, was confirmed in the in vitro conditions for the stomach cancer cells (9).

In the course of the *in vitro* examinations carried out on the T24 cancer cells of bladder in mice, it was demonstrated that, depending on the dose, capsaicin may lead to depolarisation of the mitochondrial membrane, which results in cell death.

It is suggested then that this alkaloid may be used in urine bladder cancer treatment (18). This case is similar to that of prostate cancer, for which this alkaloid shows a strong anti-proliferation influence. Capsaicin induces apoptosis both in cancer cells with an androgen receptor (AR) and without it. At the same time, it increases the concentration of p53, Bax and p21 proteins, which participate in the control of the cell cycle. The substance in question decreases the expression of PSA (prostate-specific antigen), which is the most comprehensively studied human serine protease of the kallikreins family, produced in the prostate and present in the blood at a heightened concentration in the case of, e.g., prostatitis, benign prostatic hyperplasia, or prostate cancer (11, 16).

ALT (U/l)			ALP (U/l)			Urea (mg/dl)			Creatynine (mg/dl)			Total bilirubine (mg/dl)		
D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180	D 0	D 28	D 180
16	27	22	63	44	79	32.2	29.4	26.4	1.33	1.24	1.29	0.31	0.67	0.36
27	59	82	121	315	288	40.5	36.6	27.1	1.17	1.27	1.67	0.16	0.41	0.26
31	50	46	33	118	145	33.8	34.3	43.1	1.25	1.53	1.50	0.42	0.79	0.37
46	42	38	48	68	95	26.4	30.0	37.3	1.44	1.37	1.26	0.36	0.39	0.33
39	29	40	96	85	58	33.2	29.7	21.0	1.68	1.12	1.40	0.54	0.42	0.29
50	74	63	54	190	243	41.6	43.5	38.2	1.19	1.06	1.42	0.33	0.94	0.48
39	44	28	22	38	105	25.0	37.1	42.6	1.23	1.50	1.39	0.12	0.38	0.22
15	30	33	71	115	88	28.6	32.0	44.4	1.03	1.34	1.21	0.51	0.50	0.56
22	18	37	34	79	131	32.1	30.4	36.2	1.10	1.66	1.28	0.49	0.72	0.42
28	44	50	136	117	150	44.3	38.8	40.8	1.55	1.25	1.60	0.30	0.26	0.19
16	29	21	95	249	107	40.8	43.2	40.2	1.51	1.33	1.27	0.16	0.28	0.32
14	37	33	38	47	69	31.2	29.9	31.9	1.14	1.16	1.17	0.32	0.30	0.45
15	19	26	52	39	82	25.5	37.1	22.5	1.33	1.34	1.52	0.41	0.88	0.60
23	35	30	55	63	68	27.1	23.5	37.1	1.28	1.29	1.42	0.27	0.55	0.42
26	63	78	39	206	196	31.3	25.5	35.8	1.61	1.52	1.16	0.20	0.51	0.38
20	38	28	112	94	135	44.0	28.1	34.9	1.16	1.18	1.27	0.43	0.39	0.33
33	80	74	109	89	93	28.3	39.7	37.6	1.32	1.28	1.13	0.37	0.65	0.49
41	50	35	85	122	81	24.7	39.1	41.5	1.28	1.40	1.53	0.16	0.27	0.28
48	58	48	88	249	281	32.2	35.5	36.0	1.53	1.55	1.42	0.25	0.23	0.56
16	22	31	29	48	77	39.7	41.1	37.8	1.16	1.31	1.26	0.19	0.13	0.24
3-51			20-155			20-45			1.0-1.7			≤ 0.6		

In earlier studies by the authors, it was found that capsaicin-containing habanero pepper extract exhibits both cytotoxicity towards the D-17 tumour cells of dogs (epithelial cells of osteosarcoma obtained from metastatic lung tumours) and DAN cells (fibroblasts isolated from osteosarcoma) (1). The demonstration of an anti-cancer efficacy of the tested extract in relation to the discussed cells constituted a starting point for the clinical observations presented in this paper, the results of which are a good sign for future studies on alternative preparations used in dog oncology.

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