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Investigation of the effects of pentane, hexane, heptane, and octane on bovine skin papillomatosis

ERTAN DOĞAN[1], HILMI NUHOĞLU[2]

[1]Department of Laborant and Veterinary Health, Vocational School of Nihat Delibalta Göle, University of Ardahan, 75700, Ardahan, Türkiye
[2]Department of Pathology, Faculty of Veterinary Medicine, University of Kafkas, 36000, Kars, Türkiye

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Doğan E., Nuhoğlu H.

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Summary

The purpose of this study was to determine the effects of pentane, hexane, heptane, and octane on bovine skin papillomatosis. The research was carried out on 50 head of cattle bred in the Ardahan region. The cattle that were infected with papillomatosis were divided into 5 groups of 10 animals each. All cattle in the groups were provided with the same environment and feeding conditions (they were fed ad libitum with grass and tap water from the same source). Group 1 was kept as a control group and received no treatment. Group 2 was sprayed with pentane, Group 3 with hexane, Group 4 with heptane, and Group 5 with octane twice a day (morning and evening) with an atomizer to wet and cover papilloma lesions. This treatment was continued for 15 days. All animals in the groups were clinically examined, and blood samples were collected on day 0 (prior to treatment) and on days 3, 6, 9, 12, and 15. Formula leukocyte and total leukocyte counts were performed on the blood samples. On days 3, 6, 9, 12, and 15, when blood samples were taken, it was seen that the number of lymphocytes in the hexane, heptane, and octane groups was significantly higher ($p < 0.05$) than it was in the control and pentane groups. The total leukocyte count in the hexane, heptane, and octane groups was significantly higher ($p < 0.05$) than it was in the control group on day 6 and significantly higher ($p < 0.05$) than it was in the control and pentane groups on days 9, 12, and 15. In addition, tissue sections from the lesioned areas of the sick animals were taken before and after treatment and examined histopathologically. The lesions were checked daily until day 35 after the treatment began, and the number of animals that had recovered was noted. The control and pentane group animals did not improve by the end of day 35. Improvement was observed in 70% of the hexane group, 80% of the heptane group, and 50% of the octane group. In this study, heptane, hexane, and octane were found to be effective against bovine skin papillomatosis. If the results of the research are supported by other studies, suitable preparations (such as a cream) containing these chemicals or their mixtures can be considered as an alternative method in the treatment of the disease.

Keywords: bovine papillomatosis, pentane, hexane, heptane, octane

Skin papillomatosis is an infectious viral disease that can be seen in all living things (2). Its causative agent is bovine papillomavirus (BPV), which belongs to the DNA group. Six serotypes of this virus (BPV Types 1, 2, 3, 4, 5, and 6) were initially isolated (1, 21). The number of sub-serotypes is twelve according to some studies (23) and fourteen according to others (14). Based on the latest reports, the number of sub-serotypes is 23 (5). The number of viruses responsible for the disease in bovines is thirteen. Among those, 1 to 11 subtypes cause breast warts (17). The BPV1-3 and BPV5-10 subtypes of the virus cause skin papilloma in cattle, whereas the BPV4 subtype causes tumors in the upper gastrointestinal tract (7).

Proliferation of the disease occurs in cells of the epithelial tissue. Hence, it is also known as a benign tumor. The growth of epithelial tissue leads to the occurrence of warts on the skin (2, 17, 22). The animal species most affected by the disease are cattle, horses, and dogs. It is more common in cattle under 2 years of age. The main carrier of the virus is probably cattle (24). The most common subtypes of the virus that cause disease in cattle under natural conditions are bovine papillomavirus 1 (VPB-1) and bovine papillomavirus 2 (VPB-2). These sub-types cause infection in horses as well (23). Although papillomaviruses (PV) are attracted to epithelial cells, they have also been detected in colostrum, milk, plasma, and semen (6).

The disease spreads through contact of diseased animals with healthy animals. It may also be transmitted through contaminated food or water. It has been reported that the disease may also be transmitted through tools and equipment used for milking, containment, castration, tagging, injections, and dehorning. Stinging flies and insects, as well as scratches and cuts that affect skin integrity in animals, also play an important role in the spread of the disease (2, 4, 14, 21).

The incubation period of the illness varies from one month to one year (4). The most important clinical signs of the disease are warts appearing on the skin (22, 23). They appear as hard bumps, circumscribed, cauliflower type, pedunculated, or sessile. Though their diameter usually varies from 1 to 10 cm, the lesions can also be larger. Their color is grey, white, or brownish and is unevenly distributed. Lesions are most frequent in the head, neck, eye area, chin, nose, lips, abdomen, back, legs, breasts, nipples, and tail. In addition to these areas, they were also detected in the esophagus, pharynx, stomach, and flat bowel (2, 15, 17, 22, 23). Lesions can sometimes disappear spontaneously within one year, or they can become malignant tumors (6, 15).

Economic losses in diseased animals are due to the loss of meat and milk. Warts on the surface of the body negatively affect the appearance of the animal and reduce its market value. Papillomas on the udder cause problems in milking. Calves fed from the udder with papilloma cause injuries by interfering with tissue integrity. These injuries cause mammary inflammation (mastitis) and spread viruses in the outdoor environment, which becomes the source of infection for other animals (2, 15, 17, 19, 25).

Gasoline is an oil product which contains pentane, hexane, heptane, and octane (10). Out of these, octane is used to boost agricultural production (potatoes, wheat, maize, rice, cotton) (12).

Gasoline or its metabolites may harm cells. Gasoline sprayed onto lesions has been reported to be effective in treating bovine skin papillomatosis (11). The determination of chemicals contained in gasoline that are effective against papilloma is important in the fight against the disease. The purpose of this study was to determine the effects of pentane, hexane, heptane, and octane contained in gasoline on cattle skin papillomatosis.

Material and methods

This study has been approved by the Ethics Committee of Kafkas University (decision number 2020-098 of Jun. 23, 2020) and the Ministry of Agriculture and Forestry of Turkey (letter number E.29486769-622.01-2331427 of Aug. 21, 2020). Pentane 95% (M.107176.1000), hexane 95% (M.296090.1000), heptane ≥ 99% (M.104365.2500), and octane ≥ 99% (M806910.0100) were obtained from Merck (Sigma-Aldrich, Germany).

Animals. This research was conducted on 50 head of cattle aged between 6 and 24 months in the Ardahan region. The breeds of cattle used in the study were Brown Swiss (n = 23, 16 females, 7 males) and Simmental (n = 27, 12 females, 15 males). The study included cattle that had papillomas on the head, neck, back, abdomen, and other body parts discovered during clinical examination. In order to differentiate papilloma from other skin diseases, such as dermatophytosis and external parasites, samples taken from papilloma areas were examined under a microscope in the laboratory. The cattle were diagnosed with papilloma after clinical and microscopic examination. Cattle that were not treated with any medication and had no disease other than papilloma (according to the results of clinical examination and blood analysis) were used in the study. Cattle diagnosed with papilloma by clinical and histopathological examination were divided into five groups: Group 1 (control group, n = 10, median age = 13.8 months), Group 2 (pentane group, n = 10, median age = 12.8 months), Group 3 (hexane group, n = 10, median age = 12.3 months), Group 4 (heptane group, n = 10, median age = 12.8 months), and Group 5 (octane group, n = 10, the median value of age = 13.5 months). The cattle were divided into groups without considering the diameter and number of lesions. During the study, all animals were provided with the same care and feeding conditions (grass and tap water from the same source and *ad libitum*).

Experimental design. The chemical substances were used in high concentrations, except in the untreated, control group. Group 2 was sprayed with pentane, Group 3 with hexane, Group 4 with heptane, and Group 5 with octane twice a day (morning and evening) with an atomizer to wet and cover the papilloma lesions (about 1 ml/lesion/day). This treatment was continued for 15 days. Body temperature, rumen movements, pulse, and respiratory rates of all animals were noted before treatment (day 0) and on days 3, 6, 9, 12, and 15 of treatment. In addition, 2 ml blood samples were taken from the vena jugularis of all animals in the groups before treatment (day 0) and on days 3, 6, 9, 12, and 15 of treatment into anticoagulant (EDTA) tubes (BD Vacutainer® K2E 5.4 mg). The blood samples were delivered to the laboratory (Department of Pathology, Faculty of Veterinary Medicine, University of Kafkas, Kars, Turkey) under cold conditions. Leukocyte formula and total leukocyte count in the blood samples were determined by classical methods (blood smear and Thoma slide, respectively) (27). During the experiment, the lesions were checked every day. The monitoring was continued until day 35 after the start of the experiment. The number of animals that fully recovered was noted.

Histopathological examination. Tissue samples (1-2 cm) for histopathological examination were taken from lesioned areas with a sterile scalpel under local anesthesia (Jetokain® Simplex-ADEKA) before and after treatment from all animals in the groups. The samples were placed in containers with 10% formaldehyde and delivered to the laboratory (Department of Pathology, Faculty of Veterinary Medicine, University of Kafkas, Kars, Turkey) under cold conditions. For hematoxylin-eosin staining, sections with a thickness of 4 µm were taken from paraffin blocks and deparaffinized in two different xylol series for 10 minutes. Rehydration was performed by passing the sections through a series of alcohol from high concentration to low concentration. The

nuclei were stained with Mayer hematoxylin for 10 minutes to ensure staining, and then washed with tap water, dipped once in 1% acid alcohol, washed again with tap water, and left in ammonia water for 3 minutes to dye a bright blue color. Afterwards, they was washed with distilled water for 2 minutes, immersed in 96% ethyl alcohol, and then ground dyed in Eosin solution for 5 minutes. Subsequently, dehydration was performed by passing them through an alcohol series from 70% to 100%. The tissue was cleared by treatment in two separate xylol series for 10 minutes and then closed with entellan. After closure, the preparations were examined under a light microscope (Olympus Bx53) and photographed by means of the Cell P program (Olympus Soft Imaging Solutions GmbH, 3, 4). The sections were evaluated in terms of epitalization, fibrosis tissue formation, inflammation, infection, and ulceration (18).

Statistical analysis. Statistical calculations on data obtained in this study were done with the statistical package program 20.0 (IBM SPSS Statistics®, Chicago, IL, USA). The Shapiro-Wilk test checked the normal data distribution status. One-way analysis of variance (ANOVA) was used to compare the group means. Multiple comparisons between the groups were performed using Tukey HSD and Games-Howell tests. The data were presented in the form of mean ($\bar{x}$) and standard deviation (SD). In this study, $p < 0.05$ was considered to be statistically significant.

Results and discussion

The numbers of the cattle diagnosed with papilloma before and after the application of pentane, hexane, heptane, and octane are presented in Figure 1, and the number of animals that recovered over time is presented in Table 1.

The chemicals used in the cattle groups and the numbers of animals that improved over time are shown in Table 1.

In Table 1 it is seen that at the end of the follow-up period (day 35) there was no improvement in the control and pentane groups, but there was a 70% improvement in the hexane group, an 80% improvement in the heptane group, and a 50% improvement in the octane group. Table 2 presents some general experimental findings for the cattle groups.

Tab. 1. Number of animals that recovered by days 10-35

Groups	Day 10	Day 15	Day 20	Day 25	Day 30	Day 35	%
Control	0	0	0	0	0	0	0
Pentane	0	0	0	0	0	0	0
Hexane	0	0	0	1	2	4	70
Heptane	0	0	0	1	3	4	80
Octane	0	0	0	0	2	3	50

Tab. 2. Results of the general examination of the cattle groups

Days	Parameters	Control (n = 10) ($\bar{x}$ ± SD)	Pentane (n = 10) ($\bar{x}$ ± SD)	Hexane (n = 10) ($\bar{x}$ ± SD)	Heptane (n = 10) ($\bar{x}$ ± SD)	Octane (n = 10) ($\bar{x}$ ± SD)
Day 0	Temperature / °C	38.34 ± 0.54	38.39 ± 0.47	38.33 ± 0.58	38.39 ± 0.42	38.34 ± 0.54
	Respiration / min.	16.90 ± 1.79	17.20 ± 1.68	19.10 ± 1.85	17.60 ± 2.01	17.40 ± 2.01
	Pulse / min.	76.40 ± 10.56	73.90 ± 8.06	73.80 ± 7.39	77.10 ± 8.19	75.50 ± 7.29
	Rumen / 5 min.	6.50 ± 1.71	7.80 ± 1.03	7.70 ± 1.16	7.00 ± 1.15	7.60 ± 1.17
Day 3	Temperature / °C	38.19 ± 0.55	38.13 ± 0.68	38.44 ± 0.40	38.20 ± 0.56	38.13 ± 0.57
	Respiration / min.	17.50 ± 2.06	18.20 ± 2.30	18.30 ± 2.26	18.20 ± 2.30	17.00 ± 1.24
	Pulse / min.	69.90 ± 8.27	68.00 ± 6.16	70.40 ± 8.09	69.50 ± 8.25	67.60 ± 5.71
	Rumen / 5 min.	6.20 ± 1.31	7.20 ± 1.54	7.60 ± 1.07	7.50 ± 1.08	7.40 ± 1.17
Day 6	Temperature / °C	38.19 ± 0.41	38.34 ± 0.54	38.24 ± 0.54	38.18 ± 0.30	38.54 ± 0.35
	Respiration / min.	18.30 ± 2.11	17.40 ± 2.06	18.40 ± 2.31	17.10 ± 1.91	17.50 ± 2.01
	Pulse / min.	70.00 ± 8.36	70.70 ± 3.30	69.60 ± 7.33	66.70 ± 4.94	71.30 ± 8.04
	Rumen / 5 min.	6.60 ± 1.17	7.50 ± 1.08	7.00 ± 1.49	7.60 ± 1.17	7.20 ± 1.31
Day 9	Temperature / °C	38.39 ± 0.46	38.39 ± 0.47	38.44 ± 0.40	38.23 ± 0.54	38.22 ± 0.40
	Respiration / min.	17.70 ± 2.40	18.10 ± 2.42	17.60 ± 2.50	17.50 ± 2.50	18.30 ± 2.86
	Pulse / min.	66.10 ± 3.69	66.40 ± 3.62	67.50 ± 4.95	66.60 ± 3.80	66.70 ± 4.16
	Rumen / 5 min.	6.90 ± 0.99	7.00 ± 1.15	7.50 ± 1.35	7.70 ± 0.94	7.50 ± 0.85
Day 12	Temperature / °C	38.28 ± 0.46	38.09 ± 0.37	38.34 ± 0.54	38.37 ± 0.42	38.20 ± 0.42
	Respiration / min.	18.30 ± 2.75	17.70 ± 2.40	18.30 ± 2.40	17.90 ± 2.51	17.80 ± 2.34
	Pulse / min.	67.10 ± 4.45	66.20 ± 4.13	66.60 ± 3.86	67.00 ± 3.83	67.80 ± 4.07
	Rumen / 5 min.	7.00 ± 1.24	7.60 ± 1.07	7.50 ± 1.08	7.60 ± 1.17	7.50 ± 1.08
Day 15	Temperature / °C	38.23 ± 0.64	38.34 ± 0.54	38.10 ± 0.51	38.25 ± 0.55	38.03 ± 0.44
	Respiration / min.	17.50 ± 2.50	17.70 ± 2.05	18.10 ± 2.33	17.40 ± 2.06	17.40 ± 2.22
	Pulse / min.	65.50 ± 3.97	65.50 ± 4.30	67.10 ± 4.48	66.90 ± 4.81	65.40 ± 4.03
	Rumen / 5 min.	6.90 ± 1.19	7.30 ± 1.25	7.80 ± 1.03	7.20 ± 1.31	7.50 ± 0.85

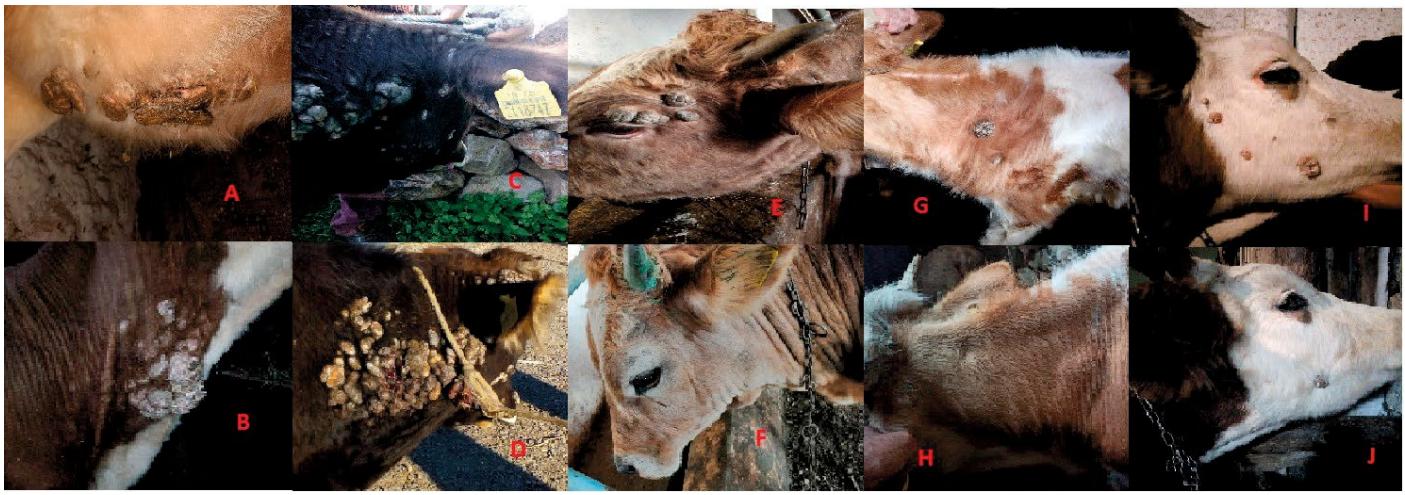

Fig. 1. Appearances of papillomas before and after treatment (A and B – unhealed animals in the control group; C – pentane before treatment; D – pentane after treatment; E – hexane before treatment; F – hexane after treatment; G – heptane before treatment, H – heptane after treatment; I – octane before treatment; J – octane after treatment)

Tab. 3. Selected blood parameters of the cattle groups

Days	Parameters	Control ($\bar{x}$ ± SD)	Pentane ($\bar{x}$ ± SD)	Hexane ($\bar{x}$ ± SD)	Heptane ($\bar{x}$ ± SD)	Octane ($\bar{x}$ ± SD)
Day 0	Neutrophil %	28.80 ± 4.73	27.40 ± 1.35	27.00 ± 2.98	26.90 ± 3.81	25.90 ± 4.01
	Eosinophil %	3.20 ± 1.54	3.30 ± 0.94	3.50 ± 1.08	3.20 ± 1.31	3.00 ± 1.24
	Monocytes %	3.10 ± 1.44	3.60 ± 1.26	4.30 ± 0.94	4.10 ± 0.99	4.60 ± 1.26
	Lymphocyte %	52.00 ± 4.87	50.80 ± 5.95	57.40 ± 8.34	54.00 ± 5.51	50.90 ± 5.08
	WBC (× 10^3/mm^3)	7.73 ± 0.38	7.95 ± 0.30	8.05 ± 0.33	8.06 ± 0.30	7.92 ± 0.28
Day 3	Neutrophil %	27.20 ± 3.08	27.40 ± 3.30	26.40 ± 4.37	26.60 ± 3.30	24.60 ± 3.30
	Eosinophil %	2.80 ± 1.22	3.50 ± 1.08	3.30 ± 1.16	3.60 ± 1.17	3.40 ± 1.35
	Monocytes %	3.30 ± 1.49	3.60 ± 1.26	4.30 ± 1.33	4.20 ± 1.31	4.10 ± 1.19
	Lymphocyte %	53.70 ± 6.14^a	54.20 ± 7.02^a	67.00 ± 6.01^b	66.60 ± 3.62^b	59.90 ± 5.56^a
	WBC (× 10^3/mm^3)	7.76 ± 0.31	8.05 ± 0.30	8.02 ± 0.27	8.03 ± 0.31	8.02 ± 0.28
Day 6	Neutrophil %	27.10 ± 2.84	26.90 ± 2.60	25.50 ± 2.71	25.30 ± 2.90	25.70 ± 3.05
	Eosinophil %	3.00 ± 1.24	3.20 ± 1.03	2.90 ± 1.19	3.10 ± 1.59	3.10 ± 1.19
	Monocytes %	3.50 ± 1.26	3.90 ± 1.19	4.10 ± 1.19	3.30 ± 0.94	3.30 ± 1.33
	Lymphocyte %	53.50 ± 5.62^a	54.20 ± 4.84^a	69.80 ± 5.26^b	67.30 ± 3.68^b	66.20 ± 3.19^b
	WBC (× 10^3/mm^3)	7.65 ± 0.36^a	8.11 ± 0.33^b	8.29 ± 0.34^b	8.24 ± 0.35^b	8.14 ± 0.28^b
Day 9	Neutrophil %	25.20 ± 7.00	24.60 ± 6.65	26.00 ± 5.69	23.90 ± 5.02	24.40 ± 5.40
	Eosinophil %	2.70 ± 1.16	3.60 ± 0.96	2.90 ± 1.37	3.10 ± 1.37	2.80 ± 1.22
	Monocytes %	3.50 ± 1.26	3.30 ± 0.94	3.90 ± 1.19	4.30 ± 1.33	4.10 ± 1.66
	Lymphocyte %	54.90 ± 3.14^a	52.90 ± 4.74^a	65.30 ± 8.90^b	66.20 ± 3.29^b	67.30 ± 3.36^b
	WBC (× 10^3/mm^3)	7.82 ± 0.42^a	8.08 ± 0.34^a	8.32 ± 0.34^b	8.34 ± 0.23^b	8.27 ± 0.24^b
Day 12	Neutrophil %	27.10 ± 3.72	25.70 ± 4.08	24.40 ± 3.43	25.50 ± 3.27	22.80 ± 4.94
	Eosinophil %	2.90 ± 1.19	2.60 ± 0.96	3.00 ± 1.24	3.20 ± 1.54	3.30 ± 1.16
	Monocytes %	3.80 ± 1.31	4.00 ± 1.05	3.50 ± 1.08	3.60 ± 1.43	3.90 ± 1.79
	Lymphocyte %	53.00 ± 4.78^a	54.40 ± 5.35^a	66.40 ± 3.06^b	65.10 ± 3.66^b	68.70 ± 2.98^b
	WBC (× 10^3/mm^3)	7.86 ± 0.36^a	8.05 ± 0.30^a	8.55 ± 0.33^b	8.79 ± 0.39^b	8.50 ± 0.21^b
Day 15	Neutrophil %	25.50 ± 4.03	24.20 ± 2.89	23.90 ± 3.28	24.10 ± 3.60	20.80 ± 5.77
	Eosinophil %	3.10 ± 0.99	2.90 ± 1.19	2.60 ± 0.96	3.30 ± 1.49	3.30 ± 1.33
	Monocytes %	3.60 ± 0.96	3.90 ± 1.19	4.20 ± 1.13	3.80 ± 1.31	4.50 ± 1.58
	Lymphocyte %	54.80 ± 4.21^a	55.20 ± 7.61^a	66.50 ± 3.40^b	64.70 ± 3.88^b	66.20 ± 3.42^b
	WBC (× 10^3/mm^3)	7.95 ± 0.30^a	8.19 ± 0.34^a	8.56 ± 0.32^b	8.87 ± 0.49^b	8.62 ± 0.22^b

Explanations: a, b – Values within the same row marked with different superscript letters were found to be significantly different ($p < 0.05$).

In Table 2 it is seen that the body temperature, respiration, pulse, and rumen movements of the cattle groups were within physiological limits. Selected blood parameters of the animals are shown in Table 3.

In Table 3 it is seen that the number of lymphocytes in the hexane, heptane, and octane groups was significantly higher ($p < 0.05$) than it was in the control and pentane groups on days 3, 6, 9, 12 and, 15. The total leukocyte count in the hexane, heptane, and octane groups was significantly higher ($p < 0.05$) than it was the control group on day 6 day, and significantly higher ($p < 0.05$) than in the control and pentane groups on days 9, 12, and 15. The numbers of neutrophils, eosinophils, and monocytes in the groups were not significantly changed on days 0, 3, 6, 9, 12, and 15, when blood samples were collected.

The tissue samples taken from papilloma cases showed acanthosis in the epidermis, dermal papillae extending towards the dermis, increased keratohyalin granules, hyperkeratosis and parakeratotic hyperkeratosis in some areas. In addition, choriocysts were found (Fig. 2A, 3A, 4A, and 5A). In tissue samples taken after treatment, it was observed that fibrocyte, fibroblast, and collagen synthesis, neovascularization, and fibrosis occurred in the dermis whereas epithelialization was complete in the hexane and heptane treatment groups (Fig. 3B, 4B). In addition, lymphocyte infiltrations were found in the dermis of the hexane group (Fig. 3B). In animals treated with pentane and octane, epithelialization was incomplete, and ulceration occurred in the epidermis (Fig. 2B, 5B). In the control group, an increase in stratum spinosum cells and hyperkeratosis were observed (Fig. 6A, 6B).

Papillomatosis is a common disease in cattle. It leads to significant economic losses through the loss of meat and milk. Furthermore, it adversely affects the leather industry because of the damage it causes to the skin of animals (25). Cutaneous lesions negatively impact the commercial value of affected animals, and their extension to the udder may interfere with milking and routine husbandry procedures (2, 17). That is why the diagnosis and treatment of the disease is of great importance to prevent the loss of productivity. Skin papillomatosis is

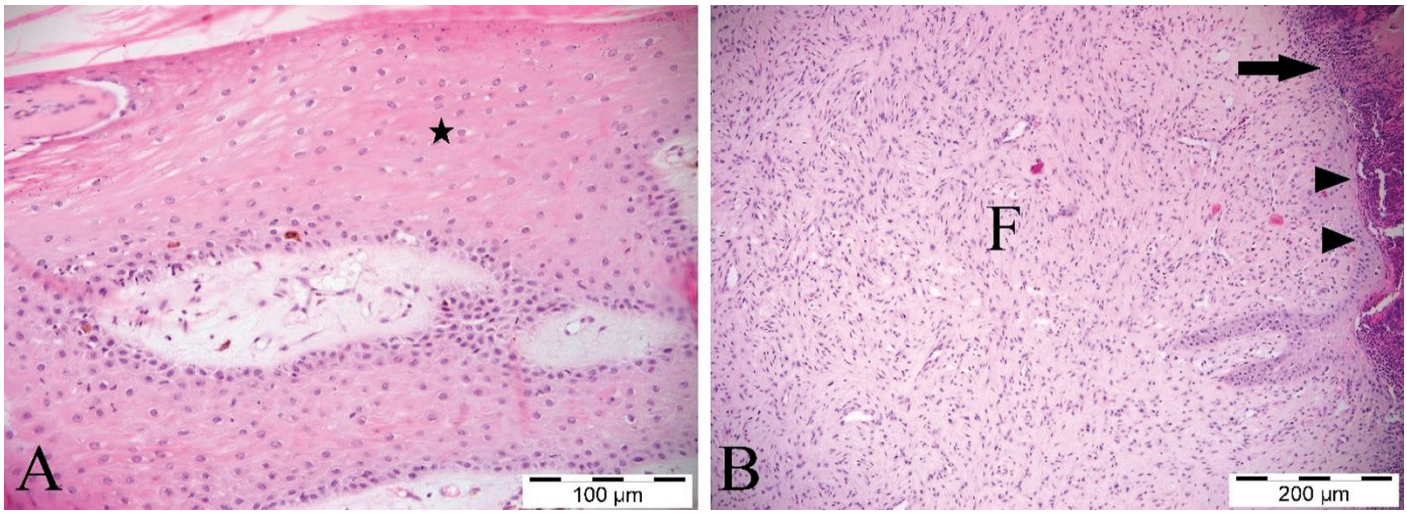

Fig. 2. A – Pentane tumor group (pre-treatment), acanthosis (star), H&E, 100 µm. B – Pentane tumor group (post-treatment), fibrosis tissue formation (F), Epithelialization (arrowhead), ulceration in the epidermis (arrow), H&E, 200 µm

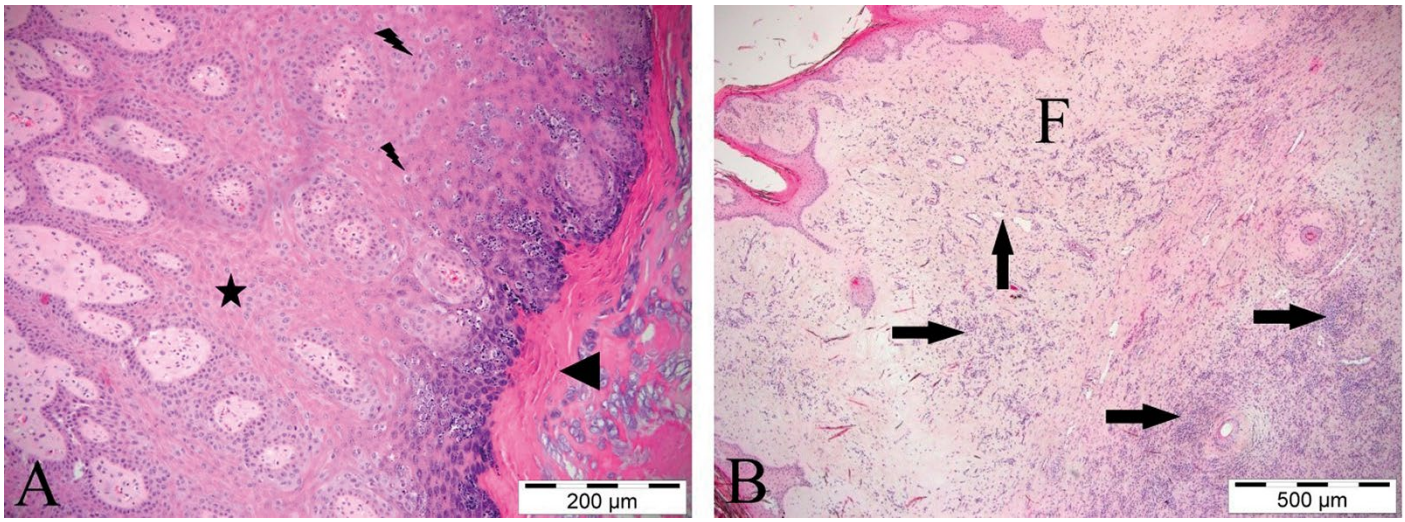

Fig. 3. A – Hexane tumor group (pre-treatment), increase in stratum spinozum cells (star), hyperkeratosis (arrowhead), increase in choriocytes (lightning form), H&E, 200 µm. B – Hexane tumor group (post-treatment), fibrosis tissue formation (F), mononuclear cell infiltration (side arrows), neovascularization (vertical arrow), H&E, 500 µm

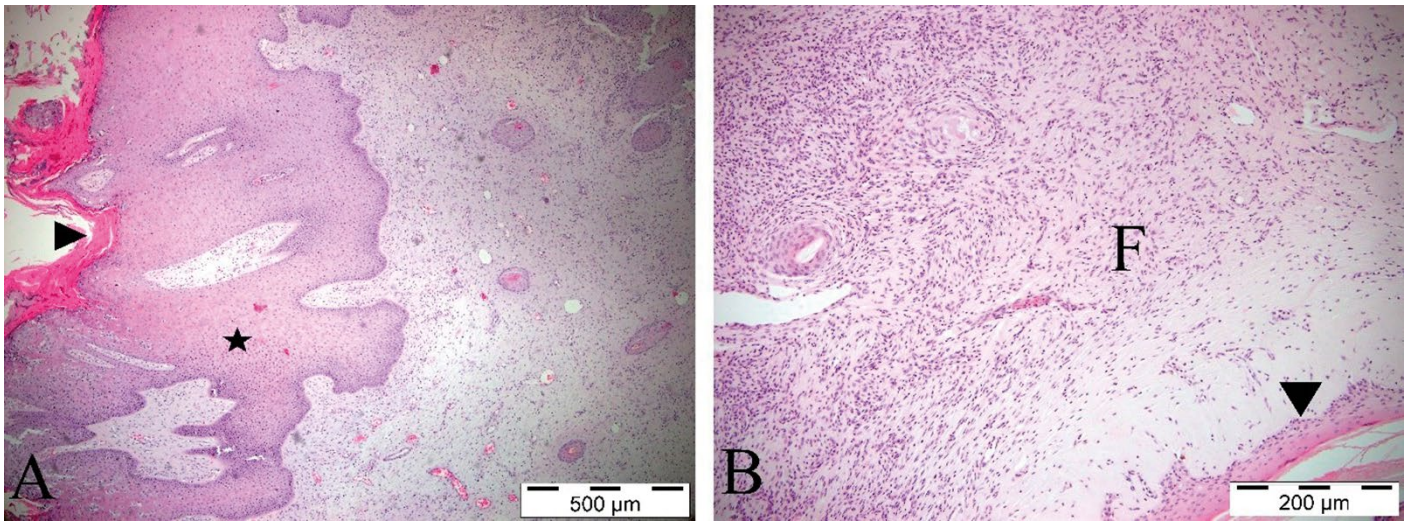

Fig. 4. A – Heptane tumor group (pre-treatment), increase in stratum spinozum cells (star), hyperkeratosis (arrowhead), H&E, 500 µm, B – Heptane tumor group (post-treatment), fibrous tissue formation (F), Epithelialization (arrowhead), H&E, 200 µm

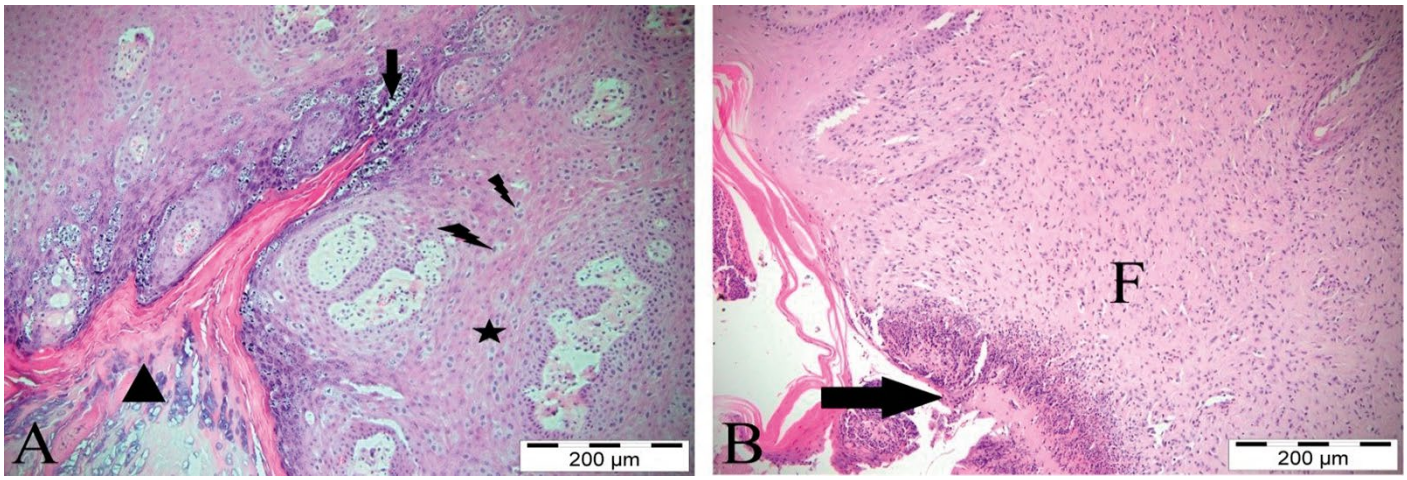

Fig. 5. A – Octane tumor group (pre-treatment), increase in stratum spinozum cells (star), hyperkeratosis (arrowhead), increase in choriocytes (lightning shape), keratohyaline granules (arrow), H&E, 200 µm. B – Octane tumor group (post-treatment), fibrous tissue formation (F), ulceration in the epidermis (arrow), H&E, 200 µm

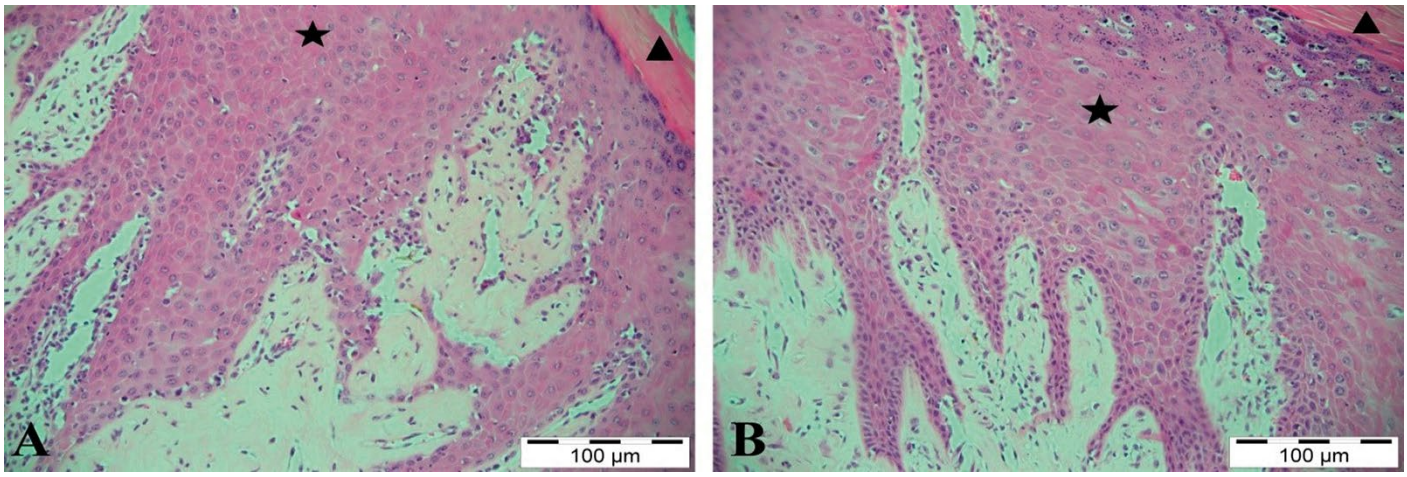

Fig. 6. A – Tumor control group (untreated). Increase in stratum spinozum cells (star), hyperkeratosis (arrowhead), H&E, 100 µm. B – Tumor control group: An animal that remained untreated during the treatment of other animals. Increase in stratum spinozum cells (star), hyperkeratosis (arrowhead), H&E, 100 µm

easy to diagnose by examination of its clinical signs. Additionally, histopathological results, electron microscopic examinations, and polymerase chain reaction (PCR) are also used (25).

The most important factor determining successful treatment of the disease is the localization of papillomas and their spread. While localized and pedunculated papillomas can be treated surgically, surgical treatment

of sessile papillomas spread in large numbers over the body surface is unsuccessful. In the latter case, various treatment options are used. Treatment options include mechanical removal of warts, allowing spontaneous regression, enhancing the immune response, and administering pharmacological agents such as azithromycin, lithium antimony thiomalate, and vincristine sulphate (24-26, 28).

Many methods have been tried to deal with papillomatosis. For example, 0.2 mg/kg ivermectin has been reported to be effective (9, 16). Stimulation of the immune system may also produce positive results in treating the disease. It is suggested that the cellular immune system is more effective than the humourous immune system against this disease (9). According to one study, ivermectin and levamisole, as well as autogenous vaccination and autohemotherapy were effective against chin papilloma (14). According to other studies, Autologous blood therapy, prepared from 20 mL of the patient's own blood and administered either as a single dose or in four weekly applications (10 mL subcutaneously and 10 mL intramuscularly), has demonstrated efficacy in the treatment of mammary papillomatosis (3, 17). Also, a 60% success rate was achieved with a 10% pomade prepared from the plant called *Mukia maderaspatana* (22).

Autogenous vaccines are also used to control bovine cutaneous papillomatosis. In the treatment of mammary papilloma, positive results were obtained by a combined use of podophylline resin and autogenic vaccine (15). In one study, autogenic vaccine is reported to have been effective against papillomatosis when given four times at weekly intervals (8, 13). It is claimed that better results are obtained when autogenic vaccination and auto-hemotherapy are used together (8). Moreover, antiomaline administered five times at 48-hour intervals has been reported to be effective in managing cases involving cutaneous dissemination (26). It is claimed that a 100% success rate was achieved when substances that stimulate the immune system (levamisole, vitamin E and selenium) were used together with an autogenous vaccine in papillomatosis cases treated by surgical methods (28). Another study reports that the combined use of lithium antimony thiomalate and levamisole was more effective in the treatment of papillomatosis than the use of lithium antimony thiomalate alone (7). In another study, autovaccination proved ineffective, whereas success rates for ivermectin, autohemotherapy, and lithium antimony thiomalate amounted to 20%, 60%, and 80%, respectively (20).

As can be seen above, many different methods have been used by researchers in the treatment of bovine skin papillomatosis, and different degrees of success have been achieved. According to Doğan et al. (11), gasoline sprayed on papillomas (enough to wet the lesions) for 15 days in the morning and evening was 90% effective at the end of day 50. No improvement was observed in the control and pentane groups in the present study. The rate of success was 70% in the hexane group, 80% in the heptane group, and 50% in the octane group (Tab. 1). This study shows that the most effective chemicals against bovine skin papilloma are hexane and heptane. The findings of this study are similar to those reported by Doğan et al. (11). Pentane, hexane, heptane, and octane are light liquid hydrocarbons containing 5, 6, 7, and 8 carbons, respectively. These hydrocarbon-structured chemicals are oxidized in the cells and are first converted into alcohols and then into aldehydes, such as hexanon (hexane). The resultant alcohols and aldehyde damage cellular structures. In addition, alcohols may cause inflammatory reactions by irritating surfaces with which they come into contact (10). It may be argued that the toxic effects of these chemicals on papillomas may be caused by alcohol and aldehyde derivatives released after their metabolism.

As the number of hydrocarbons increases, their volatility and absorption decreases. As carbon numbers increase, so do their toxic effects on cells (10). The ineffectiveness of pentane against papillomas may be due to its low carbon content (it is less toxic compared to other hydrocarbons). Low carbon numbers increase their volatility. As a result, contact with the affected area was reduced. These properties may explain why pentane was ineffective against papillomatosis. The weaker effect of octane compared with hexane and heptane may be due to its higher carbon count. The large number of carbons in the octane structure decreases its absorption and increases its toxic effect on tumor cells. This may explain why its absorption from the application site reduces overall toxicity, whereas at the same time it has a small effect on the tumor. In this study, hexane and heptane may have been more effective than pentane and octane because of the number of carbons in their structures. It can be argued that hexane and heptane are sufficiently absorbed from the lesioned area to reach an effective concentration, penetrate tumor cells well, and metabolize effective compounds, such as alcohols and aldehydes (their volatility is also important).

In this study, the number of lymphocytes was significantly higher ($p < 0.05$) in the hexane, heptane, and octane groups than in the control and pentane groups on days 3, 6, 9, 12, and 15 of treatment (Tab. 3). This increase in lymphocytes may be due to the toxic effect of hexane, heptane, and octane on papilloma cells. The production of lymphocytes may be argued to be stimulated by the action of these chemicals. This conclusion is supported by the presence of lymphocyte infiltrations found by histopathological examination in the dermis of the hexane group animals (Fig. 3B). The increase in the number of lymphocytes in cattle treated with hexane, heptane, and octane explains the effect of these substances on papillomas (cellular and/or humoral immunity) (Tab. 1, Fig. 1F, 1H, 1J). The results of this study support the research findings of Börkü et al. (9), who emphasize the importance of the cellular immune system in papillomatosis. The total leukocyte count in the hexane, heptane, and octane groups was significantly

higher ($p < 0.05$) than it was in the control group on day 6 and significantly higher ($p < 0.05$) than in the control and pentane groups on days 9, 12, and 15 (Tab. 3). The increased total number of leukocytes is thought to be due to the increased number of lymphocytes. This is because there were no significant changes in neutrophil, eosinophil, or monocyte counts in blood samples taken on days 0, 3, 6, 9, 12, and 15 (Tab. 3). It is thought that the absence of significant changes in the numbers of neutrophils, eosinophils, and monocytes may be related to the absorption of pentane, hexane, heptane, and octane.

The general examination of the cattle groups on days 0, 3, 6, 9, 12, and 15 found that body temperature, rumen movements, pulse, and respiratory rates were within physiological limits (Tab. 2). The findings of this research are similar to those of Doğan et al. (11). The fact that the results of the general examination of the cattle groups were within the range of normal values may suggest that the locally applied chemicals themselves and/or their metabolites are not absorbed or detoxified at levels that cause systemic effects.

On histopathological examination, epithelialization was incomplete in the pentane- and octane-treated animals, and ulceration was seen in the epidermis (Fig. 2B, 5B). In the hexane and heptane groups, fibrosis occurred with fibrocyte, fibroblast, and collagen synthesis, as well as neovascularization in the dermis where epithelialization was complete (Fig. 3B, 4B). Pathology results also confirmed the clinical inefficacy of the pentane treatment (Fig. 1D). Although the clinical success rate of octane in this study was 50%, the pathological results show that it caused ulcerations in the epidermis (Fig. 5B).

In conclusion, pentane applied to lesions of bovine skin papillomatosis was found to be ineffective, whereas the success rates for hexane, heptane, and octane were 70%, 80%, and 50%, respectively. It can be argued that hexane, heptane, and octane may be used in the future in the treatment of sessile papilloma that has spread to different parts of the body and cannot be treated by surgical interventions. It is therefore very important that these research findings be verified by further studies. Among the limitations of this study is the lack of evaluation regarding the potential risks that the chemicals used – namely pentane, hexane, heptane, and octane – may pose to the safety of animal-derived food products (such as meat and milk) and human exposure. As such, these aspects remain outside the scope of the present research.

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Corresponding author: Assist. Prof. Dr. Ertan Doğan, University of Ardahan, Department of Laborant and Veterinary Health, Vocational School of Nihat Delibalta Göle, 75700, Ardahan, Türkiye; e-mail: ertandogan@ardahan.edu.tr