

TOXIC MYODEGENERATION IN GOATS PRODUCED BY FEEDING MATURE FRUITS FROM THE COYOTILLO PLANT (*KARWINSKIA HUMBOLDTIANA*)

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The purpose of this paper is to describe cardiac and skeletal myodegenerations produced by experimental feeding of the fruits of the coyotillo plant (*Karwinskia humboldtiana*) to goats.

The coyotillo plant is indigeneous to Southwest Texas and Mexico and is abundant in many areas.¹ It is a spineless shrub with pinnately veined leaves, small greenish flowers and brownish black fruits which are ovoid shaped drupes. A disease syndrome called "limberleg" has been recognized in sheep and goats for many years in areas where coyotillo is prevalent. The clinical syndrome is characterized by progressive leg weakness with associated incoordination and ataxia. Finally, the animals become recumbent and die. The ingestion of coyotillo fruits has been shown to be the etiologic agent.² In addition, Marsh, Clawson and Roe² have shown that the fruits were toxic for a number of animal species.

Several writers have indicated that the fruits were toxic for humans. Clavigero³ in 1789 was the first to mention the toxic properties of *Karwinskia humboldtiana* when he wrote that Indian children became poisoned after eating the fruits. Standley⁴ stated that the seeds were oily and contained some principle which paralyzed the motor nerves. He further indicated that the fruits had been used in Mexico as anti-convulsants, particularly in the case of tetanus. Marsh and co-workers² said that a Dr. Palmer stated in 1901 that several children poisoned by consumption of coyotillo fruits had been brought to him and that one girl lost the use of her limbs. Vandرسال⁵ and Muenschner⁶ reported that children had been fatally poisoned by eating the fruits.

MATERIAL AND METHODS

Sixteen mature goats of both sexes were divided into 3 groups. In 2 of these there were 4 treated and 2 control animals; 1 group contained 3 treated and 1 control animal (Table I). The animals were maintained on pasture until ready for use. Those in group A were then placed in metabolism cages; the other groups were kept

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TABLE I
COYOTILLO POISONING

Group	Animal no.	Daily dosage (% body wt.)	Total amount fed (% body wt.)	SGOT *		SGPT *	
				Pre-treatment range	Final value	Pre-treatment range	Final value
A	1	0.15	0.59	—	—	—	—
	2	0.15	0.45	—	—	—	—
	3	0.15	0.50	—	—	—	—
	4	control	—	—	—	—	—
B	5	0.075	0.50	120-132	2,000	22-31	40
	6	0.075	0.43	132-144	1,440	22-24	34
	7	control	—	120-144	150	18-28	28
	8	0.075	0.40	128-130	1,620	30-33	86
	9	0.075	0.41	130-140	1,300	24-33	76
	10	control	—	102-140	122	30-43	33
C	11	0.025	0.17	130-144	440	22-36	50
	12	0.025	0.18	144-164	650	19-28	94
	13	control	—	108-144	130	19-24	24
	14	0.025	0.15	130-144	500	16-26	50
	15	0.025	0.43	122-140	450	24-30	45
	16	control	—	112-120	112	20-26	20

* Serum glutamic oxalacetic and pyruvic transaminase.

in 20-foot-square pens. All were fed water and prairie hay *ad libitum* and were given alfalfa hay daily.

Mature coyotillo fruits were ground to a powder in a Wiley mill and given to the treated animals as a bolus. Sufficient water was added to the powder to form a stiff paste and the material placed at the base of the tongue with an oral gelatin capsule administrator. The controls were fed ground hay. The treated animals in group A received 0.15 per cent of body weight of the ground fruit; group B received 0.075 per cent and group C, 0.025 per cent of body weight. The coyotillo was given daily until the animals were observed to have clinical signs of intoxication. These included slight hyperreactivity and mild ataxia. The administration of the plant then was discontinued. The total amount of the fruit fed to each goat is given in Table I as a percentage of the total body weight. All animals except the 4 in group A were exercised once daily for a minimum of 5 and a maximum of 10 minutes by being driven around a large pen at a pace which varied from a fast walk to a run.

The goats were placed in the treatment pens a week prior to the initial feeding. Blood was collected via the jugular vein from each animal on each of 3 days before the plant material was administered and daily or every other day after treatment began.

Levels of serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were determined by the modified Rietman-Frankel method.^{7,8} Reagents used in these tests were purchased from Dade Reagents, Inc., Miami, Florida.

All animals were examined by necropsy within 2 to 3 hours after death and most were examined within an hour. Four poisoned goats were killed *in extremis* by the intravenous injection of a concentrated pentobarbital sodium solution. Tissues were taken from all organs at necropsy and fixed in neutral isotonic 10 per cent formalin. Representative portions of various skeletal and cardiac muscles were wrapped in gauze and labeled as to their origin for identification. The tissues were embedded in paraffin, sectioned at 6 μ and stained with hematoxylin and eosin.⁹ Frozen sections of muscle were stained for fat with oil red O.⁹ Only the changes in cardiac and skeletal muscle and the liver will be described here.

RESULTS

Gross Observations

No uniformly detectable gross lesions were evident in the muscles. The supraspinatus muscles in one goat which received the 0.15 per cent dosage (group A) and one which received the 0.075 per cent dosage (group B) were mottled in appearance; scattered portions were paler than normal and the muscle was soft and slightly gelatinous. One goat which received the 0.025 per cent dosage (group C) had similar but unilateral lesions in the left gastrocnemius muscle.

There were no grossly detectable changes in the cardiac muscle.

Microscopic Lesions

None of the control goats had the muscular or hepatic lesions to be described below.

Heart. The lesions in the goats receiving the 0.075 and 0.15 per cent dosages varied somewhat in appearance regardless of the dosage levels. Some of the animals had fatty degeneration involving all cardiac fibers.

In sections stained with hematoxylin and eosin these fibers had a diffuse granular appearance throughout which tended to obscure both cross and longitudinal striations. Other hearts exhibited only irregular focal areas of granular degeneration. Some of the fibers in these areas had more advanced changes characterized by greater eosinophilia of the sarcoplasm which often was fragmented and contained pyknotic nuclei.

The microscopic lesions in the goats given the 0.025 per cent regimen were disseminated, small focal areas of proliferating muscle nuclei forming loose pleomorphic collections. These nuclei were often larger and more plump than normal with slightly folded nuclear membranes and a more than normal vesicular appearance. In some instances the sarcolemmal tube persisted with complete loss of sarcoplasm or a small residue of eosinophilic debris. In other instances the sarcoplasm persisted but had a granular appearance. In no instance were the lesions very extensive.

Skeletal Muscles. All the poisoned animals had lesions in the skeletal muscles although the amount and degree of involvement varied both within a given group as well as between groups. There seemed to be no predilection for a specific muscle or muscle group. The distribution of lesions did not depend upon dosage but the severity of the changes and type of response present were different in groups B and C. The latter 2 groups were those that were exercised. The animals in group C lived longer than those in the other groups (Table II).

TABLE II
COYOTILLO POISONING
TRANSAMINASE LEVEL AND CLINICAL MANIFESTATIONS

Group	Animal no.	Daily dosage (% body wt.)	Days after initial feeding first sign appeared	Days after initial feeding SGOT * first increased	Days after initial feeding animal died or killed
A	1	0.15	4	—	5
	2	0.15 †	3	—	4
	3	0.15	5	—	7
	4	control	—	—	—
B	5	0.075 †	6	5	7
	6	0.075	6	5	8
	7	control	—	—	—
	8	0.075 †	5	5	7
	9	0.075	5	4	6
	10	control	—	—	—
C	11	0.025 †	7	7	12
	12	0.025	7	9	18
	13	control	—	—	—
	14	0.025	7	8	14
	15	0.025	20	21	23
	16	control	—	—	—

* Serum glutamic oxalacetic transaminase.

† These poisoned goats were killed when *in extremis*.

The skeletal muscle lesions in the group fed the 0.15 per cent body weight dosage (group A) were not extensive except in the case of one animal (no. 1). The lesions here were as severe as those in the group B animals (0.075 per cent body weight dosage and exercise). The other two goats in group A had involvement of a part of a single or several muscle fibers with a random distribution in a single muscle. The changes consisted either of a fiber or fibers with granular appearance and proliferation and migration of sarcolemmal nuclei. The latter were distributed at random in the fragmented sarcoplasm.

In group B (0.075 per cent dosage), the distribution of lesions was generalized and included ocular muscles and diaphragm. The pattern was not uniform, there being only occasional groups of affected fibers interspersed among normal appearing fibers (Fig. 1). Again, the entire length of the fibers was not involved. The changes were characterized by a hyaline or granular appearance and loss of both longitudinal and cross striations. Many fibers were fragmented and exhibited retraction caps and clots or curdy masses of muscle protoplasm with interspersed empty clefts (Fig. 2). In the more severely affected fibers, the sarcolemmal nuclei appeared to be necrotic as evidenced by pyknosis and a folded, distorted appearance. In the less severely affected fibers or portions of fibers sarcolemmal nuclei became rounded, vesicular and appeared to proliferate; some moved into the center of the fiber.

In group C (0.025 per cent dosage) the predominant change in the muscle was characterized by activation of sarcolemmal nuclei; hyaline, granular or fragmented fibers were, however, also present. The involved areas were composed of one or several fibers; the lesions varied in severity and were distributed in a random manner. Occasional fibers exhibited vacuolar degeneration. Others showed fragmentation with marked proliferation of sarcolemmal nuclei at the extremity of the normal portion of the fiber. Still other fibers contained many proliferated sarcolemmal nuclei within the sarcolemmal tube. In the more severely affected portions of muscle, especially where several fibers were involved, the proliferation of sarcolemmal nuclei was accompanied by macrophages and scattered lymphocytes. These cells often formed irregular masses between normal or regenerating fibers (Fig. 3). The fibers were narrow and exhibited numerous elongated, rather vesicular sarcolemmal nuclei in a thin column of sarcoplasm. The latter often appeared slightly basophilic. In severely affected fibers removal of muscle debris by macrophages was evident. There were a few mitotic figures at the center of some fibers; whether these were sarcolemmal cells or macrophages could not be determined.

Liver. The hepatic changes were essentially the same in all poisoned

goats although the degree of involvement varied. The lesions were more severe in groups A and B but differed from the animals in group C only in degree. In groups A and B the hepatic changes were approximately the same. There was mild fatty degeneration and congestion. Necrosis of a small number of hepatic cells was evidenced by deep cytoplasmic eosinophilia, karyorrhexis and pyknosis. These cells were scattered individually in the centrilobular regions with no detectable relationship to one another. Approximately one-fourth to one-third of the lobules were affected.

Transaminase Values

The final SGOT levels in the poisoned goats were elevated when compared to pretreatment levels and to the levels found in the control animals. The degree of elevation in group B was about 10 times the pretreatment value, in group C it was approximately 4 times pretreatment level (Table I).

In Table II the initial rise in transaminase is compared to the onset of clinical signs and the time after initial feeding that death occurred. It can be seen that in group B elevation occurred 1 to 2 days before clinical signs appeared. The initial elevation in group C coincided, however, with the onset of clinical signs or occurred 1 to 2 days afterward. In all the poisoned goats the SGOT level continued to rise until death occurred or the animals were killed *in extremis*.

The final SGPT values were elevated slightly in all of the poisoned goats (Table I). There were no significant differences among the animals fed the different dosages.

DISCUSSION

Myodegeneration occurs in animals spontaneously and in a number of experimental conditions. Paramount among these in veterinary medicine is "white muscle disease."¹⁰ Selenium, tocopherol or their interaction apparently play an important role in the genesis of this condition.¹¹ In addition, certain viral disorders (coxsackie infection in mice) and such toxic substances as plasmocid¹² and monoiodoacetic acid¹² produce muscular lesions.

The alterations in cardiac muscle in this study were more generalized than those in the skeletal muscle but the latter were usually more severe. The affected fibers were not completely involved there being normal appearing areas interspersed between obviously damaged segments in the same fiber. Adams, Denny-Brown and Pearson¹² have indicated that the focal nature of skeletal muscle lesions might be related to the distribution of mitochondria.

Exercise seemed to play a role in determining the severity of the

skeletal muscle alterations. This was indicated by the fact that the lesions in all but one goat in group A, which was not exercised, were less severe than those in group B receiving lower doses but given exercise. A somewhat similar situation was reported by Young and Keeler¹⁸ in lambs with white muscle disease. The continuous cardiac activity may explain the generalized nature of the myocardial changes in all groups.

The skeletal muscle lesions in coyotillo poisoning are similar to those in white muscle disease and plasmocid poisoning.¹² Plasmocid (an 8-aminoquinoline) has a selective effect on the cardiac and skeletal muscles. Six to 8 hours after injection of this substance, focal granular and hyaline degenerations appear in muscles with the highest metabolic activity. Sarcoplasmic substance including myofibrils is affected but the nuclei and sarcolemmal sheaths are spared. Regeneration begins after approximately 24 hours. Electron microscopic studies^{14,15} have revealed the initial alteration in the diaphragmatic muscle of plasmocid poisoned rats to be a degeneration of the Z-band followed by loss of both actin filaments in the I-band and myosin filaments in the A-band. The earliest myocardial alterations were in the mitochondria with later involvement of the myofilaments. Among the features observed were the occurrence of lipid bodies separating myofibrils. There is thus considerable similarity between the lesions of plasmocid and coyotillo poisoning.

The skeletal muscle lesions in white muscle disease¹⁰ are characterized by hyaline degeneration either of entire fibers or multiple segments. The fibers undergo lumpy fragmentation and granular disintegration. Proliferation of muscle nuclei becomes prominent and calcification of necrotic fibers or portions of fibers occurs but is not constant. The cardiac muscle lesions are varied; some fibers are swollen, eosinophilic and granular, others are necrotic and fragmented. Pyknosis of muscle nuclei occurs, as does calcification of necrotic fibers.

Although the lesions in coyotillo poisoning resemble those in white muscle disease, calcification of necrotic fibers was not observed in the poisoned goats. The similarities of the lesions in coyotillo poisoning, white muscle disease and plasmocid poisoning probably reflect the limited ability of muscle to respond to injuries of various types. Although the possibility that these three conditions have a similar biochemical genesis can not be refuted, limited studies indicate the selenium-tocopheral injections which provide protection in white muscle disease¹¹ have no effect in goats with fatal coyotillo intoxication.

The studies reported here indicate the presence of a naturally occurring myotoxic substance or substances in the mature fruits of the coyotillo plant.

SUMMARY

Mature fruit of the coyotillo plant (*Karwinskia humboldtiana*) was ground and fed to goats in dosages of 0.025, 0.075 and 0.15 per cent of total body weight. Widespread skeletal and cardiac muscular degeneration was produced at each dosage level. The microscopic lesions resembled those described in plasmocid poisoning and white muscle disease. The studies indicated the existence of a naturally occurring myotoxic substance in coyotillo fruit.

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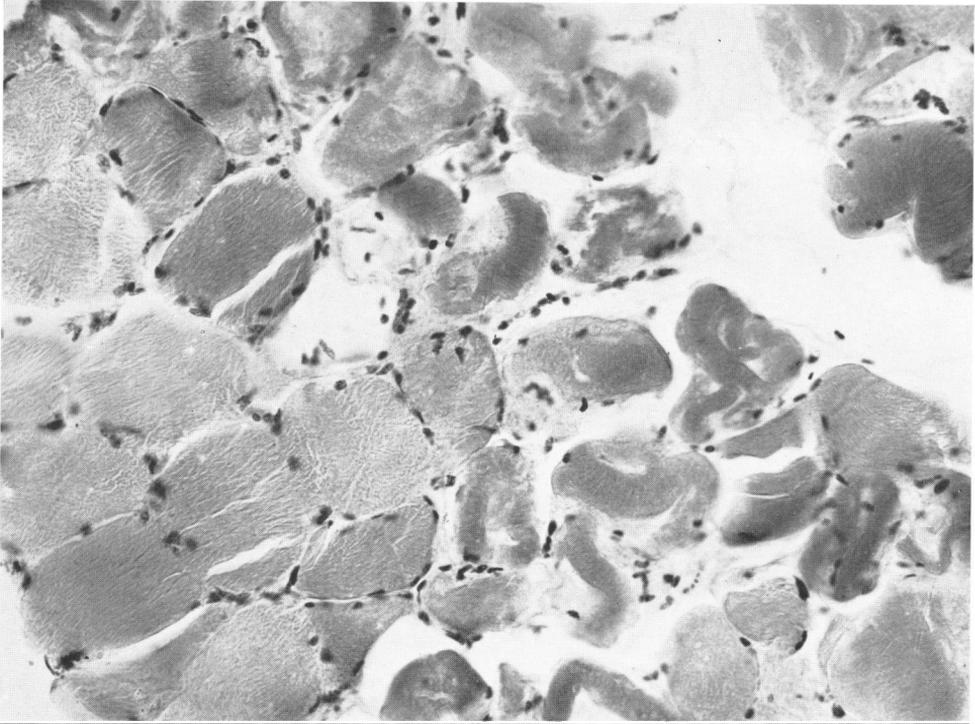
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[*Illustrations follow*]

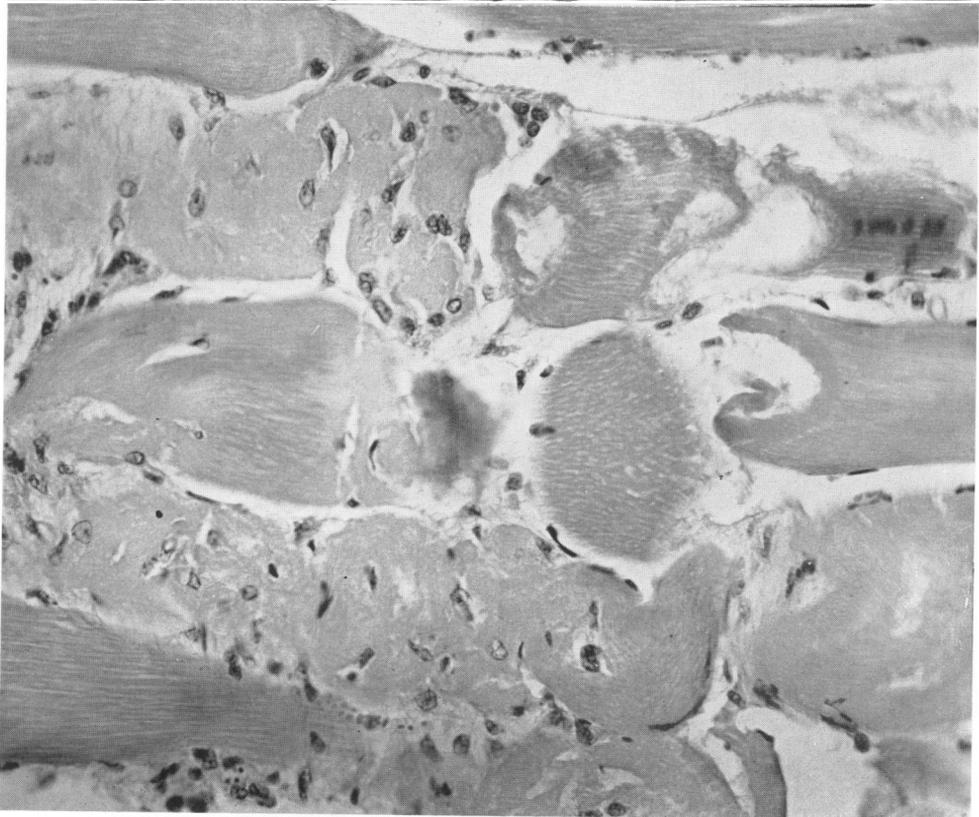
LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

- FIG. 1. Vastus medialis muscle, goat fed 0.075 per cent body weight of coyotillo fruit. Necrosis affects part of the fibers in a muscle bundle, adjacent fibers in the same bundle are normal. $\times 475$.
- FIG. 2. Supraspinatus muscle, goat fed coyotillo, 0.075 per cent body weight. Fibers exhibit fragmentation and granular degeneration. There is associated activation of sarcolemmal nuclei and macrophage infiltration. $\times 600$.



1



2

3

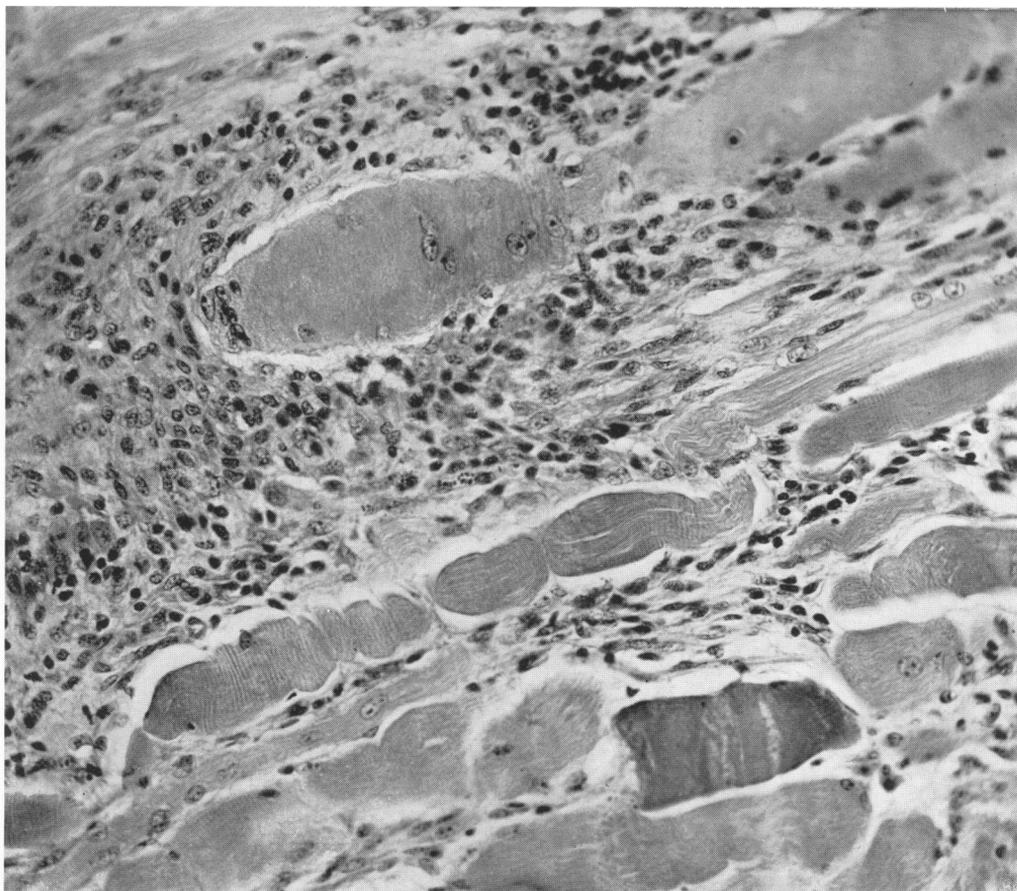


FIG. 3. Skeletal muscle, goat fed coyotillo, 0.025 per cent body weight. Both loss of fibers and various stages of regeneration are associated with lymphocyte and macrophage infiltration. $\times 350$.