PHG 322

Lab#

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Qualitative Identification of some toxic plants constituent

PHG 322 Practical course

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Background:

TLC and Extraction techniques.

Objectives

- ✓ Detection of ricinine alkaloids in castor beans.
- ✓ Understanding the effect of ricinine and ricine on human.
- ✓ Detection of cardiac Glycosides in *Nerium oleander*.
- ✓ Understanding the effect of cadiac glycosides from *Nerium oleander* on human.
- ✓ Showing : Castor plants and all parts and *Nerium oleander* plant.
- ✓ To learn some analytic laboratory skills

Equipments:

Some of them:

- Soxhlet.
- Rotary evaporator
- Water Bath (Boiled)

1. Detection of ricinine alkaloid in castor beans

1. Introductions

Castor bean (*Ricinus communis* L.) is one of the ten major oilseed crops in the world. It is highly valuable and an important raw material for chemical industry. The remains after castor oil extraction are known as Castor pomace. They contain large amounts of proteins that have been used as animal feed after removal of toxin.

The castor pomace usually contains several toxins, including ricin, allergens, hemagglutinins and ricinine. The presence of these toxins limits the usage of castor pomace as a food source. Removal of the toxin from ricinine was shown to be more difficult than the others.

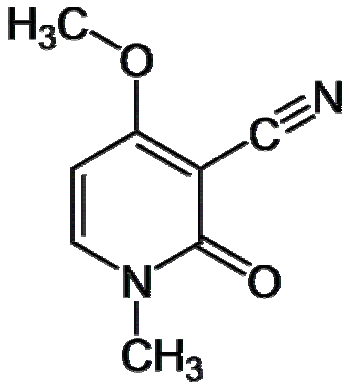
The toxicity of raw castor beans due to the presence of ricin is well-known. Although the lethal dose in adults is considered to be 4 to 8 seeds, reports of actual poisoning are relatively rare.. According to the 2007 edition of the Guinness Book of World Records, this plant is the most poisonous in the world.

Ricin is a glycoprotein, presents in seeds coat . which is also present in lower concentrations throughout castor seeds contain about 1% to 5% of ricin. Pure ricin is a white powder that is water soluble and stable over a wide pH range. Ricin can be fatal when ingested, inhaled, or given intravenously. Ricin is an N-glycosidase that affects an RNA subunit and interferes with protein synthesis, causing cell death. It may also cause toxicity by inducing apoptosis (cell death), direct cell membrane damage, changing cell membrane structure and function, and release of cytokine inflammatory mediators.

Preliminary research suggests ricin might be useful in cancer treatment, targeted specifically to cancer cells.

Hulled castor seeds, with the outer coat carefully removed, seem to be well-tolerated, and without toxic side effects; however, some patients taking hulled seeds can have transient anorexia and weight loss.

Ricinine, known as an alkaloid, exhibited certain biological activity and was used as an animal model for convulsive seizures, as insecticidal agent and as a promising cognition-enhancing drug that may be used for the treatment of human amnesias. Ricinine (1,2-dihydro-4-methoxy-1-methyl-2-oxo-3-pyridinenitrile) has a molecule formula of C8H8N2O2, molecule weight of 164 Dalton and its chemical structure is shown down.



2. Procedure

1- Collect 10 seeds of *Ricinus communis*, dry, reduce to a coarse powder.
2- De-fat the powder with 100 ml of n-hexane (or petroleum ether) by soxhlet, percolation or maceration
3- Extract on BWB with 300 ml of acidulated alcohol (70%) at PH 3 (use 10-15 drops dil. HCl).
4- Filter, concentrate to 50 ml under vacuum (Rotary evaporator) , and remove non-alkaloidal matter with two portions of CH_2Cl_2 (2X20) in a separating funnel then discard the lower layer.
5- The aqueous layer from "step 4" is made alkaline with dilute ammonia solutiont then extract with organic solvent (e.g. $CHCl_3$).
6- The solvent is dried over anhydrous Na_2SO_4, concentrated under vacuum (Rotary evaporator) to 1 ml.
7- Apply spots from the purified alkaloidal extract to silica gel pre-coated TLC plates, develop with a suitable solvent (e.g. CH_2Cl_2/MeOH, 9:1) alongside authentic ricinine sample (if available). .

8- Dry the plates in a vacuum hood, examine under UV light (254 nm), and spray with dragenddff's reagent.
9- Record your observations.

3. Precautions:

1) Each student should wear gloves during the experiment.
2) Avoid inhaling any vapors (use masks).
3) Hands should be washed thoroughly after each step.
4) The residual seed powder should be kept away from water because it contains the highly toxic "ricin" protein (soluble in water), and should be discarded properly.

2. Detection of cardiac glycosides in Nerium leaves (oleanderin)

2.1 Introduction:

Nerium

All parts of the oleander plant (***Nerium oleander*** F. APOCYNACEAE) contain the cardiac glycosides. The toxic principles are two potent cardiac glycosides (cardenolides), **oleanderin** and **neriine**, and can be isolated from all parts of the plants, which are very similar to the toxin in foxglove (Digitalis) , oleanderin and neriine have positive inotropic and negative chronotropic actions. They bind to sodium- and potassium-sensitive membrane-bound enzymes called ATPases and inhibit enzyme activities, resulting in increased intracellular sodium ions and calcium ions and increased extracellular potassium levels. At toxic levels, the sodium and calcium ions depolarize the cell after repolarization, causing late after depolarization and increased automaticity. Severe toxicity produces bradycardia and heart block.

Oleander leaf also contains other biologically active constituents that have antimitotic and insecticidal properties. Oleander is also reported to have emetogenic, cathartic, insecticidic, parasiticidic, anthelmintic, menstrual stimulant, and abortifacient activities.

2. Principle of test:

In general cardenolides form with aromatic nitro compounds in alkaline solution (e.g. Kedd's reagent) typical and very specific reagent, violet-red or blue-violet products.

3. Procedure

1. Collect 100 gm of *Nerium oleander* leaves, dry, reduce to a coarse powder.
2. Extract with alcohol (70%) on BWB for 15-30 minutes, then filtrate.
3. Dilute the filtrate with twice volume with distilled H_2O, add 1-2 ml strong lead acetate(to ppt resins, tannins and pigments).
4. Extract the filtrate with 15 ml $CHCl_3$
5. Divide the extract into 2 portions (I and II),

Extract I (Keller Kielianin Test)

6. evaporate extract I to dryness (rotary evaporator).
7. Dissolve extract I in 2 ml of 3.5% $FeCl_3$ in glacial acetic acid, transfer to a clean dry test tube.
8. Pour 2 ml conc. H_2SO_4 carefully on the wall of the test tube.
9. Notice the color that will develop in the upper layer and the reddish brown ring at the junction between two layers and record your observation.

Extract II (Kedd's test)

10. Evaporate extract II to dryness, dissolve in few drops of alcohol, add 3,5 dinitrobenzoic acid **(Kedd's A)** followed by NaOH (**Kedd's B**), then notice the color.
11. Write your conclusion.

Treatment and Medication for Castor Bean

Medical Care

- The first priority in treating a patient with castor bean poisoning is to establish that the patient's airway is patent and that breathing and circulation are adequate.
- Supportive care that is based on clinical symptoms is the primary therapy. Replace GI fluid losses with intravenous fluids.
- Whole bowel irrigation (WBI) has been suggested to ensure rapid and complete decontamination of the GI tract; however, the clinical use of WBI has not been demonstrated. In theory, rapid elimination of the bean before erosion of the outer shell may decrease or prevent the release of potent toxins. Consult the nearest regional poison control center before undertaking WBI.
- Count beans to assure complete recovery.
- Patients should remain under observation for at least 4-6 hours. Asymptomatic patients may be discharged safely after this period.
- Once the patient is symptomatic, supportive care involves attention to fluid, glucose, and electrolyte replacement.

Consultations

- Report all exposures to the regional poison control center.
- Expert consultation with a trained toxicologist is also recommended and can be obtained at the regional poison control center.

Medication

Medication is not currently a component of care for this condition

Treatment and Medication for Glycosides-Cardiac plant poisning

Prehospital Care

- Advanced life support (ALS) should transport patients who have ingested herbal cardiac glycosides or significant amounts of plants known to contain cardiac glycosides.
- Prehospital care should focus on ABCs, with special emphasis on supporting respiratory and cardiac function.
- During transport, the patient should receive supplemental oxygen and an IV line. Cardiac and pulse oximeter monitoring should be continuous.
- In patients with protected airway and normal mental status, activated charcoal can be administered.
- Atropine should be given to patients with clinically significant bradycardia (eg, hypotension, change of mental status).

Emergency Department Care

Address principles of care for toxicologic emergencies, including providing general supportive care, preventing further exposure and absorption, administering antidote (eg, fragment antigen binding [Fab] fragments), and treating complications. Management is very similar to that for digoxin/digitoxin poisoning.

- General supportive care: Attention to ABCs is paramount. Treat life-threatening conditions in accordance with advanced cardiac life support (ACLS) principles, except as outlined below.
    - Administer oxygen and start an IV line. Place patient on continuous cardiac monitoring and pulse oximeter.
    - Treat patients with altered mental status in accordance with standard protocols based on a fingerstick glucose determination and primary survey.
- Prevent further exposure: Remove plant parts or any medications brought with patient from treatment area, particularly if patient is suicidal.
- Prevent further absorption: Oral administration of activated charcoal is recommended if no contraindications exist.
- Administer antidote: Sheep-derived digoxin antibody Fab fragments reportedly are effective for some plant cardiac glycosides. Consider use in life-threatening complications, such as ventricular dysrhythmias, hyperkalemia, high degree heart block, and cardiac arrest that do not respond rapidly to conventional treatment. Indications for digoxin antibody Fab fragments are the same for both pharmaceutical as well as nonpharmaceutical cardiac glycoside toxicity and include the following:
    - Hyperkalemia (>5.0 mEq/L) in acute toxicity
    - Life-threatening supraventricular and ventricular dysrhythmias
    - Hemodynamically significant bradycardia unresponsive to atropine
    - Chronic digoxin toxicity with dysrhythmias, significant GI symptoms, acute altered mental status, or renal insufficiency
    - Serum digoxin level >15 ng/mL at any time
    - Ingestion of 10 mg in an adult or 4 mg in a child
    - Poisoning by nondigoxin cardiac glycoside
    - To aid in treatment of suspected cardiac glycoside poisoning without a confirmatory level
- Since onset of action of Fab fragments may take 30-60 minutes, intervening treatment of significant complications should occur.
    - Bradydysrhythmias: Atropine and cardiac pacing may be tried. If atropine is not rapidly successful, consider administration of Fab fragments. Patients requiring transcutaneous cardiac pacing should receive Fab fragments prior to it. Transvenous pacing and use of isoproterenol have resulted in degeneration of cardiac rhythms and both of these should be avoided. Do not delay administration of Fab fragments because of pacemaker placement. Do not use overdrive pacing for the control of ventricular dysrhythmias.
    - Phenytoin and lidocaine may be used as antidysrhythmics if Fab fragments are not immediately available. However, it should be remembered that Fab fragments are the definitive antidote to cardiac glycoside poisoning.
    - Tachydysrhythmias: Phenytoin and lidocaine (which decrease automaticity without slowing AV nodal conduction and increase fibrillation threshold) may be used to treat ventricular dysrhythmias.
    - Magnesium has been reported to reverse digoxin-induced dysrhythmias and may be useful as long as anuric renal failure is not present.
    - Use cardioversion only as a last resort, since it may induce intractable ventricular fibrillation. Fab fragments should be given with cardioversion.
    - If time permits, cardioversion should be attempted after a loading dose of phenytoin and at a significantly reduced initial power setting of 5-10 J.
    - Quinidine and procainamide may enhance cardiac glycoside toxicity by slowing conduction across AV node; both should be avoided.
    - Beta-blockers and calcium channel blockers have questionable value.

- Hyperkalemia: Life-threatening hyperkalemia (>6.5 mEq/L) may be seen with acute toxicity and results from a redistribution phenomenon rather than increased body stores.
- Glucose, insulin, sodium bicarbonate, and albuterol may be used to facilitate redistribution of potassium intracellularly. However, albuterol may precipitate cardiac dysrhythmias.
- Calcium should be avoided to prevent overloading myocytes with calcium, which is associated with development of a "stone heart," increased dysrhythmias, and a higher rate of death. A recent pilot study in a porcine model shows that, in contrast to earlier studies, IV calcium administration to treat hyperkalemia secondary to cardiac glycoside toxicity resulted in no benefit or harm. However, the authors do not recommend its use in the clinical setting at this time until more definitive studies are undertaken.[5] Theoretically calcium can be used after administration of Fab fragments and reversal of cardiac-glycosides toxicity.
- Life-threatening hyperkalemia should be treated with Fab fragments.
- Forced diuresis, hemoperfusion, and hemodialysis are ineffective in enhancing the elimination of digoxin because of its large volume of distribution. Hemodialysis will efficiently remove potassium from extracellular fluid.
- Cardiac arrest: Give 10-20 vials of Fab and continue to treat with standard ACLS protocols. Prolonged efforts at resuscitation may be warranted until Fab fragments begin to work. Phenytoin and lidocaine are antidysrhythmics of choice in patients poisoned with cardiac glycosides.

Consultations

- Poison center and toxicology: Consider consultation for any question regarding management (strongly recommended if use of Fab fragments is considered or if symptoms and signs of toxicity are severe).
- Cardiology
    - Consider consultation for advice regarding treatment of cardiac manifestations of toxicity, as needed.
    - Consider consultation if use of Fab fragments is contemplated and a toxicologist is unavailable.
- Psychiatry: Consultation is recommended for any patients with suspected intentional ingestions.
- Primary care physician: Consult for admission or for information regarding patient's medical histories.
- Botanist: Consultation with a botanist may facilitate plant identification.

Medication

Categories of drugs used to manage cardiac glycoside plant toxicity include drugs to minimize absorption and increase excretion, drugs that lower extracellular potassium, antidysrhythmics, and antidotes (eg, digoxin Fab fragments).

GI decontaminants

Activated charcoal is used to bind toxin within the GI tract. Due to enterohepatic/enteroenteric recirculation of cardiac glycosides, multiple doses can be given to help enhance elimination.