1 **Introduction to Toxicology of Natural Compounds**

2 **Characteristics of poisons**

- Ambiguous definition
- Poison is eachever compound, which is able to trigger harmfull response of biological sytem, seriously damage its function or cause death.
- Toxins:
  - Compounds strange to organism qualitatively or quantitatively, causing damage chemically or physico-chemically
  - It is necessary to know conditions and amounts, which can cause effect
- From greece words:
  - Toxon – a bow for shooting of poisonous arrows
  - Toxoema – poisonous arrow
- Paracelsus : *Dosis sola facit ut venenum non sit*.
  - Philippus Aureolus Theophrastus Bombastus von Hohenheim (1490 - 1541)
  - Compounds different in size of poisonous dose
- Acute toxicity LD_{50} is not only one measure of toxic effect
- Toxinosis, toxicosis:
  - Pathologic state caused by toxines (internal, external)

3 **History**

- Inorganic substances:
  - Pb, Hg, As, HCN
  -
  -
- Organic substances:
  - extracts from plants:
    - Deadly nightshade, henbane, thorn apple, autumn crocus, hemlock, thuja, aconite, sea onion, poppy, mushrooms

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- Ancient times:
  - 4500 B.C. – Gula – protective diety of medicinal knowledge of Mesopotamia
  - Shennung 2500 B.C. – mythic founder of Chinese herbal medicine
  - Ebers papyrus 1550 B.C.
    - 800 receipts (opium, henbane, hemlock)
  - Greek mythology:
    - Helene used opium to get Menelaos and Thelemachos sleeping
    - Hecate – aconite
    - Medea – autumn crocus
    - Hercules was killed by shirt impregnated by poisona
  - Greek scientists:
    - Essays about poisons:
      - Theophrastus 370 - 286 B.C. - *De Historia Plantarum*.
      - Nicander of Colophon 204 - 138 B.C. – essay about plant poisons - Theriaca,

therapy of intoxications - Alexipharmaca – vomiting
- First antidotes:
  - Mithridates VI. 114-63 B.C. – Mithridatium
- Aconitine (*Aconitum* – Ranunculaceae)
- Coniine (*Conium maculatum*) - Sokrates

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- Possible participitation of mycotoxins on some wonders described in Bible.
  - „Plagues of Egypt",
  - „death of first-born" possible mycotoxicosis...
  - Job disease reminds trichothecene intoxication

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- Sulla – 81 B.C. – first law about toxins *Lex Cornelia*
- Cleopatra 69-30 B.C. – intoxication by bite of cobra

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- Atropin (*Atropa belladona*) – caesar Augustus (Livia and figs on the tree)
- Amatoxins (*Amanita phaloides*) – Cladius, Agrippina and Locusta, Xenophon (alkaloids of *Citrullus colocynthis,* desert mellon – cucurbitacines (hepathotoxic, abortive, effect on glycaemia)

- Andromach´s Theriac – in period of caesar Nero 37 – 68 A. D.
  - Assay of food by slaves

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- Andromach´s Theriac – period of caesar Nero 37 – 68 A. D.
  - 64 components
  - Assaying of food by slaves

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- Dioscorides 40-80 A.D.
  - *Materia medica*
    - Sorting of poisons to group of mineral, plant and animal
- Galenos 129-200 A.D.
  - About antidotes

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- Medivial ages:
  - Mostly arabic world
    - Bezoars against intoxication, Ibn Uashiija 801 A.D. – book about poisons
    - Charles IX king of France (1550-1574), Ambroise Paré (1510-1590) – experiments on prisoners, against myths
    - Jacob I king of England (1566-1625)
  - Horn of unicorn – narwhale (*Monodon monoceros*), *Monodontidae*
    - Henry II (1519-1559) king of France married Catherine de Médicis (1519-1589) 1533, pope Clement VII dedicated to family horn of unicorn
  - *Terra Sigillata* – earth from hill at island of Lemnos

- Reasons for using antidotes:
  - Adsorption properties of animal coal – roasted frog
  - Neutralization of magnesium milk
  - Black tea contents tannin
- Known poisoner´s affairs:
  - 1035 Scotish people lead by king Duncan against troops of Norwegian king Sven Canut used deadly nightshade.
  - Pope Alexandr VI., Caesar and Lukretia Borgia – *La Cantarella* (toad frog was killed by arsenic and other poisons, cadaver was rotten and „juice" was evaporated to get powder).
  - Leonardo´s technique of passaging – animal killed by poison, organs impregnated by poisons administered to other animal and so on – for increase of concentration. The same at plants – injection of cyanide into bark of tree – poisonous fruits

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- William Piso 1611 -1678
  - Study of root of *Ipeca*
- Catherine Deshayes 1680
  - poisoner, aconitum
- Felice Fontana 1730 -1805
  - Scientific study about poisonous snakes
- Phillip Physick 1767-1837
  - Lavage of stomach
- BONAVENTURE ORFILA 1787-1853
  - Founder of modern toxicology
  - "TRAITE DE POISONS"
- FRANCOIS MAGENDIE 1783-1855
  - Discovery of emetine and its properties
- JAMES MARSH 1794-1846
  - Marsh test for discovering of arsenic presence
  - 1839 MARIE LEFARGE - first Marsh test
- CLAUDE BERNARD 1813-1878
  - Mechanism of curare poisoning
- P. TOUERY 1831
  - Proof of effect of adsorbents in strychnine intoxication
- 1860 ALBERT NIEMAN
  - Isolation of cocaine
- 1854-1918 RUDOLF KOBERT
  - Study of digitalis and ergot alkaloids

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- Medicines using poisons:
  - 1855 WILLIAM PALMER, MD
    - strychnine
  - 1863 EDMOND DE LA POMMARAIS, MD
    - digitalis
  - 1881 GEORGE HENRY LAMSON, MD
    - *Aconitum*

- 1891 THOMAS NEVILLE CREAM, MD
  - Poisoned prostitutes with strychnine
- 1974-1998 HAROLD SHIPMAN, MD
  - Murdered 250 patients with heroine and morphine

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- Lycanthropes:
  - Ergot intoxication – mass halucination, paranoia, psychosis, influence on whole town and villages
  - Rabies, porphyria, intoxication with trichothecenes and further mycotoxines, bacterial infections
- Witchcraft:
  - Ergotism - Salem, Great French Revolution

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- France 1918 – Girard (*Amanita phalloides*)
- Intoxication of Benin president Soglo 1991
- Intoxication of journalist Markov 1978
- Intoxication of Ukrain president Juščenko
- Actions of Israel Mossad or CIA, poisons and poisoners are not far history:
  - ricine
  - botuline
  - dioxine

16 **Definice toxikologie**

- Interdisciplinar branch, study of toxic effect of chemical compounds on living organisms.
- Toxinology: scientific branch touching study of toxins produced by microbes, poisonous animals and plants, also innorganic sources, touching also sources, chemical composition and antidotes.
  - Discipline about effect of poison
  - Discipline about proof and identification of poison
  - Discipline about absorption, biotransformation and excretion of poison in/from organism

17 **Disciplines of toxicology**

- Experimental
- Clinical
- Forenzic
- Ecotoxikology
- Enviromental
- Industrial
- Military
- Behavioral

18 **Intoxications**

- General damage of organism caused by effect of absorbed compound.
- Secondary intoxication:
  - Intoxication of organism by another previously intoxicated one.
- Late symptoms of intoxication:
  - Observed at many types of intoxication, after fade away of acute symptoms

19 **Organotropic properties of poisons and their components**

- Cytotoxins
- Hemoragins
- Hemotoxins
- Hepatotoxins
- Myotoxins
- Nefrotoxins
- Necrotoxins
- Neurotoxins

20 **Cytotoxins**

- Overall compounds, which damage or destroy living cells
- Compounds, which supress or damage cellular processes or are toxic in different manner

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Blood composition: blood plasma 55 %, blood elements 45 %

Blood plasma

- water
- electrolytes - Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, CO_3^{2-}, ...
- plasmatic proteins (PP) - albumin, globulin, fibrinogen,..
- Transported compounds - nutritients, waste compounds, gases, hormones, ...

Blood elements

- erythrocytes
- leucocytes
- Blood platelets

Erythrocytes

- nuclei free cells
- developed from myeloid stem cells in bone marrow
- life cycle 120 days, death in spleen
- 90 % of mass is hemoglobin

Leucocytes

- granulocytes - neutrophils, eozinophils, bazophils
- agranulocytes - lymphocytes, monocytes
- Participitate on immune response of organism

Blood platelets

- thrombocytes
- coagulability of blood

Hemoglobin

- 2 subunits α and 2 subunits β
-
- In each subunit Fe^{2+} possible to bind O_2
-

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Toxic compound causes change of number and/or function of blood cells

Anemia

- lack of erythrocytes
  - aplastic – connected with malfunction of bone marrow
  - hemolytic – lysis of erythrocytes
  - nutritional – insufficience of Fe, vitamin B_{12} and folic acid

- salts of Pb, Cr^{6+}, Cu, Pt, Au, As, Cd.....
- benzene, alkylation agents, hydrazine
- animal and plant toxins
  - Podophyllotoxin
  - Cephalosporines
  - Trichothecenes
  - *Vinca* alkaloids
  - Taxans
  - Snake poisons

Leukaemia
- Malign growth of blood cells
- benzene, PAH

Methemoglobinemia
- Fe^{2+} in haemoglobin is oxidized on Fe^{3+} (production of methemoglobine)
- Fe^{3+} is not able to bind O_2 – blockade of oxygen transportation
- produced by effect of NO_3^-, NO_2^-, nitro- and nitroso- substances, O_3, ...
- antidote – methylene blue - reduction of metHb to Hb
- Production of methemoglobine is required during poisoning with CN^-
  - antidote amyl nitrit $(CH_3)_2CHCH_2CH_2ONO$
-

Carboxyhemoglobine
- emerges during reaction of CO with Hb (CO possesses 230× higher affinity to Hb than O_2)
- oxygen transporatation blocked
- antidote is O_2

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- Cytolysins – cause hydrolysis of cell membrane phospholipids at eucaryotic cells
  - Hemolysins
  - Leucotoxins
-
- Hemoragins – desintegration of endothelium of capilaries and small vessels, as consequence leak of blood and haemorhage
  - *Ricinus communis*
  - Snakes Crotalidae
  - Microbes
- Hemotoxin – decay of blood elements
  - Hemolytic exotoxin - cobratoxins

24 **Hepatotoxic substances**

Liver function

Production of bile
- absorption of fat from intestine, metabolism of fat
- stimulation of gut peristaltic
- transportation of liver metabolism products

Biotransformation of toxic compound

- increase of molecular weight and polarity of toxic compounds
  - products - hydrophilic, more easy excreted via bile and urine

Metabolism of nutritients

- synthesis, storage and decomposition of glycogen
- metabolism of fats - β-oxidation of lipids (ATP), synthesis of phospholipids, production of cholesterol and lipoproteins
- metabolism of proteins – synthesis of blood proteins and coagulation factors
- elimination of hormones, bilirubine, ammonia.....

Immunity

- Cupfer cells – phagocytosis of erythrocytes, bacteria and coagulates
- Synthesis of immunoglobulins
-

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Manifestation of toxic effect

Liver steatosis

- accumulation of fat in hepatocytes as a consequence of metabolic disorders (lipoproteins and lipids)
- Especially result of acute exposition to:
  - ethanol, methanol, hydrazine, DDT, hexachlorcyclohexane, As, Cr, chloroform, halothane, colchicine
-

Hepatic necrosis

- decay of liver cells
- Especially result of acute one-time exposition to:
  - halothan (CF_3-CHBrCl), methoxyfluoran ($CHCl_2$-CF_2-O-CH_3), chloroform
  - *Amanita phalloides* death cap – phalloidin – centrilobular necrosis – disturbs depolimerisation of microfilaments
  - Griseofulvine – disrupts metabolism of hem – store of porphirynes
  - Galactosamine – disturb conjugation processes
  - Pyrrolizidine alkaloids – disruption of vasculature
  - Phomopsin - *Phomopsis leptostromiformis,* mould

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Cholestasis

- Disorders in bile metabolism
  - Organometalic compounds of Sn and As
  - Sporodesmin – product of moulds
  - Lantadens – *Lantana camera*
-

Liver fibrosis, cirhosis

- formation of fibrous tissue in liver, nodules
- Usualy as a consequence of chronic exposition, but also as consequence of acute damage
  - methanol, ethanol, aldehydes, ketones, vinylchloride, As
  - pyrollizidine alkaloids

Malignant tumours of liver

- Butter yellow pigment (4-dimethylaminoazobenzene) , nitrosamines, As, vinylchloride,

PAH, halogenated aromatics
- aphlatoxines, safrol
- MAM (methylazoxymethanol) – *Cycadales*
- Pyrrolizidines

27 **Nephrotoxicity**

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- Kidneys
  - 1% of body weight
    - 25 % of cardial output
  - High amounts of toxins
    - Direct effect
    - Metabolisation
  - Concentration
    - Reabsorpton
      - Passive
      - Active
  - Differences in damage of medulla and cortex
    - cortex 80 % of flow
  - Damage during large change of filtered liquid volume

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Heavy metals

- Pb, Cd and Hg during acute intoxication usually damage proximal tubulus and cause acute renal failure (polyuria, glucosuria, proteinuria $\rightarrow$ anuria)
-
- Pb, Cd and Hg are during chronic intoxication deposited in kidneys and cause different forms of chronic nephritis
-
- Cd decrease re-absorption of Ca and P - osteoporosis (Itai - Itai)
-
- Next nephrotoxic elements: As, Cr, Pt, U, Au, Sb, Th, Fe
-

Halogenated aliphatic carbohydrates

- Especially with short chain CCl_4 , $CHCl_3$ - acute renal failure
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Ethylenglycol

-
- Metabolized to oxalic acid, its salts form crystals

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Cyclosporin
Aminoglycosides
- Limits in usage
- Up to 20 % usage show marks of damage

- Acumulation in proximal tubulus
  - –Tubular necrosis

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- β-lactam antibiotics
  - – Cephaloridin
  - – Cephaloglycin
  - – Imipenem
    - High nephrotoxicity –concentration in proximal tubule
    - R2 ≠ H
      - –Tubular damage
      - –Glycosuria
      - –Proteinuria

34 **Dermatotoxic substances**

Skin functions

- Protection against infections and toxic effects of compounds
- Water elimination, NaCl, urea,....., waste compounds
- Termoregulation organ
- Organ of snse of touch, perception of heat, cold, pain
- Production of vitamin D
- Glucose depot

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Anatomy of skin

- epidermis - keratinocytes (keratine - fibrous protein repulsing water and resistant to enzymatic digestion, melanocytes (production of pigment)
- dermis – colagene fibers, smooth muscles
- subcutaneous fibrous tissue – including hypodermic fat
- cutaneous organs – perspiratory and sebaceous glandulas, hairs, nails etc.
-

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Iritation contact dermatitidis

- acute
  - – strong acids – formation of crust in the site of contact, decelaration of further invasion
  - – strong bases - saponification (reaction of base with phospholipids), deep penetration
  - – Organometalic substances Sn, P, CaO
- chronic
  - – soaps, detergents, mineral oils
  - – organic solvents – disruption of protective lipidic layer on the skin surface, secondary infection

Alergic contact dermatitidis

- Non-adequate immune response on repeated contact with alergenic substances
- Haptenes
- *Rhus* - pyrocatechols

Phototoxic compounds

- Effect of UV irradiation (280 - 400 nm) causes formation of reactive toxic species from

precursors – often formation of free radicals (influence of O_2)

- PAH, 8-methoxypsolaren (lemons, clove, figs, celery,....), tetracyclines, porphirynes

Chloracne

- Result of acute PCB intoxication, dioxines,
- Badly healing skin ulceration, after longer time hyperpigmentation, brownish nails, conjunctivitidis

Falling out of hairs, depillation

- Salts of thalium, cancerostatics

Malignant tumours

- Result of PAH phototoxicity, than toxicity of As (also oral administration)

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- Damage of neurons bodies and dendrites
  - Free radicals
    - Lipid membrane
    - Weak defensive system
    - High content of iron
  - Excitotoxins
    - Ibotenic acid
    - Domoic acid
      - *Nitzchia pungens* (alga), kontamination of shells in Canada
    - β-*N*-oxalylaminoalanine
      - *Cycas cercinalis,* Guam disease
    - MAM
  - DNA damage
    - Methylazoxymethanol (MAM)
      - *Cycas*
    -

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- Axonopathy
  - Axons damage only
    - Colchicine
    - Vincristine
    - Taxol
- Myelinopathy
  - Diphteric toxins – *Corynebacterium diphterie*
- Toxins of ion channels
  - Na+, K+, Ca2+
  - Tetrodotoxine
    - *Tetraodon* sp.
  - Saxitoxine
    - *Alexandrium tamarense*
    - *Gymnodinium catenatum*
    - *Pyrodinium bahamense*

- Batrachotoxine
  - *Phyllobates terribilis*
- Aconitine
- Pyrethrines
- Toxins of scorpios
  - *Androctonus, Buthus, Hottentotta, Leiurus*

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- Toxins of synapses
  - *Clostridium*
    - *Botulotoxin*
    - *Tetanic toxins*
  - α-latrotoxine
    - *Latrodectus mactans*
  - Convulsants
    - Usually interactions with GABA
      - Picrotoxine
        - *Anamirta cocculus*
      - β-carbolines
    - Interaction with glycine receptor
      - strychnine

- Astrocyte lesions
  - Fluoroacetate
    - *Dichopetalum* (Apiaceae)

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- Necrotoxins – death of tissue cells
  - Toxins of staphylococci
  - Tarantula
- Myotoxins – damage muscular tissue
  - Myolysis
  - Myoglobinuria
  - Renal failure
  - Toxins of venomous snakes

44  **Antitoxin**

- Antibody produced in organism as reaction on the presence of toxin
- Serum or globulin fraction from serum of animals immunized with corresponding toxin
- Antibody produced in xenogeneic organism
-
- Heterolog
- Homolog
- Monoclonal
- Biologic engineering