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Experimental Oriental Hybrid Lilies (*Lilium* Hybrids) Poisoning in Cats

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Abstract

The pathophysiology of Oriental hybrid lilies poisoning in cats was studied. Clinically normal eighteen domestic shorthair cats were orally dosed with 0, 1.5, 2.5 g wet weight of homogenate lily flower petals per kg body weight by a nasogastric tube in the study (n=3/sex/dose level). Blood and urine samples were collected before and after dosing. The cats of all treated groups presented anorexia, vomiting, lethargy and depression within 0.5 h after dosing. Serum levels of Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Glutamyl transferase (GGT), Lactate dehydrogenase (LDH), Creatinine (CREA), Blood urea nitrogen (BUN) and Creatine kinase (CK) were increased in all treated groups in a dose-dependent manner. Severe hepatocellular vacuolation was present in the cats of 2.5 g/kg group. Mild and diffuse vacuolar degeneration was observed in the renal tubule epithelium of cortex and medulla in the cats of the same group. This study indicated that oriental hybrid lily is hepatotoxic to cats, associated with some effects on myocardium and kidneys.

Keywords: Oriental hybrid lilies; Intoxication; Domestic shorthair cat; Hepatotoxicity; Liver function tests

Introduction

Lilium spp. is a common herbaceous flowering plant with more than 100 species including tiger lilies (*Lilium tigrinum Britton and Brow.*), daily lilies (*Hemerocallis* spp.), Easter lilies (*Lilium longiflorum Thun.*) and Oriental hybrid [1]. They had been reported to cause intoxication in cats which consumed incidentally [2]. Lily poisoning in cats is characterized by vomiting, anorexia, depression [3], and acute renal failure [4]. Although there were many case reports of lily poisoning in cats, the pathophysiology of lilies toxicosis has not been studied systematically and experimentally.

In order to study the mechanism of lily poisoning, we fed domestic shorthair cats the petals of Oriental hybrid lilies at different dosages in two phases. Hematology, serum biochemistry and urinalysis were performed before and after feeding lily flowers. This study provides a basis for the diagnosis and treatment of lily poisoning in clinical practice.

Material and Methods

Animals and treatment

Eighteen adult, clinically normal domestic shorthair cats (9/sex, 3 ± 1 years of age; 3.5 ± 0.3 kg) were purchased from a commercial cattery. The cats were individually housed in metal cages at room temperature (25°C) and were provided one ball or a toy mouse as environment enrichment. They were fed a commercial cat food (Innovet, Pedigree, Beijing, China) and offered free access to water. All cats were subjected to clinical examination, hematology and biochemistry analyses and antibodies to *Toxoplasma gondii* (Nicolle and Manceaux) two weeks before dosing with lily petal powder. They were vaccinated against Feline Panleukopenia, Feline calicivirus and Feline Viral Rhinotracheitis (Nobivac Vaccination, Intervet International B.V., Boxmeer, The Netherlands) at the cattery at least one month before the experiments. The experiments were approved by the University Animal Ethics Committee.

The cats were randomly divided into three groups (3/sex/group). Two groups (B and C) were given 1.5 g/kg and 2.5 g/kg lily petals WT/kg BW (35 ml) and one control group (A) received distilled water (35 ml).

Flowers of Oriental hybrid lily which were produced from Yunnan province of China were collected from a local flower shop near the university. The fresh flower petals were homogenized with distilled water after the stamen was removed. The flower homogenate was orally administered to the animals using a nasogastric tube within 5 minutes of homogenate preparation. Food was withheld for 12 hours before dosing. All cats were intramuscularly injected with 1 mg/kg bodyweight metoclopramide (Tianjin Jiaozuo Pharmaceutical Co., Ltd, Wuzhi, Henan, China) prior to dosing.

Clinical examination and necropsy

The cats were clinically examined before and after lily homogenate administration. They were weighed daily. Blood samples were collected from the cephalic vein or a lateral saphenous vein immediately before dosing and 2, 4, 6, 8, 12, 24, 48, 72 and 96 h after flower administration. Blood samples (2.2 ml/time) were anticoagulated by sodium heparin (6,250 IU/ml) and centrifuged at 1000 g for 10 min (low speed centrifuge, XiangYi Centrifuge Instrument CO., Ltd, Changsha, Hunan, China). Laboratory tests were performed within 12 hours of blood collection or stored at 20°C until analysis. Urine was collected either by cystocentesis or volunteer urination for urinalysis.

All cats recovered after study and were euthanased 96h after dosing by intravenous injection with 10% KCl injection solution (Shandong Shenglu pharmaceutical co., LTD, Shandong, China) in the study and subjected to a complete necropsy. Heart, liver, spleen, lung, kidneys, pancreas and muscle tissues were fixed in 4% formalin solution,

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dehydrated and paraffin embedded, stained with hematoxylin and eosin for microscopic examination.

Laboratory tests

Blood counts were measured using an automatic blood cell analyzer (MEK-6318k, Nihon-kohden, Shinjuku-ku, Tokyo, Japan). Urinary specific gravity, urobilinogen, occult blood, urobilirubin, urine acetone bodies, glucose, nitrite, leukocyte and protein were determined using SIMENS Multistix10SG urine test strips (Simens corporation, Munich, Germany) and Bayer CliniTek 50 urinalysis meter (Bayer corporation, Leverkusen, Germany).

Serum levels of Potassium (K), Sodium (Na), Chloride (Cl) and total CO_2, pCO_2, pH, HCO_3^-, Base Excess (BE) were determined by EC8+ cartridges (Abbott point of care Inc., Nepean, Ontario, Canada) using an I-STAT biochemical analyzer (I-STAT, ABAXIS, Abbott point of care Inc., Union city, California, USA). Serum concentrations of Calcium (Ca), Inorganic phosphorus (IP), total protein, albumin, Blood Urea Nitrogen (BUN), Creatinine (CRE), bilirubin (total and direct), ALT, AST, ALP, total cholesterol (TCHO), CK, LDH, amylase and γ-glutamyl transferase (GGT) were measured by a Tecfinicon automated biochemical analyzer (TECFINICON RA500/1000, Bayer Corporation, Pittsburgh, Pennsylvania, USA). The serum concentration of glucose was assayed by test strips and the Eukare XFH-588a blood glucose meter (Zhenzhou xinfuhua industry Co., Ltd, Henan, China).

Statistical analysis

The data were analyzed by one-way ANOVA and LSD multiple comparison using the software of SPSS (Statistical Package for Social Science, 12.0, SPSS Incorporation, Chicago, Illinois, USA).

Results

All group C (2.5 mg/kg) cats were inactive, depressed and lethargic at 30 min after dosing, and 5 of them returned to normal after 12 h. One cat had a coma and recovered 96 h after dosing. In group B (1.5 mg/kg), 5 cats presented depression and lethargy at 40 min after dosing, and recovered at 4h; one showed no abnormalities. All lilium-treated cats were anorexic, and most of them started eating at 8h in the low dose group B and by 24 h in the high dose group C. Most cats had variable degrees of vomiting (low dose group: 2-4 times; high dose group: 4-14 times). All control cats appeared normal. All treated groups appeared anorexia.

The heart rate of group c was depressed at 24 h and 48 h ($P<0.05$), but the decrease was small. The WBC counts of both treated groups were higher than that of the control group ($P<0.05$) from 4 h to 12 h. ALT and AST activities of both treated groups were significantly increased ($P<0.01$) compared to the control group and pre-treatment values. Serum levels of total bilirubin of group C and B tended to be higher than that of the control group at 24 h to 72 h after lilium dosing ($P<0.05$). The concentration of creatinine of group C peaked at 24 h ($P<0.05$) after administration, while LDH activity in both dosed groups rose dramatically from 4 h to 24 h ($P<0.01$). The level of CK of the treated groups started to rise from 4h after dosing ($P<0.05$) and peaked at 24 h ($P<0.01$) (Table 1).

Necropsy of group C cats showed mild hepatomegaly.

parameters	Groups/(dose)	time(h)							
		0	4	8	12	24	48	72	96
Heart rate	C	142.40 ± 29.50	138.20 ± 28.25	140.80 ± 26.29	130.00 ± 7.21	132.40 ± 19.46*	137.80 ± 13.60*	142.80 ± 17.81	132.80 ± 15.84
	B	141.67 ± 22.46	156.33 ± 17.17	145.33 ± 24.12	148.00 ± 35.44	156.00 ± 23.15	148.00 ± 13.56	149.33 ± 23.31	148.67 ± 22.72
	A	155.00 ± 19.00	144.83 ± 13.51	149.33 ± 20.18	147.67 ± 16.65	164.67 ± 18.67	167.33 ± 20.57	153.33 ± 18.48	140.00 ± 31.01
WBC	C	22.81 ± 2.33	29.19 ± 2.26*#	38.51 ± 5.51*#	40.46 ± 4.70*#	22.80 ± 2.09	20.21 ± 0.84	19.15 ± 1.21	18.96 ± 1.54
	B	21.7 ± 2.06	21.95 ± 1.59#	25.95 ± 1.77#	25.61 ± 2.633#	19.53 ± 2.37	17.61 ± 1.12	17.7 ± 1.39	16.98 ± 1.28
	A	19.68 ± 2.00	21.81 ± 1.42	19.65 ± 2.34	20.21 ± 3.75	20.03 ± 4.31	18.85 ± 3.18	15.90 ± 1.95	15.88 ± 1.25
ALT (U/L)	C	54.56 ± 15.06	104.08 ± 26.96**#	112.30 ± 25.68**#	116.14 ± 24.19**#	103.62 ± 17.60**	83.02 ± 20.64*	73.64 ± 22.79*	56.88 ± 24.54
	B	55.16 ± 17.38	69.87 ± 22.56#	74.57 ± 24.70#	77.08 ± 26.36#	81.77 ± 38.17*	64.65 ± 29.26	51.05 ± 13.61	49.88 ± 14.57
	A	55.05 ± 13.12	52.97 ± 13.53	52.17 ± 12.67	51.92 ± 9.28	43.41 ± 11.08	46.02 ± 13.33	45.93 ± 16.48	42.67 ± 15.64
AST (U/L)	C	33.26 ± 7.35	214.67 ± 108.28**	488.33 ± 249.41**	413.40 ± 212.33**	210.85 ± 141.74*	65.67 ± 73.83	44.43 ± 48.32	22.75 ± 7.52
	B	28.97 ± 6.90	168.08 ± 125.33*	327.02 ± 250.03*	292.02 ± 280.55**	140.33 ± 183.85	31.75 ± 21.06	19.80 ± 3.67	19.35 ± 3.06
	A	32.53 ± 8.66	34.85 ± 9.97	35.28 ± 9.58	34.02 ± 6.06	22.02 ± 5.94	21.55 ± 5.40	20.07 ± 6.73	23.22 ± 6.86
TBIL (μmol/L)	C	3.62 ± 0.50	3.47 ± 0.46	3.98 ± 1.17	3.98 ± 1.19	4.69 ± 1.34*	4.10 ± 0.61	3.67 ± 0.42#	3.80 ± 0.68
	B	3.75 ± 0.76	3.37 ± 0.61	3.73 ± 0.73	3.73 ± 0.82	4.28 ± 1.01	4.42 ± 0.45*	4.35 ± 0.39*#	4.23 ± 0.27
	A	3.53 ± 0.71	3.37 ± 0.36	3.67 ± 0.38	3.30 ± 0.60	3.26 ± 0.42	3.42 ± 0.65	3.76 ± 0.48	3.67 ± 0.29
CREA (μmol/L)	C	122.85 ± 8.69	141.96 ± 9.30	138.88 ± 7.62	143.41 ± 8.41	168.95 ± 13.41*	157.10 ± 9.09	142.18 ± 4.31	136.18 ± 6.95
	B	121.31 ± 4.77	127.99 ± 8.14	126.81 ± 8.44	139.54 ± 12.44	155.14 ± 12.50*	135.80 ± 9.22	120.19 ± 8.18	118.41 ± 7.12
	A	124.68 ± 7.60	124.09 ± 5.166	129.37 ± 4.73	131.51 ± 4.54	120.34 ± 6.72	132.50 ± 6.41	131.98 ± 8.03	131.18 ± 6.86
LDH (U/L)	C	122.00 ± 18.23	1040.17 ± 475.47**	2271.00 ± 658.04**	2412.50 ± 676.63**	2325.00 ± 1022.26**	877.50 ± 1017.30	266.33 ± 362.12	103.67 ± 47.72
	B	93.33 ± 28.87	798.00 ± 469.14**	1701.17 ± 897.21**	1889.66 ± 1260.04**	1593.00 ± 1771.49**	577.83 ± 956.64	102.00 ± 19.98	81.67 ± 10.91
	A	114.67 ± 30.63	125.50 ± 31.58	112.67 ± 23.07	121.67 ± 26.42	80.50 ± 26.75	78.50 ± 24.85	148.78 ± 215.19	79.25 ± 27.07
CK (U/L)	C	1021.23 ± 743.63	2911.23 ± 1964.31*	3270.43 ± 1805.35*#	3518.48 ± 2036.64*#	3268.08 ± 2092.15**#	1297.10 ± 1188.05*#	473.22 ± 512.40	127.70 ± 52.17
	B	975.37 ± 848.57	1334.60 ± 903.48	1545.82 ± 1094.05#	1549.25 ± 955.78#	1336.57 ± 951.31#	391.05 ± 235.24#	167.78 ± 120.44	97.63 ± 29.18
	A	1051.68 ± 374.34	1261.10 ± 757.72	1375.90 ± 983.30	1361.80 ± 969.11	1684.22 ± 1745.32	143.43 ± 77.89	110.05 ± 61.35	123.35 ± 49.56
CK-MB (U/L)	C	58.80 ± 8.14	426.80 ± 163.75**#	229.20 ± 78.78**	181.20 ± 51.50**	139.80 ± 51.72	71.40 ± 31.34	38.40 ± 7.89	41.20 ± 13.25
	B	65.20 ± 44.71	208.00 ± 148.17#	147.00 ± 73.43	120.00 ± 67.02	97.60 ± 76.43	38.40 ± 11.61	50.60 ± 39.88	36.20 ± 12.46
	A	78.50 ± 42.07	83.33 ± 59.90	90.17 ± 73.23	66.00 ± 26.69	74.67 ± 79.22	53.17 ± 56.44	42.12 ± 25.73	37.00 ± 11.73
Glucose (μmol/L)	C	5.58 ± 2.79	4.32 ± 1.05*	4.86 ± 2.38	4.00 ± 1.74	4.28 ± 1.53	4.68 ± 1.29#	4.44 ± 0.80#	4.66 ± 1.02
	B	5.18 ± 0.73	5.10 ± 0.33	5.36 ± 2.23	5.26 ± 1.30	5.72 ± 1.57	6.24 ± 0.79*#	5.78 ± 0.58#	4.78 ± 0.46
	A	6.26 ± 1.86	5.37 ± 0.58	5.67 ± 1.38	5.56 ± 1.14	5.30 ± 1.37	4.90 ± 0.93	5.20 ± 1.04	5.20 ± 1.04

* $P<0.05$, ** $P<0.01$ (compared to the control group A). #$P<0.05$, ##$P<0.01$ (B compared with C). CK, Creatine Kinase;TBIL, Total bilirubin; CREA,Creatinine. C=2.5g/kg; B=1.5g/kg; A= 0g/kg

Table 1: Study findings *

Histopathological lesions were observed in the kidneys, spleen, pancreas, cardiac muscle, skeletal muscle and lungs.

Most of liver lobules were indistinguishable, and central veins still exist, the structure of hepatic cord was absent, and most of hepatocytes were still swollen, the nucleus had been pushed to the edge, and cytoplasma were spilt, accompanied by severe hepatocellular vacuolation (Figures 1 and 2).

The morphological structures of glomeruli were normal. Mild and diffuse vacuolar degeneration and diffuse cellular swelling were seen in the tubular epithelium of renal cortex and medulla, the nucleus had been pushed to the edge, and cytoplasma were spilt (Figures 3 and 4). Cardiac muscle fibers were stumpy and had branches that formed the net structure.

Discussion

Oriental hybrid lilies are toxic to cats if a large amount of petals are ingested. The whole lily plant-petals, stamen, leaves, and pollen are toxic [3]. One study proved the most toxic fraction of the Easter lily was the flower [5]. Our study showed that 5 g/kg and 10 g/kg Oriental hybrid lily petals were lethal to cats. All cats receiving a high dose of lilies had salivation, vomiting, depression, hypothermia, bradycardia, bradypnea, seizure, shock and incontinence. Although the lily-dosed cats were administered intramuscularly metoclopramide, vomiting continued. It has been reported that clinical signs of depression, lethargy, and hypothermia might be a result of hypoglycemia [6], which hypoglycemia might also cause bradycardia [7]. Bradycardia and bradypnea might lead to hypoventilation and low cardiac output, leading to hypoxic hepatitis [8]. In the cats dosed with lily flower petals, serum aminotransferase (ALT and AST) levels increased sharply. Hypoglycemia would stimulate and accelerate hepatic glycogenolysis through the activation of glycogen phosphorylase [9]; however, glycogen metabolism was blocked by hepatic damage from lily poisoning. Severe hypoglycemia would cause seizure or shock [10].

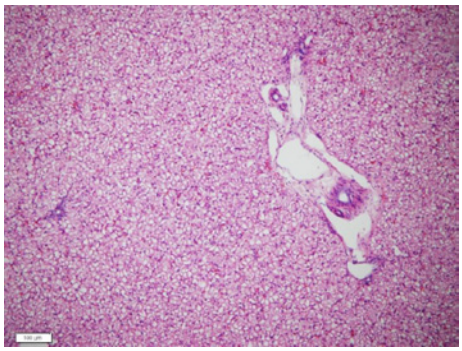

Figure 1: Most of liver lobules were indistinguishable, and the structure of hepatic cord was absent (dosage=2.5 g lilies/kg). Hematoxylin-eosin staining. Bar, 100 μm.

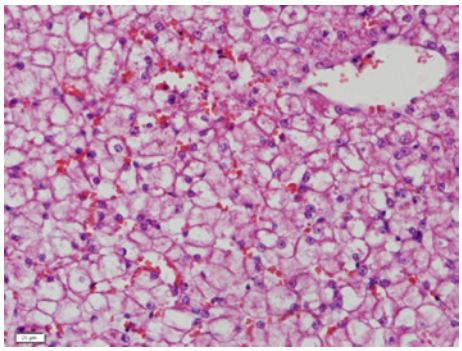

Figure 2: Severe hepatocellular vacuolation (dosage=2.5 g lilies/kg). Hematoxylin-eosin staining. Bar, 20 μm.

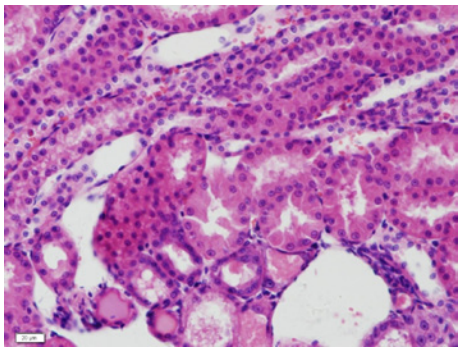

Figure 3: Diffuse cellular swelling was seen in the tubular epithelium of renal cortex and medulla (dosage=2.5 g lilies/kg). Hematoxylin-eosin staining. Bar, 20 μm.

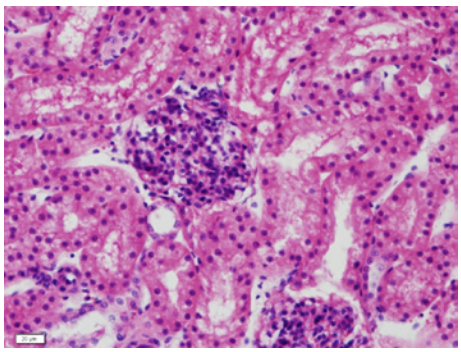

Figure 4: Mild and diffuse vacuolar degeneration of renal cortex and medulla (dosage=2.5 g lilies/kg). Hematoxylin-eosin staining. Bar, 20 μm.

We showed that the levels of total blood CO_2 and HCO_3^- decreased dramatically from 2 h after dosing, associated with a slight decrease in blood pH and increase in base excess, suggesting a combination of metabolic acidosis [11] and respiratory alkalosis. It has been reported that H^+ would move from extracellular fluid into the intracellular apartment and K^+ in the opposite direction in metabolic acidosis, leading to hypokalemia [12]. Bradycardia and anoxia might lead to myocardial injury [13,14], disturbance of muscle cell integrity [15], and elevated myocardial zymogram. We also observed increased CK, CK-MB and LDH, indicating myocardial muscle damage, possibly a result of bradycardia and anoxia.

Some papers reported nephrotoxicity caused by lily poisoning [4,16,17], indicated by increased serum urea and creatinine levels [2]. However, our study showed only mild effects on the kidneys. Serum urea and creatinine levels were unaffected, urinalysis showed no evidence of renal toxicity, and the cats presented no signs of acute renal failure although kidney lesions were present by microscopic examination. In contrast, increased aminotransferases (ALT and AST) and histopathology indicated liver damage. The differences between our study and other published studies might be related to lily breeds and thus different toxins in different lilies. It has been proposed that

colchicines might be the main toxin in *Lilium* spp.. Colchicine is a natural pseudo-alkaloid found in plants such as the autumn crocus (*Colchicum autumnale Linnaeus*) and glory lily (*Gloriosa superb Linnaeus)*, and could cause severe diarrhea, cardiovascular shock and multi-organ system failure [18]. Wang and his colleagues [19] extracted 5 to 50 μg/ml colchicine from Lanzhou lily (*Lilium davidii hoog*) samples. It is unknown whether the flower of Oriental hybrid lilies contains colchicines.

Successful treatment might be administered during early decontamination and support therapy when cats were consumed significant amounts flowers of Oriental hybrid lilies. If the symptoms were severe, peritoneal dialysis or hemodialysis was taken [3].

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