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Potential use of acetogenins as adjuvants in the treatment of canine transmissible venereal tumor. Case report

Uso potencial de acetogeninas como coadyuvante en el tratamiento del tumor venéreo transmisible canino. Reporte de caso

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ABSTRACT

Vincristine, an antimicrotubular alkaloid, is the standard treatment for transmissible venereal tumor in dogs due to its high rate of clinical remission. However, recent studies have explored its combination with natural bioactive compounds as a strategy to enhance therapeutic efficacy and reduce adverse effects. This study evaluated the clinical effectiveness of vincristine in combination with acetogenins phytochemicals derived from the leaves of *Annona muricata* in two female dogs with cytologically confirmed genital transmissible venereal tumor. Both patients received vincristine at a dose of 0.5 mg·m^{-2} intravenously, once weekly for six weeks. In one of the dogs, a daily dose of 7.5 to 10 mg of acetogenins was administered from the first chemotherapy session for the initial 15 days. From the third vincristine application onward, the dose was increased to 14.5 to 20 mg·day^{-1} of acetogenins, divided into two oral administrations (one capsule in the morning and one in the evening) with food, and maintained for a total of four months. The dog treated with the combined protocol exhibited early tumor regression, with a significant reduction from the second week and a clinical remission of 99.85% by the end of treatment. No adverse effects associated with acetogenin administration were observed. In contrast, the dog treated with vincristine alone showed a partial response, with persistent tumor tissue at the end of the protocol. The combination of vincristine and acetogenins appears to be a promising therapeutic strategy that may enhance the efficacy of conventional transmissible venereal tumor treatment in dogs. However, these preliminary findings require validation through controlled clinical trials with a higher number of experimental units, as well as histopathological and molecular markers assessments to elucidate the underlying mechanisms of action and support the development of standardized therapeutic protocols.

Key words: Transmissible venereal tumor; acetogenins; alternative therapies; vincristine sulfate; *Annona muricata*

RESUMEN

La vincristina, un alcaloide antimicrotubular, es el tratamiento estándar para el tumor venéreo transmisible en caninos debido a su elevada tasa de remisión clínica; sin embargo, recientes investigaciones han explorado su combinación con compuestos naturales bioactivos como estrategia para mejorar la eficacia terapéutica y reducir efectos adversos. El presente estudio evaluó la eficacia clínica de la vincristina en combinación con acetogeninas, fitoquímicos derivados de hojas de *Annona muricata*, en dos perras con diagnóstico citológico confirmado de tumor venéreo transmisible genital. Ambas pacientes recibieron Vincristina a 0,5 mg·m^{-2} por vía intravenosa, una vez por semana durante seis semanas. En una de las pacientes, desde la primera sesión de quimioterapia, se administró una dosis diaria de 7,5 a 10 mg de acetogeninas durante los primeros 15 días. A partir de la tercera aplicación, la dosis se incrementó a 14,5 a 20 mg·día^{-1} de acetogeninas, distribuida en dos tomas (una cápsula en la mañana y otra en la noche) junto con la alimentación, y se mantuvo hasta completar cuatro meses de tratamiento. La hembra canina que recibió la terapia combinada presentó una regresión tumoral temprana, con una reducción significativa desde la segunda semana y una remisión clínica del 99,85 % al finalizar el protocolo. No se observaron efectos adversos atribuibles a las acetogeninas durante su administración. En contraste, la paciente tratada únicamente con vincristina mostró una respuesta parcial, con persistencia del tejido tumoral. La combinación de vincristina con acetogeninas se perfila como una estrategia terapéutica prometedora que podría mejorar la eficacia del tratamiento convencional del tumor venéreo transmisible canino. No obstante, estos resultados deben ser validados mediante estudios clínicos controlados, con mayor número de unidades experimentales, evaluaciones histopatológicas y de marcadores moleculares que permitan esclarecer los mecanismos de acción implicados y establecer protocolos estandarizados.

Palabras clave: Tumor venéreo transmisible; acetogeninas; terapias alternativas; sulfato de vincristina; *Anona muricata*

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INTRODUCTION

Vincristine sulfate is widely recognized as one of the most effective chemotherapeutic agents for the treatment of canine transmissible venereal tumor (TVT), with complete remission rates consistently exceeding 90% across various clinical settings [1]. This efficacy is primarily attributed to its mechanism of action as a microtubule polymerization inhibitor, an essential process for mitotic spindle formation and subsequent cell division [1, 2, 3]. Structurally, vincristine is a bis–indole alkaloid composed of vindoline and catharanthine subunits, both featuring indole rings and polar functional groups that confer a high binding affinity to α/β–tubulin heterodimers (dissociation constant Kd ≈ 10^{-6} M) [4, 5, 6]. This interaction disrupts microtubule dynamics, leading to spindle destabilization, cell cycle arrest at the G2/M phase, and apoptosis in rapidly proliferating neoplastic cells [6, 7].

The administration of vincristine is not devoid of significant adverse effects. myelosuppression particularly leukopenia and neutropenia substantially increase the risk of opportunistic infections, thus requiring rigorous hematologic monitoring during treatment [8, 9]. Gastrointestinal side effects such as anorexia, vomiting, diarrhea, and weight loss have also been reported, attributable to both direct toxicities on intestinal mucosa and stimulation of the central emetic center [10, 11, 12]. Peripheral neurotoxicity represents another notable adverse manifestation, characterized by muscular weakness, ataxia, hyporeflexia, myalgia, tremors, and muscle spasticity, all resulting from disrupted axonal transport mediated by microtubules [10].

In addition, accidental extravasation during intravenous administration can cause severe local reactions, including inflammation, tissue necrosis, and skin ulceration [9]. Cases of thrombocytopenia, transient hepatic dysfunction, and localized alopecia in areas of friction have also been documented [8, 10].

Several phytotherapeutic compounds have been investigated in both Veternary and Human oncology, demonstrating notable cytotoxic effects against malignant cells. Among these, flavonoid–rich extracts from *Curcuma longa* (curcumin) [13], *Nigella sativa* (thymoquinone) [14, 15], and acetogenins isolated from the leaves and fruits of *Annona muricata* [16, 17, 18, 19]. Acetogenins have shown selective cytotoxicity against cancer cells *in vitro* [20, 21] and *in vivo* experimental models [22, 23], highlighting their potential as adjuvant agents in integrative oncologic therapies. Beyond their antineoplastic properties, these natural compounds also exhibit hypoglycemic [24], acaricidal [25], antimicrobial [26], and antioxidant activities [27].

The objective of the present study is to evaluate the adjuvant effect of acetogenins extracted from *A. muricata* leaves in a clinical case of canine TVT treated with vincristine, by assessing their impact on clinical progression, treatment tolerance, and the occurrence of adverse effects based on clinical, hematological, and tumor response parameters. Additionally, this study aims to discuss the therapeutic potential of these bioactive compounds within the veterinary oncology context.

MATERIALS AND METHODS

Plant material selection

A total of 20 healthy *A. muricata* L. plants, cultivated without any fertilization treatment, were selected from the canton of Chone, Manabí Province, Ecuador. Leaves were randomly harvested from both the lower and upper canopy of each plant, ensuring that only fully green and undamaged specimens were collected. The leaves were subsequently washed with distilled water and gently dried using absorbent paper to remove excess moisture without compromising foliar structure. Thereafter, the samples were placed in a convection oven (Memmert®, model UN30, Germany) at 30°C for 24 h. The dried leaves were then pulverized using a hammer mill (Hosokawa Micron®, Mikro Pulverizer® Hammer & Screen Mill, USA), equipped with a 5 hp motor and a maximum grinding capacity of 100 kg. The resulting powder was packaged into 50 g plastic bags, properly labeled, and stored at room temperature in a dry, light–protected environment.

Preparation of ethanolic extracts

Ethanolic extracts were prepared from pulverized dry leaves of *A. muricata.* The extraction was performed using a 1:8 (w/w) leaf–to–ethanol ratio. Dried leaf material was mixed with ethanol (Merck, Darmstadt, Germany) in a glass homogenizer (Thomas No. A3528, USA). The resulting homogenates were macerated for 24 h, followed by centrifugation at 3000× g for 10 min using a BHG Optima II centrifuge (BHG Systems, USA). The supernatants were then carefully filtered and clarified. The resulting fractions were stored in 10 mL amber polypropylene screw–cap tubes (Eppendorf®, Germany), suitable for preserving photosensitive compounds, thus ensuring the stability of the extracts until further analysis.

Determination of acetogenins, capsule preparation and production

The total acetogenin content in pulverized *A. muricata* leaves was quantified by high–performance liquid chromatography (HPLC) using an Agilent 1260 Infinity system (Agilent Technologies, USA) with a C18 reverse–phase column. Separation employed a water–acetonitrile gradient at 1.0 mL·min^{-1}, and detection was performed at 220 nm using a diode array detector (DAD). Quantification relied on calibration curves generated from certified acetogenin standards (Sigma–Aldrich®, USA). Capsules were formulated with 500 mg of standardized leaf powder, containing approximately 7.5–10 mg of acetogenins per capsule.

Encapsulation was carried out under controlled hygienic conditions using a semi–automatic capsule–filling machine (CapsulCN® CN-100, USA) for size "0" hard gelatin capsules (Quali–G®, CapsCanada®, USA), ensuring dosage uniformity and pharmaceutical–grade quality suitable for human use.

Animals clinical description and diagnosis

Two mixed–breed female dogs (*Canis lupus familiaris*), one approximately 3 years and 8 months old and the other 2 years and 4 months old, each weighing 12 kg as measured using a OHAUS Scout Pro SP202 digital balance (Made in USA), were included in this clinical study following a confirmed diagnosis of TVT. In both patients, the neoplasm was located at the level of the external

genitalia. The diagnosis was confirmed by cytological evaluation, a method consistent with standard Veterinary oncology protocols [28]. Veterinary inspection of the lesion revealed a friable mass with an irregular surface, ranging in color from pink to bright red, and with a tendency for spontaneous bleeding clinical features characteristic of TVT (FIG. 1).

FIGURE 1. Initial diagnosis of canine TVT through veterinary examination. Representative image showing the friable, irregular, and hemorrhagic mass located on the external genitalia, characteristic of TVT

These gross findings were consistent with the microscopic evaluation of a smear obtained via genital swab, performed using a Leica DM100 microscope (Made in USA). The cytological analysis revealed cellular features compatible with canine TVT, which are illustrated in the following (FIG. 2).

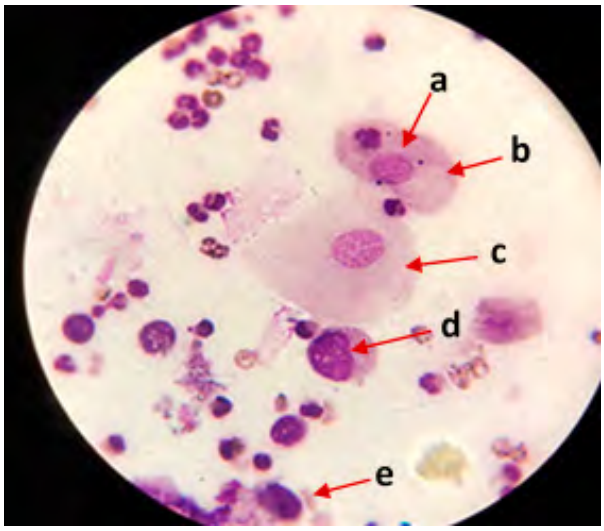

FIGURE 2. Cytological diagnosis of canine TVT using imprint technique. Microscopic image of a cytological imprint stained with Wright–Giemsa at 400× magnification, revealing abundant round to oval tumor cells (a), with distinct oval nuclei (b), finely granular chromatin (c), and multiple prominent cytoplasmic vacuoles (d), cells are dispersed individually without forming clusters (e), features characteristic of TVT

The second case evaluated exhibited clinical manifestations and cytological findings consistent with TVT, confirmed through physical examination, lesion swab, and imprint cytology (FIG. 3A, 3B).

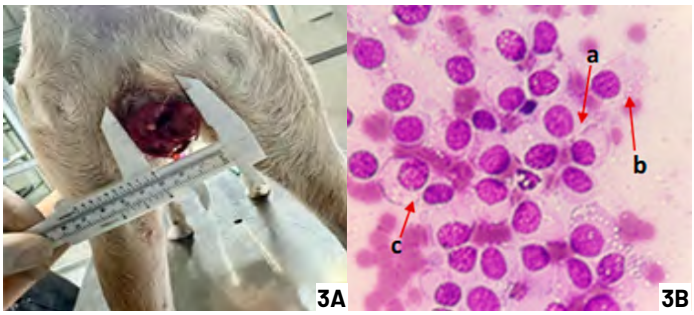

FIGURE 3A. Veterinary inspection and cytological evaluation in a canine case of TVT. Gross appearance of a friable, hemorrhagic genital mass located at the vaginal vestibule. FIGURE 3B. Cytological smear obtained from a swab sample of the lesion, stained with Giemsa, 1000× magnification, showing round neoplastic cells with prominent nucleoli (a), coarse chromatin, vacuolated cytoplasm (b), and occasional binucleation (c). These cytomorphological features are characteristic of TVT and support a reliable diagnosis. Scattered polymorphonuclear leukocytes are also observed, indicating a secondary inflammatory component commonly associated with this neoplasm

Prior to chemotherapy, both animals underwent internal and external deworming and received supplementation with a commercial multivitamin (Geriosan® Complex Multivitamin, Agrovet Market Laboratories, Peru) to optimize health status. Hematological parameters including hematocrit, hemoglobin, red and white blood cell counts, leukocyte differential, and platelet count were evaluated using an automated hematology analyzer (Mindray BC-2800Vet, China) to confirm systemic readiness for oncological treatment. The hematological parameters evaluated in the canine subject were within the normal reference ranges for the species. Hematocrit (45%) and hemoglobin (15 $g \cdot dL^{-1}$) indicate adequate oxygen–carrying capacity and absence of anemia. Red blood cell count ($6.8 \times 10^6 \cdot \mu L^{-1}$) remained within expected values, confirming the integrity of the erythrocyte series. The leukocyte series showed a total white blood cell count ($10.2 \times 10^3 \cdot \mu L^{-1}$) within reference ranges, with a balanced differential: neutrophils $6.1 \times 10^3 \cdot \mu L^{-1}$, lymphocytes $3.2 \times 10^3 \cdot \mu L^{-1}$, monocytes $0.8 \times 10^3 \cdot \mu L^{-1}$, eosinophils $0.6 \times 10^3 \cdot \mu L^{-1}$, and basophils $0.05 \times 10^3 \cdot \mu L^{-1}$. These values indicate no evidence of infectious, inflammatory, or immune–mediated processes.

Platelet count ($320 \times 10^3 \cdot \mu L^{-1}$) was within the normal range, suggesting adequate hemostatic function and absence of thrombocytopenia. Overall, the hematological findings demonstrate that the canine subject was systemically healthy and suitable for oncological treatment, without alterations that could compromise tolerance to combined vincristine and acetogenin therapy.

Treatment efficacy, adverse effects, and the total vincristine sulfate doses required for remission were monitored under a standardized clinical protocol. This study was conducted at the Veterinary Clinic of Escuela Superior Politécnica Agropecuaria de Manabí Manuel Félix López, in accordance with approved ethical standards.

Drug administration protocol

Reference standards of vincristine sulfate and vinblastine sulfate (internal standard, IS) were obtained from Sigma–Aldrich (St. Louis, MO, USA). The therapeutic vincristine sulfate formulation (V.C.S.®; 1 mg·mL^{-1}) was sourced from Boryung Pharmaceutical Co., Ltd. (Ansan, Korea). All reagents were of analytical grade. Vincristine doses were calculated based on body surface area (mg·m^{-2}) following established veterinary oncology protocols. The injectable solution was diluted in 25–50 mL of 0.9% saline, adjusted to animal size, prepared under light protection, and administered intravenously via peripheral catheter over 1–10 min.

In one of the cases, natural acetogenins were administered as a coadjuvant therapy, beginning with a daily capsule containing 7.5 to 10 mg (equivalent to 500 mg of pulverized *A. muricata* dried leaves) during the 15 d prior to the initiation of chemotherapy, in order to assess tolerance and potential adverse effects. This dosage regimen was maintained during the first two applications of the vincristine protocol. From the third application onward, the dose was increased to a range of 14.5 to 20 mg·day^{-1}, divided into two administrations (one capsule in the morning and another in the evening) given with food, and continued until completing four months of treatment. This scheme allowed for the evaluation of a potential synergistic effect of acetogenins when used as adjuvants to low–dose vincristine therapy.

Clinical monitoring and evaluation of tumor response

Tumor progression was monitored weekly through systematic physical examinations, supplemented with cytology by imprint Tumor dimensions were measured using a high–precision vernier caliper (Truper®, model CAL-6P, Mexico), allowing for accurate documentation of tumor size at each assessment. These serial measurements were essential for tracking the progressive reduction of neoplastic lesions throughout the course of treatment. During clinical evaluations, relevant gross morphological features were assessed, including changes in tumor consistency, signs of neovascularization, ulceration, and spontaneous bleeding. The integration of clinical monitoring, microscopic evaluation, and the gradual introduction of the phytotherapeutic adjuvant enabled a comprehensive assessment of therapeutic efficacy, while also facilitating early detection of relapse or emerging treatment resistance.

RESULTS AND DISCUSSION

Case 1: Clinical response of transmissible venereal tumor to combined Vincristine–acetogenin treatment

TABLE I summarizes the response to the combined treatment of vincristine sulfate and acetogenins. During the first week, the initial tumor size was 39.2 cm², which was considered the baseline value for calculating cumulative reduction. From the second week onward, a progressive and clinically significant decrease in tumor volume was observed, reaching a 29.85% reduction. This downward trend continued in subsequent weeks, with a reduction of 73.98% by week three and 94.39% by week four. By the fifth week, the tumor had a residual size of only 0.06 cm², corresponding to a cumulative reduction of 99.85% relative to the initial measurement; consequently, administration of the sixth vincristine sulfate dose was deemed unnecessary.

TABLE I
Progressive reduction in TVT size in a canine patient during six weeks of treatment with vincristine sulfate in combination with acetogenins as adjuvant

Treatment Week	Vincristine Sulfate Dose (mg·m^{-2})*	Tumor Size (cm^2)**	Cumulative Reduction (%)
1	0.5	39.2	0.00
2	0.5	27.5	29.85
3	0.5	10.20	73.98
4	0.5	2.2	94.39
5	0.5	0.06	99.85
6***	–	–	–

*: A daily dose of 500 mg of pulverized dry leaf powder was administered for fifteen days, increasing to 1000 mg daily from the second chemotherapy session until the fourth month. **: Tumor height and width measurements were recorded. ***: Administration of the sixth vincristine dose was not necessary due to a cumulative tumor regression of 99.85%

A photographic sequence was documented (FIG. 4) illustrating the progressive macroscopic regression of the TVT during five weeks of combined treatment with vincristine sulfate and acetogenins.

Weekly images visually corroborate the continuous reduction in tumor volume, consistent with the measurements reported in TABLE I. From the extensive tumor mass observed in the initial week to its near disappearance by the fifth week, this photographic series provides complementary graphical evidence supporting the observed therapeutic efficacy. The correlation between objective measurements and clinical evolution documented through imaging strengthens the validity of the findings and highlights the potential utility of this combined therapy in veterinary clinical practice.

The FIG. 5, shows absence of characteristic TVT cells large, round cells with prominent nuclei and open chromatin. Instead, cellular

FIGURE 4. Photographic sequence illustrating the clinical progression of a genital TVT in a female dog undergoing combined treatment with vincristine sulfate and acetogenins

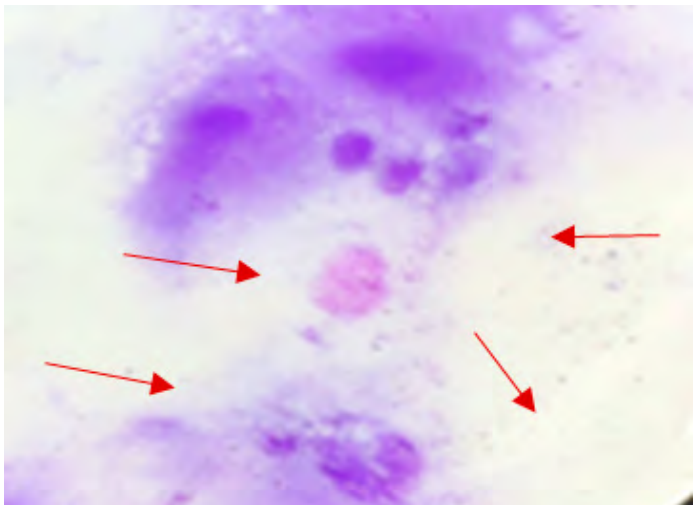

FIGURE 5. Cytological imprint showing complete remission of TVT after combined vincristine sulfate and acetogenin therapy. The smear displays a uniform population of non–neoplastic cells with regular nuclei, homogeneous cytoplasm, and absence of atypia or mitotic figures (Giemsa, 1000×). No cytomorphological features of malignancy, including anisokaryosis or pleomorphism, are observed

debris and amorphous violet–stained material, likely degenerating inflammatory cells, predominate. The lack of neoplastic cells confirms complete cytological remission, correlating with clinical tumor regression; supporting the efficacy of combined vincristine sulfate and acetogenins therapy, suggesting a synergistic effect in TVT resolution.

Finally, four months after completion of the acetogenin therapeutic protocol in a canine patient diagnosed with TVT, external evaluation (FIG. 6) revealed a fully healed genital region, with no evidence of tumor recurrence, inflammation, or lesions indicative of active neoplastic processes.

Acetogenins are secondary metabolites exclusively found in the Annonaceae family, particularly abundant in *A. muricata*, which have been extensively investigated for their potent antineoplastic activity

FIGURE 6. Four–month post–treatment clinical evaluation in a female dog showing complete remission of TVT

[20, 21, 22, 23]. These compounds demonstrate high selective cytotoxicity against neoplastic cells, as evidenced in both *in vitro* and *in vivo* models, inducing apoptosis through activation of the Bax–Bak pathway and caspase–3, as well as inhibition of the mitochondrial NADH–ubiquinone oxidoreductase enzyme (complex I). This inhibition disrupts the bioenergetic metabolism of tumor cells while sparing normal healthy cells [29]. This mechanism is especially pertinent to TVT, given the established mitochondrial dependency of these cells for sustained proliferation.

In a murine model of chemically induced carcinogenesis, annonacin, a major acetogenin, elicited a significant reduction in tumor incidence and volume without causing systemic toxicity. This favorable safety profile, combined with selective cytotoxicity, highlights the potential of acetogenins as adjuvant agents to enhance conventional chemotherapeutic protocols in veterinary oncology [30].

The chloroform fraction of the methanolic extract of *A. muricata* seeds (CMAM) was evaluated against triple–negative breast cancer cell lines (MDA–MB–231 and BT–549), yielding IC_{50} values of 4.5 ± 0.16 μg·mL^{-1} and 4.8 ± 0.3 μg·mL^{-1}, respectively, with a selectivity index of 32 for MDA–MB–231, indicating both potent cytotoxicity and antitumor specificity [31]. CMAM induced S–phase cell cycle arrest and mitochondria–mediated apoptosis triggered by reactive oxygen species (ROS), suggesting a pharmacodynamic profile compatible with synergistic use alongside vincristine in canine TVT treatment.

Acetogenins isolated from *Annona montana*, specifically annonacin and muricin P, demonstrated synergistic activity with sorafenib against hepatocellular carcinoma. *In vitro* studies showed decreased intracellular ATP levels and increased apoptosis, while *in vivo* combination therapy enhanced tumor growth inhibition [32]. Transcriptomic analysis identified SLC33A1 as a potential molecular target mediating this synergistic effect. Cryo–electron microscopy studies elucidated the atomic–level mechanism by which acetogenins inhibit mitochondrial complex I. These compounds fully occupy the ubiquinone binding channel, forming multiple hydrophilic interactions that effectively block electron transfer [33].

During oral administration of acetogenins, no clinically relevant adverse effects either gastrointestinal or systemic, were observed, indicating good tolerability and supporting their safety profile. These findings are consistent with preclinical studies reporting low toxicity in murine models.

This observation is consistent with recent preclinical studies in rats (*Rattus norvegicus*), which have demonstrated a promising safety profile. Single doses of up to 300 mg·kg^{-1} of hydro–methanolic leaf extract of *A. muricata* have been shown not to induce clinically relevant alterations in hepatic function parameters (Alanine Aminotransferase, Aspartate Aminotransferase and Alkaline Phosphatase) or renal biomarkers such as urea and creatinine [34]. Furthermore, in a rat model exposed to 7,12-dimethylbenz[a]anthracene (DMBA) to induce hepatorenal damage, treatment with *A. muricata* extract restored renal function, reduced oxidative stress, and normalized the expression of inflammatory markers (TNF–α, IL-1β, CYP2E1), with no histological evidence of hepatic or renal necrosis [35].

This therapeutic synergy may result from complementary mechanisms of action: vincristine disrupts microtubule polymerization, while acetogenins impair mitochondrial energy

metabolism by decreasing ATP production and triggering apoptotic signaling pathways. The concurrent modulation of these critical cellular targets may underlie the enhanced and sustained tumor regression observed in treated female dog. Further validation of these findings requires histopathological examination, molecular assessment of apoptosis–related markers (Bax, caspase-3, cytochrome c), and pharmacokinetic studies in a larger canine cohort to characterize the in vivo behavior of acetogenins.

Case 2: Tumor Response to Vincristine Sulfate Therapy

TABLE II presents the progression of tumor size in a case of genital–located TVT in a female dog treated with a fixed weekly dose of vincristine sulfate (0.5 mg·m^{-2}) over a six–week period. At the beginning of the treatment, the tumor area measured 23 cm^2. Throughout the therapeutic protocol, a progressive reduction in tumor volume was observed, reaching 1.9 cm^2 by the end of the cycle, representing a cumulative decrease of 91.74% relative to the baseline value. This pattern of sustained regression indicates a favorable clinical response to vincristine–based chemotherapy, with notable improvement evident as early as the second week. The continued reduction in tumor size during subsequent weeks suggests high sensitivity of the TVT to the administered regimen.

FIGURE 7. Photographic sequence depicting the clinical progression of a genital TVT in a female dog treated with vincristine sulfate. Images correspond to weekly evaluations throughout six weeks of chemotherapy. Progressive tumor regression is visually evident, particularly after the third dose

TABLE II
Progressive reduction in the size of genital TVT in a canine treated with a fixed weekly dose of vincristine sulfate (0.5 mg·m^{-2})

Treatment Week	Vincristine Sulfate Dose (mg·m^{-2})	Tumor Size (cm^2)	Cumulative Reduction (%)
1	0.5	23	0
2	0.5	20	13.04
3	0.5	15.6	32.17
4	0.5	11.4	50.43
5	0.5	4.4	80.87
6	0.5	1.9	91.74

The table shows the weekly administered dose of vincristine sulfate, the corresponding tumor size (in cm^2), and the cumulative percentage of tumor reduction over the treatment period. Tumor area was estimated through direct caliper measurements and calculated using the ellipsoid formula

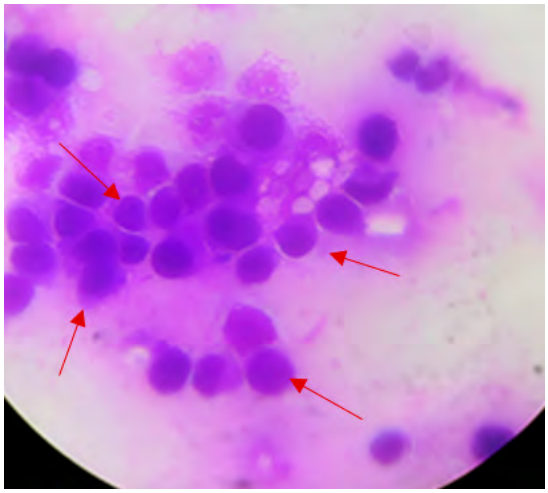

FIGURE 8. Cytological imprint of a genital lesion in a two–year–old female dog post–treatment with vincristine sulfate, still showing a considerable number of large round cells with nuclear features consistent with regressing TVT (Giemsa stain, 1000×)

The clinical progression documented in TABLE II is complemented by a photographic sequence (FIG. 7) that visually illustrates the progressive regression of a genital TVT in a female dog undergoing vincristine sulfate treatment. Over the six–week therapeutic period, the images reveal a consistent reduction in tumor volume, accompanied by gradual improvement in the appearance of the affected tissues, including decreased erythema, superficial hemorrhage, and vascularization. However, by the end of the treatment cycle, residual tissue consistent with tumor mass remained visible, indicating that complete remission was not achieved.

The image observed under light microscopy (FIG. 8) reveals a cellular cluster composed of round cells with morphological features consistent with TVT. These findings are consistent with a partial therapeutic response following six weeks of vincristine sulfate administration. The persistence of neoplastic cells suggests that, despite clear clinical regression, complete cytological remission has not been achieved, thereby supporting the need for continued or adjusted therapeutic intervention.

Vincristine has been firmly established as the first–line therapeutic agent for the management of canine TVT. The most widely supported standard protocol involves weekly intravenous administration of vincristine at doses ranging from 0.5 to 0.75 mg·m^{-2} for a variable number of cycles typically between six and eight until complete regression of the tumor mass is achieved. In this regard, a recent meta–analysis of clinical studies published between 2015 and 2023 reported that this therapeutic regimen achieves complete remission rates exceeding 93.1%, reaching

up to 98.9% when extended to eight cycles, without the need for adjuvant therapy in most cases [1].

Several studies on dogs with TVT have reported that a Vincristine sulfate dose of 0.7 mg·m^{-2} achieves therapeutically effective plasma concentrations, favorable tissue distribution, and minimal systemic accumulation [4, 5, 6]. Collectively, these findings confirm that this specific dosage is both clinically effective and well–tolerated in routine veterinary oncology practice.

Comparative analyses between combined vincristine–ivermectin regimens and vincristine monotherapy reveal comparable therapeutic outcomes contingent upon the administration of vincristine at efficacious dosages [36]. Current evidence suggests that subtherapeutic vincristine dosing may induce only partial tumor regression in canine TVT, failing to achieve complete remission.

It is important to highlight that the dog initially treated only with vincristine had her protocol extended by an additional week at 0.7 mg·m^{-2} and received a daily capsule for four weeks, each containing 500 mg of powdered leaf, equivalent to a dose of 7.5 to 10 mg of acetogenins. Complete clinical remission was observed at the end of the protocol, suggesting a potential synergistic effect between vincristine sulfate and these phytochemicals, which have been extensively documented for their antineoplastic activity [22, 29, 30, 31, 32, 33, 34, 35, 36].

CONCLUSION

The combined administration of vincristine sulfate (0.5 mg·m^{-2} weekly) and acetogenins (7.5–10 mg·day^{-1} during the first 15 days, increased to 14.5–20 mg·day^{-1} from the third administration, divided into two doses) in the studied canine subject resulted in early and nearly complete tumor regression, achieving a clinical remission of 99.85%, with no evidence of adverse effects attributable to the acetogenins. This research provides preliminary evidence of the potential of acetogenins as adjuvants to conventional chemotherapy, offering a more effective and safer integrative therapeutic strategy for the treatment of TVT in dogs. However, controlled clinical studies with a higher number of experimental units, as well as histopathological and molecular assessments, are required to confirm the safety, efficacy, and mechanisms of action of this therapeutic strategy and to establish standardized application protocols.

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Conflict of interest

The authors declare that they have no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Pimentel PAB, Giuliano A, Odatzoglou P, Ignatenko N, Wenceslau RR, Almeida IO, da Silva PHS, Costa MP, Horta RDS. Clinical guidelines for canine transmissible venereal tumour treatment: systematic review and meta–analysis. Vet. Comp. Oncol. [Internet]. 2025; 23(2):125-140. doi: https://doi.org/pprn

[2] Costa TS, Paiva FN, Manier BSML, Araújo DC, Ribeiro GB, Fernandes JI. Epidemiological, clinical, and therapeutic aspects of canine transmissible venereal tumor in Rio de Janeiro, Brazil (2015-2020). Pesq. Vet. Bras. [Internet]. 2023; 43:e07189. doi: https://doi.org/p3wj

[3] Franco–Molina MA, Santamaría–Martínez EA, Santana Krimskaya SE, Zarate–Triviño DG, Kawas JR, Ramos–Zayas Y, Palacios–Estrada N, Prado–García H, García–Coronado PL, Rodríguez–Padilla C. *In vitro* chemosensitivity of a canine tumor venereal transmissible cancer cell line. Front. Vet. Sci. [Internet]. 2022; 9:972185. doi: https://doi.org/p3wk

[4] Hantrakul S, Klangkaew N, Kunakornsawat S, Tansatit T, Poapolathep A, Kumagai S, Poapolathep S. Clinical pharmacokinetics and effects of vincristine sulfate in dogs with transmissible venereal tumor (TVT). *J. Vet. Med. Sci.* [Internet]. 2014; 76(12):1549–1553. doi: https://doi.org/f6vksm

[5] Paranzini CS, Sant’anna MC, di Santis GW, Martins MIM. Prevalence of different cytomorphological types of transmissible venereal tumours and the association with prognosis in dogs treated with vincristine sulphate. Retrospective study. Semina Cienc. Agrar. [Internet]. 2015; 36(6):3795-3800. doi: https://doi.org/p3wm

[6] Antonov A. Successful treatment of canine transmissible venereal tumor using vincristine sulfate. Adv. Res. [Internet]. 2015; 5(5):1-5. doi: https://doi.org/p3wn

[7] Leil AZA, El–Hallawany HA, Abd El–Rahman HMA. Clinical response of dogs affected with transmissible venereal tumor (TVT) to the chemotherapeutic regime with regard to cytomorphology and histopathology. J. Appl. Vet. Sci. [Internet]. 2022; 7(2):58-65. doi: https://doi.org/p3wp

[8] Sztukowski KE, Yaufman Z, Cook MR, Aarnes TK, Husbands BD. Vincristine–induced adverse events related to body weight in dogs treated for lymphoma. J. Vet. Intern. Med. [Internet]. 2024; 38(3): 1686-1692. doi: https://doi.org/p3wq

[9] Mealey KL, Fidel J, Gay JM, Impellizeri JA, Clifford CA, Bergman PJ. ABCB1-1Δ polymorphism can predict hematologic toxicity in dogs treated with vincristine. J. Vet. Intern. Med. [Internet]. 2008; 22(4):825-1073. doi: https://doi.org/dzz5n9

[10] Poirier M, Blong AE, Walton RAL. Successful management and recovery of a dog with immune–mediated thrombocytopenia following vincristine overdose. J. Vet. Emerg. Crit. Care [Internet]. 2023; 33(4): 539-544. doi: https://doi.org/p3wr

[11] Mason SL, Grant IA, Elliott J, Cripps P, Blackwood L. Gastrointestinal toxicity after vincristine or cyclophosphamide administered with or without maropitant in dogs: a prospective randomised controlled study. J. Small Anim. Pract. [Internet]. 2014; 55(8):391–398. doi: https://doi.org/f6ckbt

[12] Tsukamoto A, Ohno K, Tsukagoshi T, Maeda S, Nakashima K, Fukushima K, Fujino Y, Takeuchi A, Tsujimoto H. Ultrasonographic evaluation of vincristine–induced gastric hypomotility and the prokinetic effect of mosapride in dogs. J. Vet. Intern. Med. [Internet]. 2011; 25(6):1195-1515. doi: https://doi.org/b5bg35

[13] Kelly B, Thamm D, Rosengren RJ. Utility of the second–generation curcumin analogue RL71 in canine histiocytic sarcoma. Vet. Res. Commun. [Internet]. 2024; 48(1):563–568. doi: https://doi.org/p3ws

[14] Çetinkaya S, Çınar Ayan İ, Dursun HG, Süntar İ, Taban K, Gök HN, Atak M. Enhanced apoptosis in pancreatic cancer cells through thymoquinone–rich Nigella sativa L. methanol extract: targeting NRF2/HO-1 and TNF–α pathways. Anticancer Agents Med. Chem. [Internet]. 2025; 25(20):1607-1621. doi: https://doi.org/p3wt

[15] Ansary J, Giampieri F, Forbes–Hernandez TY, Regolo L, Quinzi D, Gracia–Villar S, Garcia–Villena E, Tutusaus–Pifarre K, Alvarez–Suarez JM, Battino M, Cianciosi D. Nutritional value and preventive role of *Nigella sativa* L. and its main component thymoquinone in cancer: an evidenced–based review of preclinical and clinical studies. Molecules [Internet]. 2021; 26(8):2108. doi: https://doi.org/gpggt8

[16] Fallon–Adido HE, Silva–Chagas CK, Ferreira GG, Carmo–Bastos ML, Dolabela MF. In silico studies on cytotoxicity and antitumoral activity of acetogenins from *Annona muricata* L. Front. Chem. [Internet]. 2023; 11:1316779. doi: https://doi.org/p3ww

[17] Olas B. The antioxidant potential of graviola and its potential medicinal application. Nutrients [Internet]. 2023; 15(2):402. doi: https://doi.org/p3wx

[18] Rady I, Bloch MB, Chamcheu RCN, Banang–Mbeumi S, Anwar MR, Mohamed H, Babatunde AS, Kuiate JR, Noubissi FK, El Sayed KA, Whitfield GK, Chamcheu JC. Anticancer properties of graviola (*Annona muricata*): a comprehensive mechanistic review. Oxid. Med. Cell. Longev. [Internet]. 2018; 1826170:1-39. doi: https://doi.org/p3wz

[19] Qazi AK, Siddiqui JA, Jahan R, Chaudhary S, Walker LA, Sayed Z, Jones DT, Batra SK, Macha MA. Emerging therapeutic potential of graviola and its constituents in cancers. Carcinogenesis [Internet]. 2018; 39(4):522–533. doi: https://doi.org/gdbcsw

[20] Manoharan JP, Palanisamy H, Vidyalakshmi S. Overcoming multi drug resistance mediated by ABC transporters by a novel acetogenin – annonacin from *Annona muricata* L. J. Ethnopharmacol. [Internet]. 2024; 322:117598. doi: https://doi.org/p3w2

[21] Periyasamy L, Muruganantham B, Deivasigamani M, Lakshmanan H, Muthusami S. Acetogenin extracted from *Annona muricata* prevented the actions of EGF in PA-1 ovarian cancer cells. Protein Pept Lett. [Internet]. 2021; 28(3):304–314. doi: https://doi.org/p3w6

[22] Naik AV, Dessai SN, Sellappan K. Antitumour activity of *Annona muricata* L. leaf methanol extracts against Ehrlich Ascites Carcinoma and Dalton's Lymphoma Ascites mediated tumours in Swiss albino mice. Libyan J. Med. [Internet]. 2020; 16(1):1846862. doi: https://doi.org/p3w7

[23] . Ogbu P, Ugota E, Onwuka R, Ogbu I, Aloke Ch. Effect of acetogenin fraction of *Annona muricata* leaves on antioxidant status and some indices of benign prostatic hyperplasia in rats. Redox Rep. [Internet]. 2020; 25(1):80–86. doi: https://doi.org/g6wpdb

[24] Zubaidi SN, Mohd–Nani H, Ahmad–Kamal MS, Abdul–Qayyum T, Maarof S, Afzan A, Mohmad–Misnan N, Hamezah HS, Baharum SN, Mediani A. *Annona muricata*: comprehensive review on the ethnomedicinal, phytochemistry, and pharmacological aspects focusing on antidiabetic properties. Life [Internet]. 2023; 13(2):353. doi: https://doi.org/p3w8

[25] Vijay M, Rajitha M, Krishnagaanth M, Jadhav ND, Chigure G, Srivastava A. Controlling deltamethrin–resistant *Rhipicephalus microplus* with a phytoformulation of *Annona muricata* and *Piper longum*. Acta Trop. [Internet]. 2025; 266:107644. doi: https://doi.org/p3w9

[26] Castro–Hernández CL, Ayasta–Senmache JG, Santa Cruz–López CY, Carrasco–Solano FA, Moreno–Mantilla M. Efecto antibacteriano del extracto etanólico de *Annona muricata* sobre microorganismos de importancia clínica. Gac. Méd. Bolívar [Internet]. 2021; 44(1):29-33. doi: https://doi.org/p3xb

[27] Coria–Téllez AV, Montalvo–González E, Yahia EM, Obledo–Vázquez EN. *Annona muricata*: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arab. J. Chem. [Internet]. 2018; 11(5):662–691. doi: https://doi.org/gpbc6m

[28] Setthawongsin C, Teewasutrakul P, Tangkawattana S, Techangamsuwan S, Rungsipipat A. Conventional–vincristine sulfate vs. modified protocol of vincristine sulfate and L–asparaginase in canine transmissible venereal tumor. Front. Vet. Sci. [Internet]. 2019; 6:300. doi: https://doi.org/p3xd

[29] Suhail P, Venkatachalam VV, Balasubramanian T, Christapher PV. A review on the *in vitro* anticancer potentials of acetogenins from *Annona muricata* Linn.: a potential inducer of Bax–Bak and Caspase-3 related pathways. Indian J. Pharm. Educ. Res. [Internet]. 2024; 58(3Suppl):S693. doi: https://doi.org/p3xf

[30] Md Roduan MR, Abd Hamid R, Mohtarrudin N. Modulation of cancer signalling pathway(s) in two–stage mouse skin tumorigenesis by annonacin. BMC Complement. Med. Ther. [Internet]. 2019; 19:238. doi: https://doi.org/p3xg

[31] Kariyil BJ, Ayyappan UPT, Gopalakrishnan A, George AJ. Chloroform fraction of methanolic extract of seeds of *Annona muricata* induce S phase arrest and ROS dependent caspase activated mitochondria–mediated apoptosis in triple–negative breast cancer. Anticancer Agents Med. Chem. [Internet]. 2021; 21(10):1250–1265. doi: https://doi.org/p3xh

[32] Li RS, Li LY, Zhu XF, Li X, Wang CY, Qiu SJ, Zhou J, Fan J, Hu B, Mu Q *Annonaceous acetogenins* synergistically inhibit hepatocellular carcinoma with sorafenib. J. Nat. Prod. [Internet]. 2024; 87(1):14-27. doi: https://doi.org/p3xj

[33] Grba DN, Blaza JN, Bridges HR, Agip ANA, Yin Z, Murai M, Miyoshi H, Hirst J. Cryo–electron microscopy reveals how acetogenins inhibit mitochondrial respiratory complex I. J. Biol. Chem. [Internet]. 2022; 298(3):101602. doi: https://doi.org/p3xm

[34] Chauhan S, Srivastava V. Evaluation acute toxicity studies of hydro alcoholic extract of soursop leaves (*Annona muricata*) on brain, liver and kidney of Wister rats. J. Clin. Toxicol. 2024 [cited May 14 2025]; 14(1):556. Available in: https://goo.su/SLzAQS

[35] Zeweil MM, Khafaga AF, Mahmoud SF, Wasef L, Saleh H, Elrehim AMA, Bassuoni NF, Alwaili MA, Saeedi NH, Ghoneim HA. *Annona muricata* L. extract restores renal function, oxidative stress, immunohistochemical structure, and gene expression of TNF–α, IL–β1, and CYP2E1 in the kidney of DMBA–intoxicated rats. Front. Pharmacol. [Internet]. 2024; 15:1348145. doi: https://doi.org/gt33nw

[36] Ferreira–Bulhosa L, Estrela–Lima A, da Silva Solcà M, Diniz Gonçalves GS, Larangeira DF, de Pinho FA, Barrouin–Melo SM. Vincristine and ivermectin combination chemotherapy in dogs with natural transmissible venereal tumor of different cyto–morphological patterns: a prospective outcome evaluation. Anim. Reprod. Sci. [Internet]. 2020; 216:106358. doi: https://doi.org/p3xn