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Renal Histopathology and Serum Biochemistry of Cadmium-exposed Rabbit Bucks Administered Methanolic Extract of *Phoenix dactylifera* Fruit

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Abstract

Cadmium (Cd), a prevalent heavy metal pollutant, is known for its harmful effects on physiological functions within the biological system. In this study, the effects of Cd exposure on some kidney related biochemical and histopathological parameters in rabbits were investigated, alongside exploring the potential protective role of methanolic extract from *Phoenix dactylifera* (MEPD) on the kidney. The study included 45 male rabbits, aged 24-28 weeks and weighing approximately 1.41-1.43 kg were assigned into five (5) treatment groups (control, Cd-only, Cd + 300 mg MEPD, Cd + 600 mg MEPD and Cd + 900 mg MEPD) in a completely randomized design. The rabbits received a 7-day dose of 3 mg CdCl₂/kg feed, followed by MEPD every 72 hours for 28 days. Results revealed higher significant ($P < 0.05$) differences in serum glucose, urea and creatinine in Cd-only treated group when compared with the control group. MEPD treatment significantly ($P < 0.05$) lowered serum glucose levels in Cd-exposed rabbits, indicating a potential glycemic modulating activity, but had no significant ($P > 0.05$) effect on urea and creatinine concentrations. High density lipoprotein (HDL), low-density lipoprotein (LDL) and cholesterol ratios were not significantly different but showed marginal improvement in the Cd+MEPD groups compared to the Cd-only group. The findings also revealed significant differences in both absolute and relative kidney weights between the group treated with Cd alone and the control group, indicating potential renal injury induced by Cd. Histopathological examination revealed kidney damage in Cd-exposed rabbits, with observed changes such as disruption of Bowman's capsule, glomerular shrinkage, degeneration of renal tubules and haemorrhage. The administration of MEPD did not ameliorate the observed histopathological alterations. In conclusion, this study suggests that Cd exposure may induce hyperglycemia and kidney injury in rabbits. MEPD treatment demonstrated some protective effects against Cd-induced hyperglycemia but did not significantly mitigate kidney damage.

Keywords: Blood, cadmium, date, nephrotoxicity, phytochemistry.

INTRODUCTION

There have been growing concerns in industrial activities and human interventions which have caused a surge in environmental pollution, significantly threatening ecosystems and organisms (Özkara and Akyıl, 2019; Bashir et al., 2020; Siddiqua et al., 2022). Among these pollutants, heavy metals like cadmium have emerged as potent nephrotoxic agents, causing detrimental effects on renal function in exposed organisms (Genchi et al., 2020). The kidneys, being primary sites of cadmium accumulation, are highly susceptible to its detrimental impact, leading to oxidative stress, inflammation, and impaired serum biochemistry (Satarug, 2018).

Cadmium toxicity is a multifaceted issue, and understanding the intricate biochemical responses is imperative for developing effective interventions. The quest for novel, safe, and natural compounds has led to the exploration of plant-based remedies. *Phoenix dactylifera*, commonly known as date palm, has emerged as a potential candidate due to its rich composition of bioactive compounds with antioxidant properties (Al-Alawi et al., 2017). The methanolic extract derived from the fruits of

Phoenix dactylifera has demonstrated significant antioxidant and cytoprotective effects, making it a subject of interest (Ferdosh et al., 2023).

This study seeks to bridge the gap between environmental toxicology and natural interventions by investigating the kidney and serum biochemistry response of cadmium-exposed rabbit bucks administered with the methanolic extract of *Phoenix dactylifera* fruit. The intricate interplay between cadmium-induced renal damage and the potential protective effects of the date palm extract will be explored to provide valuable insights into the therapeutic strategies against heavy metal toxicity.

This investigation holds significance not only in advancing our understanding of the potential therapeutic applications of *Phoenix dactylifera* in combating heavy metal toxicity but also in contributing valuable data to environmental toxicology. The findings may lead to the development of novel strategies to prevent or alleviate the detrimental effects of cadmium exposure on renal health, benefiting both animal and human populations exposed to environmental pollutants.

MATERIALS AND METHODS

Ethical consideration

This experiment was approved and conducted according to the provisions of the Ethical Committee on the use of animals for biomedical research at the University of Benin, Benin City, Nigeria.

Location of study

The study was conducted at the Rabbitary Unit within the Teaching and Research Farm of the University of Benin, Benin City, Edo State, Nigeria.

Acquisition of *Phoenix dactylifera* fruits

Dates from *Phoenix dactylifera* were obtained from the Nigerian Institute for Oil Palm Research (NIFOR) in Benin City, Edo State, Nigeria. A botanist authenticated the acquired fruits, which were then split, air-dried, finely ground using an electric blender, and stored in an airtight container.

Preparation of fruit extracts

A quantity of 0.5 kg of ground *Phoenix dactylifera* fruits was measured and subjected to extraction using 99 % methanol in a soxhlet apparatus. The resulting extract underwent concentration through the recovery of methanol using a rotary vacuum evaporator, and the concentrated extract was then stored in an airtight container.

Acute toxicity evaluation

Acute toxicity of MEPD fruit was studied using the method outlined by Lorke (1983). Twenty five (20) matured albino rats of both sexes with body weight range of 25 to 30 g were randomized into five groups of four rats per group. Each group received a single dose of NBL extract at 0, 500, 1000, 2000 and 3000 mg/kg body weight, respectively, through oral routes, while the control group received normal saline at 10 ml/kg body weight. The rats were closely observed for signs of toxicity for 72 hours.

Experimental materials and management

In this study, a total of forty-five (45) composite rabbit bucks, aged 24 to 28 weeks and weighing between 1.41-1.43 kg, were used. The rabbits were individually housed in cages measuring 50×55×40 cm, each equipped with a feeder and a drinker. Before the commencement of the experiment, the rabbits underwent a two-week quarantine period during which they received Ivmec® injections to control haemoparasites, internal, and external parasites. Throughout the study, the rabbits were provided with *ad libitum* access to both water and a commercial growers' diet (15% crude protein; 2700 kcal/kg metabolisable energy).

Experimental design

The treatment procedures involved the randomization of rabbit bucks into five groups: group 1 (control), group 2 (3 mg of CdCl₂/kg feed/day for 7 days), group 3 (CdCl₂/kg feed/day for 7 days + 300 mg/kg body weight of MEPD fruits for 28 days), group 4 (CdCl₂/kg feed/day for 7 days + 600 mg/kg body weight of MEPD fruits for 28 days), and group 5 (CdCl₂/kg feed/day for 7 days + 900 mg/kg body weight of MEPD fruits for 28 days). Each treatment group was replicated three times, with three bucks per replicate in a completely randomized design.

Blood collection

At the end of the 28-day experiment, a 5 ml blood sample was collected from the rabbit bucks via the ear vein using a needle and syringe between 0600-0900 hours. The drawn blood was transferred into plain sample bottles for serum biochemical analysis.

Determination of serum biochemical

Serum contents of glucose, creatinine and urea were measured spectrophotometrically (Photometer 5010®-Boehringer Mannheim) following the procedures defined in the commercial test kits (Biolabo, France), while serum Na⁺, K⁺, HCO₃⁻, and Cl⁻ were analysed on an ion selective device (ISE®-Medica). Serum triglycerides, total cholesterol, high density lipoprotein (HDL), and low density lipoprotein (LDL) were determined by using the diagnostic kit (Pars Azmoon Kit, IRI) on an automatic analyser (Abbot, model Alcyon 300, USA).

Evaluation of kidney weight

Following the blood sample collection, the experimental rabbit bucks were humanely sacrificed by stunning using a captive bolt, immediately followed by exsanguination. The kidneys were harvested and weighed using an electronic scale.

Renal histopathological study

The kidneys obtained from the assessment of kidney weight were transversely cut and immersed in Bouin's fluid for 24 hours. The tissues were washed in ascending ethanol concentrations (50%, 75%, and 100%) and were then cleared with xylene. Following embedment in paraffin wax, the tissues were sectioned using a microtome at a thickness of 4-5 µ. Dewaxed sections were stained with Hematoxylin and Eosin (H&E). DPX mountant (Distyrene, Plasticizer, and Xylene) was applied to enhance the refractive index of the stained preparation, and cover slips were added to prevent scratches. Examination of all sections was conducted under a light microscope at ×40 magnification. Olympus photomicroscope was utilized for capturing photomicrographs of the renal tissues to observe and document histopathology.

Statistical analysis

Obtained data were subjected to statistical analysis using the Analysis of Variance (ANOVA) procedure in GenStat 12th edition at a 5% probability level. Significant mean differences were identified through the application of the Duncan Multiple Range Test (DMRT) within the same statistical software.

RESULTS

Table 1 presents the mean values of glucose, electrolyte, urea, creatinine and lipid profile of cadmium exposed rabbit bucks treated with methanolic extract of *Phoenix dactylifera* fruit.

The result presented in Table 1 indicates significant differences (P<0.05) in serum levels of glucose, urea and creatinine. The serum glucose level in the Cd-only group was significantly higher than that in the control group. Additionally, the control group exhibited significantly lower levels of serum urea and creatinine (P<0.05) compared to the Cd-exposed groups. Nevertheless, treatment with MEPD significantly (P<0.05) decreased the serum glucose level, although no significant reduction was observed in urea and creatinine concentrations compared

to the control group. Notwithstanding that the mean values of the lipid panel, including HDL, LDL, and cholesterol ratios (TC/HDL and LDL/HDL) were not significantly ($P > 0.05$) different in the Cd+extract treated groups, they showed marginal improvement in their

respective ratios when compared to the Cd-only exposed rabbits.

The results of performance, absolute and relative kidney weight of rabbit bucks studied in this finding are presented in Table 2.

Table 1. Glucose, electrolytes, urea, creatinine, and lipid profile of cadmium exposed rabbits administered MEPD

Parameters	Control	Cd	Cd + 300 MEPD	Cd + 600 MEPD	Cd + 900 MEPD	SEM
Glucose (mg/dl)	113.30 ^b	139.00 ^a	110.70 ^b	116.70 ^b	101.70 ^b	7.28
Urea (mg/dl)	20.67 ^c	25.33 ^b	27.33 ^b	30.00 ^a	26.67 ^b	2.80
Na ⁺ (mmol/L)	144.00	143.30	150.00	144.70	147.70	2.32
K ⁺ (mmol/L)	6.37	5.63	6.57	5.63	5.40	0.38
Cl ⁻ (mmol/L)	105.00	103.00	104.33	102.67	102.00	1.80
HCO (mmol/L)	19.33	19.33	18.33	19.00	19.33	1.42
Creatinine (mg/dl)	0.67 ^b	0.94 ^a	0.91 ^a	0.80 ^a	0.80 ^a	0.09
Total cholesterol (mg/dl)	49.33	55.00	55.67	51.67	54.67	7.28
HDL (mg/dl)	30.00	30.67	40.00	35.00	33.67	3.90
LDL (mg/dl)	14.40	13.67	11.73	9.00	12.90	4.83
TC/HDL	1.63	1.87	1.38	1.49	1.65	0.17
LDL/HDL	0.50	0.46	0.28	0.27	0.39	0.13

^{a,b,c} Means bearing different letters of superscript within the same row differ significantly ($P < 0.05$). HDL- High density lipoprotein; LDL- Low density lipoprotein; TC- Total cholesterol.

Table 2. Performance, absolute and relative kidney weight of cadmium exposed rabbits administered MEPD

Parameters	Control	Cd	Cd + 300 MEPD	Cd + 600 MEPD	Cd + 900 MEPD	SEM
Initial weight (kg)	1.43	1.42	1.43	1.41	1.42	0.08
Final weight (kg)	1.67	1.53	1.57	1.62	1.62	0.07
Weight gain (kg)	0.23	0.12	0.13	0.18	0.20	0.04
Feed intake (g/day)	89.30	73.00	82.3	85.3	92.0	6.68
Mortality	0.00	1.00	0.33	1.00	0.67	0.42
Kidney (g)	7.60 ^b	8.83 ^a	9.10 ^a	9.23 ^a	8.63 ^a	0.31
Relative kidney weight (expressed as percentage of body weight)						
Kidney	0.46 ^b	0.59 ^a	0.55 ^{ab}	0.56 ^{ab}	0.54 ^{ab}	0.04

^{a,b} Means bearing different letters of superscript within the same row differ significantly ($P < 0.05$).

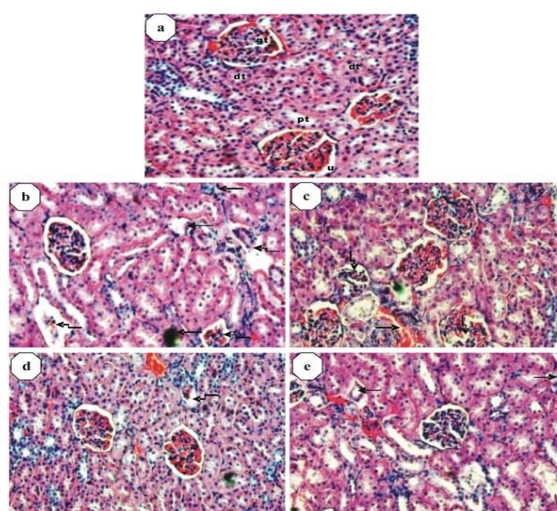


Figure 1. A photomicrograph of the kidney of cadmium exposed rabbits administered MEPD fruit stained with H&E.

(a) Renal tissue of the control group demonstrating normal appearance of glomerular tuft (gt), urinary space (U), Bowman's capsule, proximal tubule (pt), distal tubule (dt) with their nuclei. (b) Renal tissue of Cd-only treated rabbit showing; Disruption of Bowman's capsule, shrinking and degeneration of some glomeruli (elbow arrow), degenerated cytoplasm of some cells of the renal tubules (thin arrow). (c – e): Renal tissues of rabbit treated with Cd+300, 600 and 900 mg/kg MEPD showing. (c) Shrinking and degeneration of some glomeruli (elbow arrow), haemorrhage (thin arrow) (d) shrinking and degeneration of some glomeruli (elbow arrow) (e) degenerated cytoplasm of some cells of the renal tubules (thin arrow).

The results of performance, absolute and relative kidney weight of rabbit bucks in Table 2 revealed that significant ($P < 0.05$) differences existed in absolute and relative kidney weight between Cd-only treated and the control rabbit groups. The administration of MEPD did not significantly ($P > 0.05$) moderate the higher mean values of absolute and relative kidney weights recorded in the Cd-treated groups.

The result of histopathological study of kidney is presented in Figure 1.

DISCUSSION AND CONCLUSION

The assessment of acute oral toxicity in this study revealed that the MEPD fruit caused neither mortality nor observable health concerns in the test rats, even when given at the highest dose of 3000 mg/kg over the 72-hour monitoring period. The rats exhibited typical behaviour, retained their regular feeding patterns, and showed no variation in the consistency of their droppings compared to the control group.

Results on glucose, electrolyte, urea, creatinine and lipid profile in this study shows that serum levels of fasting blood glucose in the Cd-only treated group was significantly higher ($P < 0.05$) when compared to the normal control group. The Cd-MEPD treated groups showed marked reduction in serum blood glucose and the mean values were in agreement with the normal reference value of 122 ± 15 mg/dl reported by Hewitt et al., (1989). The outcome of this study indicates that Cd has the capability to induce hyperglycemia in rabbits, and that the detrimental effect can be mitigated through the use of date extract. Cd is believed to bring about hyperglycemia through various mechanisms. According to Shanbaky et al., (1978), Cd prompts an increase in catecholamine release, indicating an epinephrine-mediated response to carbohydrate metabolism. Epinephrine, in turn, stimulates hepatic glycogenesis and inhibits insulin release (Merali and Singhal, 1980). Additionally, Lei et al., (2007) demonstrated that Cd leads to the destruction of β -cell-rich pancreatic islets, reducing the number of functional β -cells. This supports an earlier study by Bell et al., (1990) documenting Cd-induced atrophy of islets and a decline in insulin secretory activity. Hence, it could be conceived that the increased glucose in this study following cadmium administration might be attributed to reduced insulin levels resulting from the cytotoxic effects on the pancreas caused by cadmium. Nevertheless, the co-administration of date extract after cadmium exposure resulted in a decrease in the mean glucose concentration in the experimental rabbit bucks. This suggests that date extract possesses potential glycermic modulating properties.

Results of serum urea, electrolytes (Na^+ , K^+ , Cl^- , HCO_3^-) and creatinine were not significantly different ($P > 0.05$) and the mean values obtained conformed with the range of normal serum biochemical parameters investigated by Hewitt et al., (1989), Burnett et al., (2006) and Özkan et al., (2012) for healthy rabbits. The higher significant ($P < 0.05$) concentrations of urea and creatinine in the Cd-only and Cd-extract treated groups revealed that Cd may have impaired the functional integrity of the kidney of rabbits used in this study.

Although serum lipid panel (Total cholesterol, HDL and LDL) screening in this study showed no significant ($P > 0.05$) differences, however, in an attempt to optimize the predictive capacity of lipid profile with greater predictive value than isolated parameters used independently, total cholesterol/HDL cholesterol and

LDL/HDL cholesterol ratios as defined by Millan et al., (2009) is here adopted. Hence, individuals with elevated total cholesterol/HDL cholesterol or LDL/HDL cholesterol ratios have an increased cardiovascular risk due to an imbalance between atherogenic and protective lipoproteins (Criqui and Golom, 1998). This imbalance may result from a rise in the atherogenic component in the numerator and a reduction in the anti-atherosclerotic trait of the denominator, or a combination of both. Despite the lipid profile mean values falling within the normal range as reported by Burnett et al., (2006), the observation of the numerical decline in the total cholesterol/HDL and LDL/HDL ratios within the Cd-extract groups, compared to the Cd-only group, suggests that MEPD might have a modulatory effect on cadmium-induced hyperlipidemia in rabbits.

Clinical observations indicated that rabbits exposed to feed contaminated with Cd exhibited a slight reduction in appetite and were docile compared to those in the control group. Within the 7 days of Cd administration, nine (9) cases of mortality were recorded, each preceded by audible gasping indicative of respiratory distress.

The kidney, being a well-perfused organ, eliminates large quantities of toxic substances from the blood through urine. Occasionally, the proximal convoluted tubule of the kidney becomes a target for exposure to toxic substances. Changes in organ weight are commonly linked to treatment-related effects. This study reveals a significant ($P < 0.05$) difference in both the absolute and relative mean weight of the kidney. Comparing the results of key kidney function indices (urea and creatinine) with the kidney weight obtained, it suggests potential injury to the renal architecture of rabbits in the Cd-only and Cd+extract treated groups.

Previous research by Zeng et al., (2003) and Amara et al., (2008) had shown a decrease in body weight as a primary sign of CdCl_2 administration in rats due to appetite loss. Also, Sajjad, (2014) documented a significant ($P < 0.05$) reduction in body weight in male rabbits orally administered with 1.3 mg/kg body weight of CdCl_2 for 5 weeks. Similar effects were observed by Sant 'Ana et al., (2005) in Japanese quail exposed to 100 ppm CdCl_2 for 28 days. de Souza Predes et al., (2010) noted a temporary reduction in kidney weight after 7 days, which normalized after 56 days post-cadmium administration, suggesting that the initial weight loss in the kidney was a temporary change overcome by the natural defenses of the animal. Contrastingly, Abduljaleel and Shuhaimi-Othman, (2013) found that dietary Cd at concentrations of 5, 25, and 100 ppm did not significantly ($P > 0.05$) affect the final body weight of chicks in their study. Haouem and El-Hani, (2013) reported that rats fed 1.1 mg of Cd/g of diet showed body weight gain identical to control rats. Chapatwala et al., (1982) reported a non-significant increase in total kidney weight when exposed to different doses of cadmium for four weeks. Similarly, Dwivedi, (2015) observed no significant ($P > 0.05$) effects on heart, kidney, and liver weights in albino rats treated for 30 days with a dose of 2.6 mg/kg.b.wt. CdCl_2 .

In this study, the administration of Cd to rabbit bucks resulted in nephrotoxicity, particularly evident in glomerular and tubular changes. The observed effects included the shrinking and degeneration of glomeruli, as well as pyknosis and degenerated cytoplasm in certain renal tubule cells. These findings align with the research conducted by El-Refaey and Eissa, (2013), who noted Cd-induced impact on glomeruli, specifically affecting

glomerular capillaries, favouring Bowman's space, and leading to the atrophy of some glomeruli. Various histopathological studies have consistently shown the toxic effects of Cd on the kidney, including edema (Choi and Rhee, 2003), proximal tubular necrosis, apoptosis, and tubular degeneration (Damek-Poprawa and Sawicka-Kapusta, 2004). The nephrotoxicity induced by Cd in this study may have been mediated through the formation of the cadmium metallothionein (Cd-Mt) complex. This complex, synthesized in the liver, is released into circulation, taken up by renal proximal tubule cells, and, when insufficiently bound by metallothionein, causes injury to hepatocytes and is released into the bloodstream. Subsequently, the complex passes through the glomeruli, reaching the proximal tubule and leading to a gradual loss of kidney function (El-Refaiy and Eissa, 2013). On the other hand, the observed histopathological results may be a direct consequence of the toxic effect of Cd on the kidney. Renugadevi and Prabu, (2009) proposed that histopathological changes contributing to nephrotoxicity may result from the accumulation of free radicals due to increased lipid peroxidation caused by free Cd ions in renal tissues of Cd-treated rats.

Furthermore, the histopathological results in the kidney tissue are consistent with the elevated levels of urea and creatinine (refer to Table 1) detected in rabbits subjected to Cd exposure in this investigation. Although Al-Qarawi et al., (2008) and Hammed, (2015) reported that the administration of date extract can mitigate renal histopathological effects by reducing plasma creatinine and urea concentrations, as well as ameliorating damage to the proximal tubular regions of kidneys; the altered histomorphological structures in this study remained unaffected by MEPD treatment.

The current results indicate that short term exposure to Cd could lead to kidney toxicity. There are indications that MEPD holds potential for regulating blood glucose levels, yet, may not offer kidney protection against Cd-induced dysfunction.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Research Design: A.A., Data Analysis/interpretation: A.A., Data Collection: A.A., O.A., Literature Search: A.A., O.A., Writing: A.A.

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Ethical Approval

This experiment was approved and conducted according to the provisions of the Ethical Committee on the use of animals for biomedical research at the University of Benin, Benin City, Nigeria.

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A Retrospective Study on The Evaluation of Hind Limb Bone Fractures in Cats

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Abstract

As a result of many traumatic factors such as traffic accidents, falls from height, gunshot wounds and animal fights with each other, orthopedic and soft tissue damage, especially fractures, occur in the musculoskeletal system. Among the fractures within these lesions, the incidence of hind limb long bone fractures is higher than other bones. While the incidence of femur fractures among other fractures in cats is 20-26%, tibia fractures constitute a significant proportion of approximately 20% of other fractures. This study aimed to retrospectively evaluate the distribution, etiology, treatment, and treatment results of fractures in cats with hind limb bone fractures (n=92). In addition to the use of intramedullary nails and plates, external fixators are also used for the treatment of hind limb bone fractures. Despite the advantages and disadvantages of each technique, most of the time no problems are observed during the application of these techniques and in the postoperative period. A functional recovery was observed in all cats at postoperative follow-up. As a result, it can be said that the method to be used in hind limb bone fractures in cats varies according to the surgeon's preference, experience, and existing orthopedic material inventory. However, anatomical locking plates have the advantages of not using postoperative bandages and allowing the limb to be used early.

Keywords: Cats, external fixator, femur, fracture, intramedullary pins, plate, tibia.

INTRODUCTION

Lesions that occur due to disruption of the integrity of bone tissue are defined as fractures. Fractures and their treatment constitute the most important part of veterinary orthopedic practice. Many different fixation techniques have been developed in fracture treatment. The main goal in all of these is to ensure fracture healing in the most accurate way in the shortest time (Şen et al., 2015).

Traumatic situations such as traffic accidents, falls from height, gunshot wounds and other animal attacks are the main cause of fractures in cats, as in many animals (McDonald et al., 2017; Sağlam et al., 2019). Among all fracture cases, femur fractures constitute 20 to 26 percent, while tibia fractures represent 20 percent (Langley-Hobbs et al., 1996; Sancak et al., 2014).

Traumatic fractures vary depending on the intensity of the impacting force and the ability to absorb them. It can vary from a small crack to a fracture in one or more bones to dislocation in the adjacent joints (dislocation with fracture). The force that creates a fracture may not only cause a fracture in the bone, but also injure the skin, muscle, vessel, tendon, ligament, nerve and adjacent tissues and organs around the bone (Sağlam et al., 2019).

The reason for fractures in the hind limbs in cats can be instinctively associated with survival. The risk of mortality is low in cases where the caudal part of the body is exposed to trauma, and cats tend to use their

hindquarters to absorb the force of the traumatic factor at the time of trauma (Cardoso et al., 2016).

The basic approach to a patient exposed to trauma should include rapid assessment of respiratory, circulatory and neurological conditions without stressing the patient (Zurita M, 2022).

Clinical, neurological and radiological examinations of hind limb-related cases in cats must be performed carefully and in detail. While a traumatic effect creates a lesion in the area it directly affects, each case should be systematically examined for other possible lesions. The treatment method should be decided by taking into consideration the type of fractures, their localization, displacement status, and the temperament of the patient (Sağlam et al., 2021).

This study aimed to retrospectively evaluate the distribution, etiology, treatment, and treatment results of fractures in cats with hind limb bone fractures (n=92).

MATERIALS AND METHODS

In this study, 92 cats of different ages, breeds and genders with hind limb fractures were evaluated between 2021 and 2023 at Dicle University Faculty of Veterinary Medicine Department of Surgery.

The study was conducted based on clinical examination findings, radiographic findings and treatment results. A clinical follow-up form was created for each

patient, and these forms included the patient's description, the cause of the fracture, treatment, and outcome information. First, a detailed clinical examination was performed on each patient, and then bilateral (mediolateral or laterolateral and ventrodorsal or craniocaudal) radiographs were taken. Following the identification of the fracture(s) on the radiograms, a surgical treatment was planned. Since fracture patients had a history of trauma, a general trauma protocol (Airways-breathing-circulation (ABC) assessment) was applied and patients who needed oxygen support were supported with an oxygen cage or intensive care cabin.

All operations were performed under general anesthesia. 1 mg/kg IM (intramuscular) xylazine was injected as a premedication and 15 mg/kg IM

(intramuscular) ketamine was injected as a general anesthetic. Additionally, maintenance anesthetic (ketamine) was administered at half the initial dose for long operations. For pain management, meloxicam SC (subcutaneous) was administered preemptively as a single dose of 0.3 mg/kg. In addition, 0.9% NaCl solution was given IV at a dose of 10 ml/kg/hour to each anesthetized animal during the operation period. In addition, cats were routinely monitored with VAS and Glasgow (as previously defined Teixeira et al., 2020) pain scale in the first 24 hours postoperatively, and 0.3 mg/kg meloxicam was administered postoperatively to those requiring analgesics. In the postoperative period, antibiotics (35 mg clavulanic acid, 140 mg amoxicillin) were recommended to the patients for 5 days.

Table 1. The types of fractures and the treatments applied.

Broken bone	Fracture type		U/B, M	Applied treatment	Treatment result			
					+++	++	+	-
Femur	Proximal n=7	3 caput	3 U	Resection	3	-	-	-
		1 collum	1 U	Resection	1	-	-	-
		1 trochanteric	1 U	K wire tension band	1	-	-	-
	Diaphyseal n=14	2 subtrahonteric	2 U	2 Pin	1	-	1	-
		7 spiral	7 U, 3 M	7 Pin	3	2	1	1
		2 transversal	2 U, 1 M	1 Pin	-	1	-	-
				1 plate	1	-	-	-
		3 oblique	3 U	1 Pin	-	1	-	-
				1 plate	-	1	-	-
				1 external	-	1	-	-
		2 multiple	2 B	2 Pin+ cerclage	-	2	-	-
	Distal n=33	19 supracondylar	4 B, 15 U, 3 M	5 Pin (rush or cross)	-	3	2	-
				14 Plate	7	4	3	-
		14 condylar (Salter Harris I-IV)	14 U	6 Pin (rush or cross)	1	2	3	-
				8 L plate	3	5	-	-
Patella	-	-	-	-	-	-	-	-
Tibia/fibula	Proximal n=3	3	3 U	2 cross pin	2	-	-	-
				1 Plate	1	-	-	-
	Diaphyseal n=27	3 spiral	1 B, 2U	3 plate	2	1	-	-
		7 oblique	7U	4 plate	2	2	-	-
				3 External	2	1	-	-
		8 transversal	8U	8 plate	3	4	1	-
		9 multiple	7 U, 2B	6 pin	3	2	1	-
				3 plate	1	2	-	-
	Distal n=14	14	13U, 1B	6 cross pin	1	4	1	-
				7 T-L plate	6	1	-	-
				1 pantarsal arthrodesis	-	1	-	-
Tarsus		1 calcaneus	1 U	1 K wire tension band	-	1	-	-
		6 talus	5U, 1B	6 pantarsal arthrodesis	4	2	-	-
Metatarsus		3	3U	3 Pin	-	3	-	-
Phalanx		4	4U, 2M	4 Pin	-	3	1	-
Total	112 fractures (92 cats)		23 bilateral, 69 unilateral	Pin: 45, Plate: 50, External: 4, Tension band:2	+++ 48, ++ 49, + 14, -:			

U: Unilateral, B: bilateral, M: Multiple, +++: very good recovery, ++: good recovery, +: poor recovery, -: fair recovery

*Multiple unilateral or bilateral fractures may occur in an animal at the same time.

After the preparation for the operation was completed, the patients included in the study were placed in an appropriate position according to the location and condition of the fracture. Asepsis-antisepsis of the area was ensured and the area was limited with sterile drapes. Then, a suitable technique among intramedullary pin application (Figure 1A), plate osteosynthesis (Figure 1B) and external fixation techniques (Figure 1C) was performed and records were taken. In intramedullary pin applications, the extremity was kept in a protective bandage for 10 days.

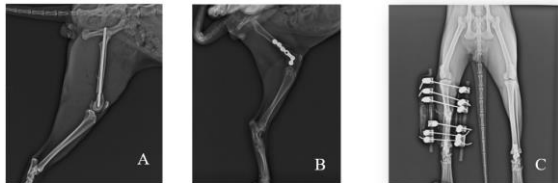


Figure 1. Image of some of the cases; A-intramedullary pin application, B-plate osteosynthesis and C- external fixation techniques.

Postoperative outcomes were followed and results were very good (+++: no lameness and normal gait), good recovery (++: mild lameness after intense exercise), poor recovery (+: stepping on but moderate or mild lameness), and fair recovery (-: non-bearing lameness). Additionally, postoperative complications were categorized as complications related to the applied technique, complications related to the implant, and complications related to the patient.

RESULTS

The average age of the cats evaluated within the scope of the study was determined to be 1.89 ± 1.66 (min: 035, max 11) years old. Their breeds were 67 tabby cats, 1 Persian cat, 5 Van cats, 2 Angora cats, 6 British Shorthairs and 9 Scottish Folds. Additionally, 48 of these cats were female and 44 were male (castrated or not). The weight of the cats was calculated as 3.46 ± 1.50 . According to the information in the study records, the etiologies were observed as falling from height in 62 cases, traffic or vehicle accidents in 21 cases, other animal attacks in 3 cases, and unknown causes in the remaining 6 cases.

The depth and duration of anesthesia during the operation were sufficient to complete the operations.

According to the information obtained from the study data, the types of fractures and the treatments applied are given in Table 1.

Postoperative follow-up showed poor recovery in 11 cats, and 3 of them had bilateral fractures. Additionally, only unilateral pin migration was observed in 1 cat whose bilateral condylar fracture was treated with rush pin application, and a functional recovery was observed after re-fixation with an L plate. Nonunion was observed in 1 cat with diaphyseal femur fracture, and clinical recovery was poor. No complications such as fracture or bending of the implant were observed in all cats, and no complications related to infection or wound area were observed. However, there were 8 cats that had regional atrophy and mild circulatory impairment due to the bandage. Additionally, pin base infection was observed in 2 of 4 cats that received external fixator.

DISCUSSION AND CONCLUSION

Recently, especially after the pandemic, there has been a significant increase in the number of animals being cared for at home. Parallel to this increase, there is a significant increase in the number of animals exposed to trauma. Although bone fractures are one of the lesions seen in animals exposed to trauma, it is also a frequently encountered condition in domestic animals (Sağlam et al., 2021; Özden, 2022). Long bone fractures in cats occur most often in the hind limbs. In cases where the caudal part of the body is exposed to trauma, immediate trauma management is required, even if the risk of mortality is low. In addition, cats tend to use their hindquarters to absorb the force of the traumatic agent at the time of trauma (Scott, H., 2005; Cardoso et al., 2016, Gülaydın and Alkan, 2023). The incidence of fractures is especially high in the femur and tibia bones that form the hind extremity. The distribution of observed femur fractures constitutes 20-26% of all fracture cases. Tibia and fibula fractures are encountered in 20% of general fracture cases. This is due to the fact that the area is surrounded by anatomically weak soft tissue and the body weight is given to the rear extremity at a higher rate (Yardımcı and Çetinkaya, 2007). In this study, it was aimed to retrospectively evaluate the fracture cases occurring in the hind limb bones in cats using different osteosynthesis techniques such as intramedullary pin, plate osteosynthesis, external fixator and tension band, and evaluating the clinical and radiographic findings in the postoperative period, the degree of healing and the complications that occurred.

Many factors that directly affect cats are known to cause trauma, especially traffic accidents and falls from height. It has also been reported that animal fights with each other and traumas of unknown origin are also effective in the formation of fractures (Sağlam et al., 2021). In this study, the cause of hind limb fracture was determined as 67.39% falling from height, 22.83% traffic accident, 3.26% other animal attack and 6.52% trauma of unknown cause. It is thought that traffic accidents, high-rise buildings and hot climate in a big city like Diyarbakır are effective on this situation.

Fractures occurring in the femur can be classified as proximal, diaphyseal and distal fractures. Caput femoris, collum femoris, trochanteric and subtrochanteric fractures can be considered as proximal fractures. It is possible to classify diaphyseal fractures as spiral, transversal, oblique, simple and comminuted fractures. Supracondylar and condylar fractures are included in the classification of distal fractures and can be classified in their own way (T, Y or Salter Harris I-V) (Robetson and Meeson, 2022). In a study on the subject (Piermattei et al., 2006), it was reported that femur fractures were more common than other bone fractures in the whole body. However, the classification of fractures in the tibia is simpler and is generally classified as proximal, diaphyseal and distal (Sancak et al., 2014). In the study, a total of 112 fractures, unilateral or bilateral, were observed in 92 cats. These fractures were 48.21% in the femur, 39.29% in the tibia, 6.25% in the tarsus, 2.68% in the metatarsus, and 3.57% in the phalanx. Of the 54 femur fractures, 12.96% occurred in the proximal femur, 25.93% occurred in the diaphysis of the femur, and 61.11% occurred in the distal femur. It was observed that 6.82% of the tibia fractures occurred in the proximal tibia, 61.36% in the diaphysis of the tibia, and 31.81% in the distal tibia.

Fractures that may occur in bones will lead to structural and functional changes in the musculoskeletal structure and may lead to permanent defects if appropriate treatment is not applied. A number of factors, such as the condition of the fracture, the surgeon's experience, the structure of the broken bone, the patient's age, weight, and soft tissue damage accompanying trauma in the area, have an impact on the choice of fixation technique for treatment. The selected technique should provide adequate stabilization of the fracture fragments throughout the healing process and cause minimal adverse effects during the healing period, and the extremity should return to normal or near-normal function after the fracture has healed (Scott H, 2005; Yayla et al., 2022; El-shafey et al., 2022). Both conservative and surgical methods have been developed to treat hind limb fractures in cats. Surgical methods include external and internal operation techniques using different materials. In general, in patients with intramedullary pin application, the pin cannot resist rotational forces and a supported bandage is needed to prevent pin migration (Yayla et al., 2022). On the other hand, external fixators are easy to apply, durable and easily available, and the operation time is shorter than internal fixation methods, but since they are applied percutaneously, the incidence of pin base infection and osteomyelitis in the area where the pins are located is high (Sağlam et al., 2019). In this study, the external fixation method was applied in 4 cases (%13.39), while the internal fixation method (50 plates, 45 pins, 2 tension bands, 7 arthrodesis) was applied in a much larger number of cases. For internal fixation, plate osteosynthesis was applied in 44.64%, intramedullar pin in 40.18%, tension band in 1.29% and pantarsal arthrodesis in 5.36%. Apart from these procedures, caput resection was deemed appropriate for 4 patients (3.57%) with unilateral caput or collum fractures located on the proximal femur. While planning the method, the type and location of the fracture in the bone, the experience of the surgeon, the age of the patient, the preference of the patient owner and the economic situation were taken into consideration. In addition, PVC-supported bandage was applied to 45 patients who received intramedullary pins for 10 days in order to prevent pin migration and to provide the stability of the bone ends. During clinical examination, bandage complications such as muscle atrophy and edema due to mild circulatory disorders were detected in 8 of 45 patients (17.78%). For this reason, in cases where bandage application is required, necessary precautions should be taken considering the possible complications that may be encountered after the bandage.

While the craniolateral approach is preferred in the treatment of femur fractures, the medial approach is frequently used in the treatment of tibia and tarsus fractures. Although approaching the femur from the craniolateral side is beneficial in reducing the risk of complications by providing easier access to the region and minimal tissue damage, if a medial approach is preferred for the tibia, it is especially for the protection of vessels and nerves and to minimize the destruction of thin muscle tissues. In addition, in case of fractures of the distal bones, it is very important not to cause vascular damage during transportation to the region in terms of local blood supply and nutrition of the region in the postoperative period (Piermattei et al., 2006). We also preferred a craniolateral incision for the femur in our surgeries. The tibia and talus were approached through a medial incision. For other

distal bones of the extremity, they were approached from the cranial side. It was extremely easy to reach the fracture line during the operations. In the operations performed in our study, no abnormal bleeding, anesthesia complications, or any complications related to technical equipment were observed during the intraoperative period. In the 50 patients to whom the plate was applied, it was observed that the patients used the relevant extremity and started walking within the first 2 postoperative days. No complications related to screws, plates or gait were encountered in these patients, who were called for control examinations at regular intervals. Bandage-related complications were observed in 8 of 45 patients to whom PVC-supported bandage was applied to prevent pin migration during pin applications. Although these complications have been eliminated, the need to support postoperative period with a bandage can be considered a significant disadvantage compared to plate. In addition, pin base infection developed in 2 of 4 cases in which external fixation was used, and as noted in the literature, this situation should be taken into consideration in external fixator applications. For this purpose, the patient owner should be informed about complications such as pin root infection and osteomyelitis in the postoperative period and asepsis of the area should be ensured. Additionally, in one of our cases with bilateral distal femur fractures, both fractures were intervened with a rush pin, but it was determined that nonunion developed as a result of pin migration in one of the fractures. In this fracture, the migrating pin was removed and a locking L plate was placed in its place, and healing was achieved without any problems.

In some studies (Gorse et al., 1991; Johnson et al., 2005), arthrodesis is defined as 'irreversible surgical fusion of two or more bones in a joint'; Indications for pantarsal arthrodesis include irreparable joint fractures of the distal tibia or talus, unstageable tarsocrural luxations, severe instability of the tarsus, and end-stage degenerative joint disease. In this study, for the pantarsal arthrodesis applied to the tarsus, the angle of the tarsus was adjusted to 100-125 degrees and the tarsus was immobilized with a locked 'V' plate, which is an internal fixation method. During the examination of the patients who were called for control, their gait was evaluated and no complications were found.

As a result, many different methods are used in hind extremity fractures depending on the location of the fracture, the patient's condition and the surgeon's preference. Among these, it is possible to use external and internal osteosynthesis techniques or their combinations. However, it can be said that plate osteosynthesis provides a significant advantage in terms of fewer complications and earlier recovery compared to bandage applications applied with external fixators and pins.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: R.C., S.Y., Design: S.Y., E.C., B.E.K., N.S., S.A., Data Collection or Processing: R.C., S.Y., N.S., Analyses or interpretation: R.C., S.Y., Literature Search: R.C., Writing: R.C., S.Y.

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The Effects of Propolis-Incorporated Nanofibers Produced by the Electrospinning Method on Skin Wounds in Rats

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Abstract

Electrospinning is a widely used process in various industries to create polymeric fibers with unique properties. In the context of wound healing, electrospun nanofibers can mimic the extracellular matrix structure, promote tissue regeneration, and enhance the wound healing process. Propolis, a natural substance with various biological properties, has shown potential in promoting healthy skin and wound healing. It has antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral effects. The study was conducted on male Wistar albino rats. The rats were divided into three groups. The nanopropolis group received nanopropolis applied once daily, while the ethanol extracted propolis group received applied once daily. The control group did not receive any application after the wound was formed. The researchers evaluated the wound sizes throughout the study period. Macroscopically, a gradual healing was observed in all three groups. On the 11th day, the wounds in the nanopropolis and propolis groups healed completely, while the wounds in the control group healed on the 14th day. When the wound sizes were analyzed, the nanopropolis group showed a significant decrease in wound size compared to the control group. Histopathological analysis was performed on the wound samples collected at the end of the study. Microscopically, it was observed that the epidermis layer was more regular in the propolis and nanopropolis groups compared to the control group. In conclusion, the results of this study suggest that propolis-incorporated nanofibers produced by electrospinning (nanopropolis) have a positive effect on wound healing compared to propolis alone and the control group. The nanopropolis group showed a significant reduction in wound size and improved histopathological parameters. These findings highlight the potential of nanopropolis in promoting wound healing and tissue regeneration.

Keywords: Experimental, healing, nanopropolis, polyethylene oxide.

INTRODUCTION

Electrospinning is a commonly used electrohydrodynamic technique in a variety of industries to create polymeric fibers with diameters ranging from a few nanometers to a few microns (Luraghi et al., 2021). The technique involves the application of an electric field to a polymer solution or melt, which leads to the formation of ultrafine fibers through a process of electrostatic stretching and solidification. The process starts with a polymer either in the form of a solution or melt, is prepared. Polyvinyl alcohol, chitosan, polyurethane, poly (lactide-co-glycolic acid), silk fibroin, polyvinylpyrrolidone (PVP), cellulose acetate, and some other polymers have been used in production of nano/microfibers by electrospinning to formulate wound dressings and similar products (Tan et al., 2015). The polymer solution is essential for the formation of the fibers (Liu et al., 2021).

Electrospun nanofibers find applications in various fields, including biomedical, energy, environmental, and filtration. They have shown promise in tissue engineering scaffolds, drug delivery systems, supercapacitors, sensors, and air filtration, among others (Li and Xia, 2004). Electrospinning has shown great potential in the field of

wound healing by fabricating nanofibrous scaffolds that mimic the extracellular matrix (ECM) structure of natural tissues. These scaffolds can support cell growth, tissue regeneration, and wound healing processes. Electrospun nanofibers can be designed to have a high surface area and porosity, which facilitates the absorption of wound exudates and creates a moist environment, known to be beneficial for wound healing. The nanofiber matrices can be functionalized with bioactive molecules like growth factors, antimicrobial agents, and extracellular matrix proteins to enhance the wound healing process (Sill and Von Recum, 2008). The porous structure of electrospun nanofiber scaffolds facilitates the diffusion of nutrients and oxygen, promoting angiogenesis and tissue regeneration at the wound site. This property is crucial for wound healing and tissue repair (Deeken and White, 2011).

Propolis is an important bee product created by honeybees by collecting resins and secretions of plants and mixing them with wax, pollen and their own enzymes. It contains more than 300 active compounds and these compounds give propolis many biological activities. Propolis is used as a building material in the hive and protects the hive against bacterial and fungal infections. In

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vivo studies on propolis have reported that propolis contains many components including flavanoids and hydroxycinnamic acids. Thanks to this content, propolis has been found to have antifungal and antibacterial properties. Propolis has been used in the treatment of wounds since ancient times (Bonvehi and Coll, 2000; Oryan et al., 2018).

Wound healing is a multi-stage process that depends on both internal and external factors. During the healing process, bacterial infections accompany the skin damage and delay the healing time. To eliminate these negative effects, antibiotics are often included in the treatment. However, recently, antibiotic-resistant strains have been developing as a result of excessive and incorrect use of antibiotics. Propolis, on the other hand, is effective on wound healing because it is a natural substance produced from bees and has antibacterial properties. In addition, propolis accelerates the process of reepithelialisation in histology (Medellin-Luna, 2019; Rojczyk, 2020).

Propolis has been used in skincare for its potential benefits in promoting healthy skin and addressing various skin concerns. According to studies by Schnitzler et al. (2010); Seven et al., (2011); Funakoshi-Tago et al., (2015); Gul Baykalir et al., (2016) and Shokri et al., (2017) propolis has a number of biological properties, including antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral effects. Due to its tissue-regenerating properties, propolis can aid in wound healing and promote tissue repair. It can be applied to minor cuts, scrapes, and wounds to support the healing process (Przybyłek and Karpiński, 2019).

The aim of this study is to investigate the effects of propolis-incorporated nanofibers produced by the electrospinning method on skin wounds in rats and to explore the potential benefits of propolis in wound healing. The study aims to compare the effects of propolis and nanofiber-based propolis on experimentally created wounds to identify any differences in their impact on wound healing.

MATERIALS AND METHODS

Animals

For the study, a total of 24 male Wistar albino rats weighing 230-250 g were used, with 8 animals in each group. The Bursa Uludag University Experimental Animals Application and Research Center provided the rats. The study was conducted with the approval of the Bursa Uludag University Animal Experiments Local Ethics Committee under permit number 2019-06/02. The rats were kept in a 22°C, 12-hour cycle of light and darkness, with unlimited access to food and water.

The Preparation Propolis

The propolis utilized for this investigation was propolis number 30, collected using a propolis trap in summer from the village of Edime-Uzunköprü-Aşlıhan village. Propolis that had been collected was reddish in hue. Prior to being ground into a powder using a mill (Lavion grain mill), the entire propolis was first frozen at a temperature of -20°C. Propolis was extracted using ethanol (70%) and water (30%). 400 grams of propolis were combined with one liter of a 70% ethanolic solvent, and the mixture was then allowed to stand in an orbital shaker for ten days. It was submerged in an ultrasonic bath twice daily for 30 minutes each during its time in the orbital shaker. To obtain the extract, the obtained mixture was filtered through Whatman No. 1 filter paper (Sorucu and Oruc, 2019). The

extract was sent to the Department of Textile Engineering at Bursa Uludag University order to produce nano-tissue.

Techniques for Electrospinning and Nanopropolis

In this work, polyethylene oxide (PEO) (Mw: 900 kDa, Sigma Aldrich) was used as the polymer and propolis was used as the active ingredient to be added to the polymer solution. There was no pre-treatment applied to the materials used in the investigation. A polymer solution was first created, and 3% PEO by weight was added to it. For this, the PEO polymer was thoroughly dissolved in a 1:1 mixture of ethanol and water using a magnetic stirrer. The prepared solution was then combined with a propolis: ethanol solution, with propolis constituting 1% of the total weight of the PEO solution. On a magnetic stirrer, it was mixed for roughly 24 hours to produce the final product.

Measurements and Model for Wounds

Rats were anesthetized with 10 mg/kg of Xylazine hydrochloride (Rompun, Bayer 23.32 mg/mL) before receiving injections of 70 mg/kg of Ketamin hydrochloride (Ketalar, Parke-Davis, 50 mg/mL) intramuscularly to create a skin wound model. On the dorsal side, a 3 cm line of shaving encircled the intrascapular area. After cleaning the shaving area with povidone-iodine, a full-thickness skin wound model was created using a 5mm punch trephine. Up until the day of sacrifice, the wound site was measured and photographed after the procedure. The images were imported into a computer environment and loaded into the "Image j" (Wayne Rasband National Institutes of Health, USA) program to calculate the wound surface areas. The wound areas was calculated in square millimetres.

Experimental Design

The animals were divided into three groups of eight each. The applications were filled out in the groups in the manner described below; Control Group: Until the day of the sacrifice, no application was made after the wound had formed. Nanopropolis Group: Following the development of wounds, nanopropolis was applied just once every day up until the day of sacrifice. Ethanol extracted propolis: From the time the wound first appeared, it was applied once daily until the day of the sacrifice.

Histopathologic Analyses

The rats were put to death by cervical dislocation under anesthesia 15 days after the corneal wound was made. Each euthanized rat had a sample of tissue from a wound removed. The panniculus layer and the surrounding full-thickness skin were also removed from the body for this reason. For histopathological analysis, the collected samples were fixed in a 10% formaldehyde solution. Following fixation, the samples were dehydrated using a series of alcohol and xylene before being placed on paraffin blocks. Hematoxylin-eosin was used to stain 4 m sections from these paraffin blocks, which were then examined under a microscope.

Statistical analysis

Statistical evaluation of the data was performed using SPSS 26 statistical package programme (Inc., Chicago, IL, USA). The conformity of the variables to normal distribution was analysed by analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). One-way ANOVA test was used for comparison between groups for wound area. In cases where there was a significant

difference between groups in variables, pairwise post-hoc comparisons were made using the Tukey test. Kruskal-Wallis test was used for histo-pathological examination. Paired comparisons of variables were performed using the Mann-Whitney U test and evaluated using Bonferroni correction. P-values below 0.05 were considered as statistically significant results.

RESULTS

The Content of Propolis

The LC-MS/MS method was used to analyze 15 phenolic compounds in propolis. The phenolic compounds which were contained in propolis; apigenin 8419 µg/ml, caffeic acid phenethyl ester 5731 µg/ml, trans-ferulic acid 4380 µg/ml, gallic acid (not detected), caffeic acid 6967 µg/ml, kaemferol 443 µg/ml, quercetin 5956 µg/ml, lutein 590 µg/ml, p-coumaric acid 945 µg/ml, naringenin 1333 µg/ml, pinosembrin 10736 µg/ml, p-coumaric acid 2869 µg/ml, rutin (not detected), trans-cinnamic acid 2300 µg/ml (Sorucu and Oruc, 2019).

Wound Sizes

The wounds were followed macroscopically throughout the study. All rats were photographed daily throughout the study (15 days) and the wound area was calculated in square millimetres (Figure 1).

When compared macroscopically, a gradual healing was observed in 3 groups. On the 11th day, it was observed that the wounds of the rats in the ethanol extracted propolis and nanapropolis group healed completely, while the wounds in the control group healed on the 14th day (Table 1).

When the wound sizes were analysed, there was no significant difference between the groups on day 0 ($p>0.05$). Wound sizes in the nanopropolis group decreased more than the control group in the first 10 days. It also decreased more than the propolis group on days 1 ($p<0.01$), 2 ($p<0.001$), 3 ($p<0.001$), 4 ($p<0.01$), 5 ($p<0.001$) and 7 ($p<0.01$) (Table 1).

The average values of the wound sizes of the groups were taken and the percentage healing amounts were calculated. According to the percentage healing amounts, the nanopropolis group consistently healed more than the other groups. When the control and propolis groups were compared within themselves, the propolis group healed more in percentage after the 3rd day (Figure 2).

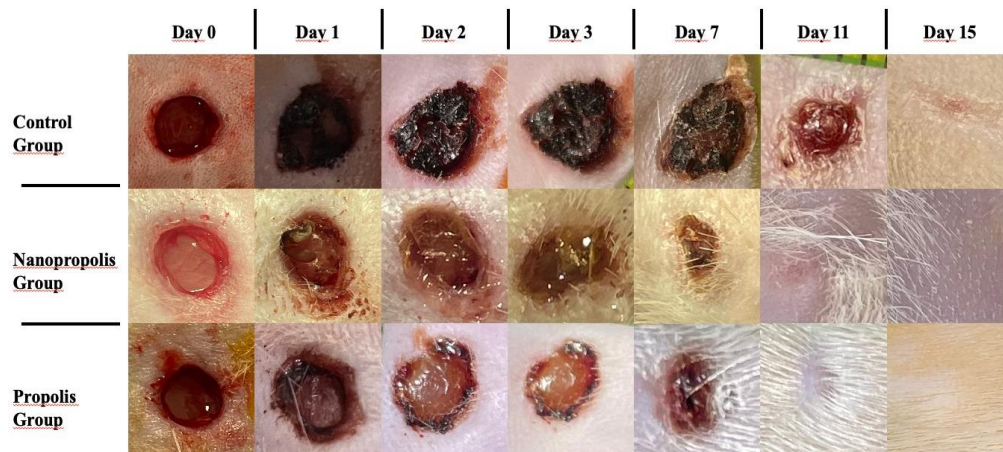


Figure 1. Macroscopic wound size.

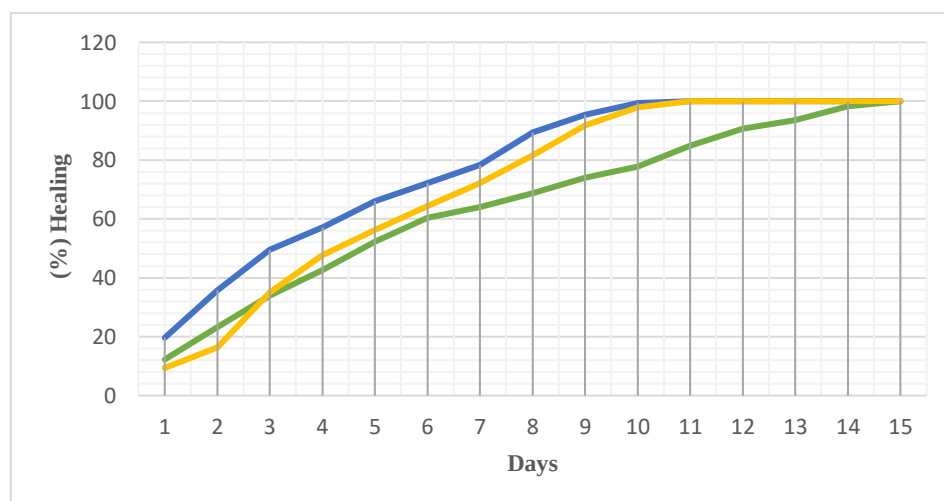


Figure 2. Healing percentages of wound sizes.

Table 1. Wound areas (wound areas was calculated in square millimetres).

Days	n	Control		Nanopropolis		Propolis		P-value
		Mean	S.E	Mean	S.E	Mean	S.E	
Day 0	8	20.71	0.46	20.42	0.68	20.48	0.61	0.935
Day 1	8	18.17 ^a	0.45	16.41 ^b	0.45	18.59 ^a	0.09	0.001
Day 2	8	15.90 ^a	0.31	13.12 ^b	0.50	17.14 ^a	0.25	<0.0001
Day 3	8	13.66 ^a	0.41	10.31 ^b	0.48	13.32 ^a	0.70	<0.0001
Day 4	8	11.88 ^a	0.60	8.75 ^b	0.63	10.70 ^a	0.33	0.002
Day 5	8	9.88 ^a	0.43	6.93 ^b	0.46	8.96 ^a	0.31	<0.0001
Day 6	8	8.19 ^a	0.60	5.69 ^b	0.47	7.31 ^{ab}	0.26	0.003
Day 7	8	7.45 ^a	0.59	4.42 ^b	0.53	5.70 ^a	0.27	0.001
Day 8	8	6.38 ^a	0.68	2.15 ^b	0.73	3.78 ^b	0.36	<0.0001
Day 9	8	5.38 ^a	0.72	0.95 ^b	0.50	1.69 ^b	0.45	<0.0001
Day 10	8	4.58 ^a	0.68	0.13 ^b	0.13	0.43 ^b	0.30	<0.0001
Day 11	8	3.13	0.68	0.00	0.00	0.00	0.00	-
Day 12	8	1.93	0.64	0.00	0.00	0.00	0.00	-
Day 13	8	1.34	0.48	0.00	0.00	0.00	0.00	-
Day 14	8	0.35	0.23	0.00	0.00	0.00	0.00	-
Day 15	8	0.00	0.00	0.00	0.00	0.00	0.00	-

^{a,b}: Different letters in the same line are statistically significant ($p < 0.05$).

Histopathologic Results

At the end of the 15th day, wound samples were collected and microscopically analysed. The samples evaluated in terms of epithelialisation, inflammatory cell infiltration and granulation cell formation were given numbers from 1 to 3 (0: none, 1: mild, 2: moderate, 3: severe).

Microscopically, it was observed that the epidermis layer was more regular in propolis and nanopropolis groups compared to the control group. In the control and propolis groups, there are ulcerated areas close to the epidermis layer. Granulation cells are observed in the dermis layer in the nanopropolis group (Figure 3).

Histopathological parameters such as epithelialisation ($p < 0.001$), inflammatory cell infiltration and granulation tissue formation increased in nanopropolis and propolis

groups ($p < 0.01$), whereas there was no difference between the experimental groups ($p > 0.05$) (Table 2).

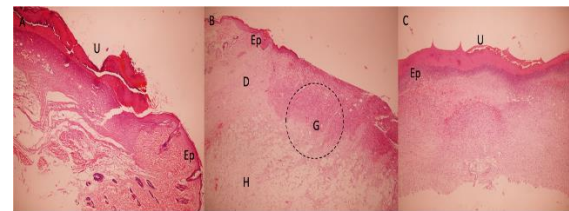


Figure 3. Histopathological images (A) control group, U: ulceration, Ep: epidermis. (B) nanopropolis group, Ep: epidermis, D: dermis, G: granulation cells, H: hypodermis. (C) propolis group, U: ulceration, Ep: epidermis

Table 2. Histopathologic evaluation. The findings were evaluated as follows: 0; none, 1; mild, 2; moderate, 3; severe.

Histopathologic Parameters	n	Control		Nanopropolis		Propolis		P-value
		Mean	S.E	Mean	S.E	Mean	S.E	
Epithelization	8	0.88 ^b	0.23	2.75 ^a	0.16	2.38 ^a	0.18	<0.0001
Inflammatory cell infiltration	8	0.75 ^b	0.25	2.13 ^a	0.23	1.88 ^a	0.23	0.004
Granulation tissue formation	8	0.88 ^b	0.29	2.25 ^a	0.16	2.00 ^a	0.27	0.007

^{a,b}: Different letters in the same line are statistically significant ($p < 0.05$).

S.E: Standard error.

DISCUSSION AND CONCLUSION

Wound healing is the body's physiological response to tissue damage, which causes living tissue to replace dead tissue and restore the integrity of the injured tissue. It entails a carefully orchestrated series of cellular reactions that involve the interaction of numerous cell types over extended periods (Pillai et al., 2010; Anjum et al., 2019). The degree of tissue damage, the tissue's capacity for repair, and the overall health of the tissue are the main determining wound contracture, which develops during the healing process (Balata et al., 2018). Debridement, irrigation, antibiotics, tissue grafting, and protein hydrolases are some of the current methods used to treat wounds; however, all of these methods have significant disadvantages and these treatments have limitations in

terms of cost, treatment time, and toxicity (side effects) (Ahmad et al., 2021). Due to its accessibility and low price, traditional medicine is growing in popularity worldwide (Dwita et al., 2019). Natural products are crucial to this process. Natural product therapy could provide a fresh approach to treating skin wounds (Dong et al., 2018). With the advancement of technology, new approaches in wound healing have emerged in the field of medicine. Nanotechnological products produced through various methods have been increasingly used in wound care, especially in recent years. The combination of natural and technological products can eliminate the disadvantages of current treatment methods. Since ancient times, propolis has been employed in traditional medicine (Gavanji and Larki, 2017). Propolis is a suitable component of

biomaterials because it has been demonstrated to have wound healing and antimicrobial properties, making it useful for use in wound and skin dressings (Lesmana et al., 2022). The purpose of this study was to assess the efficacy of propolis and nanopropolis in treating wounds at the sites of experimental rats' wounds.

Bees gather propolis from tree buds or other plant sources such as poplar, willow, birch, elm, alder, beech, conifer, and horse chestnut in order to create a mixture of natural resinous substances, pollen, waxes, and enzymes (Sforcin, 2016). In terms of pharmacological effects, various biological components have been shown to have antibacterial (Afrouzan et al., 2018), antioxidant (Cao et al., 2017), anti-inflammatory (Moura et al., 2020), accelerated tissue repair (Olczyk et al., 2013), antitumor (Alserbiny et al., 2021), liver protection (Badr et al., 2023), oral health (Sakaba et al., 2013), anti-radiation (Anjaly and Tiku, 2022), anti-ulcer effects (Paulino et al., 2015) and hypoglycemic (Rivera-Yanez et al., 2018) as well as gastrointestinal disorders (Song et al., 2020) properties. Propolis samples from various regions have yielded more than 300 chemical components, the majority of which are rich in flavonoids, terpenes, phenolic acids, amino acids, as well as different hydrocarbons, minerals, trace elements, vitamins, and enzymes (Huang et al., 2014). In addition to preventing or delaying the onset of cell necrosis, flavonoids are known to improve vascularity and lower lipid peroxidation. Therefore, it is thought that any medication that inhibits lipid peroxidation will increase the viability of collagen fibrils by increasing their strength, circulation, preventing cell damage, and promoting DNA synthesis (Getie et al., 2002). Triterpenoids and flavonoids are also known to hasten the healing of wounds primarily because of their astringent and antimicrobial properties that appear to be responsible for wound contraction and accelerated epithelialization (Carvalho et al., 2021). According to our results propolis and nanopropolis showed an improvement skin wound, for the first 5 days after the injury. This effect comes from the flavonoids contained in propolis.

A natural nano-material called nano-propolis can be helpful for veterinary medicine in terms of performance, health, and dependable food production. Because of their smaller size, nanoparticles are more readily absorbed by the body (Sahlan et al., 2017), whereas nano-propolis has greater antibacterial and antifungal activity than propolis (Afrouzan et al., 2012). In their study, Patil and Ark, (2015) reported that silver nanopropolis loaded with propolis could be used as a potential product for burn wounds in rats. Adomaviciute et al., (2016) stated in their in vitro study that silver and propolis-loaded wound dressings demonstrated antibacterial and antifungal properties and supported wound healing. Cavalu and Ark, (2019) stated in their in vitro study that encapsulated propolis in nano formulation supports the healing of cutaneous wounds. Saharraf and El Nagggar, (2018) reported in their in vitro study that propolis and cellulose acetate loaded nanofibers are effective in wound healing and have antibacterial properties. Eskandarinia et al., (2020) demonstrated the antibacterial properties of biodegradable gelatin-based nanofibers with polyurethane and propolis additives in their in vitro and in vivo study. They also stated that the biomaterial they produced created a moderate exudate and low microbial load in the wound area during the in vivo study.

Within a few hours of injury, inflammatory cells begin to invade the wound site. Monocytes, macrophages,

fibroblasts, lymphocytes, and neutrophils are the next to arrive (Yang et al., 2023). This phase can last up to 4 days after an injury. The blood vessels become leaky as a result of the inflammatory response, releasing plasma and polymorphonucleocytes into the surrounding tissue. The neutrophils act as the body's first line of defense against infection by phagocytizing debris and microorganisms with the help of mast cells. Bacteria can be phagocytosed by macrophages, which act as an additional line of defense. Additionally, they secrete a variety of chemotactic and growth factors, including granulation tissue formation-initiating interleukin-1 (IL-1), transforming growth factor beta (TGF- β), fibroblast growth factor (FGF), and epidermal growth factor (EGF) (Broughton et al., 2006). In our study, an increase in the level of cell infiltration was observed in the propolis and nanopropolis groups compared to the control group, which was statistically significant. This finding occurred at the expected level during a healthy wound healing phase and contributed to wound healing.

The granulation phase is identified by the presence of pebbled red tissue at the wound base and involves replacement of dermal tissues, and in deeper wounds, subcutaneous tissues as well as wound contraction. Reparatory cells, such as fibroblasts, endothelial cells, pericytes, and keratinocytes, become more prevalent in the wound site as the granulation phase develops. These cells are in charge of creating the new matrix required for the structure and function repair of injured tissue (Pillai et al., 2010). In our study, statistically significant differences were found in terms of granulation tissue formation between the propolis and nanopropolis groups compared to the control group. The wounds that had been treated with propolis and nanopropolis application were tidy and had strong granulation tissue. The data gathered for this study are consistent with the conclusions made above.

Through regulation of skin extracellular matrix components, expression of transforming growth factor, and improved skin cell growth and remodeling, propolis promotes re-epithelialization (Toreti et al., 2013). This process involves fibroblast adhesion, migration, proliferation of keratinocytes and epidermal cells, as well as contraction of extracellular matrix components in the skin (Olczyk et al., 2014). Reduced granulation tissue maturation time and wound contraction due to the presence of biofilm in propolis suggest that it enhances re-epithelialization and has a significant impact on inflammatory infiltration and fibroblast population in a time-dependent manner. In rats with excisional wounds, nanopropolis significantly accelerates full-thickness wound healing (Abbaszadeh et al., 2019). In our study, it was determined with histopathological data that the level of epithelialization in the wound area was statistically significantly higher in the propolis and nanopropolis groups compared to the control group. The obtained data were found to be consistent with the literature data.

An important parameter to consider when evaluating the wound healing process is the amount of time needed for the excision wound to completely epithelialize (Behyari et al., 2021). Yang et al., (2023) stated in their study on mice that nanopropolis applications provided the fastest wound healing, with 48% of the wound healed on the first day. They also mentioned that this rate was 40% in the propolis group. Another study suggested that topical administration of a nano-emulsion containing nanovitamins C, E, and propolis gel in the mouth cavity during surgical procedures under local anesthesia might

aid in wound healing, especially in the first three days following the procedure. It provided efficient healing and anti-inflammatory effects in addition to safeguarding patients undergoing oral surgical procedures from the early complications of wound healing (such as bleeding) (Furukawa et al., 2021). Yang et al., (2022) have stated in their study on wounds in mice that nanopropolis provides faster wound healing compared to propolis and other experimental groups. The healing parameters examined changed significantly, clearly demonstrating the pro-healing activity of nanopropolis and propolis. Epithelial reorganization occurred very slowly in control wounds. According to the findings of the study, both propolis groups had wound contraction rates close to 100% within 10 days, accelerating wound closure from the early stages. In the nanopropolis group, the wound healing rate was found to be higher compared to other groups, and on the third day, this rate was detected at a level of 49.51%. The topical application of propolis and nanopropolis at the wound site elicited significant wound healing activity due to their angiogenic and mitogenic potential.

This study was conducted to investigate the effects and potential benefits of propolis-incorporated nanofibers produced by the electrospinning method and ethanol-extracted propolis on skin wounds in rats. At the end of the study, it was revealed that the application of propolis and nanopropolis had a positive contribution to wound healing at a statistically significant level in terms of histopathology. It was determined that nanopropolis exhibited a faster healing effect on the wounds compared to propolis and the control group in terms of wound healing rate. As a result of the study, it was concluded that further research is needed to determine the effects of propolis and nanopropolis obtained with different extraction methods and materials, and that new and more effective wound healing methods and materials could be developed based on the obtained data. It was also concluded that more clinical studies are needed for conclusive results.

Conflict of Interest

The authors declare that they have no competing interests.

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Authorship contributions

Concept: O.B., Design: O.B., T.M.D., Data Collection or Processing: O.B., A.S., T.M.D., Z.A., Analysis or Interpretation: O.B., A.S., T.M.D., Z.A., Literature Search: O.B., T.M.D., Writing: O.B.

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Ethical Approval

The study was conducted with the approval of the Bursa Uludag University Animal Experiments Local Ethics Committee under permit number 2019-06/02.

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Treatment of Diaphyseal Tibial Fractures of Cats with Using Minimal Invasive Plate Osteosynthesis and Evaluation of Outcomes Postoperatively

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Abstract

In the present study, it was aimed to apply minimally invasive plate osteosynthesis (MIPO) in the treatment of diaphyseal tibia fractures in cats and to evaluate recovery and complications postoperatively. Minimally invasive fracture repair preserves the blood supply of fragments and periosteal tissues which help to result faster healing, less morbidity, and rapid recovery of limb function. The study was conducted on 12 cats with diaphyseal tibia fracture. After closed reduction of the fractures of the cats included in the study, two small incisions were made from the proximal and distal tibia to expose the bone tissue. Plate placement was performed percutaneously through these incision areas. The plate was fixed with two screws from the proximal and distal incision line and the fixation of the fracture line was ensured. Soft bandage was applied for 5 days postoperatively and animals caged to restrict movements for 3 weeks. X-rays were taken at regular intervals postoperatively and fracture healing was evaluated. In the controls, it was seen that the animals started to use their legs after the bandage was removed. There were no complications related to the very small operation wound and bone tissue. Healing times were determined as 35 days on average. As a result, it was determined that earlier healing was performed and less complication rate compared to open operational techniques.

Keywords: Cat, fracture, plate osteosynthesis, tibia.

INTRODUCTION

AO principles recommend complete anatomical reduction of fracture fragments prior to the application of internal fixation methods (Schutz and Sudkamp, 2003). Complete exposure and manipulation of the fracture site are required for complete anatomical reduction (Schatzker, 1995). The rigid fixation and interfragmental compression created support for primary fracture healing with minimal callus formation (Palmer, 1999). Although open reduction techniques allow reduction of fragments by direct manipulation, fracture hematoma in the region and disruption of regional extraosseous blood supply may pose problems in fracture healing (Farouk et al., 1998; Field and Tomkvist, 2001; Borrelli et al., 2002). The iatrogenic trauma created can slow the rate of new bone formation and cause devitalization of the fracture fragments (Mizuno et al., 1990).

In order to maximize the biological healing potential in fracture treatment, indirect stabilization techniques that cause minimal damage to soft tissue and biological osteosynthesis principles that provide adequate reduction have been developed (Schatzker, 1995; Palmer, 1999; Field and Tomkvist, 2001). Minimally invasive fracture repair preserves the blood supply of fragments and periosteal tissues, resulting in faster healing, less morbidity, and rapid recovery of limb function. (Johnson et al., 1998; Schmokel et al., 2003; Hudson et al., 2009) Minimally invasive surgical procedures can be performed using an external fixator, interlocking screw, plate-rod combination, clamp-rod internal fixation, and plate-screw

(Johnson et al., 1998; Schwarz, 2005; Piermattei et al., 2006; Tong and Bavonratanavech, 2007; Guiot and Dejardin, 2011). Minimally invasive plate osteosynthesis (MIPO) is the procedure of applying a bone plate without an open approach to the fracture site. In MIPO, only the intact bone cortices of proximal and distal fracture fragments are exposed to position the plaque and fix the screws. Thus, the osteogenic tissues surrounding the fracture are preserved. MIPO decreases the duration of the surgery, therefore reducing the risk of infection (Eugster et al., 2004; Hudson et al., 2009). In noninfectious fracture healing; the incidence of complications such as loss of fixation or delayed union is reduced (Krettek et al., 1997).

Tibia-fibula and radius-ulna fractures are common fractures in cats and dogs (Harasen, 2003; Nolte et al., 2005). Especially in distal tibia fractures, the risk of open fractures or vascular damage increases due to the weak amount of muscle on the medial surface of the tibia. It has been reported that 11/18 of cat diaphyseal tibia fractures turn into non-union fractures (Nolte et al., 2005). The cause of delayed / non-union fractures is due to insufficient biological compensation (insufficient blood supply due to limited extraosseous soft tissue) and mechanical compliance (insufficient stabilization of the fracture line, large space between fragments and soft tissues entering between the fracture fragments) (DeAngelis, 1975). Therefore, the rate of complications such as osteomyelitis, non-union or implant failure in the tibia is high in open reduction techniques (Boone et al., 1986; Dudley et al., 1997; Nolte et al., 2005).

This study aimed to treat diaphyseal tibia fracture of cats using minimal invasive plate osteosynthesis and evaluate outcomes postoperatively.

MATERIALS AND METHODS

Ethics committee approval was received from the Kırıkkale University Clinical Practices Ethics Committee. The study was conducted on 12 cats with different breeds, ages, and sexes brought to Kırıkkale University Animal Hospital with complaints of diaphyseal tibia fracture. Owners were informed about the operation and postoperative process to be performed on the animal.

Food restrictions started 12 hours prior to operation, and no water restrictions were applied. In preparation for the operation, an intravenous (IV) cannula was placed into the cephalic vein. For pre-anesthesia, animals were premedicated with IV 20 mcg/kg medetomidine (Domitor, Zoetis, USA) and 0.2 mg/kg butorphanol (Butomidor, Richter Pharma, Austria) and anesthesia induced with 5-7.5 mg/kg dose of ketamine (Ketasol, Interhas, Turkey). After the induction, animals were intubated and anesthesia was maintained with 1-2% isoflurane (Isoflurane, Piramal, USA) using a semicircular inhalation device (SMS Company, Turkey). The extremity was prepared for aseptic surgery from the tarsal joint to the lumbosacral region. Intravenous 22 mg/kg Cefazolin sodium (Eqizolin, Tüm Ekip Ilac AS, Turkey) was administered at induction and every 90 minutes intraoperatively. In the postoperative period, amoxicillin clavulanic acid (Synulox, Pfizer, USA) subcutan at a dose of 12.5 mg/kg for a week and meloxicam (Maxicam, Sanovel, Turkey) sc at a dose of 0.2 ml/kg for 3 days.

A medial approach was used for the surgery. The cats were positioned in lateral recumbency to expose the medial surface of the affected tibia. Proximal and distal tibia palpated and 1 cm incision was made to approach to tibia. The proximal tibia is exposed after incising the tendons of insertion of the semitendinosus, gracilis, and sartorius muscles. Care is taken to preserve the medial saphenous artery and vein for the distal approach of tibia. The epiperiosteal tunnel is created by protecting the subcutaneous tissues and muscles by entering through the proximal and distal incision lines, and the plate was inserted through this tunnel. The plate is fixed to the bone with a minimum of two screws from the proximal and distal incision lines (Figure 1). The incision lines that were opened are ligated and closed. Postoperative radiographs were taken, and bandage is applied for 5 days and activities were restricted for 3 weeks (Figure 2).



Figure 1. Mediolateral and craniocaudal radiographic views of tibia fracture preoperatively.

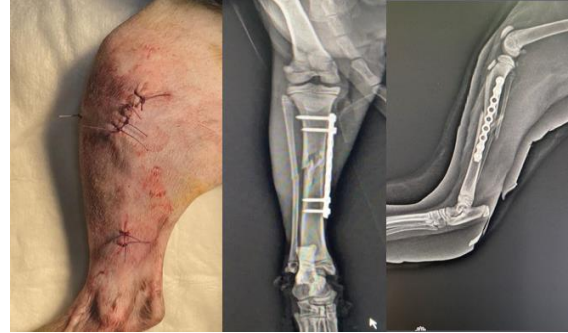


Figure 2. Immediately after postoperative views of incision area and radiographic views of tibia fracture fixation.

RESULTS

Animal ages and body weight were determined as 26 ± 15.30 (ranged from 5 to 52 months) and 3.7 ± 0.87 (mean $\pm$ SD), respectively (Table 1). All fractures were treated with 1.5/2.0 mm plates. Nine cats could be vetted again. Wound dehiscence was detected in 3 cats in postoperative period. These were treated with local wound care and parenteral antibiotic administration. There was no pain in the fracture area's palpation; there was no abnormality in the cat's walking and movements. Healing occurred without further complications 35 days after the initial surgery. Clinical examinations and radiological evaluations were performed during the follow-up period, ranging from 1 to 6 months. Follow-up x-rays were examined to assess the healing progress of fractures and to identify issues such as malunion, nonunion, and implant failures including as bending or breakage (Figure 3-4). There was no radiographic abnormality observed.

Table 1. Details and clinical outcomes of 12 cats postoperatively.

Age at injury (month)	Breed	Weight(kg)	Fracture type	Complication
5	Crossbreed	2.0	Transverse	---
18	British short hair	3.4	Transverse	Wound dehiscence
48	British short hair	3.9	Comminuted	---
37	Crossbreed	3.9	Oblique	---
52	Crossbreed	4.8	Transverse	Wound dehiscence
32	Siyam	3.0	Oblique	---
36	Crossbreed	4.5	Comminuted	---
11	British short hair	4.1	Comminuted	Wound dehiscence
17	Persian	5.0	Oblique	---
8	Crossbreed	2.6	Transverse	---
21	Crossbreed	3.4	Transverse	---
25	Crossbreed	3.6	Comminuted	---

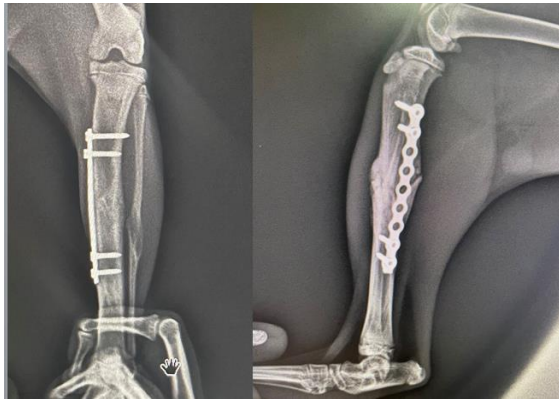


Figure 3. Radiographic image of tibia fracture repair with MIPO on postoperative 33. day.

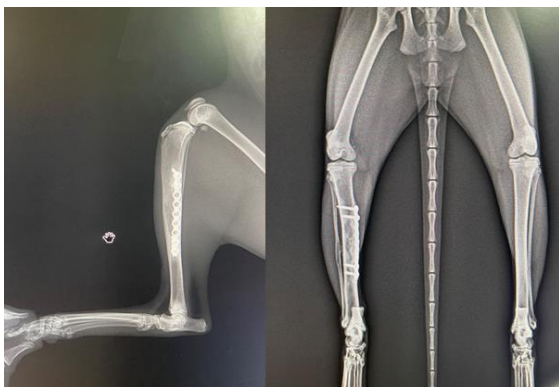


Figure 4. Radiographic image of tibia fracture repair with MIPO on postoperative views of tibia fracture postoperative 6th month.

DISCUSSION AND CONCLUSION

It is stated that there is no need for full anatomical reduction of the fracture line in MIPO application. The aim of this application is to bring the fracture fragments together in the correct alignment and restore bone function. Fracture reduction is performed with an indirect technique; thus, this technique causes minimal discomfort to the fracture hematoma and preserves periosteal blood flow to the bone. In recent years, it has been reported that leaving the fracture hematoma and soft tissues around the fracture untouched helps fracture healing and in this way, it is stated that fracture healing is rapid and the risk of contamination is minimal (Farouk et al., 1997; Nikalou et al., 2008; Baroncelli 2012; Peirone, 2020). Anatomically reducing comminuted fractures is not a primary goal in fracture treatment with minimally invasive plate osteosynthesis, a kind of biological internal fixing. The purpose of biological fixation is to fix the bone at its original length and prevent the movement of the fragments by resisting the resulting axial and torsional forces (Gautier and Ganz, 1994; Wenda et al., 1997). It is reported in previous studies (Wenda et al., 1997; Conzemius and Swainson, 1999) that the minimally invasive plate osteosynthesis procedure has an advantage over the traditional plate application technique, as the procedure is shorter (36-45 days) and bone healing is faster on radiological examination. On the other hand, it is stated that there is no significant difference in the degree of healing between MIPO and the open reduction internal

fixation method (Baroncelli et al., 2012). It is stated in a previous study that healing time was 87-121 days using open reduction internal fixation technique with a bone plate. In the presented study, MIPO was applied to diaphyseal tibia fractures of cats and biological healing was aimed, as stated in the literature. It is aimed to bring the fracture lines on the same alignment and the plate is placed in this way for biological healing. Since the tissue around the fracture line was not damaged when applying mipo after bone reduction, circulatory damage was kept to a minimum and recovery was short (35 days) and infection-free. Due to the absence of a control group in the study, it was not suitable to conclusively mention on the recovery duration.

In MIPO application, the entire bone line may not be visible when the plate is placed, which can lead to shifts in the fracture line reduction after screwing due to incorrect plate positioning and misalignment of screw holes. It is reported that caution should be exercised during the screw tightening process, as tightening the screw too much or too little will have negative effects. To avoid problems, radiological imaging modalities should be used and fixation should be checked with palpation during MIPO administration (Baroncelli et al., 2012; Peirone, 2020). Although the use of long plates and a limited number of screws is useful in comminuted fractures, should be avoided in transverse or short oblique fractures as it will increase the interfragmentary stress. In such fractures, it is recommended to place plate screws close to the fracture lines to increase local stability and create sufficient durability (Stoffel et al., 2003). In the study, intraoperative radiological imaging was used for both fracture reduction and fracture fixation. Images were obtained prior to the plate being positioned on the fracture line, and the final condition of the fracture line was examined following its placement.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: B.K., M.B., Design: B.K., M.B., Data Collection or Processing: B.K., M.B., Analysis or Interpretation: B.K., M.B., Literature Search: B.K., M.B., Writing: B.K., M.B.

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Ethical Approval

Ethics committee approval was received from the Kırkkale University Clinical Practices Ethics Committee.

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Poisoning Plants in Cats and Dogs

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Abstract

Today, cats and dogs are common pets among domestic animals. These animals are exposed to many diseases or poisonings in their lives. In cases of poisoning, plants have an important role. In this respect, it should not be forgotten that plants at home or outside can cause poisoning in animals. In this review, some plants that can pose a potential danger to cats and dogs, their properties, and the treatment options that can be made with clinical signs in case of poisoning are mentioned.

Keywords: Cat, dog, poisoning plant, therapy.

INTRODUCTION

Plants are the most important creatures of the food chain. They produce their food by photosynthesis, and at the same time, they keep the carbon dioxide in the air in balance by cleaning it. They cling to the soil with their roots and reproduce with their seeds. Substances found in plants play a role in the defense of the plant and cause beneficial or toxic effects for animals that consume them (Gökkür and Doğan, 2018).

Plant poisoning is unusual in dogs and cats as it is not ordinarily part of their diet, but it is still possible. For example, plants mostly used to adorn homes, gardens, and parks are likely to cause poisoning for cats and dogs. Knowing the circumstances under which these poisonings

may consist and taking all necessary preventive measures can reduce the incidence of poisoning. For this purpose, it is necessary to identify plants that cause clinical symptoms in animals; In addition, knowing the places where the plant grows (closed areas such as parks, gardens, and ornamental plants) and the places where animals live or go regularly are important elements for diagnosis (Severino, 2009; Kızıl and Çiftçi, 2018).

In this review, some plants that show poisoning for cats and dogs (Table 1), the responsible parts for poisoning for each plant, toxic effects, clinical symptoms following ingestion of plants, and therapeutic applications in such poisonings are included.

Table 1. Poisoning plants in cats and dogs.

Plants	Poisoning parts	Clinical symptoms	Therapeutic approach
Allium species (onion, garlic, green onion, leek, etc.)	Bulbus	Hemolytic anemia; gastroenteritis and are associated with vomiting, diarrhoea, pain, loss of appetite, depression and dehydration	Blood transfusions may be helpful, especially in severe anaemia cases
Atatürk or Christmas flower	Leaves, stem, and red bracts	Gastrointestinal system diseases such as sialorrhea, dermatitis, and stomatitis.	Symptomatic treatment is recommended and it is a good practice to clean the affected areas with warm water
Azalea	Leaves	Hypersalivation, depression, anorexia, vomiting, colic, tachypnea, and tenesmus	Treatment is performed for the symptoms
Breadfruit	Leaves and stems	Edema and dermatitis in the oral and mucous membranes.	Giving antihistamines and rinsing the mouth with calcium gluconate
Boxwood	Leaves	Heart failure	Symptomatic treatment

Table 1. Continues.

Plants	Poisoning parts	Clinical symptoms	Therapeutic approach
Castor bean	Seeds	Vomiting, diarrhoea, digestive disorders, bleeding in the gastrointestinal tract, abdominal pain, increased dehydration, body temperature, intense thirst, and colic	Symptomatic treatment
Croton	Leaves and seeds	Localized eczema, diarrhea, colic, and vomiting	Symptomatic treatment
Cyclamen	All parts	Salivation, gastrointestinal symptoms, heart rhythm abnormalities	Symptomatic treatment
Hall ivy	Stem and arms	Cane tongue and include symptoms affecting the digestive system (rough edema and stomatitis), skin (vesicular rash or toxic erythema), lungs, and kidneys	Giving antihistamines in addition to general treatment applications
Holly	Leaves	Gastrointestinal disturbances, vomiting and diarrhoea	Symptomatic treatment
Hollyhock	Leaves and seeds	Agitation, shortness of breath, convulsions, vomiting, and tachycardia	Symptomatic treatment
Juniper	All parts	Salivation, diarrhoea (occasionally hemorrhagic), and shortness of breath	Symptomatic treatment
Lily	Flowers or leaves	Ingestion and include vomiting, depression, polyurea, proteinuria, polydipsia, glucosuria, and azotemia	Emetic drugs within at least 2 hours after ingestion or administration of activated charcoal and liquid therapy as soon as possible and this should be continued for at least 48 hours
Meadow	Leaves and stems	Gastrointestinal disorders, neurological and cardiovascular symptoms	Symptomatic treatment
Narcissus	Bulb parts of the plant	Vomiting, diarrhea, anorexia, abdominal pain, and sialorrhea. However, if the amount ingested is high, animals may show lethargy, ataxia, bradycardia, hypothermia, depression, and hypotension	Symptomatic treatment
Oleander	Leaves	Diarrhea, vomiting, heart block, hypercalcemia, hyperkalemia, and hypomagnesemia.	Treatment is symptomatic and administration of ephedrine, atropine, and propranolol
Rhododendron	Leaves	Anorexia, vomiting, hypersalivation, colic, tachypnea, and tenesmus	Symptomatic treatment
Rosaceae	Seeds and leaves	Tremor or ataxia, foaming at the mouth, shortness of breath and convulsions	Sodium nitrate and sodium thiosulfate
Rubber	Leaves and stem	Mucosal, skin and digestive system lesions	Symptomatic treatment
Silk tree	Seeds	Vomiting, colic, hemorrhagic diarrhoea, tetanic spasms, tachycardia, and convulsions	Symptomatic treatment
Theobroma cacao fruit	Fruit	Cardiovascular, gastrointestinal, and nervous effects	Propranolol or metoprolol can be used for tachyarrhythmias and hypertension, atropine for bradyarrhythmias, and lidocaine for refractory ventricular arrhythmias

Table 1. Continues.

Plants	Poisoning parts	Clinical symptoms	Therapeutic approach
Tongue cane	Leaves, stem, and roots	Digestive, respiratory system, and kidney	Symptomatic treatment is performed and it is recommended to give antihistamine, to rinse the mouth with water or sodium bicarbonate solution
Yew	Seeds, leaves, and bark	Agitation and muscle tremors, a state of depression is observed with extinction of respiratory frequency	Heptaminol, analeptics and sliders

Allium species

Allium species (onion, garlic, green onion, leek, etc.) are especially toxic to domestic cats and dogs (Kobayashi, 1981). Among these species, especially onions contain toxic substances that cause hemolytic anemia in many pet species, including cats and dogs. The use of children's foods to stimulate their appetite, especially due to anorexia problems, and the fact that these foods contain onion products cause poisoning cases (Aslani et al., 2005). Allium species contain organosulfoxides, particularly cysteine sulfoxides, which are responsible for their characteristic odor. Crushing the plant (chewing, chopping, etc.) causes the conversion of these organosulfoxides to sulfur-containing compounds, creating negative effects on animals. Of these, dipropylsulfide and allylpropylsulfide are substances associated with hemolytic anemia (Yamato et al., 1999). Sodium-n-propylthiosulfite, isolated from boiled onions, increases the damage to the erythrocytes of dogs and cats and stimulates the susceptibility to hemolytic anaemia in the following period. In the blood samples taken on different days, it was determined that the number of red blood cells, haemoglobin and hematocrit decreased significantly compared to the first day, the number of Heinz bodies in the erythrocytes increased and other blood parameters changed. Garlic is less toxic to dogs than onions (Tang et al., 2008). In particular, the main effect of the organic sulfur compounds in it is oxidative hemolysis, which occurs when the oxidant level in erythrocytes exceeds the antioxidant capacity. This oxidative damage seems to be related to the amount of glutathione found in red cells (Yamato et al., 1999). The antioxidant activity of catalase in the erythrocytes of dogs is low (Nakamura et al., 1998). In addition, the hemoglobin of cats is 2-3 times more sensitive to oxidative damage than the hemoglobin of other species (Cope, 2005). Consumption of garlic at doses of 5 g/kg body weight in cats and 15-30 g/kg body weight in dogs results in clinically significant haematological changes (Slater et al., 2011). The first striking findings in poisoning cases in cats and dogs are usually related to gastroenteritis and are associated with vomiting, diarrhoea, pain, loss of appetite, depression and dehydration. Within a few days of ingestion, dogs show signs of anaemia, pale mucous membranes, rapid and difficult breathing, weakness, dark urine (red or brown), jaundice, weakness, and tachycardia, which are symptoms associated with erythrocyte loss. In addition, cats and dogs who have recently consumed these foods will have an odour of onions or garlic. For the therapeutic approach, blood transfusions may be helpful, especially in severe anaemia cases (Slater et al., 2011; Bates, 2018).

Atatürk or Christmas flower (*Euphorbia pulcherrima*)

Atatürk or Christmas flower is in the Euphorbiaceae family. The toxic compounds are found in the leaves, stem, and red bracts containing cyclic tetraterpenes that may cause conjunctivitis, lacrimation, photophobia, and keratitis when in contact with the eyes. Rarely, this plant can cause gastrointestinal system diseases such as sialorrhea, dermatitis, and stomatitis. Symptomatic treatment is recommended and it is a good practice to clean the affected areas with warm water (Bilgili et al., 2020).

Azalea (*Rhododendron simsii*)

Azalea is in the Ericaceae family and has different coloring flowers. Its leaves are intoxicating parts and cause symptoms that cause hypersalivation, depression, anorexia, vomiting, colic, tachypnea, and tenesmus. It can also cause kidney and liver failure. There is no specific treatment available, and treatment is performed for the symptoms (Pischon et al., 2018).

Breadfruit (*Monstera deliciosa*)

Breadfruit is in the Araceae family and is called *Philodendron pertusum* because of the holes found in the leaves. It is used in office and home decoration and can reach large sizes. The leaves and stems of the plant include irritating liquid too dangerous to pets. With direct contact, it can cause edema and dermatitis in the oral and mucous membranes. Giving antihistamines and rinsing the mouth with calcium gluconate may be beneficial in the treatment (Colombo et al., 2010).

Boxwood (*Buxus sempervirens*)

Boxwood is an evergreen shrub species belonging to the Buxaceae family, spreading in different areas of Europe, Asia, America and Africa. While it sometimes causes poisoning in pigs, poisoning is rarely seen in pets. The leaves of the plant contain alkaloids that cause sudden heart failure in animals. Symptomatic treatment is recommended in poisoning (Bertero et al., 2021).

Castor bean (*Ricinus communis*)

Castor beans belong to the Euphorbiaceae family. Although it is used as a decorative plant in many countries, it mostly grows as a wild plant extending from the coasts to the mountains. Its toxic substance is a lectin called ricin found in the seeds, and a few seeds can kill pets or a child (Bradberry et al., 2003; Milewski and Khan, 2006). While symptoms differ between species, humans and horses are highly susceptible. Symptoms of poisoning occur approximately 6-42 hours after ingestion and include

vomiting, diarrhoea, digestive disorders, bleeding in the gastrointestinal tract, abdominal pain, increased dehydration, body temperature, intense thirst, and colic (Doan, 2004). One day after ingestion, other symptoms such as tachycardia, bloody diarrhoea, and convulsions may appear. Symptomatic treatment is recommended in poisoning (Audi et al., 2005; Mouser et al., 2007).

Croton (*Codiaeum variegatum pictum*)

Croton is in the Euphorbiaceae family and has large and variegated leaves. The leaves of the plant include caustic latex rich in calcium oxalate. Also, the seeds of plant contain a phytotoxin that may be dangerous to pets. When latex comes into direct contact with the skin at first, it causes localized eczema; then the lesion extends to areas of the body. Also, latex may cause proteinuria and hyperthermia in animals. Ingestion of the seeds causes bloody diarrhea, colic, and vomiting. Symptomatic treatment is recommended in the treatment (Severino, 2009).

Cyclamen (*Cyclamen persicum*)

Cyclamen (Primulaceae family) is a genus including more than 20 plant species as wild and cultivated flowering perennials. The best-known species, *Cyclamen persicum*, has red or white flowers and is often grown in homes. After flowering, a capsule-shaped fruit develops. Leaves of cyclamen are long petiolate, kidney or round shaped. These plants are comprised of toxic terpenoid saponins (saxifragifolin B and cyclamine) found in all parts of them, particularly in their tubers and roots (Van der Kolk, 2000). Cyclamen stems have been used aim of sedative, diarrheal, anthelmintic, and abortive effects in medicine (Robertson et al., 1998; Van der Kolk, 2000; Aslani et al., 2005; Tang et al., 2008). Moreover, cyclamen stem extracts have been shown to exhibit various biological activities such as cytotoxicity and antimicrobial activity (Van der Kolk, 2000). Increased salivation (sialorrhea) and onset of gastrointestinal symptoms (vomiting and diarrhoea) are seen following ingestion of the plant, and seizures, heart rhythm abnormalities, and death may also occur when consumed in large quantities. In case of poisoning, symptomatic treatment is performed (Houston and Myers, 1993).

Hall ivy (*Philodendron scandens*)

Hall ivy is a plant in the Araceae family and is often responsible for fatal poisoning in cats. The stem and arms of plants are responsible for the dangerous part and contain proteolytic enzymes and toxic substances such as calcium oxalate. Poisoning symptoms are similar to those caused by a cane tongue and include symptoms affecting the digestive system (rough edema and stomatitis), skin (vesicular rash or toxic erythema), lungs, and kidneys. For treatment, it is recommended to give antihistamines in addition to general treatment applications (Beasley, 1999).

Holly (*Ilex aquifolium*)

Holly is in the Aquifoliaceae family and dogs are the more commonly poisoned animal species by this plant. Its leaves contain ilicin, ilexanthin, tannins, and ilex acid while its fruits include toxins that cause both emetic and diarrheal effects. Gastrointestinal disturbances, vomiting and diarrhoea are often seen in poisoning. In terms of treatment, symptomatic treatment is recommended (Caloni et al., 2013).

Hollyhock (*Cytisus laburnum*)

The plant, also known as hollyhock or golden chain, is in the Leguminosae family. In some countries, it is grown to decorate gardens or parks. The yellow flowers of the plant, which form in clusters, have the appearance of a bush reaching the size of a tree, and the fruits are black or small dark brown. Cattle and horses are often poisoned by this plant, while dogs are rarely poisoned. The toxic substances of the plant are the leaves and seeds, which contain toxic alkaloids such as methylcystine, laburnine, cystine, and laburnamine. Clinical symptoms include agitation, shortness of breath, convulsions, vomiting, and tachycardia. Animal deaths occur only in the most severe cases. There is no specific treatment available, and symptomatic treatment is recommended (Lorgue et al., 1999; Bates, 2018).

Juniper (*Juniperus sabina*)

Juniper is in the Cupressaceae family and is an evergreen shrub that reaches the size of a green-leaved tree. It is a self-developing plant that is used as an ornamental outdoor plant. Poisoning is scarce due to the bitter taste of the plant. While ingestion poisoning may occur in cattle, poisoning in dogs and cats is extremely rare. The substances responsible for poisoning are the essential oil, gums, and resins found in the plant. Symptoms include salivation, diarrhoea (occasionally hemorrhagic), and shortness of breath. No specific treatment is available; administering activated charcoal to prevent absorption of the ingested herb may be helpful for treatment (Pages et al., 1989; Vostinaru et al., 2020).

Lily (*Lilium spp.*)

Lily is in the Liliaceae family and has colourful flowers. Many varieties are available, such as tiger lily, Easter lily, and Japanese lily. Just a few flowers or leaves pose a health risk to cats, the most susceptible species. Especially, the aqueous extract of flowers and leaves is nephrotoxic and toxic to the pancreas (Rumbeih et al., 2004). The bulb parts of the plant contain alkaloids that cause kidney failure, especially in cats, while dogs are more resistant to this condition. Lily can cause acute kidney failure leading to death of animals (Langston, 2002). Symptoms begin within 24 hours of ingestion and include vomiting, depression, polyurea, proteinuria, polydipsia, glucosuria, and azotemia. Histologically, acute necrosis in proximal convoluted tubules and degeneration in pancreatic acinar cells occur in the kidneys. In case of poisoning, early decontamination of the plant with emetic drugs within at least 2 hours after ingestion or administration of activated charcoal and liquid therapy as soon as possible and this should be continued for at least 48 hours (Brady and Janovitz, 2000).

Meadow (*Iris sibirica*)

Meadow iris is in the Iridaceae family and is found in gardens. The flowers are yellow, violet or white. The poisonous substances in the plant are the alkaloids found in the bulbs and the glycoside iridin, which is an irritant and causes diarrhoea. Clinically, symptoms specific to gastroenteritis with hemorrhagic diarrhoea are observed and symptomatic treatment is recommended in case of poisoning (Severino, 2009).

Mistletoe (*Viscum album*)

The plant is in the Viscaceae family. This plant has green leaves and translucent white fruits that remain on the plant

throughout the winter. The leaves and stems contain many compounds such as alkaloids, glycosides, and saponins. In addition, it contains viscotoxins that exhibit cardiac and neurotoxic effects. Characteristic poisoning symptoms are gastrointestinal disorders such as vomiting, diarrhea, and sialorrhea. Also, neurological symptoms include mydriasis, ataxia, fatigue, and hypersensitivity, while effects on cardiovascular function include hypotension. In case of poisoning, symptomatic and supportive treatment is recommended (Valle and de Carvalho, 2021).

Narcissus (*Narcissus spp.*)

Narcissus is a decorative plant with yellow or white flowers belonging to the Amaryllidaceae family. The dog is a commonly affected species as it can chew or ingest the plant, but calendula toxicosis can also occur in cats (Saxon-Bury, 2004). The bulb parts of the plant are the most poisonous as they include alkaloids such as galantamine and lycorine. However flowers and leaves can also pose a risk to pets. In poisoning, symptoms appear early and include vomiting, diarrhea, anorexia, abdominal pain, and sialorrhea. However, if the amount ingested is high, animals may show lethargy, ataxia, bradycardia, hypothermia, depression, and hypotension until they fall into a coma. Large amounts of around 15g can kill a dog. There is no specific treatment and it is recommended to apply symptomatic treatment and induce vomiting (Bilgili et al., 2020).

Oleander (*Nerium oleander*)

Oleander is in the Apocynaceae family and is a shrub plant widely grown in the Mediterranean basin. Oleander is a quite poisonous plant and all parts of it, especially its leaves, are poisonous to pets. Consuming a few leaves is lethal to pets. However, poisoning is not common in cats and dogs. Oleander includes many glycosides such as rosagenin, neriatoside, nerioside, and oleandroside which have cardiotoxic effects similar to digital glycoside. Poisoned animals show diarrhea, vomiting, heart block, hypercalcemia, hyperkalemia, and hypomagnesemia. Treatment is symptomatic and administration of ephedrine, atropine, and propranolol gives good results (Langford and Boor, 1996; Caloni et al., 2013).

Rhododendron (*Rhododendron ferrugineum*)

Rhododendron is in the Ericaceae family and is widely used to decorative in gardens and parks. Rarely, cats and dogs are poisoned by this plant, while sheep and goats are the most frequently poisoned species. The toxic parts of the plant are the leaves, which contain a grayanotoxin that causes anorexia, vomiting, hypersalivation, colic, tachypnea, and tenesmus followed by bradypnea in animals. Poisoned animals may also have kidney and liver failure. Symptomatic treatment is recommended in poisoning. (Puschner et al., 2001; Jansen et al., 2012).

Rosaceae family

This family has many of the most common fruit trees such as peach, cherry, apricot, apple, and plum. They can rarely pose a risk to pets; this is due to the seeds or seeds of the fruit being swallowed by pets playing with them. The seeds and leaves of the fruits may be toxic because they contain cyanogenetic glycosides (such as prunasin, amygdalin, and prunelaurasin). Clinical signs such as tremor or ataxia, foaming at the mouth, shortness of breath and convulsions are observed a few minutes after ingestion. Cyanide inhibits cytochrome oxidase and cellular

respiration; As a result, the administration of sodium nitrate, which forms methemoglobin, which binds with cyanide-forming cyan-methemoglobin, and sodium thiosulfate, which converts cyanides to thiocyanate, may be beneficial in cases of poisoning (Severino, 2009; Bates, 2018).

Rubber (*Ficus elastika*)

The rubber is in the Moraceae family and has oval glossy green leaves. Rubber is used not only in the decoration of houses but also in the decoration of gardens that can grow as large as a tree. Toxic compounds are found in the leaves and stem and form latex that causes digestive disorders such as vomiting and diarrhea. Sometimes kidney damage is present in addition to mucosal, skin and digestive system lesions. The prognosis is generally favorable and treatment is symptomatic (Lucia, 2021).

Silk tree (*Albizia julibrissin*)

The silk tree or mimosa is in the Leguminosae family. Pets may accidentally eat the seeds, in such cases, different symptoms such as vomiting, colic, hemorrhagic diarrhoea, tetanic spasms, tachycardia, and convulsions may occur. Cylindricaluria and proteinuria may also be available. The poisonous part in the plant is the lectin phytohemagglutinin. Symptomatic treatment is recommended in poisoning (Severino, 2009).

Theobroma cacao fruit

Theobroma cacao fruit in the Sterculiaceae family and includes theobromine, which primarily affects the heart. Cardiovascular effects (hypertension, tachycardia, and arrhythmias), gastrointestinal effects (diarrhea and vomiting), and nervous effects such as tremors, agitation, seizures, and hyperactivity may occurred following ingestion of large quantities of the fruit. In severe poisonings, death may occur due to heart failure (Cortinovis and Caloni, 2016). Stabilization is important in animals. Methocarbamol or diazepam can be used for tremor and mild contractions, and barbiturates can be used for severe contractions. Propranolol or metoprolol can be used for tachyarrhythmias and hypertension, atropine for bradyarrhythmias, and lidocaine for refractory ventricular arrhythmias. Diuresis can be applied to preserve cardiovascular functions and accelerate urinary excretion of methylxanthines (Yurdakök-Dikmen, 2020).

Tongue cane (*Dieffenbachia picta*)

Tongue cane, which is one of the ornamental plants that is often used for decorative plant at home, can pose a risk to the health of pets. The plant originates from South America and takes part in the Araceae family. Its large elliptical leaves are green with a white variegation. The poisonous parts of the plant are the leaves, stem, and roots and contain a very irritating liquid rich in calcium oxalate crystals (Loretti et al., 2003). Calcium oxalate crystals facilitate histamine release due to damage to mast cells. However, calcium oxalates are irritating to the mucous membranes and cause severe pain in the mouth, paralysis of the tongue, salivation, and dysphagia. Symptoms mainly affect the digestive system (stomatitis and rough edema), skin (toxic erythema or vesicular rash), respiratory system, and kidney, resulting in the death of the animals following ingestion of many quantities of herbs. In the treatment, symptomatic treatment is performed and it is recommended to give antihistamine, to rinse the mouth with water or sodium bicarbonate solution. It is also good

practice to remove plant residues from the animal's stomach by administering emetics if ingestion has occurred within the last two hours (Pedaci et al., 1999; Dip et al., 2004).

Yew (*Taxus baccata*)

Yew is a shrub plant growing wild in mountain and sub-mountain regions. It is the most dangerous plants for pets. A cardiotoxic alkaloid called taxine, found in seeds, leaves, and bark is responsible for its toxic effect. Although large animals are the most commonly affected species, pets may also be affected by the toxic substances of the plant. Most characteristic symptoms are the found in nervous system. After an early period of arousal characterized by agitation and muscle tremors, a state of depression is observed with extinction of respiratory frequency. Often pets die without showing any symptoms, but postmortem diagnosis is easy due to the presence of leaf fragments in the stomach contents. Since the development of poisoning is very rapid, there is no effective treatment. Also, heptaminol (an amino alcohol classified as a cardiac stimulant) given at an early stage can be an effective measure. In addition, the administration of analeptics and sliders may be beneficial (Cope et al., 2004; Tiwary et al., 2005).

CONCLUSION

Toxicity may vary depending on the types of plants as well as the parts taken, the vegetative period, and environmental conditions (irrigation, soil type, temperature, etc.) in which the plant is located. Therefore, it is important to consider these aspects when approaching the patient in case of poisoning to establish an appropriate therapeutic strategy. There is usually no specific treatment for plant poisonings, but knowledge of the plant species involved is very useful for rapid decontamination and symptomatic/supportive care and medical attention. However, knowing the plant species can provide information that helps to initiate a rapid and focused response. It also makes a difference in determining the survival rate in case of poisoning and the quality and speed of recovery.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

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