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Case report

TREATMENT OF MALIGNANT FIBROUS HISTIOCYTOMA IN A DONKEY WITH *CATHARANTHUS ROSEUS* EXTRACT - A CASE REPORT

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Abstract

Malignant fibrous histiocytoma (MFH) is a pleomorphic sarcoma of unknown etiology that rarely occurs in equines (horses and donkeys). In September 2021, a case of an 8-year-old female donkey with a tumor mass in the right eye was admitted to Animal Care (ACE) Hospital in Luxor, Egypt. The case underwent both clinicopathological and radiological examination. Based on microscopic examination, the morphological diagnosis was malignant fibrous histiocytoma. Radiological assessment (X-ray) revealed a non-metastatic tumor mass. An aqueous extract of *Catharanthus roseus* leaves and flowers was (ESC16) prepared by cold maceration and characterized by GC-MS analysis. The tumor was treated with 0.9 ml of an intratumoral injection of extract ESC16 and received one

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injection every other day for 50 days due to the large size of the tumor mass. The tumor showed macroscopic regression over the study period. To our knowledge, this is the first time that MFH in a donkey has been treated with *C. roseus* extract. ESC16 showed efficacy in treating MFH in that female donkey. Thus, it may be concluded that the intralesional injection of aqueous extract of *C. roseus* could be a promising antitumor agent for equine MFH in the Egyptian field.

Keywords: Malignant fibrous histiocytoma, donkeys, *Catharanthus roseus*, eyes, histopathology, radiology, gas chromatography, mass spectrometry.

INTRODUCTION

Malignant fibrous histiocytoma (MFH) is a pleomorphic sarcoma of unknown etiology that rarely occurs in equines (horses and donkeys) (Hamir, 1989; Schneider et al., 1999a; Valentine, 2006; Geburek et al., 2007). MFH is often manifested as a single solid or soft tissue mass with a diameter ranging from one to several centimeters. It is characterized by invasive growth and is not well separated from the surrounding tissue. Although MFH can develop anywhere on the body, it most frequently affects the neck, upper hind legs, knees, and proximal parts of all limbs (Geburek et al., 2007). Fibroblastic cells, histiocytic round or polygonal, and multinucleated giant cells make up MFH under a microscope (Hatano et al., 1999).

A survey conducted in the Pacific Northwest of the USA revealed that the incidence of MFH was 1.3% in all equine cutaneous neoplasms (Knottenbelt et al., 2015). Having developed from undifferentiated mesenchymal cells, it was known as an undifferentiated sarcoma and a giant cell tumor (Schneider et al., 1999b). Reliable and available information about MFH in Egypt is scarce. Previous investigations have denied an age- or breed-specific predilection for tumor development (Gilger, 2013). Other research found this tumor type in animals over 10 years of age with a male propensity; six cases of equine MFH were reported at ages ranging from 3 to 12 years, with a mean age of 6.8 years (Hamir 1989; Knottenbelt et al. 2015). The most common complication reported was recurrence in the ocular structures, which occurred in 10% to 51% animals, or in approximately 30% on average (Plummer et al., 2007). Many surgical and therapeutic interventions were utilized, including surgical excision of the third eyelid, keratectomy, enucleation, or exenteration (Elce et al., 2011); cryotherapy; radiofrequency hyperthermia; radiotherapy; topical and intralesional chemotherapy with cisplatin or 5-fluorouracil, Bacillus Calmette-Guerin (BCG) cell wall extract; and carbon dioxide laser ablation (Rabo et al., 2000; Chahory et al., 2002; Montiani-Ferreira et al., 2008; Cian et al., 2016).

C. roseus is a perennial herb species of the *Apocynaceae* family, originally native to Madagascar (Goyal et al., 2008). Its seeds, stems, leaves, and flowers contain various bioactive chemicals, including alkaloids, particularly vincristine and vinblastine (Kabesh et al., 2015; Sharma et al., 2019), steroids, phenolic compounds, flavonoids, saponins, terpenes, and anthocyanins (Ferreres et al.,

2008; El Enshasy, 2019). Owing to its phytoconstituents that possess a wide range of important pharmacodynamical properties, *C. roseus* extract can be used to combat various diseases, for example, Hodgkin's disease, malaria, and diabetes. Vincristine and vinblastine, two essential anticancer agents, are solely present in this plant species, which generates significant interest in cancer therapy (Sharma et al., 2019; Chunarkar-Patil et al., 2024).

Here, a case of a female donkey histologically diagnosed with MFH in Luxor, Egypt, is presented. As a treatment option, an extract of *Catharanthus roseus* was prepared, used for intralesional injection, and monitored to evaluate its therapeutic efficacy. Given the clinical regression of the tumor, a successful treatment of MFH is reported.

CASE PRESENTATION

Anamnestic Data

In September 2021, an 8-year-old female donkey was admitted to Animal Care Hospital (ACE) in Luxor, Egypt, for further diagnosis and treatment. The animal had a tumor mass in the right eye, which was perceived by its owner a month and a half before the presentation as increasing in size over time. The owner stated that the donkey was a hard-working animal, housed in a sunny area, exposed to solar radiation. Palpation of the mass revealed its solid, soft consistency. An ocular neoplasm was suspected and confirmed by clinicopathological examination. Ophthalmic examination of the affected eye revealed a 5 x 3 cm tumor mass originating from the palpebral conjunctiva of the lower eyelid, with purulent discharge and epiphora leading to blackening skin under the eye, and moderate swelling of the mandibular lymph node (Figure 1). For histopathological examination, an 8-mm biopsy sample was collected. Moreover, X-ray images were captured before and after treatment.

Histopathological examination

Tissue biopsies from the affected eye with ocular neoplasm were collected and fixed in 10% neutral, buffered formalin, processed routinely in paraffin wax to form blocks, sectioned into 5-µm-thick slices, and stained with hematoxylin and eosin (H&E**).** The second biopsy was obtained after the swelling had resolved. The evaluation of mitotic count (MC) via Aperio image scope for counting the mitotic figures was performed as follows: the hotspot area of high mitotic activity, free from necrosis, hemorrhage, or inflammation was identified, and a contiguous field of view (FOV) in an area of 2.37 mm^2 defined; at the highest power for the scanned slide, 22 FOV were selected, and the mitotic figures (MF) were counted within the area (Romansik et al., 2007).

Figure 1. A female 8-year-old donkey with malignant fibrous histiocytoma (MFH) in the right eye, admitted to the hospital in September 2021.

Radiological assessment

Radiography was used to determine if the tumor had metastasized into the bones of the eye socket. The examination was done with an Agfa computed radiography (CR) apparatus. An X-ray plate was held against the side of the donkey's face while a camera on a tripod took the pictures. Since the donkey is very sensitive about its head, the animal was sedated with xylazine to keep its head perfectly still. Several pictures were taken from different angles to "see around" the eye: lateral, oblique, and dorsoventral views.

C. roseus extract preparation

C. roseus leaves and flowers were collected from the garden of the Animal Care Hospital (25°40'45.3"N 32°39'13.3"E) (Figure 2). The plant parts were screened, washed, dried, and ground into a fine powder with a high-speed mixer (Moulinex Egypt, Al-Araby Co., Egypt). The material that passed through an 80-mesh sieve was retained for use. The plant species was authenticated by the Botany Department of the Faculty of Science, Aswan University. The aqueous extract of *C. roseus* leaves and flowers was prepared by maceration of 150 g of dried powder in 1 L of distilled water at 37°C for 72 h (Da Silva et al., 2018). The supernatant was filtered using Whatman filter paper No. 1, and its moisture was removed in a rotary evaporator.

The extract was sterilized by autoclaving at 220°C for 1 h. The resultant extract was named ESC16.

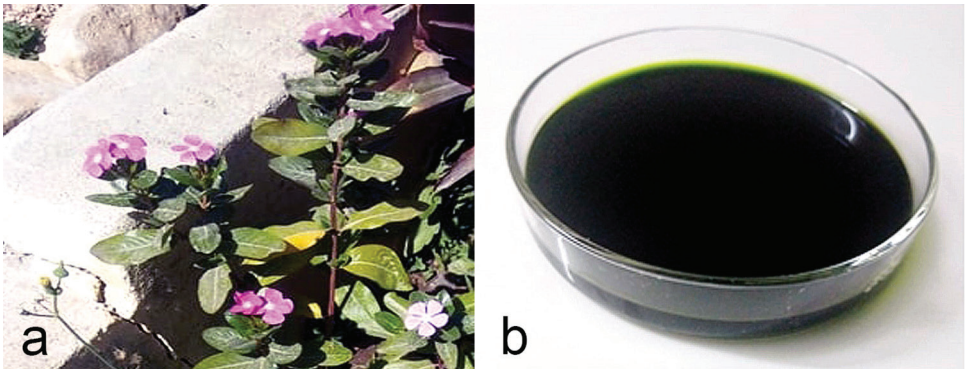

Figure 2. a) *Catharanthus roseus* plant. b) *Catharanthus roseus* aqueous extract

GC-MS analysis

The GC-MS analysis was carried out using a gas chromatography-mass spectrometry instrument TRACE GC Ultra Gas Chromatograph (Thermo Scientific Corp., USA), coupled with a thermo-mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). The analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 in the following temperature program: 60°C for 1 min, rising at 4.0°C/min to 240°C, and held for 1 min. The injector and detector were held at 210°C, and diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. The identification of the chemical constituents of the essential oil was deconvoluted using AMDIS software (www.amdis.net) and identified based on their retention indices (relative to n-alkanes C8-C22), mass spectrum matching to authentic standards (when available), the Wiley spectral library collection, and the NIST library database.

Treatment protocol and follow-up

The tumor was irrigated with 100 ml of saline solution, followed by the intratumoral injection of 0.9 ml of ESC16. One injection was administered every other day for 50 days due to the large size of the tumor mass. After the treatment was finished, the animal remained in the hospital for an additional month and was monitored for any signs of recurrence. In addition, a long-term follow-up

was obtained through telephone contact with the owner, who also provided the photographs of the animal.

GC-MS findings

GC-MS analysis revealed that *C. roseus* extract contained fifteen compounds (Figure 3 and Table 1). The retention time, molecular weight, and molecular formulas of the compounds are presented. The results detected the presence of alcohols, esters, acids, steroids, nitrogen-derived compounds, and other secondary metabolites. The highest peak was obtained for the following compounds: 9,12,15-octadecatrienoic acid, methyl ester, (Z, Z, Z)-, 6,9,12,15-docosatetraenoic acid, methyl ester, with a retention time of 23.45.

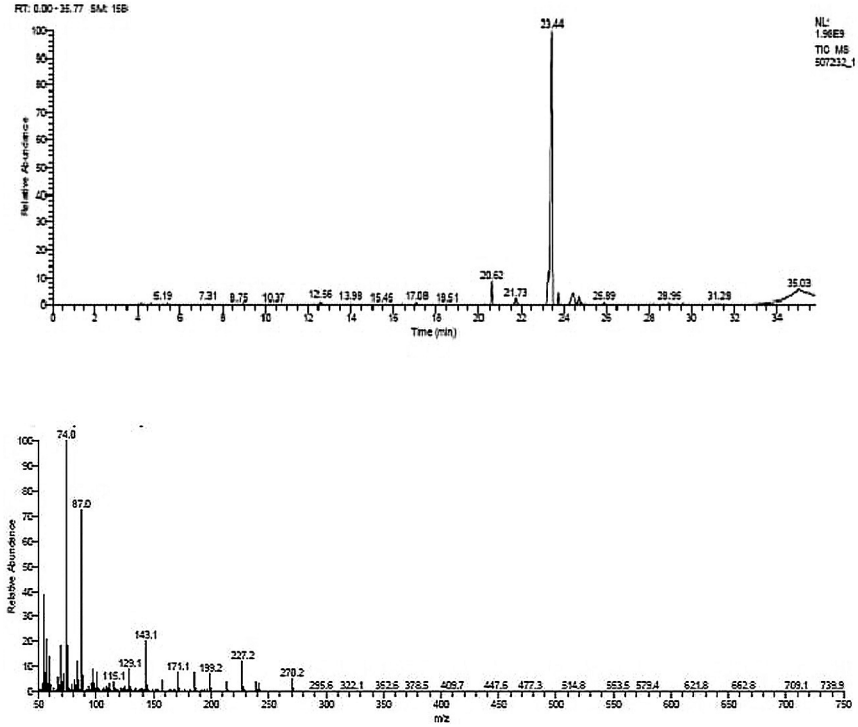

Figure 3. Chromatogram of *Catharanthus roseus* aqueous extract

GC-MS analysis of *C. roseus* revealed certain chemical compounds that have anticancer and antioxidant effects, such as 9,12,15-octadecatrienoic acid, methyl ester, (Z, Z, Z)- [Linolenic acid, methyl ester].

Table 1. GC-MS analysis of *C. roseus* aqueous extract

No	Compound	Molecular Formula	Rt	Molecular Weight	Area %
1	Hexadecanoic acid, methyl ester	C17H34O2	20.62	270	4.50
2	n-Hexadecanoic acid	C16H32O2	21.74	256	1.32
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	23.26	294	4.01
4	Methyl 9-cis,11-trans-octadecadienoate	C19H34O2	23.26	294	4.01
5	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	23.45	292	81.91
6	6,9,12,15-Docosatetraenoic acid, methyl ester	C23H38O2	23.45	346	81.91
8	Methyl stearate	C19H38O2	23.77	298	2.16
9	Trans-13-Octadecenoic acid	C18H34O2	24.43	282	3.03
10	Oleic acid	C18H34O2	24.43	282	3.03
11	Cis-Vaccenic acid	C18H34O2	24.43	282	3.03
12	Octadecanoic acid	C18H36O2	24.73	284	1.33
13	4h-1-benzopyran-4-one, 2-(3,4 dihydroxyphenyl)-6,8- Di-á-d glucopyranosyl-5,7- Dihydroxy- [6,8-DI-C-á-GLUCOSYLLUTEOLIN]	C27H30O16	35.03	610	1.74
14	1-Heptatriacotanol	C37H76O	35.03	536	1.74
15	03027205002 flavone 4'-oh,5-oh,7-di-o-glucoside	C27H30O15	35.03	594	1.74
16	Rhodopin	C40H58O	35.03	554	1.74

Clinical findings

On day 10, the tumor mass began to shrink; on day 20, it diminished, as did the swelling of the lymph node. On day 30, the tumor decreased in size by half. On day 40, the tumor was barely detected, and on day 50, it completely disappeared (Figures 4).

Histopathological findings

The microscopic examination of the tumor biopsy sample revealed a hypercellular, densely packed neoplasm (Figure 5). The mitotic figures were identified in two areas of 2.37 mm^2 and revealed numerous mitotic figures, both typical and atypical ones (Figure 5a). The average number of mitotic figures across 22 contiguous FOVs in each area was 2.14. The neoplastic cells exhibited two different cellular patterns: spindle-shaped cells with a fascicle pattern and polygonal cells with a cluster aggregation pattern (Figure 5b).

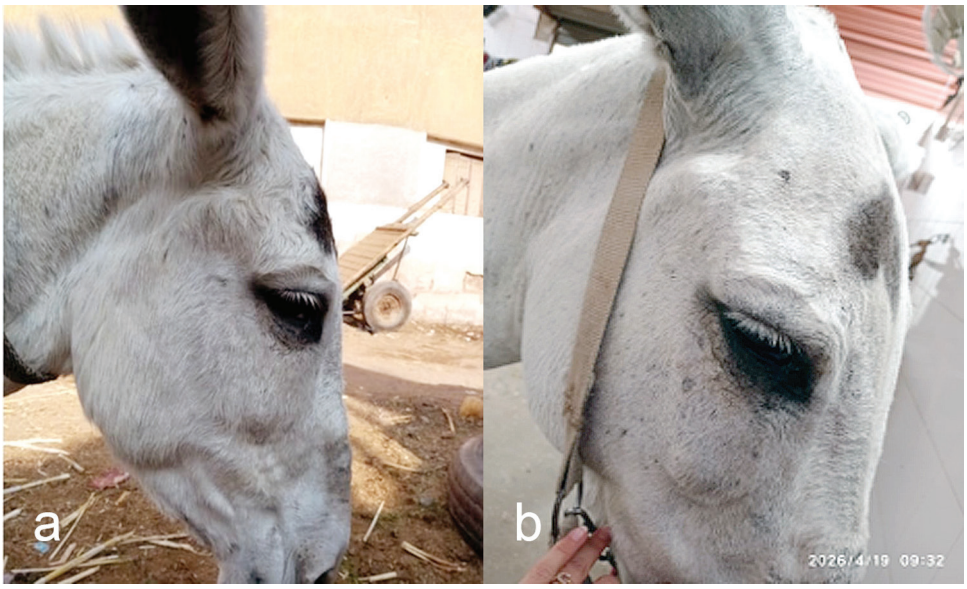

Figure 4. a) Tumor disappearance after 50-day treatment protocol with intralesional injection of Catharanthus roseus 0.9 % aqueous extract. b) At the five-year follow-up (April 2026), no tumor recurrence was found.

The neoplastic cells were of two types: polygonal and spindle. The spindle cell fibroblasts were arranged in irregular patterns of interconnected fascicles with occasional storiform patterns and dense sheets (Figure 5c) and contained hyperchromatic nuclei (Figure 5d). These cells, admixed with a pleomorphic population of histiocytes and multinucleated tumor giant cells (MGCs) with abundant eosinophilic cytoplasm, vesicular nuclei, and prominent nucleoli, showed many xanthoma cells laden with lipid vacuoles. Generally, the histiocytes exhibited anisokaryosis, nuclear hyperchromasia, and mitoses. The inflammatory reaction of mononuclear cells was evident between neoplastic cells (Figures 5e and 5f). The phytotherapeutic treatment of the tumor mass resulted in its regression and markedly affected the neoplastic cells. The microscopic evaluation of the tumor mass after phytotherapy revealed hypocellularity, massive necrosis of neoplastic cells (Figure 5g), apoptosis of histiocytes, and necrosis of fibroblasts (Figure 5h). In addition, there were no xanthoma cells or tumor giant cells in the treated tumor. Neutrophil infiltration was observed between the neoplastic cells.

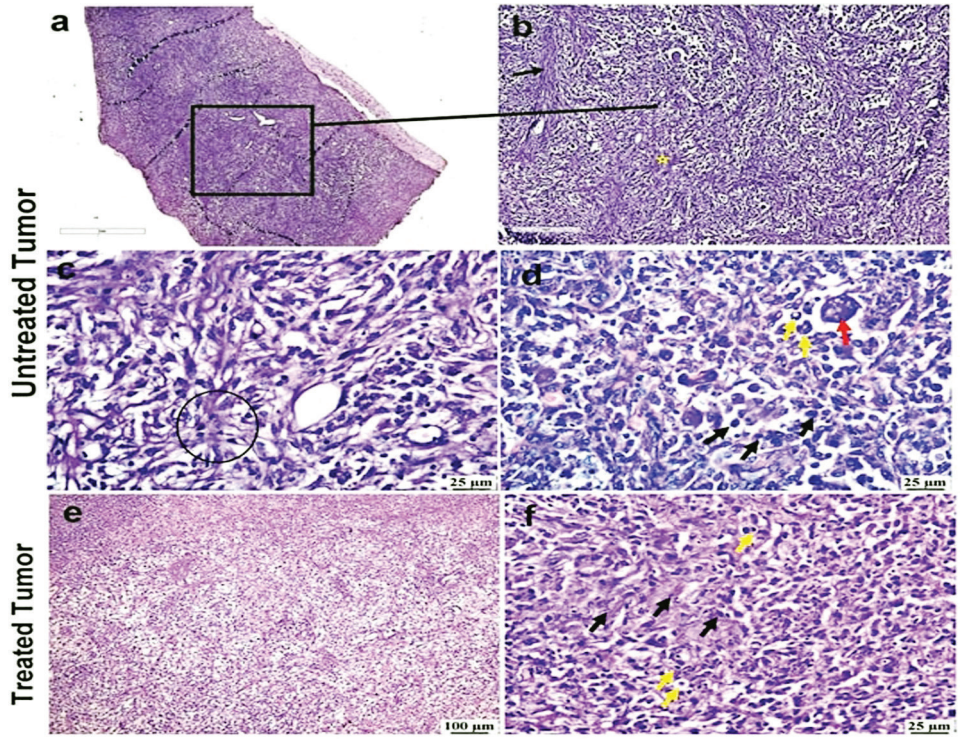

Figure 5. Histopathologic section of malignant fibrous histiocytoma, excised untreated (a-f) and treated tumor mass (g-h) showing dense cellular closely packed neoplastic cells a) with typical (black & red lines) and atypical multipolar mitotic figures (yellow line). Two different histological patterns of arrangement, irregular, interconnected, fascicles (black arrows) and clustered group population of polygonal cells (yellow arrows) (b). The spindle-shaped fibroblasts showed a storiform growth pattern (c), with spindle hyperchromatic nuclei (black arrows) associated with an inflammatory reaction in the stroma (d), The second type of pleomorphic histiocytes admixed with multinucleated giant cells (e), the neoplastic cells showing anisokaryosis, karyomegaly, and vesicular nuclei, prominent nucleoli, mitoses were evident (black arrows), xanthoma cells (yellow arrows) (f). Hypocellularity of the tumor mass (g) with necrosis of fibroblasts and apoptosis of histiocytes (black arrows) associated with an inflammatory reaction (h).

DISCUSSION

Malignant fibrous histiocytomas (MFH), now known as undifferentiated pleomorphic sarcomas (UPSs), are a group of soft tissue sarcomas that have been reported in many species, including humans, horses, dogs, cats, mice, rats, rabbits, birds, a pig and a cow (Al-Agha and Igbokwe 2008; Reesink et al. 2009). The MFH of soft parts, also referred to as a giant cell tumor, is considered rare in the horse (Marryatt, 2003). This tumor was reported in the hindquarters of

Arabian horses and mules (Hamir, 1989; Meuten, 2020) and in the forelimb quarter of a horse mare (Geburek et al. 2007). Also, previously conducted surveys confirmed the development of MFH in soft tissue in different body parts: the neck, limbs, mammary tissue, muzzle, shoulders, and trunk (Knottenbelt et al., 2015). Mammary tumors are uncommon in mares; however, MFH was documented in the mammary gland of a 6-year-old mare (Reesink et al., 2009). Ocular MFHs were recorded previously in dogs (Smith et al., 1976), cats (Demir et al., 2023), and a man (Ryan et al., 2012). The presented case revealed the development of MFH at an older age, as it was recorded at 8 years, which corresponds with previous studies (Hamir, 1989; Marryatt, 2003). Hamir (1989) stated that there is no sex bias in the development of MFH. In this case, MFH was reported in a donkey as previously documented (Valentine, 2006). Geburek et al. (2007) used conventional and laser surgery to treat MFH in the forelimb of a Quarter Horse mare. Generally, post-surgical recurrence of MFH was a variable outcome with a high rate after surgical excision. Similarly, Bellitto et al. (2023) used a harmonic scalpel to excise a large fibrosarcoma in the distal equine limb.

The tumor's histopathology validated the diagnosis of MFH. Histologically, the tumor was hypercellular and of an infiltrative growth. The neoplastic cells were composed of fibroblasts admixed with a pleomorphic population of histiocytes and multinucleated tumor giant cells with abundant eosinophilic cytoplasm and vesicular nuclei. Generally, the histiocytes exhibited anisokaryosis and nuclear hyperchromasia, and frequent mitosis. Regarding the histopathology of this tumor type, a similar microscopic description was recorded by (Marryatt et al (2003).

In the present case, we showed an 8 years-old female donkey afflicted with MFH that agrees with previous reports documented old age of animals affected with MFH (Hamir, 1989; Schneider et al., 1999a; Mair and Couto, 2006; Valentine, 2006; Knottenbelt et al., 2015). The lesion originated from the palpebral conjunctiva of the lower eyelid of the right eye, and it had a soft and solid consistency. Eye infection associated with malignant cell tumor is uncommon, according to previous studies (Geburek et al. 2007). Moreover, the lesion was assessed via radiography, which did not reveal any metastasis in the surrounding areas (Figure 6).

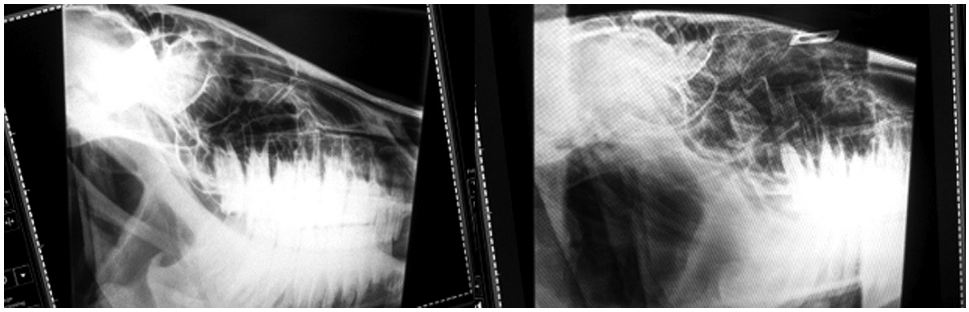

Figure 6. Radiographs revealed a non-metastatic tumor mass, as the tumor had not invaded the skull.

Catharanthus roseus (*C. roseus*) is an important medicinal plant that grows in many countries. Since *Catharanthus roseus* ("Annual Vinca") is well known for its anticancer activity (El Enshasy, 2019; Chunarkar-Patil et al., 2024), an aqueous extract of its leaves and flowers was prepared to assess its efficacy against MFH when administered via intra-tumoral injections. *C. roseus* produces the anticancer bisindole alkaloids vinblastine and vincristine, which are derived from the union of the monomeric terpenoid indole alkaloids catharanthine and vindoline (Zhu et al., 2015). The extract was characterized using GC-MS analysis. GC–MS analysis identified 15 compounds in the extract of *C. roseous*. GC-MS analysis of *C. roseous* extract showed the presence of hexadecanoic acid, methyl ester [C17H34O2], 9,12-Octadecadienoic acid (Z,Z)-, methyl ester [C19H34O2], Methyl 9-cis,11-trans-octadecadienoate [C19H34O2], 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- [C19H32O2], 6,9,12,15-Docosatetraenoic acid, methyl ester [C23H38O2].

Bansal et al. (2023) reported that the presence of hexadecanoic acid, methyl ester, as bioactive in Anther Callus of *C. roseus* L. Methyl 9-cis,11-trans-octadecadienoate is a polyunsaturated fatty acid (fatty acid ester of linoleic acid) that has anti-inflammatory and anti-carcinogenic properties (Cifuni et al., 2023). This fatty acid and its derivatives are known to exert potent antioxidant activities (El Hosry et al., 2023; Radajewska et al., 2023). 9, 12, 15-octadecatrienoic acid, methyl ester, (z, z, z)-] is an essential polyunsaturated fatty acid molecule. It was reported that 9,12,15-octadecatrienoic acid can inhibit caspase-3. It has anticancer, antibacterial, anti-inflammatory, and antioxidant properties (Vohra and Kaur, 2011; Yamunadevi et al., 2011; Akpuaka et al. ,2013; Rani et al., 2021). The extract from *C. roseus* cell cultures exhibits its most potent anticancer effects through the combined action of multiple bioactive compounds rather than any single component. Studies suggest these compounds act synergistically, enhancing their impact on cancer cells. This synergistic interaction, also noted in other plants, is currently being explored as a potential strategy for cancer therapy (Bhuyan et al., 2018).

Macroscopically, following treatment, the tumor mass has undergone regression without complications. The microscopic appearance showed hypocellularity of the tumor mass, massive necrosis involving the neoplastic cells; apoptosis of histiocytes and coagulative necrosis of fibroblasts. In addition, there were no xanthoma cells or tumor giant cells in the treated tumor. Neutrophil infiltration was observed between the neoplastic cells. It showed the direct cytotoxic effect of *C. roseus* extract on cancerous cells, resulting in tumor regression. Other previous treatments have some disadvantages; for example, cisplatin-based intralesional chemotherapy is toxic and is a hazard to personnel exposure (Théon, 1998), systemic chemotherapy is costly and leads to adverse reactions and results in potential tumor recurrence (Geburek et al., 2007). Laser treatment and its thermal effects are considered effective for inhibiting tumor growth in horses (Weigand and Gerhards, 1996). After seven weeks, a regression in the size of the lesional area was noted, as the tumor mass was initially large. Interestingly, no recurrence or relapse was reported in the female

donkey after intralesional treatment with *C. roseus* extract either during the one-month stay in the hospital or follow-up via telephone contact with the owner and photographs of the animal. Radiography confirmed that the MFH didn't extend to the hard tissues (Figure 6). MFH, in this case, was confirmed, and its metastasis to the surrounding bone was assessed through clinical and histopathological examination, and diagnostic imaging, as reported in similar cases (Reesink et al. 2009), such as in soft tissue sarcomas (Bellitto et al., 2023).

CONCLUSION

The current paper demonstrated that the aqueous extract of *Catharanthus roseus* had anti-tumor activities. *C. roseus* extract showed efficacy in the treatment of malignant fibrous histiocytoma in a donkey. Thus, it could be concluded that the intralesional injection of aqueous extract of *C. roseus* may be a potential therapeutic agent; however, this is a preliminary finding that requires further studies.

Authors' contributions

HS, AAM, AAS, FFM, MAE, and AA have equally contributed to the case description and manuscript preparation.

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The authors received no funding for this case report.

Data availability

All data generated during this study are included in this published article.

Ethical statement

The tissues were fixed and processed routinely as per any clinical ophthalmic case. This study did not influence the recommended therapy in any way. The owner provided consent for his donkey to participate in this study and for the publication of this case report and any accompanying images.

Competing interests

The authors declare no competing interests.

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TRETMAN MALIGNOG FIBROZNOG HISTIOCITOMA KOD MAGARCA EKSTRAKTOM BILJKE *CATHARANTHUS ROSEUS*– PRIKAZ SLUČAJA

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Kratak sadržaj

Maligni fibrozni histiocitom (MFH) je pleomorfni sarkom nepoznate etiologije, koji je redak kod ekvida (konja i magaraca). U septembru 2021, osam godina stara magarica sa tumorskom masom na desnom oku dovedena je u Bolnicu za životinje (ACE) u Luksoru, Egipat. Urađen je klinički i radiološki pregled životinje. Na osnovu mikroskopskog pregleda dijagnostikovan je maligni fibrozni histiocitom. Radiografijom je ustanovljena tumorska masa bez metastaza. Vodeni ekstrakt lišća i cvetova biljke *Catharanthus roseus* (ESC16) pripremljen je hladnom maceracijom i analiziran primenom GC-MS. Tumor je tretiran primenom 0,9 ml ekstrakta ESC16 koji je zbog veličine tumorske mase aplikovan intratumorskom injekcijom svaki drugi dan tokom 50 dana. Tokom perioda praćenja tumor se makroskopski smanjio. Po saznanju autora rada, ovo je prva primena ekstrakta *C. roseus* u lečenju MFH kod magarca. ESC16 se pokazao efikasnim u tretmanu MFH kod ove magarice. Na osnovu svega navedenog, može se zaključiti da vodeni ekstrakt biljke *C. roseus* primenjen u obliku injekcija u tumorsko tkivo može predstavljati obećavajući antitumorski agens protiv MFH kod ekvida u egipatskim uslovima.

Ključne reči: Maligni fibrozni histiocitom, magarci, *Catharanthus roseus*, oči, histopatologija, radiologija, gasna hromatografija, masena spektrometrija.