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COMPARATIVE THERAPEUTIC EFFICACY OF VINCRISTINE SULPHATE, VINCRISTINE-IVERMECTIN COMBINATION AND *Thuja occidentalis* FOR MANAGEMENT OF CANINE TRANSMISSIBLE VENEREAL TUMOUR

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ABSTRACT

The present study was conducted on 18 dogs at VCC, College of Veterinary Science & A.H. Mhow. The 18 dogs irrespective of, age and sex with history of bleeding from the genital region were examined for the CTVT and randomly allocated equally in following three treatment groups. Animals of group-1 were treated with Vincristine sulphate, group-2 were treated with combination of Vincristine sulphate and Ivermectin and group-3 were treated with oral drops of Thuja 200c and the observations were recorded on subsequent weeks (0, 7th, 14th and 21st day). The therapeutic efficacy was judged on the basis of the above combination of the drugs, reduction of tumour mass, stoppage of bleeding, observation on clinical side effects, haemato-biochemical parameters and cytological parameters. The minimum side effects were with thuja as compared to vincristine + ivermectin and vincristine alone administration. Comparatively higher regression of tumour mass was observed with vincristine sulphate + ivermectin (99.80%) as compared to vincristine (97.80%) alone and Thuja (43.42%).

Keywords: Chemotherapy, Ivermectin, Transmissible and Thuja.

Introduction

Canine transmissible venereal tumour (CTVT) is affection of genital system of dogs and compromises the general clinical conditions (Ganguly *et al.*, 2016). CTVT is contagious benign reticulo-endothelial tumour of the dog that mainly affects the external genitalia and occasionally the internal genitalia. CTVT is also called as transmissible venereal sarcoma, sticker's sarcoma, venereal granuloma and infectious sarcoma.

CTVT represents a unique, naturally transmissible, contagious tumour where the mutated tumour cell itself is the causative agent and spreads as a parasitic allograft in the host.

CTVT is divided into two types, i.e. genital and extra genital CTVT. Genital CTVT affects the genital area of dogs. In males, it generally affects the caudal aspect of the penis and often the prepuce area resulting in complications such as phimosis or paraphimosis. In female dogs, the lesions are common in the posterior vaginal area particularly at the vestibulovaginal junction (Milo and Snead, 2014) Because of its deep location, it is sometimes not visible until it is massive (Kabuusu *et al.*, 2010).

Definitive diagnosis of CTVT can be made on the basis of physical examination and cytological findings typical to the CTVT in exfoliated cells obtained by swabs, fine needle aspirations or imprints of the tumours (Richardson, 1981; Moulton, 1978). Various

treatments for CTVT cases are currently available, such as surgery, chemotherapy and radiotherapy.

Chemotherapy gives more promising results and upto 100% remission can be achieved. Vincristine sulphate is the first choice in the treatment of CTVT (Filho *et al.*, 2020). However, some side effects like anorexia, nausea, vomition, lethargy were observed with this drug. Vincristine and its combination with other drugs like ivermectin can be used together for treatment of CTVT (Bulhosa *et al.*, 2020).

Treatment with ivermectin can results in a reversing of the resistance to anti-neoplastic drugs by inhibiting P-glycoprotein (P-gp) production with this being a protein that promote the efflux of xenobiotics from within the tumour cells (Jiang *et al.*, 2019). Homeopathy is also a therapeutic possibility, which can be established as a single treatment or associated with chemotherapy of tumours. *Arbor vitae* (*Thuja occidentalis* L.) are a native European tree widely used in homeopathy and evidence-based phytotherapy. Alcoholic extract of thuja known as Thujone have anticancerous property (Biswas *et al.*, 2011).

Materials and Methods

Selection of animals

The present investigation was conducted on clinical cases brought to the Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Mhow. Regardless of age or sex dogs showing the history of bleeding from the genital area were examined for the cauliflower like pedunculated growth.

Technical Programme of Work

Grouping of animals

A total of 18 confirmed CTVT cases were randomly placed into the following three therapeutic groups of 6 animals in each group.

Group 1: Animals of this group were treated with injection vincristine sulphate @ 0.025 mg/kg B.wt intravenously, diluted with 10 ml 0.9 % sodium chloride on the 0, 7th and 14th day. This group was served as a control group.

Group 2: Animals of this group were treated with a combination of injection vincristine sulphate and ivermectin. The animals were administered 1% ivermectin injection @ 0.5 mg/kg B.wt subcutaneously 24 hours prior to vincristine sulphate administration at the same dose and route as in group 1 on 0, 7th and 14th day.

Group 3: Animals were treated with liquid drops of 0.5 ml of Thuja 200C (*Thuja occidentalis*) BID orally daily for 21 days.

Supportive therapy was also given to all the animal of the groups along with the treatment.

Days of observations

Animals receiving treatments of all the groups were monitored by cytological, haematological and biochemical observations on 0, 7th, 14th and 21st day.

Therapeutic efficacy of vincristine sulphate, combination of vincristine sulphate with ivermectin, and *Thuja occidentalis* was evaluated on the basis of following parameters:

Reduction of tumour mass and stoppage of bleeding

Observation on clinical side effects: Such as fatigue, in-appetence, vomition, alopecia, skin problems were recorded.

Statistical analysis

The Statistical analysis was done using two-way ANOVA as described by Snedecor and Cochran (1994).

Results and Discussion

All clinically suspected cases having history of bleeding were initially included in this study. After confirmatory diagnosis of CTVT by cytological and clinical examination the 18 animals were randomly distributed into three treatment groups. The observations were analyzed and interpreted as follows.

Reduction of the tumour mass

The result of reduction in tumour mass in different groups is presented in table 01 and figure 01.

In the present study, the mean size of tumour mass was 5.01±0.09 cm on day 0 (before the initiation of treatment) which became 3.70±0.15, 0.10±0.06 and 0.11±0.01 cm on day 7th, 14th and 21st day, respectively and the overall regression was 97.80% in group-1 (Vincristine) dogs. The statistical analysis of data revealed that there was no significant difference between day 0 and 7th, 14th and 21st day however, significant difference was observed between day 7th and 14th, day 0 and 14th.

In group-2 dogs (vincristine + ivermectin), the mean size of tumour mass was 4.55±0.06 cm on day 0. The size of tumour mass was 3.45±0.12cm, 0.040±0.02 cm and 0.010±0.00 cm on 7th, 14th and 21st day, respectively and the overall regression observed was 99.80%. The statistical analysis of data revealed that there was no significant difference between day 0 and

7th, 14th and 21st day but there was significant difference between day 7th and 14th, day 0 and 14th, day 7th and 21st.

The mean size of tumour mass was 3.50±0.08 cm on day 0. It was 3.20±0.09 cm, 2.40±0.10 cm and 1.98±0.03 cm on 7th, 14th and 21st day, respectively and the overall reduction was 43.42% in group -3 (Thuja) dogs. The statistical analysis showed non-significant difference between the days within the group.

On 7th day, the mean size of the tumour was 3.70±0.15 cm, 3.45±0.12 cm and 3.20±0.09 cm in

group-1, 2 and 3, respectively. The statistical analysis showed significant difference between the groups.

On day 14th, the mean size of the tumour was 0.10±0.06 cm, 0.04±0.02cm and 2.40±0.10 cm in group-1, 2 and 3, respectively. The statistical analysis shows significant difference between the groups.

On 21st day, the mean size of the tumour was 0.11±0.01 cm and 0.01±0.0 cm, 1.98±0.03 cm in group-1, 2 and 3, respectively. The statistical analysis showed significant difference between group 1 and group 3, group 2 and group 3 but showed non-significant difference between group 1 and group 2.

Table 1 : Reduction of tumour mass at different time intervals in different groups (Mean±SE)

Groups (n=6)	Reduction in tumour mass (cm)				
	0 day	7 th day	14 th day	21 st day	Overall regression (%)
Group 1 (Vincristine)	5.01 ^b ±0.09	3.70 ^{Cb} ±0.15	0.10 ^{Aa} ±0.06	0.11 ^{Aa} ±0.01	97.80%
Group 2 (Vincristine+ Ivermectin)	4.55 ^b ±0.06	3.45 ^{Bb} ±0.12	0.04 ^{Ba} ±0.02	0.01 ^{Aa} ±0.00	99.80%
Group 3 (Thuja)	3.50 ^a ±0.08	3.20 ^{Aa} ±0.09	2.40 ^{Ca} ±0.10	1.98 ^{Ba} ±0.03	43.42%

Values with different superscripts (a,b) (A,B) showing significant difference (P<0.05) within the group and between the group, respectively.

The findings of present study (97.80%) are more or less in accordance with Thakur (2019) who also reported 99.23 % reduction of tumour mass in vincristine sulphate treated animals. Findings are also well corroborated with Antonov (2015) and Regmi *et al.* (2020) who reported complete regression of tumour mass in dogs treated with vincristine sulphate.

Effective tumour mass regression that was observed at different time intervals is due to mitotic inhibition by vincristine. Satoskar *et al.* (1995) discovered that vincristine sulphate binds to tubulin and prevents the formation of mitotic spindles.

The findings of this study are also in agreement with the findings of Lapa *et al.* (2012) where they find significant reduction of the tumour with less administration of the protocol (vincristine + ivermectin). They also concluded that the combination of vincristine and ivermectin found to be more effective as compared to vincristine alone. The present findings are in contrast with another similar study conducted by Andrade *et al.* (2009), where reduction was not significantly varying.

Griffin *et al.* (2005) reported that P-gp marker protein in the cell membrane is a known protein involved in the mechanism for tumour resistance to chemotherapy, including vincristine. The antitumor effects of antineoplastic agent might be enhanced by combination of vincristine with ivermectin. Ivermectin inhibits the P-gp dependent drug resistance of tumour cells and also is potent substrate and inhibitor of P-gp.

Drinyaev *et al.* (2004) reported that, ivermectin when injected after vincristine, it enhances the antitumor effect of vincristine.

In this investigation the regression of tumour mass was 43.42% in Thuja treated group at the end of observation period which is quite lower than the study conducted by Umadevi *et al.* (2013) who observed complete recovery of the tumour growth in two dogs within 35 days from start of the treatment with Thuja and appreciable shrinkage in other two dogs after 25 days which might be due to difference in the duration of treatment.

The ethanolic extract of Thuja is known as Thujone. This Thujone have anticancerous properties which helps in reduction of the tumour mass by decrease in cell viability, induced inter-nucleosomal DNA fragmentation, mitochondrial transmembrane potential collapse, increase in ROS generation, and release of cytochrome c and caspase-3 activation, all of which are closely related to the induction of apoptosis (Biswas *et al.* 2011).

Stoppage of bleeding

In group-1 (vincristine) vaginal bleeding stopped after first dose in 66.6 % animals upto 7th day. After second dose, bleeding was stopped in 83.3% animals upto 14th day and after third dose bleeding was stopped in 100 % animals upto 21 days.

In group-2 (vincristine + ivermectin) vaginal bleeding stopped after first dose in 83.30% animals

upto 7th day. After second dose, bleeding was stopped in 100 % animals upto 14th day.

In group-3 (Thuja) vaginal bleeding stopped after first dose in 16.6% animals upto 7th day. After second dose, bleeding was stopped in 50 % animals upto 14th day and after third dose bleeding was stopped in 66.6% animals upto 21st day.

Table 2: Stoppage of vaginal bleeding in different groups at different time intervals (%)

Groups (n=6)	0 day	7 th day	14 th day	21 st day
Group 1 (Vincristine)	Nil	66.6% (4)	83.3% (5)	100% (6)
Group 2 (Vincristine + Ivermectin)	Nil	83.3% (5)	100% (6)	100% (6)
Group 3 (Thuja)	Nil	16.6% (1)	50.0% (3)	66.6% (4)

Note: The figures in the parenthesis indicating number of animals

Conclusively, 100% bleeding was stopped in vincristine alone and combination of vincristine with ivermectin treated groups whereas in 66.6 % cases bleeding stopped in Thuja treated animals. That means Thuja is comparatively less effective as compared to the vincristine alone and combination of vincristine with ivermectin. These results are in agreement with the results of Lapa *et al.* (2012) where significant reduction was observed with less number of protocol (vincristine + ivermectin) administration compared to vincristine alone.

Clinical side effects

Following are the adverse effects of the drugs recorded in CTVT affected male and female dogs of group-1, 2 and 3.

Symptoms of fatigue such as weakness, lethargy, unwillingness to participate in physical activity were the physical parameters observed to monitor the side effects.

In group 1, 50 % animals showed the symptoms of fatigue where as 66.7 % animals showed the symptoms of inappetence, 16% animals showed the symptoms of vomition and 66.7% animals showed alopecia.

In group 2, 33.3% animals showed the fatigue symptoms, 50% animals showed the symptoms of inappetence, 16% animals showed alopecia and skin problem.

In group 3, any of these symptoms were not evident in the animals.

Table 3: Side effects observed in different groups at different time intervals

Groups (n=6)	Fatigue	Inappetence	Vomition	Alopecia
Group 1 (Vincristine)	50% (3)	66.7% (4)	16% (1)	66.7% (4)
Group 2 (Vincristine + Ivermectin)	33.3% (2)	50% (3)	16% (1)	16% (1)
Group 3 (Thuja)	0% (0)	0% (0)	0% (0)	0% (0)

Note: The figures in the parenthesis indicating number of animals

Anorexia and vomition was the main side effects observed by Gandhimathi *et al.* (2011) and Yadav *et al.* (2018) during treatment with vincristine. Gandotra *et al.* (1993) and Amber *et al.* (1990) reported occasional anorexia in 20% cases, while Rogers (1997) reported mild anorexia in one case treated with vincristine which is more or less similar with the present study. Side effects of vincristine treatment have been reported as vomition in 8% animals by Calvert *et al.* (1982) which was quite lower with the present study which might be due to differences in methodology and sample size. Other workers like Erunalmaral *et al.* (2000) reported anorexia, vomition and diarrhea in 20% cases.

Dinesh *et al.* (1993) reported anorexia, vomition and diarrhea in 20% cases. Singh *et al.* (1996) also reported vomition, anorexia, depression and paralysis in 17% cases treated with vincristine.

Nak *et al.* (2005) observed anorexia (14%), diarrhoea (8%), weight loss (8%), generalized alopecia (24%) and localized alopecia (14%) after treatment with vincristine.

The findings of present study are in accordance with the findings of Abeka (2019), Lapa *et al.* (2012) and Bulhosa *et al.* (2020) however they found more severe side effects with vincristine as compared to the vincristine + ivermectin treatment.

The ivermectin might have enhanced bone marrow “chemoprotection”, perhaps inducing a desirable retention of vincristine within tumor cells, particularly in the more aggressive plasmacytic and mixed types of CTVT cells that have greater abundances of P-gp molecules. Defective P-gp function can cause serious adverse drug reactions due to enhanced brain penetration and/or decreased clearance. P-gp dysfunction in dogs can be intrinsic or acquired (Mealey *et al.*, 2017).

In the present investigation the least or no side effects were observed in Thuja treated group, this investigation is in agreement with Umadevi *et al.* (2013).

Group I Vincristine Sulfate

Plate 01: Gross appearance of tumour size at 0 day



Plate 02: Reduction of tumour size at 7th day



Plate 03: Tumour regressed after 14th day

Group II Combination of Vincristine and Ivermectin

Plate 04: Gross appearance of tumour mass at 0 day



Plate 05: Reduction of tumour mass at 7th day



Plate 06: Recovery after 14th day

Group III Thuja

Plate 07: Gross appearance of tumour mass at 0 day



Plate 08: Tumour mass reduced at 7th day



Plate 09: Reduction of Tumour mass at 14th day



Plate 10: Recovery after 21st day

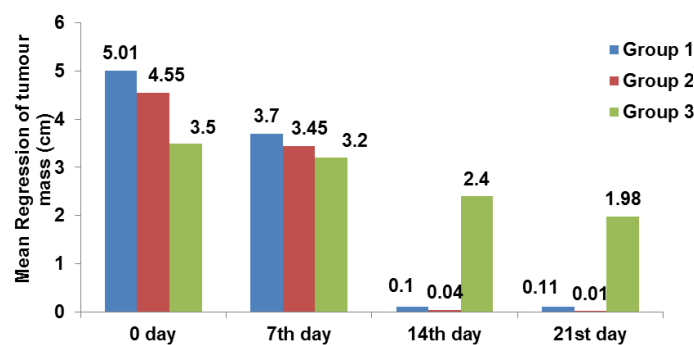


Fig. 1: Regression of tumour mass in different groups at different time intervals

Conclusion

The comparative therapeutic evaluation revealed that vincristine sulphate remains the most effective and reliable treatment for complete regression of canine transmissible venereal tumour. The combination of vincristine sulphate with ivermectin demonstrated comparable and potentially enhanced therapeutic response, suggesting a supportive role of ivermectin in CTVT management. In contrast, *Thuja occidentalis* showed comparatively slower and less consistent tumour regression when used alone. Therefore, vincristine sulphate continues to be the treatment of choice, while combination therapy may offer additional clinical advantages. Further studies are recommended to validate the adjunctive benefits of ivermectin and the role of phytotherapeutic agents in canine oncology.

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