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Effect of Propolis in Dogs with Transmissible Venereal Tumor were Treated Vincristine Sulphate: Experimental Study

Vinkristin Sülfat ile Tedavi Edilen Bulaşıcı Zührevi Tümörlü Köpeklerde Propolisin Etkisi: Deneysel Çalışma

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ABSTRACT Objective: The objective of the study was to determine mean of treatment period, some liver enzyme levels and blood parameters in dogs with transmissible venereal tumor (TVT) that were administered standard vincristine sulphate and administered propolis by different routes. **Material and Methods:** The study was permormed with 24 dogs (9 males and 15 females). The groups consist of control group, oral propolis group, local propolis group, and oral+local propolis group. Tumor regression was determined at weekly by a physical and histopathological examination. Blood samples and the smears were also collected at weekly interval. **Results:** Although there are not significant differences, mean treatment weeks in all groups that added propolis were lower than control group. Neutropenia, neutrophilia, leukopenia, and leukocytosis were confirmed most of the treated dogs. Alanine transaminase, aspartate transaminaz, alkaline phosphatase, gamma glutamyltransferase and lactate dehydrogenase levels were in the reference values in control and experimental groups. **Conclusion:** Treatment periods in groups used propolis were shorter than the standard vincristine sulphate therapy. Antitumoral effects of propolis should much more extensively study with animal and clinical experiments.

Keywords: Transmissible venereal tumor; vincristine; propolis

ÖZET Amaç: Bu çalışmada, vinkristin sülfat ile standart medikal tedavi alan bulaşıcı zührevi tümörlü [transmissible venereal tumor (TVT)] köpeklerde, farklı yollarla propolis uygulamasının, iyileşme sürelerine, bazı karaciğer enzim düzeyleri ve kan parametrelerine etkisinin incelenmesi amaçlandı. **Gereç ve Yöntemler:** Çalışmaya 24 TVT'li köpek (9 erkek, 15 dişi) dâhil edildi. Çalışma kontrol grubu, oral propolis grubu, lokal propolis grubu ve oral+lokal propolis grubu olarak 4 gruba ayrılarak yapıldı. Haftalık aralıklarla fiziksel muayene ve histopatolojik inceleme ile tümör regresyonu belirlendi. Her hafta, smear ve kan örnekleri alındı. **Bulgular:** İstatistiki olarak fark bulunmamakla birlikte propolis ilave edilen tüm gruplarda iyileşme süresinin daha kısa olduğu görüldü. Nötropeni, nötrofili ile lökopeni ve lökositoz tedavi edilen köpeklerin büyük çoğunluğunda tespit edildi. Alanin aminotransferaz, aspartat aminotransferaz, alkalen fosfataz, gama glutamil transferaz ve laktat dehidrojenaz düzeylerinin kontrol ve deney gruplarında referans değer aralığında olduğu görüldü. **Sonuç:** TVT'nin standart vinkristin sülfat ile tedavisinde propolis ilave edilen gruplarda iyileşme daha hızlı olmuştur. Propolisin antitümoral etkisi daha detaylı olarak hayvan ve klinik çalışmalarla yapılmalıdır.

Anahtar Kelimeler: Bulaşıcı zührevi tümör; vinkristin; propolis

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Canine transmissible venereal tumor (TVT) is a benign tumor affecting the mucosa of external genital organs in dogs, commonly observed in a significant population of stray dogs. The tumor's etiology involves the transplantation of tumor cells through behaviors like coitus, licking, and sniffing, leading to its transmission. Apart from genital areas, TVT may also manifest in the conjunctiva, skin, nasal and oral cavities.[1,2]

Diagnosing TVT relies on clinical and cytology/histopathological examination. Cytology, a rapid and minimally invasive method, reveals multicellu-

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lar samples with round cells exhibiting well-defined cytoplasmic borders, round nuclei of varying sizes, and granular chromatin. Nevertheless, taking into account the cytoplasmic vacuoles, cellular dimensions and morphology, as well as the ratio of nucleus to cytoplasm, TVT can be classified into plasmacytoid, lymphocytoid, and mixed types, with plasmacytoid TVT often displaying higher resistance.[3-5]

Treatment options for TVT include surgery, radiotherapy, immunotherapy, and chemotherapy. Chemotherapy, particularly vincristine sulfate, is considered the most effective and practical treatment. Other agents like cyclophosphamide, vinblastine, and methotrexate can be used alone or in combination. Doxorubicin may be employed for resistant cases.[2,6] Despite vincristine sulfate's effectiveness, it can have undesirable cytostatic effects, impacting the dog's immunity and potentially causing hematological disorders like thrombocytosis, anemia, lymphopenia, and leukopenia.[7-9] Additionally, vincristine sulfate in dogs with TVT may elevate liver enzymes.[10]

Propolis is a honey bee product which has biological and pharmacological effects such as antitumoral, antibacterial, antiviral, immunomodulatory, hepatoprotective, antiinflammatory, and tissue regeneration due to content of phenolic compounds.[11] Several studies conducted in vivo and in vitro, propolis showed antitumor properties. Previous studies have reported the anticancer effects of propolis that has shown activity against human cancer cell lines, including oral, KYSE-30 esophageal squamous carcinoma, gastric, cervical, colon, leukemia, stomach, skin, breast, and prostate cancers.[12-15] Antitumoral effect of propolis may be act with different acting mechanism such as starting apoptosis, antiangiogenic effect, cell cycle inhibition in tumor cells, and prevention of metastasis.[16-22] Propolis has immunomodulatory effect, and may also effective against tumor cells.[23] Propolis also showed antitumoral active against TVT-cells.[4] The antitumoral, hepatoprotective, and immunomodulatory effects of propolis can potentially be used for TVT therapy and to minimize the side effects of vincristine sulphate.[16,23-26] Therefore, the aim of the investigation was to determine the average duration of the treatment period, blood parameters and some liver enzyme levels in dogs with TVT that were administered standard vincristine sulphate and administered propolis by different routes.

MATERIAL AND METHODS

This study carried out between 09.01.2020 and 05.01.2021, and was approved by the Animal Ethics Research Committee of Bursa Uludağ University (date: January 07, 2020; no: 2020-01/11). The dog owners were instructed on the experimental model used and signed a consent form for dogs treatment, and was careful for animal rights. The limitation of the study was if the animal did not respond to the treatment in eight weeks and had any side effect of the treatment it would be revomed from the project. The study included 29 dogs, 10 males and 19 females at the begining of the study, from different breeds such as husky, golden retriever, anatolian sheepdog and crossbred dogs, 2-13 years of age, with naturally occurring TVT. Five animals were excluded from this study due to treatment of three dogs took more time than the usual eight weeks treatment period, a dog had high levels of liver and kidney enzymes with thirteen years old, one of them died during treatment because of the progressive worsening of the general condition with old age (thirteen years old). Death might rarely observed in dogs during the TVT treatment with vincristine sulphate.[27] As a result, the study was carried out with 24 dogs including 9 males and 15 females. TVT diagnosis was performed based on a physical examination and a positive cytological diagnosis. The physical examination included the clinical history of bleeding from penis and prepuce in males, from vagina in females, and the presence of cauliflower-like mass formation (between 1 and 5 cm diameters) in both genders (Figure 1). The cytological diagnosis was determined from the samples of smears prepared from tumoral masses by seeing typical TVT cells, shaped round, ovoid or polyhedral with eosinophylic vacuole, thin cytoplasm, round hyperchromatic nucleus and nucleolus (Figure 2). TVT classes also determined as plasmocytoid, lymphocytoid, and mixed in Figure 3, respectivly. The dogs were individually housed in cages at the Clinics of the Faculty of Veterinary Medicine, Bursa Uludağ University. The animals were allowed access to a

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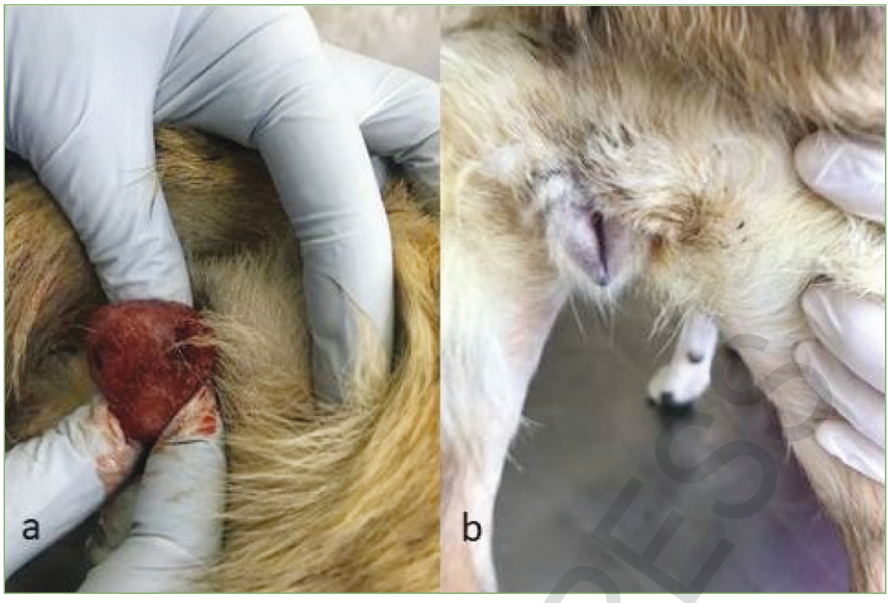

FIGURE 1: The physical examinations of transmissible venereal tumor in a female dog.

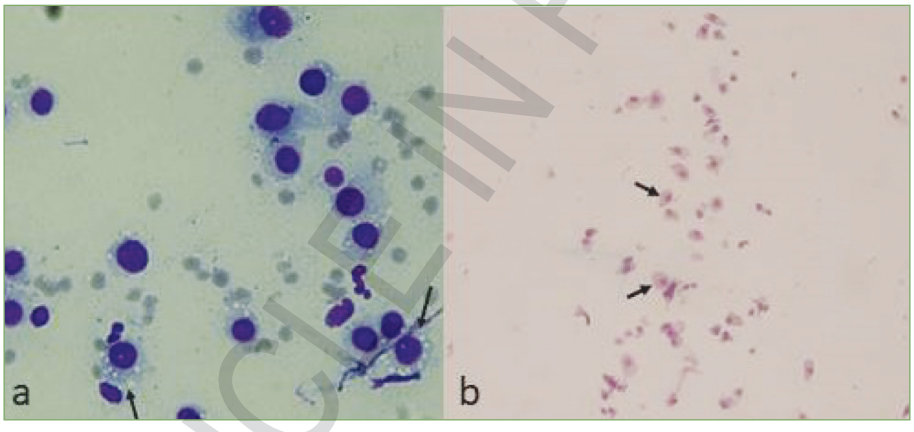

FIGURE 2: The cytological diagnosis transmissible venereal tumor cells.

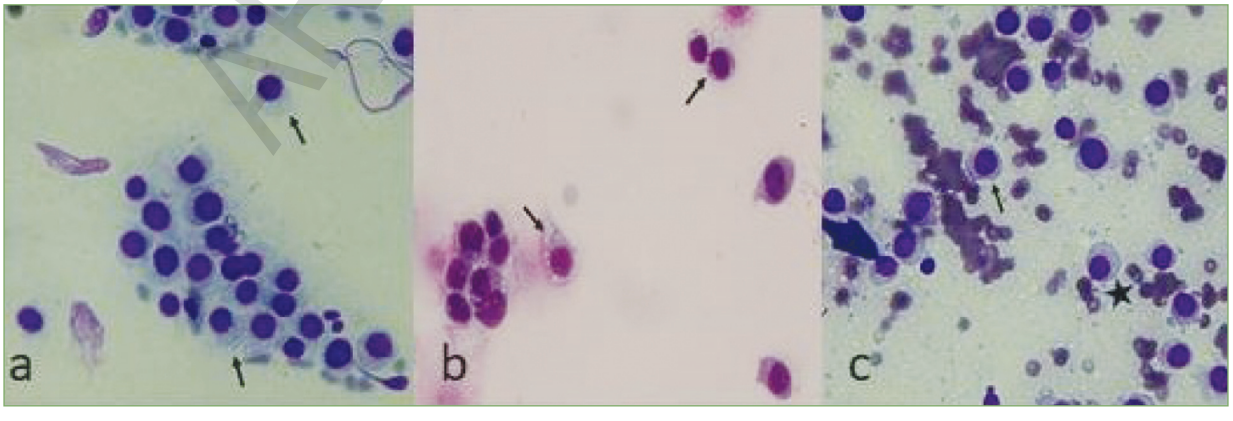

FIGURE 3: Transmissible venereal tumor classes (a-plasmocytoid, b-lymphocytoid, and c-mixed).

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standard diet and drinking water ad libitum during the experimental period.

Total 24 dogs (male n=15 and female n=9) were divided into four groups. The propolis doses were basically determined as described in Oršolić et al.[28] In first group (control group, n=6; 2 males, 4 females), dogs were treated with standard vincristine sulphate (Vincristine-Koçak, Koçak Farma, İstanbul, Türkiye) at 0.025 mg/kg body weight (BW), intravenous at weekly interval. In second group (oral group, n=7; 3 males, 4 females), the treatment was same as in first group, propolis was applied at 100 mg/kg BW, orally by enjector at daily interval as a raw propolis (as equivalent 0.25 mg/mL/kg raw propolis tincture). In third group (oral+local group, n=6; 2 males, 4 females), the treatment was same as in second group, however, propolis tincture was additionally administered at between 2 and 10 mL depend on the tumor size, that tumor diameters were between 1 and 5 cm, (2 mL sprayed for each diameter size), locally as spray at daily as raw propolis tincture. In fourth group (local group, n=5; 2 males, 3 females), the treatment was same as in first group for standard vincristine sulphate therapy, however, propolis tincture was additionally administered at between (2 and 10 mL), depend on the tumor size (2 mL sprayed for each diameter size) as group third for local therapy, locally as spray at daily as raw propolis tincture. Propolis tincture used in this study that analyzed for individual phenolic compounds are presented in Table 1. The procedures employed to assess the well-being of all canines involved a thorough physical examination, a comprehensive complete blood cell count, and a serum biochemistry profile specifically assessing hepatic function. These blood and serum samples were taken every week, before the administration of vincristine. They were performed weekly until the tumor was visibly eradicated (1b) and subsequently confirmed through cytological examination (2b), limited to a maximum of eight treatments. The cytological exam was made by imprint of the tumoral masses with a histological slide and after staining in Diff-Quik. Blood samples were collected in EDTA vacutainer and serum vacutainer for haematological and biochemical analyzis, respectively, before administration of vincristine sulphate. Serum was stored at -18 ºC until analyzed for biochemical parameters. Haematological parameters such as white blood cell (WBC), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), eosinophils (EOS), trombocytes (total platelets/PLT), haemoglobin (HGB) and hemotocrit (HCT) were analyzed by automatized blood count analyzer (HASVET VH5R, Antalya, Türkiye). Serum biochemical parameters such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) was performed by automatized clinical biochemical analyzer (Mindray, BS 800, Shenzhen Mindray Bio-Medical Electronics Co., Shenzhen, China).

The raw propolis was collected from beekeepers (İnegöl, Cumalıkızık region) in Bursa in autumn of 2019, then mixed the raw propolis samples, and extracted by hydro-alcholic solvent. The raw propolis extraction and the phenolic compounds analysis in propolis tincture was carried out as described in Oruç et al.[29] The details of extraction method were the frozen raw propolis samples were cut into small pieces and finely powdered using a coffee grinder (DeLonghi KG 49). During the extraction, the applied sample-to-solvent ratio was 1:9 (w/v). The method was based on five grams of crude propolis sample with 45 mL of 70% ethanol stirred at 55 ºC for three hours with an orbital shaker (Shel Lab, SL Shaking Incubator). Then, the sample was subjected to ultrasonication for 15 min (Bandelin, Sonorex, RK 100) and stirred again for an hour. After stirring the sample, the propolis solution was filtered by Whatman filter paper (No. 1). The hydroalcoholic solvent filtrate was filtered again through a polyvinyl difluoride syringe filter (Millipore Millex-HV, 0.45 µl) for injection to HPLC system (Shimadzu, LC-20 AD/SPD-M20A). The analyzed phenolic compounds were gallic acid, epigallocatechin gallate, caffeic acid, ferulic acid, isoferulic acid, dimethoxycinnamic acid, quercetin, cinnamic acid, naringenin, apigenin, kaempferol, chrysin, pinocembrin, galangin, caffeic acid phenylethyl ester, and chalcone.

Statistical analysis were performed with IBM SPSS Statistics 20. Since the number of data in the groups was small and did not comply with normal distribution, non-parametric tests were preferred in

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the data analysis. The Kruskal-Wallis test was used to compare continuous variables among the groups. The changes in each group over time (for five weeks) in terms of relevant variables were examined with the Friedman test. The threshold for statistical significance was set at a p-value of 0.05.

RESULTS

The concentrations of phenolic compounds in propolis tincture used in the study were presented in Table 1. Hematological parameters, serum biochemical parameters, and their levels are shown in Table 2 and Table 3, respectively. Only one dog from 24 dogs was lymphocytoid, nine dogs were plasmocytoid, and fourteen dogs were mixed as TVT classes.

There were no significant differences between the control and other propolis-added groups (p=0.423) and among the propolis-added groups (p=0.381) for the average length of treatment periods. However, the average treatment length was 3.60 weeks for the local group, 4.16 weeks for the oral+local group, 4.42 weeks for the oral group, and 5.00 weeks for the control group.

LYM, MON, EOS, basophils, HGB and HCT were generally not much influenced in most of the groups, maintaining within the reference value for the species (Table 2). However, WBC, NEU, and trombocytes (total platelets) were much influenced. Thrombocytopenia and thrombocytosis were observed in two of 24 and five of 24 treated dogs, respectively, neutropenia and neutrophilia were observed in 16 of 24 and 10 of 24 treated dogs, respectively. Leukopenia and leukocytosis were also confirmed in 14 of 24 and 10 of 24 treated dogs, respectively.

Significant differences were observed in groups for some hematological parameters for the first five weeks; for WBC, in control (p=0.011), oral (p=0.031) and oral+local group (p=0.002); for MON, only oral+local group (p=0.045); for NEU, control (p=0.030), oral (p=0.044) and oral+local group (p=0.003); for EOS, only control group (p=0.008); for PLT only oral+local group (p=0.040). However, a significant difference was not found for HGB and HCT. Bonferroni correction (α^{*}=0.005) was used to prevent Type I error in pairwise comparison of groups, and there was no significant difference. Significant differences were not observed for biochemical parameters ALT, AST, ALP, GGT, and LDH levels between groups for the first five weeks (p>0.05).

DISCUSSION

In this study, the aim of the study was to determine effects of standard vincristine sulphate and additionally propolis treatment by different routes in dogs with TVT on average of treatment period, blood parameters and liver enzyme levels.

The chemical composition of propolis is very complex and is dependent upon the source plant. Bud exudates of different poplar species are the main sources of propolis in temperate zone, including Europe, Asia and North America.[30] Samples originating from these regions are characterized by similar chemical composition. The main phenolics including flavonoid aglycones such as pinocembrin, naringenin, quercetin, galangin, kaempferol, and including hydroxycinnamic acids and their esters such as caffeic acid, CAPE, *m*-coumaric acid, *p*-coumaric acid and ferulic acid are predominant in propolis samples from Türkiye as Europe, Asia and North America.[29,31] The propolis used in this study results were also in agreement with the data as *Populus* spp. (poplar) was one of the main propolis sources determined in this study as well (Table 1). The most stud-

TABLE 1: Concentrations of phenolic compounds in propolis tincture used in the study (µg/mL).

GA	EGCG	CA	COU	FR	IFR	DMCA	QE	CINA	NR	AP	KF	CR	PN	GL	CAPE	CL
56	115	376	635	232	788	569	348	186	497	332	249	1610	4295	3686	10060	1944

GA: Gallic acid; EGCG: (-)-Epigallocatechin gallate; CA: Caffeic acid; FR: trans-Ferulic acid; IFR: trans-Isoferulic acid; DMCA: 3,4-Dimethoxycinnamic acid; QE: Quercetin; CINA: trans-Cinnamic acid; NR: Naringenin; AP: Apigenin; KF: Kaempferol; CR: Chrysin; PN: Pinocembrin; GL: Galangin; CAPE: Caffeic acid phenylethyl ester; CL: trans-Chalcone.

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TABLE 2: Effects of vincristine sulphate and propolis on haematological parameters.

Groups n	Parameter	Before drug admn Day 0 (1st dose) Median (Min-Max)/Mean±SEM	Day 7 2nd dose Median (Min-Max)/Mean±SEM	Day 14 3rd dose Median (Min-Max)/Mean±SEM	Day 21 4th dose Median (Min-Max)/Mean±SEM	Day 28 5th dose Median (Min-Max)/Mean±SEM
Control n=6	HGB (g/dL)	13.70 (9.90-20.30)/14.33±1.40	13.40 (11.10-20.00)/14.22±1.40	14.40 (11.80-18.30)/14.55±0.99	16.05 (10.90-18.50)/15.08±1.21	16.40 (11.70-21.20)/16.00±1.86
	WBC	22.58 (14.81-32.56)/23.16±3.10	6.83 (3.87-9.71)/6.76±0.86	7.64 (4.59-17.90)/9.69±2.32	8.55 (5.20-21.69)/10.13±5.97	10.40 (9.05-11.14)/10.30±0.40
	HCT	39.5 (27.10-56.20)/40.63±3.83	38.45 (32.70-55.40)/41.18±3.47	43.92 (36.60-54.16)/43.33±2.63	46.80 (31.40-49.70)/43.23±2.99	49.90 (34.70-59.70)/46.62±4.96
	LYM	1.17 (0.73-2.32)/1.30±0.22	1.45 (0.82-1.88)/1.43±0.15	1.72 (0.94-2.99)/1.81±0.35	1.97 (1.19-2.56)/1.92±0.21	1.21 (0.87-2.96)/1.55±0.38
	MON	1.57 (0.03-4.26)/1.83±0.63	1.00 (0.11-2.29)/1.08±0.29	0.79 (0.12-1.36)/0.68±0.20	1.37 (0.30-2.78)/1.42±0.34	1.30 (0.83-1.90)/1.29±0.17
	NEU	16.88 (12.54-27.78)/18.78±2.72	3.38 (2.19-6.75)/3.90±0.70	5.27 (2.36-14.42)/6.73±1.94	4.98 (1.67-15.59)/5.98±2.06	6.92 (6.21-7.68)/6.84±0.25
	EOS	1.09 (0.50-1.63)/1.06±0.18	0.24 (0.09-0.77)/0.29±0.10	0.42 (0.04-0.82)/0.38±0.11	0.58 (0.01-1.68)/0.71±0.27	0.43 (0.28-1.02)/0.51±0.13
	PLT	344.5 (106-622)/342.17±71.61	431.5 (231-602)/433.33±53.90	306.5 (132-484)/301.67±52.73	312.5 (111-549)/327.33±58.03	363 (261-449)/358.4±36.44
Oral+ local n=6	HGB (g/dL)	15.65 (12.70-16.90)/15.03±0.65	14.20 (10.50-15.80)/13.87±0.80	13.55 (9.80-15.70)/13.08±1.06	16.40 (11.30-19.80)/15.98±1.16	15.05 (14.70-16.10)/15.22±0.31
	WBC	19.05 (9.00-24.43)/17.99±2.35	5.76 (3.99-21.53)/8.16±2.73	4.76 (3.32-15.99)/6.95±1.97	6.25 (3.48-7.75)/5.99±0.62	7.26 (3.52-9.57)/6.90±1.27
	HCT	43.40 (37.00-47.80)/42.52±1.71	40.90 (32.24-45.50)/39.66±2.27	39.35 (25.90-46.00)/37.98±3.17	46.10 (34.90-55.20)/45.82±2.77	43.65 (42.60-44.00)/43.47±0.32
	LYM	1.68 (1.37-2.35)/1.80±0.17	1.59 (0.70-2.13)/1.50±0.22	1.53 (1.15-2.30)/1.62±0.17	1.62 (1.08-2.30)/1.66±0.17	2.08 (1.57-2.70)/2.10±0.24
	MON	1.54 (1.04-2.77)/1.61±0.25	0.72 (0.12-1.30)/0.65±0.19	0.54 (0.32-2.70)/0.92±0.37	0.75 (0.32-1.04)/0.68±0.12	0.72 (0.21-0.83)/0.62±0.14
	NEU	14.48 (5.27-19.10)/13.54±2.06	3.52 (2.28-17.76)/5.67±2.44	2.28 (1.32-9.92)/3.96±1.37	3.10 (1.40-5.41)/3.23±0.53	3.73 (1.62-5.47)/3.64±0.80
	EOS	0.68 (0.48-1.50)/0.87±0.18	0.22 (0.04-1.61)/0.28±0.09	0.29 (0.13-1.04)/0.40±0.14	0.34 (0.09-0.87)/0.36±0.11	0.36 (0.11-1.04)/0.46±0.20
	PLT	396.5 (267-500)/387.33±42.50	387.5 (260-501)/388.17±39.50	423.5 (245-576)/395.83±50.26	357.5 (274-452)/362.5±27.72	273 (125-427)/274.5±61.81
Oral n=7	HGB (g/dL)	16.80 (11.80-20.00)/16.33±1.01	16.30 (13.20-22.70)/17.23±1.21	15.80 (12.60-17.70)/15.26±0.66	15.80 (13.70-17.50)/15.48±0.54	14.90 (5.90-18.50)/14.53±1.55
	WBC	12.89 (9.52-21.69)/13.67±1.62	8.74 (4.61-20.12)/9.62±2.05	7.07 (3.78-11.60)/7.83±0.96	6.62 (4.51-11.07)/7.05±0.87	6.63 (4.80-11.79)/7.44±0.88
	HCT	47.20 (38.30-57.30)/46.70±2.65	44.50 (38.50-60.60)/47.06±2.93	43.80 (37.10-51.10)/42.94±1.93	44.80 (37.60-50.00)/43.67±1.54	44.62 (16.10-50.10)/41.33±4.41
	LYM	1.60 (1.00-5.17)/2.06±0.53	1.68 (1.00-5.01)/2.42±0.56	1.51 (1.05-3.73)/1.92±0.37	1.58 (1.14-4.08)/2.14±0.43	1.83 (1.25-3.84)/2.18±0.38
	MON	1.16 (0.56-2.78)/1.40±0.29	0.92 (0.48-3.47)/1.41±0.38	1.24 (0.56-1.46)/1.10±0.13	0.85 (0.56-1.73)/0.94±0.15	0.56 (0.39-1.74)/0.79±0.18
	NEU	8.63 (6.53-15.59)/9.40±1.13	4.40 (2.18-14.53)/5.38±1.60	4.21 (1.13-6.71)/4.30±0.67	3.66 (2.01-4.92)/3.57±0.39	3.78 (2.83-5.84)/4.10±0.46
	EOS	0.41 (0.08-1.39)/0.68±0.23	0.32 (0.07-0.59)/0.31±0.07	0.35 (0.06-0.97)/0.43±0.14	0.27 (0.13-0.65)/0.33±0.06	0.19 (0.12-0.67)/0.29±0.07
	PLT	283 (221-306)/269.71±11.48	303 (228-454)/328.0±35.54	279 (168-426)/298.71±35.90	263 (211-429)/293.71±32.98	210 (161-331)/217.28±20.95
Local n=5	HGB (g/dL)	16.30 (8.80-17.10)/14.46±1.54	14.90 (7.60-16.40)/13.34±1.55	14.90 (8.80-17.90)/14.14±1.49	13.75 (9.80-17.10)/13.60±1.50	14.70 (14.20-16.30)/15.07±0.63
	WBC	15.26 (7.12-38.77)/18.59±5.40	10.18 (5.87-31.09)/13.25±4.55	7.12 (3.86-10.99)/7.13±1.32	7.00 (4.95-11.86)/7.70±1.62	6.15 (5.44-7.24)/6.28±0.52
	HCT	46.60 (27.80-48.40)/41.64±4.03	41.30 (25.60-46.90)/38.62±3.75	42.80 (30.50-50.90)/41.52±3.28	39.15 (30.60-49.20)/39.52±3.80	44.50 (44.00-47.40)/45.30±1.06
	LYM	1.88 (0.87-5.60)/2.35±0.84	1.64 (1.20-6.16)/2.55±0.92	1.31 (0.96-5.29)/2.11±0.81	1.43 (1.13-3.67)/1.91±0.59	1.30 (0.88-2.16)/1.44±0.38
	MON	1.22 (0.47-3.14)/1.50±0.50	1.58 (0.97-3.35)/1.99±0.49	1.03 (0.10-1.58)/0.98±0.27	1.55 (0.45-2.43)/1.49±0.41	1.52 (0.38-2.38)/1.43±0.58
	NEU	9.99 (5.11-32.66)/14.10±5.11	4.30 (2.91-26.04)/8.27±4.46	3.86 (1.64-6.18)/3.82±0.72	2.89 (1.43-8.56)/3.94±1.58	3.15 (1.51-4.62)/3.09±0.89
	EOS	0.33 (0.13-1.03)/0.46±0.17	0.30 (0.14-0.57)/0.30±0.08	0.13 (0.08-0.30)/0.16±0.04	0.27 (0.24-0.41)/0.29±0.04	0.18 (0.15-0.39)/0.24±0.07
	PLT	297 (56-511)/297.6±81.29	400 (171-937)/426±137.62	390 (155-1105)/355.08±40.34	322 (109-1000)/347.87±36.56	263 (125-705)/303.63±31.14

HGB: Haemoglobin; WBC: White blood cell; HCT: Hematokcrit; LYM: Lymphocytes; MON: Monocytes; NEU: Neutrophils; EOS: Eosinophils; PLT: Total platelet count.

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TABLE 3: Effects of vincristine sulphate and propolis on liver enzymes.

Groups n	Liver enzy	Before drug admn Day 0 (1st dose) Median (Min-Max)/Mean±SEM	Day 7 2nd dose Median (Min-Max)/Mean±SEM	Day 14 3rd dose Median (Min-Max)/Mean±SEM	Day 21 4th dose Median (Min-Max)/Mean±SEM	Day 28 5th dose Median (Min-Max)/Mean±SEM
Control n=6	ALT	20.35 (13.1-218.6)/55.58±32.93	25.1 (15.70-50.80)/28.08±5.22	24.35 (17.10-30.20)/24.37±1.75	25.85 (17.40-30.50)/25.38±1.86	26.10 (16.00-34.90)/24.68±3.56
	AST	16.15 (10.9-95.4)/28.00±13.51	15.35 (14.50-39.80)/20.63±4.11	15.90 (13.20-26.00)/16.96±1.89	17.40 (12.20-22.40)/17.30±1.77	19.10 (15.20-29.60)/20.40±2.69
	ALP	16.6 (14.5-44.6)/22.80±4.91	19.7 (10.60-55.20)/24.95±6.54	21.15 (11.70-55.90)/27.93±6.92	22.65 (14.60-51.10)/27.30±6.05	28.50 (22.40-67.10)/40.92±9.61
	GGT	4.3 (3.0-8.0)/4.73±0.79	4.70 (2.30-7.60)/4.78±0.81	4.65 (2.50-7.10)/4.72±0.66	4.75 (3.20-7.40)/4.98±0.62	4.70 (3.90-6.30)/4.88±0.40
	LDH	92.15 (79.5-276.2)/123.1±30.87	178.15 (132-247.1)/185.88±17.51	119.45 (68.6-171.8)/117.62±14.41	167.8 (96.3-327.8)/191.55±33.11	198.7 (104.3-677.3)/290.54±107.39
Oral+local n=6	ALT	21.9 (11.3-42.9)/27.57±4.85	25.80 (13.00-34.70)/25.73±2.91	26.50 (14.40-33.10)/67.61±43.93	24.50 (15.00-59.70)/28.23±5.46	23.40 (19.40-31.20)/24.63±1.88
	AST	16.3 (11.4-32.7)/18.39±2.99	18.90 (11.50-34.70)/20.51±3.14	20.20 (9.60-110.70)/31.06±13.38	19.80 (11.40-27.00)/18.44±2.51	16.10 (10.50-33.10)/18.45±3.50
	ALP	31.3 (23.3-70.4)/36.46±5.87	31.20 (14.50-64.10)/32.57±6.08	32.70 (14.70-61.30)/36.97±5.46	34.20 (12.50-59.70)/37.01±6.43	35.80 (17.00-76.00)/40.00±8.89
	GGT	5.10 (3.60-6.0)/5.00±0.35	4.90 (3.10-6.30/4.93±0.39	5.10 (3.30-11.30)/5.76±1.02	5.70 (2.80-6.60)/5.10±0.59	5.00 (3.70-6.10)/5.10±0.36
	LDH	203.7 (76-419.3)/235.01±49.81	267.3 (95.2-963.7)/385.26±118.14	248.4 (74.5-471.6)/281.93±58.1	248.8 (68.7-518.4)/287.11±71.74	171.2 (64.8-610.6)/245.36±88.37
Oral n=7	ALT	16.1 (12.90-41.1)/20.32±4.40	19.95 (8.7-38.1)/20.23±4.54	23.45 (10.30-35.40)/23.06±3.88	19.55 (12.80-36.40)/22.48±3.57	29.35 (12.90-42.70)/28.58±6.58
	AST	12.3 (7.60-19.0)/13.55±1.77	14.45 (7.8-20.9)/14.88±2.19	16.10 (7.90-18.20)/15.07±1.50	12.80 (8.80-19.40)/13.48±1.44	13.25 (11.90-15.60)/13.50±0.78
	ALP	26.1 (18.60-60.5)/34.05±7.39	39.7 (19.3-92.3)/47.98±12.14	37.00 (19.60-84.40)/41.83±10.16	36.70 (20.70-52.40)/36.78±5.55	31.65 (22.60-46.10)/33.00±4.87
	GGT	3.2 (2.90-4.5)/3.45±0.24	4.15 (2.8-4.7)/4.01±0.27	4.40 (3.60-4.90)/4.35±0.20	4.40 (3.40-5.30)/4.38±0.25	4.55 (2.50-4.80)/4.10±0.54
	LDH	109.9 (40-378.6)/146.32±51.01	108.3 (46.6-386.4)/140.83±51.09	166.85 (30.3-287.2)/165.37±39.84	87.45 (45.9-175.9)/103.83±23.46	116.95 (57.2-125.1)/104.05±16.06
Local n=5	ALT	23.20 (19.20-34.7)/24.34±2.70	23 (15.9-29.3)/23.1±2.16	24.30 (21.10-32.90)/25.30±2.16	21.60 (18.20-30.70)/23.02±2.69	25.70 (20.80-27.60)/24.70±2.02
	AST	17.0 (11.1-21.6)/17.04±2.03	16.4 (14-20.8)/17.1±1.13	17.80 (13.50-27.90)/19.14±2.80	20.75 (14.50-25.90)/20.47±2.33	17.50 (17.30-21.80)/18.86±1.47
	ALP	30.5 (12.8-106.2)/42.84±16.61	26.30 (19.6-87.6)/36.94±12.77	29.70 (15.30-62.20)/32.82±7.93	33.95 (29.00-67.70)/41.15±8.93	32.00 (20.60-69.10)/40.57±14.64
	GGT	4.10 (2.40-5.2)/4±0.48	3.8 (3.1-5.2)/3.84±0.37	4.40 (2.50-4.70)/3.94±0.40	4.30 (2.50-5.20)/4.07±0.63	4.50 (4.10-4.60)/4.40±0.15
	LDH	91.1 (22.2-694.9)/217.52±123.81	96.6 (43.8-635.9)/222.04±109.63	391.7 (50.3-409.7)/273.08±80.37	315.35 (103-663.1)/349.2±118.77	389.5 (319.3-490.4)/399.73±49.65

Liver enzy: Liver enzymes; Median (Min-Max)/Mean±SEM: Minimum-Maximum/Mean±Standard Error of Mean; ALT Reference range: 10-88 IU/L; AST Reference range: 10-88 IU/L; ALP Reference range: 20-150 IU/L; GGT Reference range: 1-10 IU/L; LDH Reference range: 50-495 IU/L; ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; LDH: Lactate dehydrogenase.

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ied two propolis species have been identified several compounds that can show anticancer activity. Active compounds of poplar propolis are CAPE, caffeic acid, apigenin, quercetin, genistein, routine, p-coumaric acid, ferulic acid, kaempferol, naringenin. Active compounds of Baccharis (Brazil) propolis are artepillin C, baccharin, drupanin, cinnamic acid derivatives, prenylated p-coumaric acids, klerodone terpenes, benzofurans.[13,32] Similarly, major components of propolis including caffeic acid, CAPE, artepillin C, quercetin, naringenin, resveratrol, galangin, genistein, and others are considered as promising antineoplastic agents.[33] The phenolic compounds determined in the propolis used in this study were generally in agreement with the results of previous study results for antitumoral effects (Table 1).[13,32,33]

Results have shown that propolis and its polyphenolic compounds exerted an anti-metastatic and antitumour effect in mice and rats and considerable cytotoxicity without cross-resistance in both wild-type and chemoresistant human tumour cell lines.[34] Propolis orally at doses of 50 or 150 mg/kg could stimulated macrophages and reduced the number of mammary carcinoma (MCa) metastases in CBA mouse.[34] The Japanese propolis (aqueous extract) inhibited the growth of S-180 mouse sarcoma in vitro, and significantly inhibited the growth of transplanted tumor cells in Mouse.[35]

Growing resistance to chemotherapy in dogs with TVT might be observed in plasmocytoid TVT.[3-5] In this study, only one dog was lymphocytoid, nine dogs were plasmocytoid, and fourteen dogs from 24 dogs were mixed as TVT classes. The results indicated that TVT with plasmocytoid and mixed are high number (n=23) and would be need longer period than lymphocytoid TVT for treatment.

There were no significant differences between control and other propolis added groups (p=0.423), among the propolis added groups (p=0.381) for the average length of treatment periods, were 3.60 weeks for local group, 4.16 weeks for oral+local group, 4.42 weeks for oral group, and 5.00 weeks for control group. Treatment periods of all propolis added groups were shorter than control group. Although there are no any study related oral propolis use in dogs with TVT for review these results, the main reason for shorter treatment period in groups with propolis should due to the antitumoral and immunomodulatory effects of propolis, and the propolis used in this study were containing the phenolic contents related with antitumoral effects (Table 1).[13,16,23,24,32,33] The identification and quantification of certain individual phenolic compounds in propolis are essential for propolis quality, and phenolic compounds in propolis tincture used in the study was shown in Table 1. According to the study results, propolis might not potentially be used for TVT therapy, but propolis may support treatment of TVT with standard vincristine sulphate therapy. The clinical studies related with antitumoral effects of propolis in veterinary and human medicine is not carried out yet, hence these studies should extensively study in the future. However, application route and solvent of propolis tinctures should carefully choose according to our observation during the study. Oral route was not suitable for alcoholic tincture of propolis for some dogs, the dogs could not consume propolis tincture in enjector, and propolis tincture was added in their foods. Alcoholic tincture of propolis might be bleeding in local application in some dogs. Therefore, the authors sugges glycerine or olive oil tinctures of propolis should be try for local and oral application for future studies.

WBCs and NEU were much influenced. Neutropenia, neutrophilia, leukopenia, and leukocytosis were observed in most of treated dogs (Table 2). These findings including neutropenia and leukopenia were harmonious with certain previous studies.[8,9] Braz and Marinho, indicated that when performing the leukocyte differential, it was possible to notice that the animals underwent conventional chemotherapy had a reduction in the amount of segmented NEU ($p>0.05$), presenting a neutropenia and leukopenia at the end of the treatment.[9]

Vincristine sulphate in dogs with TVT and also healthy dogs could increase the liver enzymes levels.[10,36,37] No significant changes ($p>0.05$) in ALT, AST, ALP, GGT and LDH levels that were in the referans values in control and experimental groups in this study (Table 3). Therefore, vincristine sulphate was not caused to liver damage. The ALT and ALP

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concentrations were harmonious with Braz and Marinho, and Souza et al. Propolis has hepatoprotective effects, but in this study we could not observe hepatotoxicity effects according to control group enzyme results.[7,9,38,39] Therefore, hepatoprotective effects of propolis could not be evaluated.

CONCLUSION

In conclusion, TVT with plasmocytoid and mixed were high number (n=23). Although no significant differences between control and other propolis added groups, treatment periods of all propolis added groups were shorter than control group. Neutropenia, neutrophilia, leukopenia and leukocytosis were observed in most of treated dogs. No significant changes in ALT, AST, ALP, GGT and LDH levels that were in the reference values in control and experimental groups in this study, and vincristine sulphate was not caused to liver damage. Propolis may support treatment of TVT with standard vincristine sulphate therapy. Antitumoral effects of propolis should extensively study with animal and clinical experiments.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Hikmet Aysın Usta, Hasan Hüseyin Oruç;* ***Design:*** *Hikmet Aysın Usta, Hasan Hüseyin Oruç;* ***Control/Supervision:*** *Hikmet Aysın Usta, Hasan Hüseyin Oruç, Musa Özgür Özyiğit;* ***Data Collection and/or Processing:*** *Hikmet Aysın Usta, Hasan Hüseyin Oruç, Musa Özgür Özyiğit, Onur Kızılgün;* ***Analysis and/or Interpretation:*** *Hikmet Aysın Usta, Hasan Hüseyin Oruç, Onur Kızılgün, Ender Uzabacı;* ***Literature Review:*** *Hikmet Aysın Usta, Hasan Hüseyin Oruç;* ***Writing the Article:*** *Hikmet Aysın Usta, Hasan Hüseyin Oruç, Musa Özgür Özyiğit, Ender Uzabacı;* ***Critical Review:*** *Hikmet Aysın Usta, Hasan Hüseyin Oruç, Musa Özgür Özyiğit, Ender Uzabacı;* ***References and Fundings:*** *Hikmet Aysın Usta, Hasan Hüseyin Oruç;* ***Materials:*** *Hikmet Aysın Usta, Onur Kızılgün.*

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