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Anti-cancer effects of *Gynura procumbens* leaves against recurrent multiple cutaneous mast cell tumor grade III in a 7-year-old chihuahua dog

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Abstract

Background: *Gynura procumbens*, a well-known traditional herb, has been studied to be an effective chemotherapeutic agent against a wide range of cancer cell types.

Case Description: A 7-year-old chihuahua dog was presented to the veterinary teaching hospital with multiple cutaneous MCT grade III masses found on the cranial aspect of the right front leg and the dorsal aspect of the neck/back. Local excision of all cutaneous tumors was performed followed by chemotherapy (vinblastine, prednisone, and cyclophosphamide) every 21-day interval for 9 treatments. Nearby 3 months later, recurrent MCT was observed and lomustine was used instead for 21-day intervals. During the first chemotherapy, the dog had a fever, weak, lethargy, and vomiting. Alternative natural therapy is preferred, the dog has been receiving *G. procumbens* daily. The dog responded well to this herbal treatment. These recurrent masses began to shrink after a few weeks of treatment. After several months of treatment, all masses were completely gone. Moreover, no micro-metastasis to the lungs, spleen, and liver were detected.

Conclusion: This natural herbal was helpful to stop the growth of tumors, to prevent micro-metastasis, and to relieve the patient's symptoms.

Keywords: Dog, Mast cell tumor, *Gynura procumbens*.

Introduction

Canine cutaneous mast cell tumor (MCT) is one of the most common of all skin tumors in dogs, accounting for an estimated 16%–21% of all cases (London and Seguin, 2003; London and Thamm, 2020). MCT is highly invasive and metastatic, and frequently recurrent after having surgical removal (Misdorp, 2004; London and Thamm, 2020). Histopathological examination of biopsy tissue has typically been required for tumor grading as well-, intermediate-, and poor-differentiated or grade I (low grade), grade II, or grade III (moderate, high grade), respectively (Patnaik *et al.*, 1984).

Surgical removal with completely excised tumors is the curative treatment for low-grade MCT's dog (grade I and some grade II), further therapy is typically not necessary (Weisse *et al.*, 2002; Blackwood *et al.*, 2012). Grade II MCT is generally local, but some behave more like grade III MCT which has a high rate of spread for regional and distant metastasis (Thamm *et al.*, 2006; London and Thamm, 2020). Grade III MCT is a highly aggressive tumors with a high propensity for metastasis. Chemotherapy following surgery is usually indicated if the MCT is found to

grade III and some grade II with a high mitotic index due to a high rate of spread to other organs (Cooper *et al.*, 2009; Rassnick *et al.*, 2010; Vail *et al.*, 2012; Lejeune *et al.*, 2015). In addition, depending on where the tumor is located, a debulking surgery followed immediately by radiotherapy is one of the appropriate options to perform if one may not be able to make a wide excision with clean surgical margins because radiation therapy can be used to control local disease (Dobson *et al.*, 2004; Hahn *et al.*, 2004; Carlsten *et al.*, 2012). Chemotherapy is generally recommended in all cases with high-grade II or grade III MCT, even with the tumor is excised with wide margins and cases with lymph node or distant metastasis involvement.

Many patients respond very well to conventional therapies, such as chemotherapy and radiotherapy but the related side effects, long-term prognosis, quality of life, and other health issues all need to be considered when looking at treatment options. In recent years, herbal therapy can be used safely, without intervention, in conjunction with conventional treatments to reduce the toxic effects of chemo-drugs and radiation, to slow the progression of the tumor, and to improve the overall

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quality of life for cancer patients by helping mobilize the immune system to support the body itself (Maw *et al.*, 2011; Wen and Johnston, 2011). One of the medicinal herbs is *Gynura procumbens*, a well-known traditional herb in Southeast Asia, has been studied to be an effective chemotherapeutic agent against a wide range of cancer cell types and it exerts its anticancer activities via the modulation of various points of carcinogenesis including cancer initiation, cell proliferation, metastasis, and angiogenesis (Agustina *et al.*, 2006; Nurulita *et al.*, 2012; Hew *et al.*, 2013; Wang *et al.*, 2013; Gofur *et al.*, 2015; Jermnak, U *et al.*, 2022). Other therapeutic potential of this plant has also been reported to treat topical inflammation, kidney discomfort, viral ailments, diabetes mellitus, gastrointestinal disorders, and hypertension, among other ailments (Iskander *et al.*, 2002; Vejanan *et al.*, 2012; Poh *et al.*, 2013; Algariri *et al.*, 2014; Tan *et al.*, 2016). Using the animal clinical model, this is the first study to evaluate the anti-cancer effects of *G. procumbens* leaves against recurrent multiple cutaneous MCT in a chihuahua dog.

Case Details

A 7-year-old, male, intact chihuahua breed dog was presented to the Veterinary Teaching Hospital (VTH), Kasetsart University, with firm and hairless multiple masses on the cranial aspect of the right front leg and the dorsal aspect of the neck/back. The owner reported that all masses had been seen for several weeks. On physical examination, the dog was bright, alert, and responsive, well hydrated and in good body condition. Three masses on the cranial aspect of the right front leg were 0.2–0.5 cm in diameter and were firm and ulcerated. There was also a well-circumscribed 1.0 cm in diameter hairless cutaneous mass on the dorsal aspect of the neck. A similar mass had a dimension of $2.0 \times 1.5 \times 0.4 \text{ cm}^3$ was noted on the dorsal aspect of the back. The dog underwent a complete blood cell count (CBC), serum electrolytes, urea, alkaline phosphatase (ALK), alanine aminotransferase (ALT), urinalysis, and chest radiographs. All values were found to be within the normal range. Thoracic radiographs were normal. An impression smear of the ulcerated mass on the front leg revealed MCT. Fine needle aspirate of all masses showed the majority of cells of mast cells (Fig. 1). The regional lymph nodes were not palpably enlarged. Abdominal ultrasonography was made for additional diagnostics to the extent of tumor in other areas of the body. The result showed no overt evidence of neoplasia. Surgical biopsies of all skin masses were performed and definitive histopathologic diagnoses of MCT grade III were confirmed (Fig. 2). Tumors were staged according to clinical examination findings for canine MCT reported by the World Health Organization (Owen, 1980) (Table 1).

This dog was staged at III-anesthesia and was induced with propofol (4 mg/kg) and maintained with 2%

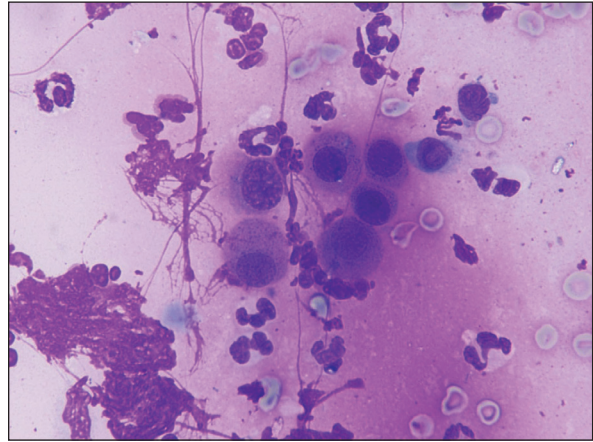


Fig. 1. Canine, skin mass (MCT): Round cells which moderate anisocytosis, anisokaryosis, moderate to high N:C ratio, and poorly metachromatic granules.

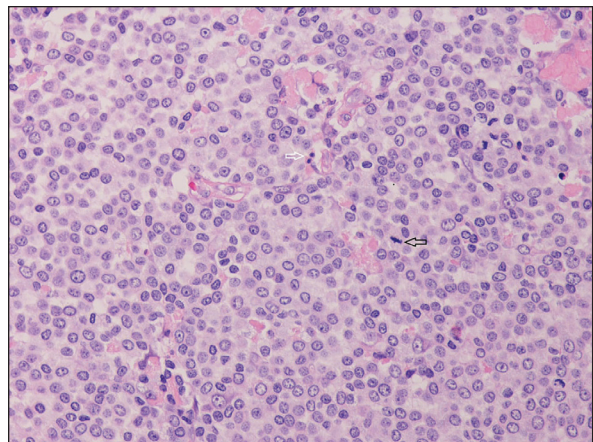


Fig. 2. Canine, skin mass (MCT grade III): Pleomorphic round to polygonal cells, nuclei are large, round to oval, and vesicular and have prominent nucleoli. Cytoplasm is moderate and variably granulated. Eosinophils and mitotic figures are found; black arrow → mitotic figure, white arrow → eosinophil.

isoflurane and oxygen. Wide excisions of masses on the dorsal aspect of the neck and back were removed, except for multiple masses on the cranial aspect of the right front leg. The owner declined amputation of the front leg for completeness of surgical margins, thus these masses were removed by local excision technique instead. To control local disease after incomplete resection at the front leg, the owner was not convenient to take the dog treated with radiation to control local recurrence. Thus, the dog was treated with surgery followed by chemotherapy using vinblastine, prednisone, and cyclophosphamide (Table 2) for a nine-dose protocol without evidence of gross disease. Nearby 3 months later, recurrent multiple cutaneous MCT was observed at the same areas, especially at the

Table 1. World Health Organization clinical staging system for mast cell tumors.

Stage	Description
I	One tumor confined to the dermis, without regional lymph node involvement 1. Without systemic signs 2. With systemic signs
II	One tumor confined to the dermis, with regional lymph node involvement 1. Without systemic signs 2. With systemic signs
III	Multiple dermal tumors; large, infiltrating tumors with or without regional lymph node involvement 1. Without systemic signs 2. With systemic signs
IV	Any tumor with distant metastasis, including blood or bone marrow involvement

Table 2. Chemotherapy protocol (vinblastine, prednisone, cyclophosphamide).

Vinblastine	2 mg/m ² intravenous, on day 1 of the 21-day protocol
Prednisone	20–40 mg/m ² oral (high dose at first, then taper over 4 months)
Cyclophosphamide	50 mg/m ² oral, on days 8,9,10, and 11 of the 21-day protocol

cranial aspect of the right front leg, without regional lymph node involvement and distant metastasis. Confirm biopsy of recurrent masses found MCT grade III. Due to no response for the previous chemotherapy protocol, it was used lomustine (50–90 mg/m² oral, every 21 days) to treat where further wide excision was not possible and radiation was not available. During the first chemotherapeutic treatment, the patient had a fever, weakness, lethargy, vomiting, loss of appetite, and itchiness. In addition, these recurrent masses still got larger not responding to chemo-drug. The patient was placed on supportive treatment controlling illness symptoms and maintaining a good quality of life.

The owner is required to stop treatment with other chemotherapies. An alternative natural therapy was selected, *G. procumbens* (Fig. 3). The fresh leaves of *G. procumbens* were washed with running tap water. Five ml of drinking water and four fresh leaves of *Gynura procumbens* were squeezed water out. The juice from these fresh leaves was fed once orally every day. A regular follow-up of this dog monthly was scheduled to evaluate for herbal response by observation size of tumors, blood sample analysis, chest radiography, abdominal ultrasonography and side effects of *G. procumbens* leaves such as diarrhea, vomiting, and neutropenia based on the criteria for toxicity described in Table 3 (Langova *et al.*, 2004). A blood sample and urine sample were taken for analysis before starting oral herbal administration and this was repeated at regular intervals, every 2–4 weeks initially, reducing to every 1–2 months with time. Four years ago to the present, the patient has still not been found



Fig. 3. *Gynura procumbens*.

any mass recurrence at the right front leg and the dorsal aspects of the neck/back regions, but the hair still does not come up in these regions (Fig. 4A, B, C, and D). The last blood tests, a CBC, and serum biochemical profiles were within normal limits (Table 4), and chest/abdominal radiography and abdominal ultrasonography (Fig. 5A, B, C, D, and E) showed no overt evidence of metastasis.

Discussion

Adjuvant chemotherapy typically is used to treat residual microscopic disease where further wide surgery is not possible and radiation therapy is not available. This dog with MCT grade III was treated with vinblastine, prednisolone, and cyclophosphamide. The aim of this treatment is either to slow down the recurrence of tumor or to prevent the progression of existing metastatic disease. After adjuvant chemotherapy for 83 days, the dog developed local recurrence in the same areas without regional/distant metastasis. As we know, MCT with either high grade II or grade III was considered to be at high risk for recurrence and distant metastasis. Thamm *et al.* (2006) reported the disease-free interval was 1,305 days in high-grade II or grade

Table 3. Criteria for toxicity.

Toxic effect and grade	Signs
Diarrhea	
I	Soft stools, response to dietary modification
II	3–7 watery stools per day, < 2 days
III	>7 watery stools a day
IV	Intractable or bloody, requires hospitalization
Vomiting	
I	sporadic, self-limiting
II	1–5 episodes a day, < 2 days
III	6–10 episodes per day
IV	Intractable, requires hospitalization
Neutropenia	
I	Neutrophils < $3.5 \times 10^9/l$
II	Neutrophils < $2.0 \times 10^9/l$
III	Neutrophils < $1.0 \times 10^9/l$
IV	Neutrophils < $0.5 \times 10^9/l$

III MCT dogs treated with vinblastine and prednisone following surgical excision, with or without radiation therapy. Compared to this previous study, the disease-free interval in this study was shorter. Because the incomplete-excised margin, especially the masses located at the cranial aspect of the right front leg was surgically limited. To make a complete-excised margin was better to do right forelimb amputation. If the first surgery was able to make wide excision, the time to local recurrence should be longer. Anyway, the treatment planning was also based on the owner's decision. While these chemo-drugs failed to prevent local recurrence, they still helped to control residual microscopic disease.

To treat the cutaneous MCT recurrence, lomustine (CCNU) at a dosage of 50–90 mg/m² orally every 3 weeks was the drug of choice used to reduce where further wide excision was not possible and radiation was not available. Unfortunately, the patient had a fever, weak, lethargy, vomiting, loss of appetite, and itchiness during this chemo-drug treatment. As we know, lomustine can be very myelosuppressive, as well as hepatotoxic (Kristal *et al.*, 2004). This dog experienced some form of stomach or intestinal discomfort and had an allergic reaction after chemotherapy treatment. Moreover, almost all recurrent masses did not respond to partial or complete remission. It apparently showed treatment failure for this oral lomustine drug used in this MCT recurrent dog. In discussions with the pet owner, the owner denied to use of other chemo-drugs



Fig. 4. (A, B, C, D): (A) A MCT grade III chihuahua dog treated *G. procumbens* leaves for several months. (B, C, D) all masses at the cranial aspect of the right front leg and the dorsal aspects of the neck and the back regions were disappeared.

or other small molecule inhibitors of tyrosine kinase inhibitors including masitinib and toceranib. The goal that owner's needed was to maintain a good quality of life for as long as possible with palliative therapy aimed at controlling symptoms caused by the MCT. Alternative natural therapy was selected, using juice out of fresh leaves of *G. procumbens* was given to this dog to drink every day. Unexpected was all up and about, as if this dog had never been sick at all. So far, the dog responded well to this herbal treatment. These recurrent masses began to shrink after a few weeks of treatment. After several months of treatment, all masses were completely gone. Moreover, no micro-metastasis to lungs, spleen, and liver was detected. This dog has been alive more than 4 years without tumor recurrence. This seemed to be an effective natural drug to inhibit

Table 4. Hematology and blood chemistry.

HGB	17.40 (10–18 gm%)	BUN	29.00 (10–26 mg%)
PCV	49.20 (35%–55%)	CREATININE	0.93 (0.5–1.3 mg%)
RBC	7.19 (5–9 × 10 ³ /cumm)	ALT SGPT	104.00 (6–70, 37°IU/l)
MCV	68.43 (60–77 fl)	ALK PHOS	50.00 (8–76 U/l)
MCHC	35.37 (32–36 gm%)		
WBC	9.71 (6–17 × 10 ³ /cumm)		
SEGS	74.00 (3,000–11,400)		
LYMPH	12.00 (1,000–4,000)		
MONO	9.00 (150–1350)		
EOS	5.00 (100–750)		
PLATELETS	214.00 (200–500 × 10 ³)		
PROTEIN (REFRACT)	7.60 (6–7.5)		

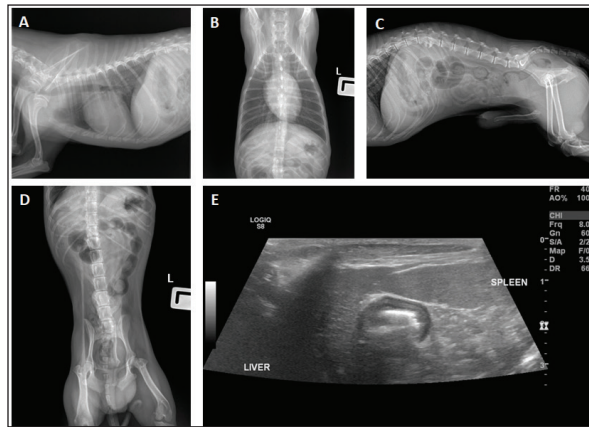


Fig. 5. (A, B, C, D, E): (A, B) Lateral and ventrodorsal thoracic radiographs revealed no evidence of lung nodules and thoracic lymph node enlargement. (C, D) Lateral and ventrodorsal radiographs demonstrated no mass effect and the sign of abdominal lymph node enlargement. (E) B-mode liver and splenic ultrasonography showed normal parenchyma and homogeneously. No evidence of metastasis based on radiography and ultrasonography is detected.

tumor growth and maintain a good quality of life. In this study, *Gynura procumbens*' leaves were a substitute for mainstream cancer care. This natural herbal was helpful in this multiple cutaneous MCT recurrent dog to stop the growth of tumor, to prevent micro-metastasis and to relieve the patient's symptoms. We hope that this study will be useful for the future to investigate the phytochemicals associated with plants for use in health product, pharmaceutical, and medical applications.

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Authors' contributions

The authors contributed equally to this study.

Conflict of interest

The author declares that there is no conflict of interest.

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None.

Data availability

All data are provided in the manuscript.

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