

Brief Report

WILD-MUSHROOM INTOXICATION
AS A CAUSE OF RHABDOMYOLYSIS

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THE growing popularity of eating wild mushrooms has led to an increase in the incidence of mushroom poisoning. Most fatalities are due to amatoxin-containing species, which cause fulminant hepatocytolysis, and to cortinari species, which lead to acute renal damage. A 1996 report described a patient with hepatic failure, encephalopathy, and myopathy related to the ingestion of *Amanita phalloides*.¹ Since 1992, 12 cases of delayed rhabdomyolysis have occurred in France after meals that included large quantities of the edible wild mushroom *Tricholoma equestre*.² The circumstances of these 12 cases clearly implicate *T. equestre* as the cause. The mushroom was positively identified, and no other cause, such as bacterial, viral, fungal, or immune disease or exposure to a toxin, was found. Three of the 12 patients died.

The implicated mushrooms were harvested from beneath pine trees on the sandy coast of southwestern France, between late fall and midwinter. *T. equestre* is widely disseminated throughout the world and is also known as *T. flavovirens* and colloquially as “bidaou” or “canari” in France, “riddarmusseron” in Sweden, “shimokoshi” in Japan, and “man on horseback” or “yellow-knight fungus” in the United States (Fig. 1).^{3,4} We investigated the rhabdomyolysis apparently induced in 12 humans by several consecutive meals of *T. equestre* by administering equivalent doses of extracts of this mushroom to mice.

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Figure 1. *Tricholoma equestre*.

The cap of this species measures 6 to 8 cm, and the stem is 7 to 10 cm long and 1.5 cm in diameter.

CASE REPORTS

Seven women (age range, 22 to 60 years) and five men (age range, 24 to 61 years) were hospitalized between 1992 and 2000 with severe rhabdomyolysis approximately one week after eating wild mushrooms. All 12 patients had eaten at least three consecutive meals that included *T. equestre*, and none had a history of trauma, other known underlying cause, or medication use that could explain the occurrence of rhabdomyolysis. All patients reported fatigue and muscle weakness accompanied by myalgia, mainly in the upper part of the legs, 24 to 72 hours after their last meal containing mushrooms. The weakness worsened over a period of three to four days, leading to stiffness of the legs and the production of dark urine. These signs were accompanied by facial erythema, mild nausea without vomiting, and profuse sweating in eight of the patients. No fever was noted, and five patients had hyperpnea. The findings on physical examination, which included pulmonary auscultation and a neurologic examination, were unremarkable.

Initial screening tests showed evidence of rhabdomyolysis, with a mean maximal serum creatine kinase activity of 226,067 U per liter in the women and 34,786 U per liter in the men (Fig. 2). No hepatic injury was evident. γ -Glutamyltransferase values were normal (range, 5 to 24 U per liter), and maximal average levels of aspartate aminotransferase and alanine aminotransferase were 8104 and 1392 U per liter, respectively, in the women and 1173 and 325 U per liter, respectively, in the men. Despite the intensity of the clinical rhabdomyolysis, electrolyte levels, including potassium values, were normal, and no renal failure occurred. Coagulation tests were normal. Additional studies were negative for parasites or other microorganisms (coxsackievirus, toxoplasma, toxocara, trichinella, hepatitis B and C viruses, and human immunodeficiency virus), as well as for systemic diseases (as assessed by complement-fixation tests and tests for circulating nuclear antibodies).

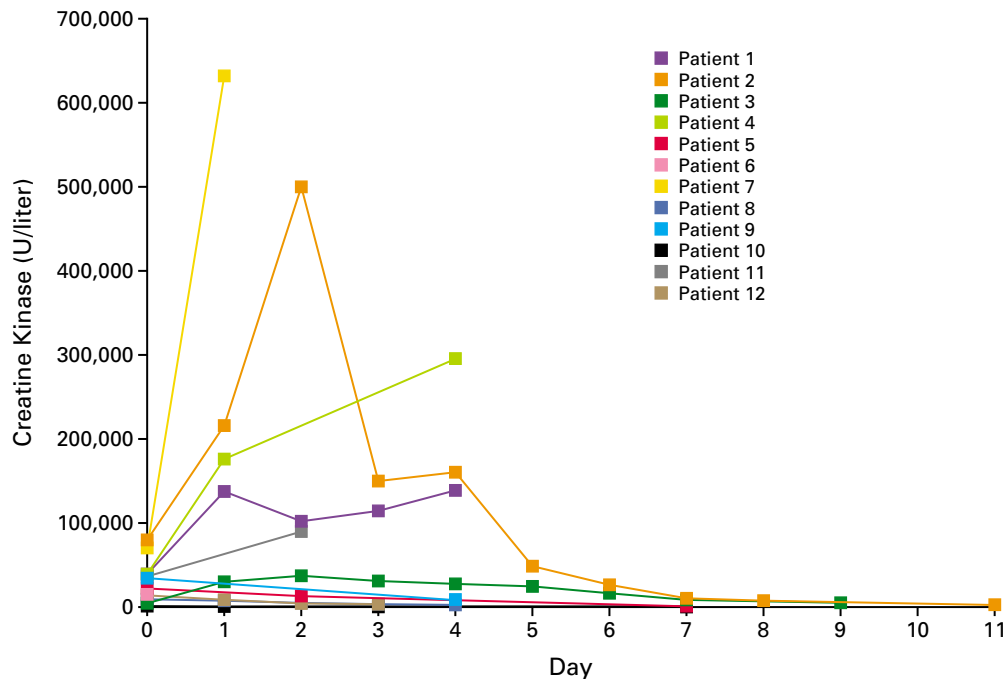


Figure 2. Serum Creatine Kinase Levels in the 12 Patients during Hospitalization. Patients 1, 4, and 7 died.

Given the absence of evidence of deliberate intoxication, the analyses focused on the hypothesis that mushroom intoxication caused the rhabdomyolysis. Electromyography was performed in four patients and revealed muscle injury without peripheral-nerve involvement. The greatest changes were in the proximal thigh muscles. Complex and generally myotonic activity was present, without fibrillation, even at rest. On stimulation, muscle contractile activity was particularly prominent. All the motor and sensory potentials were normal. Electromyography of the diaphragm was performed in one patient and showed similar findings, suggesting the presence of rhabdomyolysis of the diaphragm without phrenic involvement.

In six patients, samples of quadriceps muscle were obtained for histologic analysis. On light microscopy, the fascicular architecture of the muscles was well preserved, the myofibrils had a “nibbled” appearance, and in some cases the fibers were separated from each other by edema in the absence of vacuoles or the accumulation of glycogen or lipid — signs of a direct muscle injury. In the three patients who died, samples of the psoas and other muscles (from the arms, myocardium, and diaphragm) also showed evidence of acute myopathy.

Over the next 15 days, in all but three of the patients, the serum enzyme values gradually normalized and most symptoms disappeared, although the muscular weakness persisted for several weeks. In the three patients who died, the increasing dyspnea at rest was the first symptom of further deterioration and was followed by increasing rales at both lung bases, leading to admission to the intensive care unit. All three patients had hyperthermia (a temperature of up to 42°C); signs of acute myocarditis, including cardiac arrhythmia, cardiovascular collapse, and widening of the QRS complex without severe acidosis (pH, 7.37, with a serum bicarbonate level of 16 to 20 mmol per liter); and evidence of renal dysfunction, including elevated levels of blood urea nitrogen (30 to 52 mg per deciliter [10.7 to 18.7 mmol per liter]) and serum creatinine (1.4 to 2.5 mg per deciliter [126 to 224 μmol per liter]), with hyperkalemia (potassium, 6.0 to 7.2 mmol per liter) and hypocalcemia (cal-

cium, 5.6 to 8.3 mg per deciliter [1.4 to 2.07 mmol per liter]) and a normal total protein level. The three patients had creatine kinase values of 632,000, 138,900, and 295,700 U per liter, with isoenzyme MB making up 0.5 to 0.7 percent of the total. Despite intensive physiological care, including, in one case, continuous venovenous hemofiltration, all three patients died. Autopsy revealed myocardial lesions identical to the muscular lesions in one patient, renal lesions in one patient, and no hepatic lesions.

METHODS

Additional studies were necessary to demonstrate that *T. equestre* was the cause of the rhabdomyolysis in the 12 patients. Since it would have been unethical to administer *T. equestre* extracts to human subjects, we chose an established model of myonecrosis in mice.⁵⁻⁷ Extracts of *T. equestre* were prepared and then administered by gavage (gastric intubation) in a dose equivalent to that ingested by the patients. The animals were then assessed for evidence of rhabdomyolysis. Two protocols were employed: a dose-response study with *T. equestre* alone and one in which mice received extracts of either *T. equestre* or the nontoxic mushroom *Pleurotus ostreatus*.

Mushroom Extracts

Specimens of *T. equestre* collected in southwestern France were identified by qualified mycologists.^{2,4} The taxonomic denomination of *T. equestre* is synonymous with that of *T. flavovirens* (Fries) Lundell.³ Specimens of *P. ostreatus* obtained commercially were confirmed as such by qualified mycologists. Then 500 g of *T. equestre* was frozen, ground, and mixed with 200 ml of ultrapure water. The resulting mixture was lyophilized to yield 60 g of powder. Extracts were then obtained. A concentrate of 1.35 g of cold aqueous extract was obtained from 5 g of powdered *T. equestre*, and a concentrate of 1.65 g of boiled aqueous extract was obtained from 5 g of powdered *T. equestre*. A concentrate of 1.3 g was obtained from 10 g of powdered *T. equestre* after chloroform-methanol (vol/vol) extraction,

and a concentrate of 50 mg of chloroform-methanol lipid-free extract was then obtained from 200 mg of the chloroform-methanol extract. We obtained a cold aqueous extract, a boiled aqueous extract, and a chloroform-methanol (vol/vol) extract of *P. ostreatus* in a similar manner.

Experimental Intoxication

Adult male Swiss mice (mean [\pm SE] weight, 30 ± 5 g) (Depre), were randomly divided into several groups. In each group, *T. equestre* powder, *T. equestre* or *P. ostreatus* extract, or solvent alone was administered to each animal in a standardized fashion.

Twenty-four or 48 hours after the final dose, the mice were anesthetized with ether, and blood was collected from the retroorbital sinus and centrifuged at $2000 \times g$ for 15 minutes at 5°C to recover serum. The serum was frozen and stored at -20°C until analysis. Creatinine levels were determined by the Jaffé reaction (Merck kit 3385). Aspartate aminotransferase and alanine aminotransferase activities were determined with the use of enzyme kits (Merckotest, Merck).⁸ Creatine kinase was measured with a commercial kit (Enzyline kit, Biomérieux). The mice were then killed, and samples of striated muscle, liver, and tissues were obtained.

In the first protocol, three groups of three mice each were given 1 ml of powdered *T. equestre* suspension in water once a day by gavage for three days. The highest total dose was 6 g per kilogram of the body weight of the mouse (0.18 g per mouse), corresponding to a hypothetical toxic dose in a 60-kg person of 3 kg of fresh mushrooms eaten over the course of six meals in a period of three days (72 hours). Creatine kinase activity was determined in serum collected 48 hours after the final dose.

In the second protocol, five groups of five mice each were given 0.3 ml of tricholoma or pleurotus extract dissolved in water or dimethylsulfoxide once a day by gavage for three days. This dose corresponded to a total dose of 0.18 g of powdered *T. equestre* suspension. The positive control consisted of *p*-phenylenediamine (dose, 70 mg per kilogram per day for three days), which is a potent myotoxic compound in mice.⁶ Levels of aspartate aminotransferase, alanine aminotransferase, creatine kinase, and creatinine were determined in serum collected 96 hours after the final dose.

Statistical Analysis

Data are presented as means \pm SE. The results were analyzed with use of the Wilcoxon rank-sum test, a nonparametric statistical test chosen because of the small number of animals.

RESULTS

Mice treated with *T. equestre* powder (Table 1) had a concentration-dependent increase in the serum crea-

TABLE 1. SERUM CREATINE KINASE LEVELS IN MICE GIVEN A SUSPENSION OF *TRICHOLOMA EQUESTRE* POWDER (BY GASTRIC INTUBATION) EVERY DAY FOR THREE DAYS.*

TOTAL DOSE	CREATINE KINASE U/liter
2 g of powdered <i>T. equestre</i> /kg	210 \pm 90
4 g of powdered <i>T. equestre</i> /kg	345 \pm 120†
6 g of powdered <i>T. equestre</i> /kg	380 \pm 25†
Water (control)	145 \pm 40

*Plus-minus values are means \pm SE.

†P=0.01 for the comparison with the control.

tine kinase level (up to a mean of 380 ± 25 U per liter). At a total dose of 4 g and 6 g per kilogram, the increase was significant when compared with the levels in controls (mean, 145 ± 40 U per liter; P=0.01 for both comparisons), although there was variability between mice.

Mice treated with boiled *T. equestre* extracts, chloroform-methanol lipid-free extract, and *p*-phenylenediamine had a significant increase in serum creatine kinase activity (912 ± 425 , 883 ± 500 , and 1828 ± 450 U per liter, respectively; P=0.01 for each comparison with base-line levels) (Fig. 3). No such increase was observed in mice treated with extracts of *P. ostreatus*. Serum levels of aspartate aminotransferase, alanine aminotransferase, and creatinine were not increased significantly in mice treated with extracts of *T. equestre* or *P. ostreatus* (data not shown).

All the treated mice had tachypnea, reduced motor activity, and occasional diarrhea. Light microscopy of muscle fibers showed evident disorganization.

Two mice — one that received boiled aqueous extract and one that received chloroform-methanol lipid-free extract — died 72 hours after the last dose. These animals were not autopsied because by the time the deaths were discovered autolysis had set in.

DISCUSSION

Rhabdomyolysis is a rare but potentially fatal condition. Muscle compression is the most common cause, but neither muscle ischemia nor unconsciousness was noted before the onset of symptoms in our patients. Deliberate intoxication with substances including cocaine, amphetamines, alcohol, theophylline, phenothiazines, *p*-phenylenediamine,^{9,10} antihistamines, and antihyperlipidemic drugs was ruled out.^{11,12} Medications that could cause dermatomyositis or polymyositis (penicillamine, phenytoin, levodopa, and quinidine) were ruled out by screening tests. Finally, immunologic screening as well as muscle biopsies did not indicate the presence of other systemic disorders or McArdle's disease.¹³

Toxic rhabdomyolysis has been described after the ingestion of small wild birds that had eaten water hemlock (*Conium maculatum*), a direct muscle toxin.^{14,15} Our patients had not eaten such birds. Although mushroom poisoning is not known to produce rhabdomyolysis, this series of cases clearly associates rhabdomyolysis with the ingestion of *T. equestre*.

Since 75 percent of the patients with large increases in creatine kinase survived, a genetic muscular susceptibility may be unmasked by the direct muscle toxicant contained in *T. equestre* when the amount of mushrooms ingested exceeds a certain threshold. Therefore, physicians should be aware of the possibility of severe rhabdomyolysis after repeated consumption of *T. equestre*. At this time treatment is supportive, and hospitalization is recommended for patients with dyspnea, signs of acute myocarditis, or even mild renal failure.

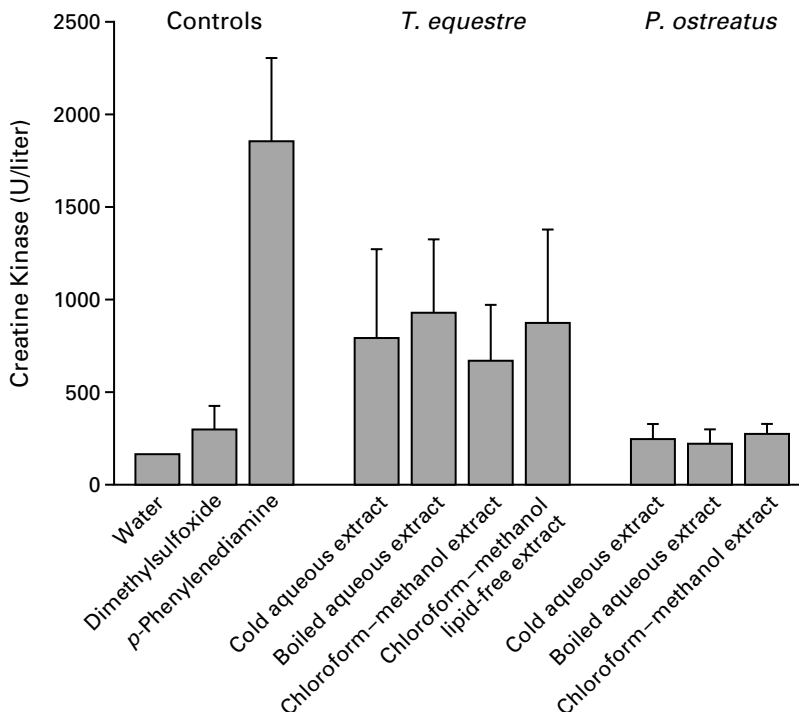


Figure 3. Mean (+SE) Serum Creatine Kinase Levels in Mice Treated by Gastric Intubation with Extracts of *Tricholoma equestre* and *Pleurotus ostreatus* Every Day for Three Days.

The total dose of *T. equestre* powder was 6 g per kilogram of body weight.

Our experiments in animals confirmed the involvement of *T. equestre* in the etiology of rhabdomyolysis. Mice receiving *T. equestre* extract had increased creatine kinase levels, whereas those receiving *P. ostreatus* extract did not.

Several metabolites have been isolated from various tricholoma species — triterpenoids,¹⁶ sterols,¹⁷ indoles,¹⁸ and acetylenic compounds¹⁹ — but their muscle toxicity is unknown. The yellow pigment of *T. equestre*, 7,7' bi-physcion, has been identified.^{20,21} However, since this pigment is minimally soluble in water, we think it is unlikely to be the toxic compound. Since all extracts of *T. equestre* were toxic to mice, inducing increases in creatine kinase levels, the toxic compound appears to be extracted equally well by water and chloroform-methanol. It remains to be identified.

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