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Der Pharmacia Lettre, 2015, 7 (6):110-115
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Doping with strychnine in Turkomenstallion race horse in Iran: A case report

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ABSTRACT

Doping is one of the major problems in the racing in veterinary medicine, not only has many risks for consumersof drugs (horse and dog), but also is the fraud, that will change the results of tournament. In competitive sports, doping refers to the use of banned athletic performance-enhancing drugs by athletic competitors, where the term doping widely used by organizations that regulate sporting competitions.In january 2015 during20th weeks of course competition, the result of doping related to Turkomen stallion reported positive with strychnine, but any sign of poisoning not seen in this case. Strychnine is a very dangrous and toxic substance that was the first alkaloid to be identified in the plants of the genus Strychnos, its oral LD50 values in dogs, cattle, horses and pigs is 0.5-1 mg/kg. In Veterinary medicine strychnine is used in form of the sulphate or nitrate salts as a palliative for oral treatment of cattle for simulation of ruminal motility. It is also used for subcutaneous and intramascular injection in horses. The claimed indication is symptomatic treatment of locomotor deficits of medullary origin,but the drug is on the list of banned drugs of the anti-doping committee and its use is illigal.

Key words: Horse race, Doping, Strychnine, anti-doping committee, Iran

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INTRODUCTION

In competitive sports, doping refers to the use of banned athletic performance-enhancing drugs by athletic competitors, where the term *doping* widely used by organizations that regulate sporting competitions. The use of drugs to enhance performance is considered unethical by most international sports organizations, such as theInternational Olympic Committee (IOC). The general trend among authorities and sporting organizations over the past several decades had been to strictly regulate the use of drugs in sport. The reasons for the ban are mainly the health risks of performance-enhancing drugs, the equality of opportunity for athletes, and the exemplary effect of drug-free sport for the public. Anti-doping authorities state that using performance-enhancing drugs goes against the "spirit of sport". The use of drugs in sports goes back centuries, about all the way back to the very invention of the concept of sports (14). In ancient times, when the fittest of a nation was selected as athlete or combatant, he was fed diets and given treatments considered beneficial. For instance, Scandinavian mythology says Berserkerscould drink a mixture called "butotens", to greatly increase their physical power at the risk of insanity. One theory is that the

mixture was prepared from the *Amanita muscaria* mushroom, though this has been disputed and other causes suggested. Hence, in Veterinary medicine, the history of doping is related to dogand horse racing.

1.1. Strychnine history and characteristics

Strychnine (fig.1) was the first alkaloid to be identified in the plants of the genus *Strychnos*, family Loganiaceae. *Strychnos*, named by Carl Linnaeus in 1753, is a genus of trees and climbing shrubs of the gentian order. The genus contains 196 various species and is distributed throughout the warm regions of Asia (58 species), America (64 species), and Africa (75 species). The seeds and bark of many plants in this genus contain the powerful poison strychnine.The toxic and medicinal effects of *Strychnos nux-vomica* have been well known from the times of ancient India, although the chemical compound itself was not identified and characterized until the 19th century. The inhabitants of these countries had historical knowledge of the species *Strychnos nux-vomica* and Saint-Ignatius bean (*Strychnos ignatii*)(1, 2). Strychnine, a white crystalline powder, is available in an alkaloid form. It has a characteristic bitter taste. Strychnine alkaloid is almost entirely insoluble in water and very stable; however, it is subject to acid-salt formation which renders it water soluble and subject to leaching in acid soils (15).

1.2.Pharmacology of strychnine

Strychnine isa terpene indole alkaloid belonging to the Strychnos family of Corynanthe alkaloids, and it is derived from tryptamine and secologanin. The enzyme, strictosidine synthase, catalyzes the condensation of tryptamine and secologanin, followed by a Pictet-Spengler reaction to form strictosidine(3). While the enzymes that catalyze the following steps have not been identified, the steps have been inferred by isolation of intermediates from Strychnos nux-vomica(4). The next step is hydrolysis of the acetal, which opens the ring by elimination of glucose (O-Glu) and provides a reactive aldehyde. The nascent aldehyde is then attacked by a secondary amine to afford geissoschizine, a common intermediate of many related compounds in the *Strychnos* family (1).Strychnine reacts the quickest (excluding fumigants) of the commonly used rodenticides. It is not cumulative, not absorbed through normal intact skin, has a very slight odor, has very high toxicity, and is somewhat variable in action against target animals. Strychnine enters the blood very rapidly and acts on the central nervous system. The time of action depends upon the condition of the stomach, that is, whether empty or full and the nature of the food present. Animals with little in their stomach react more quickly to strychnine than those that have fed recently(16). Symptoms may appear from five to thirty minutes after ingestion.Strychnine is transported by plasma and erythrocytes. Due to slight protein binding, strychnine leaves the bloodstream quickly and distributes to the tissues. Approximately 50% of the ingested dose can enter the tissues in 5 minutes. Also within a few minutes of ingestion, strychnine can be detected in the urine. Strychnine is rapidly metabolized by the liver microsomal enzyme system requiring NADPH and O_2. Strychnine competes with the inhibitory neurotransmitter glycine resulting in an excitatory state. However, the toxicokinetics after overdose have not been well described. In most severe cases of strychnine poisoning, the patient dies before reaching the hospital. The biological half-life of strychnine is about 10 hours. A few minutes after ingestion, strychnine is excreted unchanged in the urine, and accounts for about 5 to 15% of a sublethal dose given over 6 hours. Approximately 10 to 20% of the dose will be excreted unchanged in the urine in the first 24 hours (6). It primarily affects the motor nerves in the spinal cord which control muscle contraction. An impulse is triggered at one end of a nerve by the binding of neurotransmitters to the receptors. In the presence of a neuroinhibitor, such asglycine, a greater quantity of excitatory neurotransmitters must bind to receptors before there will be an action potential generated. Glycine acts primarily as an agonist of the glycine receptor, which is a ligand-gated chloride channel in neurons located in the spinal cord and in the brain. This chloride channel will allow the negatively charged chloride ions into the neuron, causing a hyperpolarization which pushes the membrane potential further from threshold. Strychnine is anantagonist of glycine, which means it binds to the same receptor, preventing the inhibitory effects of glycine on the postsynaptic neuron. Therefore, action potentialsare triggered with lower levels of excitatory neurotransmitters. When the inhibitory signals are prevented, the motor neurons are more easily activated and the victim will have spastic muscle contractions, resulting in death by asphyxiation (5,8). Structure of strychnine in complex with ACh binding protein (AChBP) (9).

1.3.Application of strychnine

Strychnine is currently registered for underground use only for controlling pocket gophers. Any agricultural use requires a Restricted Materials Permit that can be obtained through the agricultural commissioner. Historically, strychnine was used for controlling many birds and mammals, including skunks and coyotes. Since 1988 these uses have been banned. Only underground uses are allowed.In Veterinary medicine strychnine is used in form of the sulphate (approximately 78% alkaloid) or nitrate (approximately 84% alkaloid) salts as a palliative for oral treatment of cattle for simulation of ruminal motility. It is also used for subcutaneous and intramascular injection in horses.

The claimed indication is symptomatic treatment of locomotor deficits of medullary origin. The daily doses are 5 mg for young cattle, 12.5 mg for adult cattle (approximately 0.025 to 0.1 mg/kg bw) and 12.5 mg daily (approximately 0.025) in horses. Strychnine may be introduced into the body orally, by inhalation, or by injection and rapidly absorbed from the gastrointestinal tract(13).

4.1.Toxicity of strychnine

In general, strychnine is somewhat less toxic to gallinaceous birds than other life forms. LD50 values range from a low of 0.70 and 0.75 mg/kg for coyotes and desert kit fox; from 1.5 mg/kg for black tailed prairie dogs, to 27.0 mg/kg for nutria; and from 16.0 mg/kg for chuckar partridge to 24.7 mg/kg for ring-necked pheasants.Horses and hogs show no hesitation in eating strychnine baits. Cattle and sheep are more reluctant to accept baits. Geese and ducks show no reluctance in eating strychnine baits. However, gallinaceous game birds and domestic poultry are less susceptible to strychnine than most rodents (16,17).Strychnine toxicity in rats is dependent on sex. It is more toxic to females than to males when administered via subcutaneous injection or intraperitoneal injection. Differences are due to higher rates of metabolism by male rat liver microsomes. Dogs and cats are more susceptible among domestic animals, pigs are believed to be as susceptible as dogs, and horses are able to tolerate relatively large amounts of strychnine. Birds affected by strychnine poisoning exhibit wing droop,salivation, tremors, muscle tenseness and convulsions. Death occurs as a result of respiratory arrest. The clinical signs of strychnine poisoning relate to its effects on the central nervous system(16).

5.1.Turkomen breed

The Turkomen breed is more widely known as the Akhal Teke from Turkmenistan, its Persian counterpart has played an equal role in the development of all light breeds of horses in the world. Until the Russian conquest of Turkmenistan in the early 20th century Turkomen tribes roamed freely from the Alborz mountain range, over the Atrek river and through the Kopet Dagh mountains to the great sand desert. The major tribes of Goklan, Yamoud and Teke proudly bred their own strains, basically keeping them seperate.Today the Akhal Teke is primarily an amalgam of the different strains. Their Persian counterparts continued to be bred in the tribal manner and are still identifiable as individual strains although invariably mixing amongst the strains has occurred.This horse is gradually regaining recognition in the world as DNA analysis has shown its blood runs in all our modern breeds of light horse. The genetic contribution is immense; its history romantic; its form, action flowing,is beautiful and the people who raise them still live as they did 2000 years ago (http://www.turkmens.com).The Turkoman horse is noted for its endurance and race. It has a slender body, similar to a greyhound. Although refined in appearance, the breed was actually one of the toughest in the world. They have a straight profile, long neck, and sloping shoulders. Their back is long, with sloping quarters and tucked-up abdomen. They have long and muscular legs. The horses ranged from 15–16 hands. The coat of a Turkomen horse could have been of any color, and usually possessed a metallic glow. This was due to a change in the structure of the individual hair. Many theories have been formulated to explain why hair of the Turkomen and its descendants shines, but none explain why the Turkoman horses in particular benefitted from this genetic difference and why other horses would not.

Racing horse competition is held weekly in Iran annually. A large number of horses participating in this tournament, but some of them in order to enhance performanceare doping. Theresponsibilityof Equestrian Federation and Anti-Doping Committee is to identify and exclude these horsesof the competition, until the competitions to be held without cheating.It can not happen unless the Equestrian federations and anti-doping committee have a decent cooperation together.

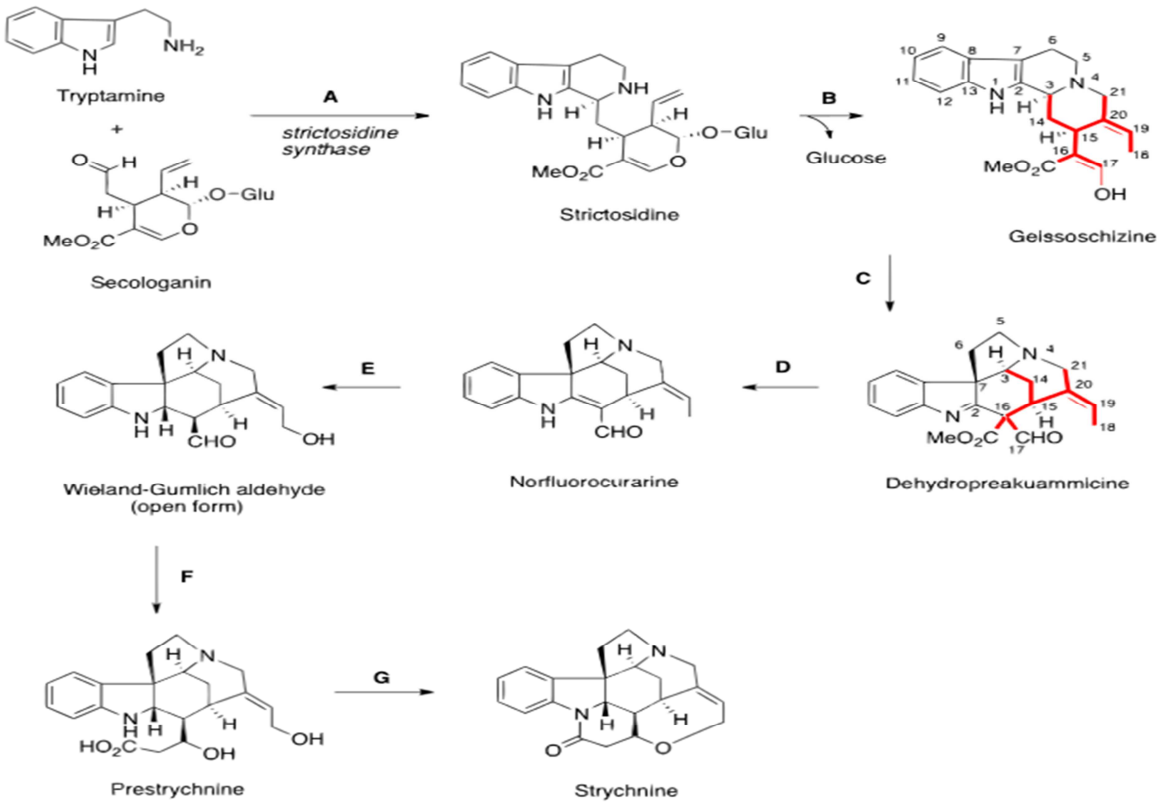


Fig 1. Chemical structure of Strychnine

2.Case description

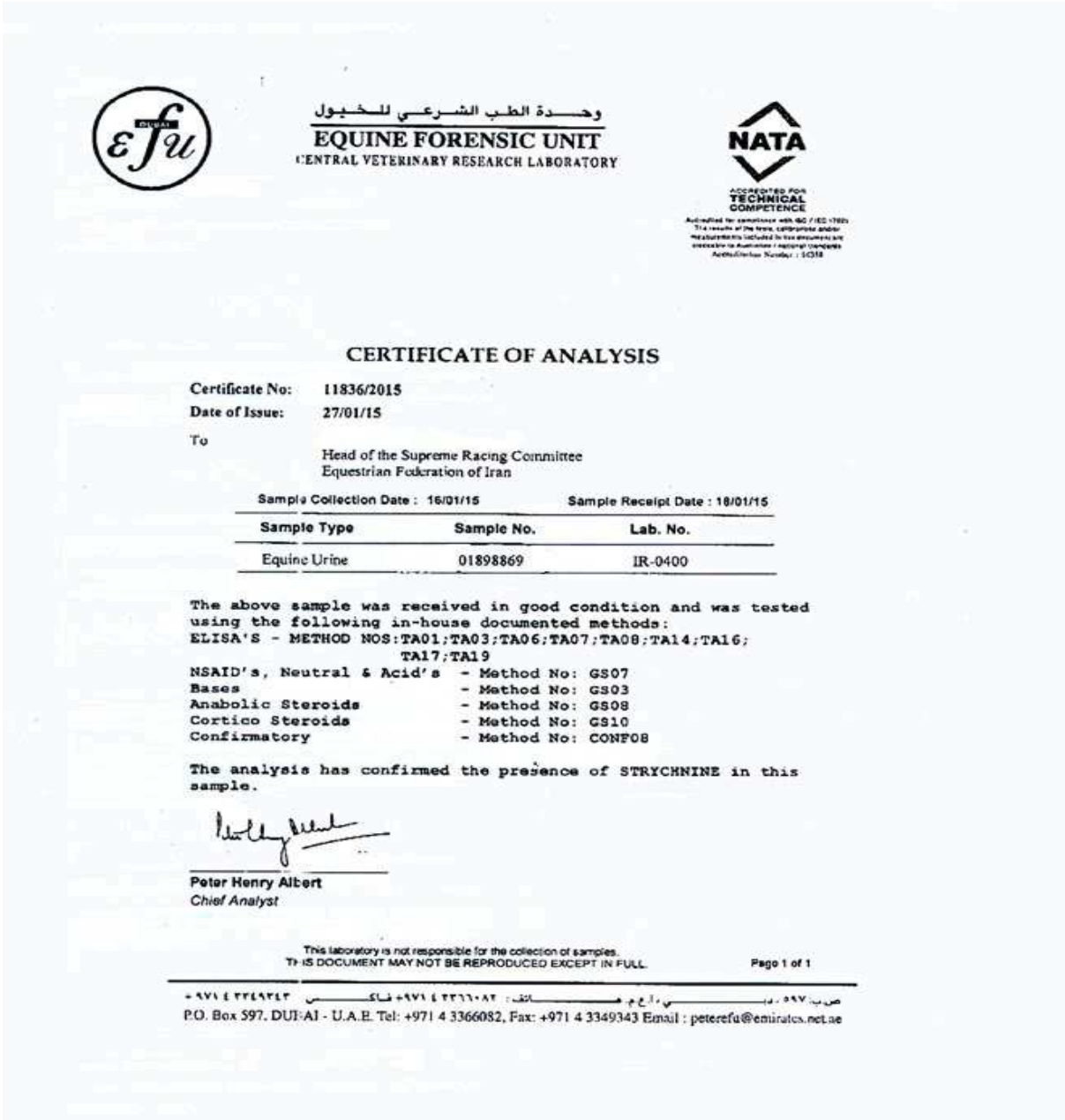
During the 20th weeks of course that fall inGonbad Kavoos, Turkomen stallion horse named Mazyar achieved second rank in the first round. After finishing the tournoment by the winner's method and just 1st& 2nd in every run are selected and tested for dope. The horses put into one of the dope testing unit's stables until a urine samples can be provided, then sampels sent to labratory for checking the point of any prohibited substances in urine samples.After physical examination, symptomes such as excitement, restlessness, loud and frequent nicker, pawing, stamping or kicking observed clearly, after 30 minutes all signs disappeared. Finally, labratory with Sample No. 01898869 and Lab. No. IR-0400 confirmed the presence of Strychnine in sent sample (fig. 2), as a result doping horse deprived and it'splace was withdrawn.

DISCUSSION

The horse racing industry and equestrian sport acknowledges thatlegitimate drug treatment must be applied when necessary and makes a clear distinction between medication control (legitimate drug) and 'doping' control (illegal substances). To detect drug exposure, sophisticated analytical techniques are used and any trace of a prohibited substance often constitutes a 'doping' offence. Although appropriate for 'doping' control, such an approach, known as the 'zero tolerance rule' is not suitable for medication control because very sensitive analytical detection methods mean irrelevant amounts of therapeutic substances may now be detected a long time after their administration(11).Different drugs used for doping included: 1) Central nervous stimulants and respiratorystimulants such as caffein and amphetamine.This group includes numerous localanaesthetics which are known to have astimulating effect on the central nervoussystem, such as procaine and benzocaine.2) Drugs with a sedative effect on the centralnervous system, such as barbiturates andhyoscine. This group includes psychomatic tranquillizers like chlorpromazineand meprobromate.3) Antihistamine drugs, some of which havea strong effect on the central nervoussystem, but usually tranquillizing, occasionallystimulating.4) Analgesic drugs whose main effect is tokill pain or to reduce the threshold to pain, such as asprin, phenacetin,cinchophen, and phenyl butazone.5) Hormones and vitamins in very largedoses (10).Even as long ago as the 3rdcentury B.C., the Greeks, inventors of democracy and the Socratic method, were known to ingest hallucinogenic mushrooms to improve athletic performance. In the

Roman erea, gladiators used stimulants in the famed Circus Maximus (circa 600 B.C.) to overcome fatigue and injury, while other athletes experimented with caffeine, alcohol, nitroglycerine, opium and even the potent stimulant, strychnine(12).It is considered one the most ancient drugs known in the history. First time in athletics sport, Thomas Hicks (won the Olympic marathon in1904) decided to inject himselfone milligram of strychnine sulphate(7). Gradually, the Strychnine was considered more as a drug doping in sport. But strychnine is very toxic to humans and many other animals, that poisoning occure by inhalation, swallowing or absorption through eyes or mouth can be fatal. There is no specific antidote for strychnine but recovery from strychnine exposure is possible with early treatment.

This is the first documented report of strychine usage for doping in race horse that improved the performance without poisoning symptoms of strychnine toxicosis.



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EQUINE FORENSIC UNIT
CENTRAL VETERINARY RESEARCH LABORATORY

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

CERTIFICATE OF ANALYSIS

Certificate No: 11836/2015
Date of Issue: 27/01/15

To
Head of the Supreme Racing Committee
Equestrian Federation of Iran

Sample Collection Date : 16/01/15 Sample Receipt Date : 18/01/15

Sample Type	Sample No.	Lab. No.
Equine Urine	01898869	IR-0400

The above sample was received in good condition and was tested using the following in-house documented methods:
ELISA'S - METHOD NOS:TA01;TA03;TA06;TA07;TA08;TA14;TA16; TA17;TA19
NSAID's, Neutral & Acid's - Method No: GS07
Bases - Method No: GS03
Anabolic Steroids - Method No: GS08
Cortico Steroids - Method No: GS10
Confirmatory - Method No: CONF08

The analysis has confirmed the presence of STRYCHNINE in this sample.

Peter Henry Albert
Chief Analyst

This laboratory is not responsible for the collection of samples.
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Fig 2.The report form of Central Veterinary Research laboratory

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