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"Herb Power for Animal Health:
From Tradition to Legislation„

Safety and Risk Assessment in Herbal Veterinary Practices

Module 3
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cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY
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Topics we will cover

- 1. Introduction to general toxicology of medicinal plants
- 2. Toxic biactive compounds in plants (e.g., alkaloids, saponins, glycosides..etc)
- 3. Species-Specific Toxicokinetics and Dynamics of Plant Bioactive Compounds
- 4. Acute, subacute, and chronic toxicity
- 5. Understanding herbal-drug interactions
- 7. Approaches to risk management in veterinary settings
- 8. Integration of herbal veterinary medicinal products into One Health approaches
- 9. General information about Testing Methods in Risk Assessment

Composition of Herbal Veterinary Products

- **Bioactive Compounds**: Derived from plant parts such as roots, leaves, seeds, and flowers. Each plant contains complex mixtures of bioactive compounds.
- **Additives**: Excipients like stabilizers or preservatives may also impact safety.
  - **Variability**: Environmental factors, harvesting methods, and processing techniques affect the consistency and potency of herbal ingredients.

Safety Concerns in HVPs

- **Toxicity**: Some herbs may contain toxic compounds (e.g., alkaloids, cyanogenic glycosides) which could harm animals if misused.
- **Contamination**: Risk of microbial contamination, heavy metals, pesticides, and adulterants.
- **Drug Interactions**: Herbal ingredients may interact with conventional veterinary drugs, leading to adverse effects.
- **Species-Specific Effects**: Different animal species metabolize herbs differently; doses safe for one species may be harmful to another.
- **Quality Issues**: Inconsistent manufacturing practices can lead to variations in product quality and efficacy.

Regulatory Challenges

- **Lack of Standardization**: Limited standards for HVPs lead to variability in quality control (one of the aim of MedPlant4Vet)
- **Insufficient Research**: Few clinical trials validate the efficacy and safety of many herbal products.
- **Labeling Issues**: Mislabeling or incomplete ingredient lists pose risks.

- So, lets start…
- Can we extrapolate pharmacology and toxicology data from available human studies ??? How? Why?

Animals are not small humans

- Cats are not small dogs

DOI: 10.1053/TVJL.2001.0578 • Corpus ID: 33866193

A horse is not a large rat: getting toxicology into perspective.

A. V. Miert • Published 2001 • Biology, Medicine • Veterinary journal

Not all dogs are the same!

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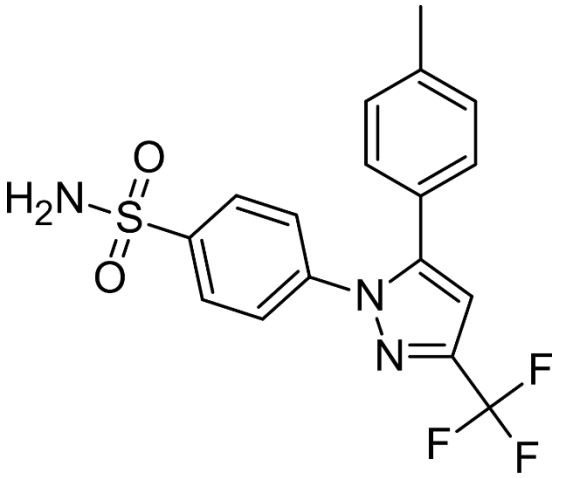
Or all cats, cattle, goats…

CYP450/drug transporters

Selective COX2 inhibitor Celecoxib- plasma clearance may be 2.5 times higher in Beagles than in other breeds.



Introduction

- Plant bioactive compounds are naturally occurring chemical constituents in plants that exert beneficial or adverse effects on living organisms and the environment.

- Understanding the mechanisms of action, exposure pathways, and physiological effects of these compounds is crucial for their therapeutic use, safety assessment, and toxicity prevention.

- Plant bioactive compounds play a significant role in pharmacology, veterinary medicine, food safety, and public health, with applications ranging from drug discovery to functional foods.

Alle Dinge sind Gift, und nichts ist ohne Gift. Allein die Dosis macht, daß ein Ding kein Gift ist.

(Paracelsus)

"All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy."

Paracelsus (1493-1541)

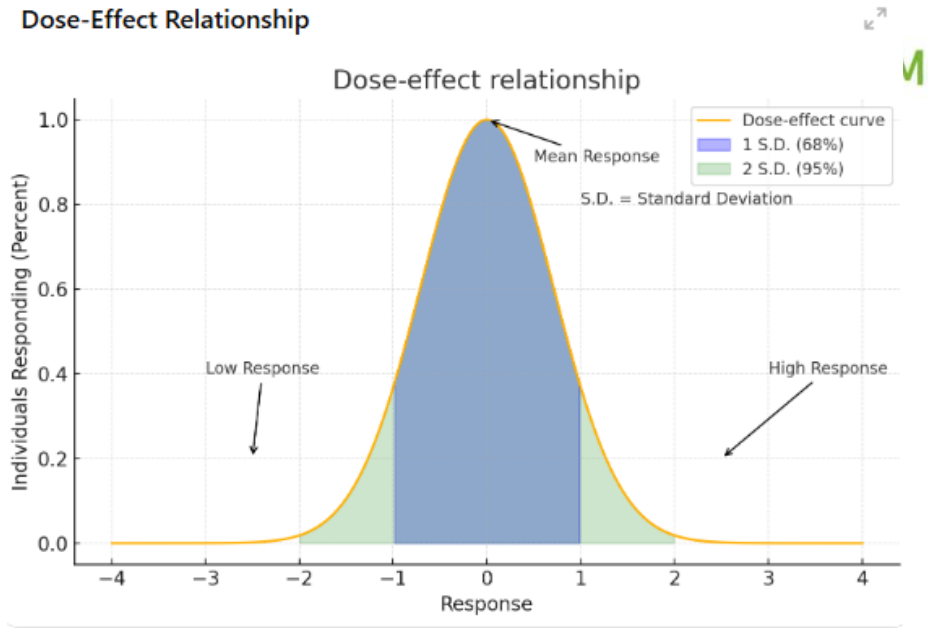
(Philippus Theophrastus Aureolus Bombastus von Hohenheim)

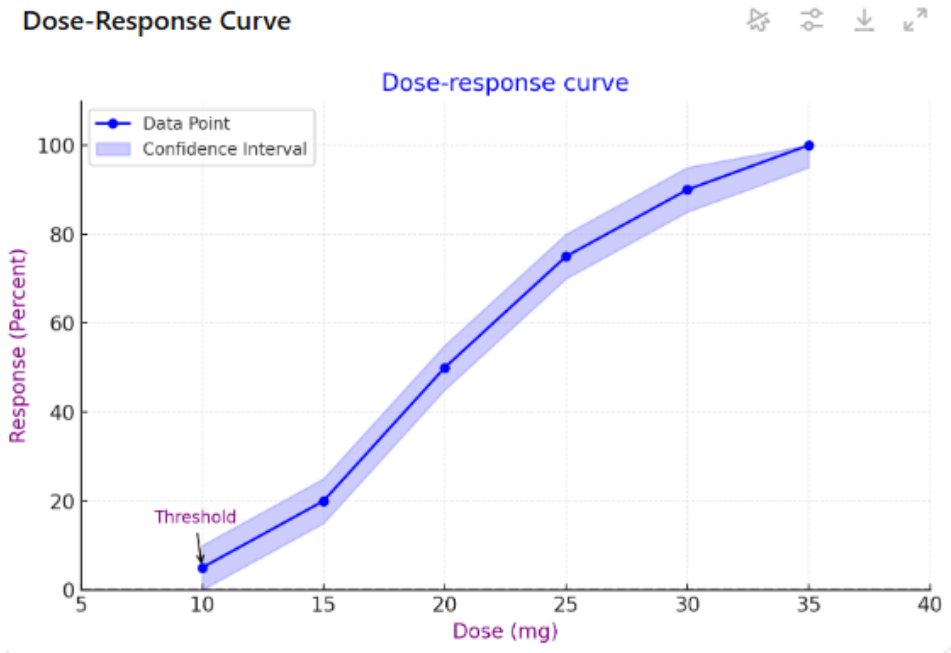
What about non-monotonic

- Toxicity - The adverse effects that a herbal bioactive compound may produce.
- Dose - The amount of a herbal bioactive compound(s) entering the body
- Response- Dependent upon the dose and organism- change from the normal state
  - Molecular, cellular, organ, organism level
  - Local/systemic

Response

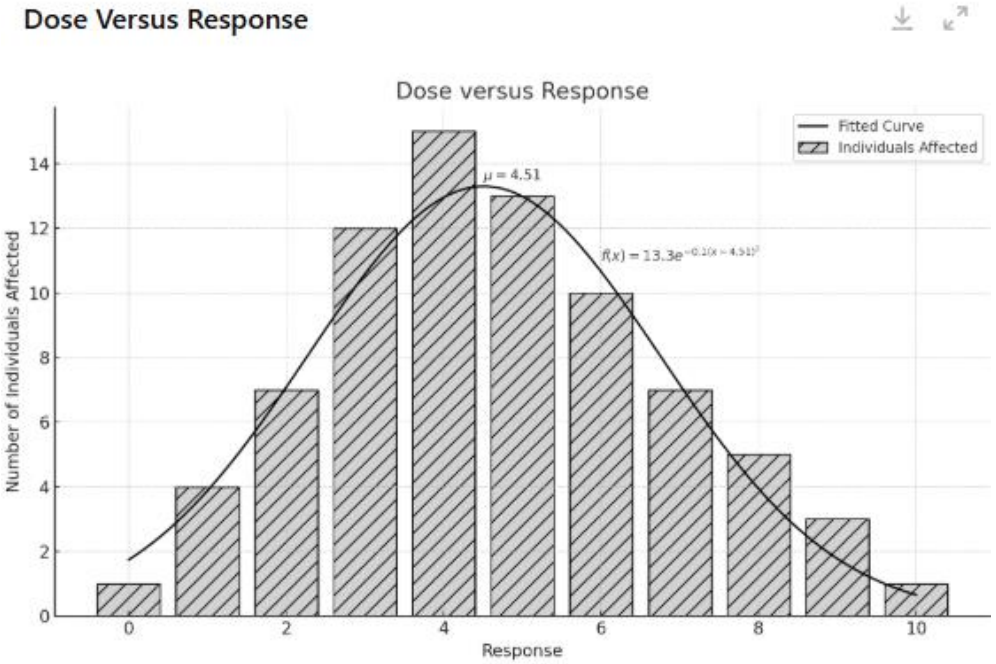
- Reversible/ Irreversible
- Delayed/ Immediate
- Dose response
  - Monotonic (response increase with dose)/Nonmonotonic (response does not increase with dose- Endocrine disrupting compounds, hormones)
    - Hormesis- beneficial effect at low conc- decreases at high
  - Treshold/no treshold
    - A threshold for toxic effects occurs at the point where the body's ability to detoxify a xenobiotic or repair toxic injury has been exceeded (cirrhosis- %50)
  - Cancer/non cancer

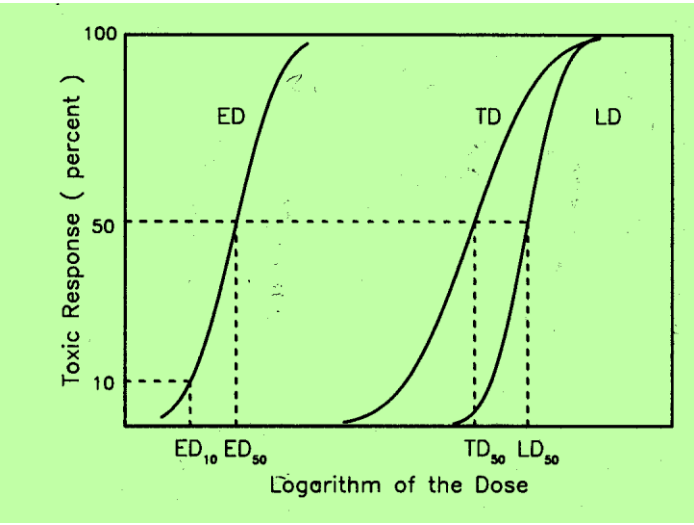




Dose versus Response

- Conduct tests on a "large" population.
- Administer the same dose of the herbal substance (often standardized to dose/body weight).
- Identify the number or fraction of individuals exhibiting a response.





Mixture interaction

- Additive
- Synergistic
- Potentiation
- Antagonism (functional, chemical, receptor= physiological, chemical, pharmacological)

Phyto-toxicology Basics

From pharmacokinetics and pharmacodynamics to toxicokinetics/dynamics

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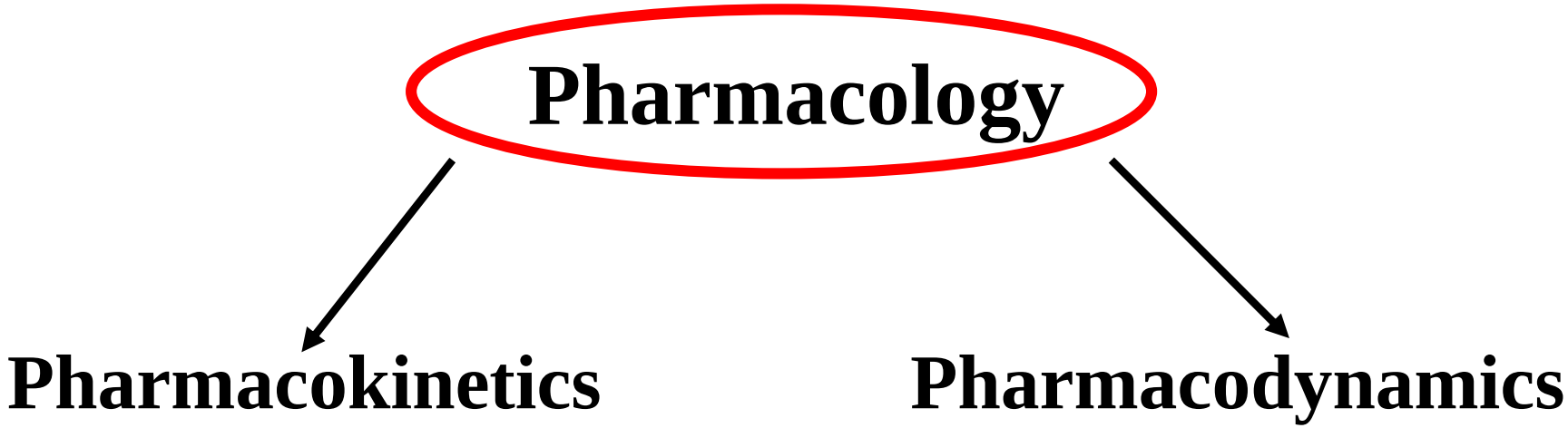
- Toxicology intersects with pharmacokinetics and pharmacodynamics by examining how the absorption, distribution, metabolism, and excretion of substances influence their toxic effects, as well as the mechanisms through which these effects manifest at cellular and systemic levels.



Pharmacokinetics (PK)

The disposition of a drug includes the processes of *ADME*

- Absorption
- Distribution
- Metabolism
- Excretion

Why Study Herbal Pharmacokinetics in Animals

- Pharmacokinetics is the study of how drugs are absorbed, distributed, metabolized, and excreted.
- Key to understanding drug dosage, efficacy, and safety.
- Pharmacokinetics (ADME) of herbs can differ significantly from synthetic drugs due to:
  - Complex chemical composition.
  - Species-specific differences in metabolism.
- Importance for ensuring therapeutic efficacy and minimizing toxicity

Absorption of Herbal Compounds

Absorption is the process by which a herbal bioactive compound enters the bloodstream or systemic circulation from its site of administration.

Challenges with Herbal Absorption:

- Poor solubility of active compounds.
- Interaction with feed or gut microbiota.
- Variability in gastrointestinal pH among species.

Factors Influencing Absorption:

Presence of plant secondary metabolites (e.g., tannins, alkaloids).

Formulation (e.g., extracts, powders, decoctions).

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1. Compound-Related Factors_1

- **a. Physicochemical Properties:**
- **Solubility**: Herbal compounds like alkaloids, flavonoids, and terpenes vary widely in water and lipid solubility. Lipophilic compounds (e.g., essential oils) are better absorbed via lipid membranes, while hydrophilic compounds may require aqueous environments.

- **Lipophilicity**: Lipid-soluble herbal constituents (e.g., curcumin from turmeric) are absorbed more easily through membranes compared to hydrophilic components.

- **Molecular Size**: Small herbal molecules (e.g., polyphenols) cross membranes easily, while larger compounds (e.g., polysaccharides) may require specific transport mechanisms.

- **Ionization**: The ionization state of herbal compounds depends on their pKa and the pH of the environment. For example, saponins are poorly absorbed in their ionized state.

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1. Compound-Related Factors_2

- **b. Herbal Formulation:**
- **Extract Type**: Crude extracts, tinctures, decoctions, and standardized extracts can have different absorption profiles. For example, ethanol-based tinctures may enhance the absorption of lipophilic compounds.
- **Particle Size**: Micronization or nanoemulsions can increase the surface area and enhance absorption of herbal compounds.
- **Carrier Systems**: Liposomal or phytosomal formulations can improve bioavailability of poorly soluble herbal compounds, such as flavonoids.

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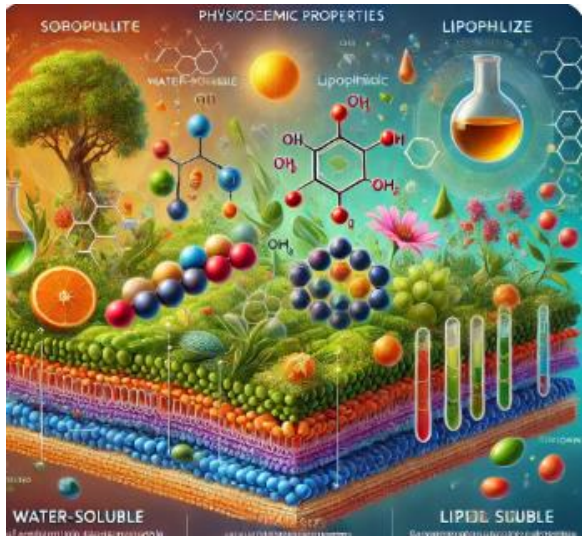
1. Compound-Related Factors_3

- **c. Chemical Stability:**
- Many herbal compounds are unstable in gastric or intestinal environments. For instance, catechins in green tea degrade in acidic environments, reducing their bioavailability.



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2. Route of Administration in Animals

- **Oral**: Most herbal remedies are administered orally in veterinary medicine. Absorption depends on gastrointestinal factors, which vary across species (e.g., monogastric animals vs. ruminants).
- **Topical**: Herbal ointments or oils (e.g., neem oil) must penetrate the skin barrier to be effective.
- **Parenteral**: Limited for herbal compounds, but injectable formulations (e.g., herbal alkaloids) may bypass absorption barriers.
- **Intramammary or Intravaginal**: Common in veterinary practice for localized infections (e.g., herbal-based mastitis treatments).
- **Inhalation**: Herbal essential oils (e.g., eucalyptus) may be absorbed through the respiratory epithelium.

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3. Physiological Factors in Animals_1

- **a. Gastrointestinal (GI) Environment:**
- **pH Variations**: The pH of the stomach and intestines influences the solubility and ionization of herbal compounds. For instance:
  - Horses have a relatively neutral stomach pH due to continuous feeding, potentially affecting absorption.
  - Ruminants (e.g., cattle) have complex stomach systems, where herbal compounds may degrade in the rumen before reaching the intestine for absorption.
- **GI Motility**: Herbal remedies for GI motility disorders (e.g., ginger) may alter the transit time of co-administered compounds.
- **Enzymatic Activity**: Enzymes in the digestive tract may degrade some herbal compounds, such as tannins or alkaloids, before absorption.

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3. Physiological Factors in Animals_2

- **b. Species Differences:**
- Monogastric animals (e.g., dogs, cats) generally absorb herbal compounds faster than ruminants (e.g., cattle, sheep), due to differences in stomach physiology and digestion time.
- **c. Presence of Food:**
- Food can delay gastric emptying or alter the solubility of herbal compounds. For example:
  - Fats can enhance the absorption of lipid-soluble herbal compounds like curcumin.
  - Fiber may bind to some herbal compounds (e.g., polyphenols) and reduce their bioavailability.

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3. Physiological Factors in Animals_3

- **d. First-Pass Metabolism:**
- Like synthetic drugs, herbal compounds may undergo extensive first-pass metabolism in the liver, reducing systemic bioavailability. For example:
  - Silymarin (from milk thistle) is heavily metabolized in the liver.
  - Species with faster hepatic metabolism (e.g., cats) may show reduced efficacy for certain herbal compounds.
- **e. Surface Area and Blood Flow:**
- The large surface area of the intestines in most animals enhances absorption, but blood flow differences (e.g., due to stress or illness) can impact the uptake of herbal compounds.

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4. Disease States in Animals

- **GI Disorders**: Conditions like diarrhea, parasitic infections, or ulcers can impair the absorption of herbal remedies.

- **Hepatic Impairment**: In animals with liver dysfunction, the first-pass metabolism of herbal compounds may be reduced, leading to increased systemic levels and potential toxicity.

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5. Drug-Drug and Herb-Drug Interactions

- Herbal compounds often interact with synthetic drugs, which may affect absorption. Examples include:
  - **P-glycoprotein Substrates**: Herbal constituents like quercetin may inhibit efflux pumps, enhancing the absorption of co-administered drugs.
  - **Binding Interactions**: Tannins or fiber-rich herbs can bind to other drugs, reducing their absorption.
  - Cytochrome p450s

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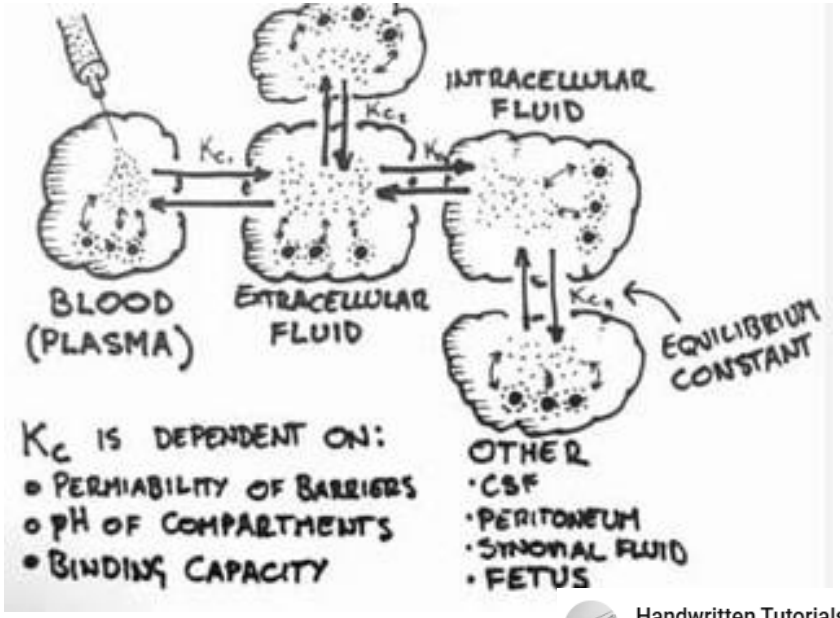
6. Environmental and Management Factors

- **Stress**: Stress can alter gut motility and pH, affecting absorption.
- **Diet**: Animals on high-fat diets may show better absorption of lipophilic herbal compounds.
- **Ration Formulation**: In ruminants, feed composition may alter rumen microbial activity, affecting the breakdown and absorption of herbal additives.

Distribution



- Transfer of plant active compounds between the blood and the extra vascular fluids and tissues

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1. Compound-Related Factors

- **a. Lipophilicity :**
- Lipid-soluble herbal compounds (e.g., curcumin, essential oils) tend to accumulate in fatty tissues, while water-soluble compounds (e.g., polysaccharides) remain in the plasma or extracellular fluid.
- Lipophilic compounds may show prolonged retention in fat-rich tissues, especially in animals with higher fat content (e.g., certain livestock breeds).
- **b. Protein Binding:**
- Many herbal compounds bind to plasma proteins (e.g., albumin). Highly protein-bound compounds have limited free (active) drug available for distribution.
  - Example: Flavonoids like quercetin exhibit strong protein binding, affecting their tissue availability.
- Changes in protein levels (e.g., due to disease or species differences) can alter distribution.
- **c. Molecular Size:**
- Small herbal molecules distribute more easily to tissues compared to larger compounds like tannins or polysaccharides.
- **d. Stability:**
- Unstable compounds (e.g., catechins or anthocyanins) may degrade before reaching target tissues, limiting their distribution.

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2. Physiological Factors in Animals_1

- **a. Blood Flow to Tissues:**
- Organs with high blood perfusion (e.g., liver, kidneys, lungs) receive herbal compounds more rapidly than poorly perfused tissues (e.g., fat, cartilage).
  - Example: Essential oils may distribute rapidly to the liver and lungs due to high perfusion.
- **b. Tissue Affinity:**
- Some herbal compounds have specific affinities for certain tissues:
  - Alkaloids like berberine accumulate in the liver due to active uptake.
  - Flavonoids may target vascular endothelial cells.

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2. Physiological Factors in Animals_2

- **c. Species Differences:**
- The distribution of herbal compounds varies across species due to anatomical and physiological differences:
  - Ruminants: The rumen and liver act as barriers to the systemic distribution of herbal compounds.
  - Birds: The avian circulatory system allows rapid distribution due to high cardiac output.
- **d. Barriers:**
- **Blood-Brain Barrier (BBB)**: Lipophilic and small herbal compounds (e.g., terpenes in essential oils) can cross the BBB, while hydrophilic compounds cannot.
- **Placental Barrier**: Some herbal compounds may cross the placenta, affecting fetal tissues.
- **e. Volume of Distribution (Vd):**
- Animals with larger extracellular fluid spaces (e.g., neonates) may show higher distribution of water-soluble herbal compounds.

3. Pathophysiological Conditions

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a. Disease States:

- **Liver Disease**: Impaired liver function can alter plasma protein levels, reducing protein binding and increasing the free fraction of herbal compounds.
- **Inflammation**: Increases capillary permeability, allowing larger or protein-bound herbal compounds to access tissues.
- **Cachexia or Malnutrition**: Decreased fat stores reduce the distribution of lipophilic compounds.

b. Species-Specific Conditions:

- Stress or dehydration in animals can reduce blood flow to peripheral tissues, altering distribution.
- Lactation increases blood flow to the mammary gland, which may affect the excretion and distribution of herbal products in milk.

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4. Herb-Drug and Herb-Herb Interactions

- **Enzyme Induction/Inhibition**: Some herbal compounds (e.g., St. John's wort) can induce or inhibit enzymes, affecting the distribution of co-administered drugs.
- **Protein Binding Competition**: Herbal compounds may displace synthetic drugs or other herbal compounds from plasma proteins, increasing free drug levels.
  - Example: Polyphenols can compete with antibiotics for plasma protein binding, altering distribution.

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5. Tissue-Specific Factors

- **a. Storage in Tissues:**
- Lipophilic herbal compounds (e.g., cannabinoids) may accumulate in adipose tissue, prolonging their elimination and altering their therapeutic profile.
- Chelation of herbal minerals (e.g., in bones) can lead to prolonged retention in skeletal tissue.
- **b. Active Transport:**
- Certain herbal compounds are transported into specific tissues via active transport mechanisms:
  - Example: Silymarin is taken up by hepatocytes, concentrating in the liver.
- **c. Efflux Mechanisms:**
- Efflux pumps like P-glycoprotein in the gut, liver, and brain can limit the distribution of herbal compounds, reducing their penetration into certain tissues.

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6. Formulation and Administration Factors

a. Route of Administration:

- **Oral**: After absorption, the liver acts as a major site for first-pass metabolism, reducing the amount of herbal compounds available for distribution.
- **Topical**: Distribution is localized unless the compound penetrates deeply into systemic circulation.
- **Parenteral**: Herbal compounds injected intravenously bypass first-pass metabolism and distribute more rapidly.

b. Formulation Enhancements:

- **Nanoformulations**: Liposomes or phytosomes improve tissue penetration of poorly distributed herbal compounds.
- **Co-administration**: Combining herbal compounds with absorption enhancers (e.g., piperine with curcumin) improves systemic distribution.

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7. Veterinary-Specific Considerations

a. Residue in Food-Producing Animals:

- Distribution to tissues like the liver, kidney, or fat is critical when considering withdrawal periods for herbal treatments in food-producing animals.
- Lipophilic herbal compounds may persist in fat tissues, requiring careful monitoring.
- Meat (edible tissues), milk, egg

Flavonoids like quercetin can accumulate in eggs or milk

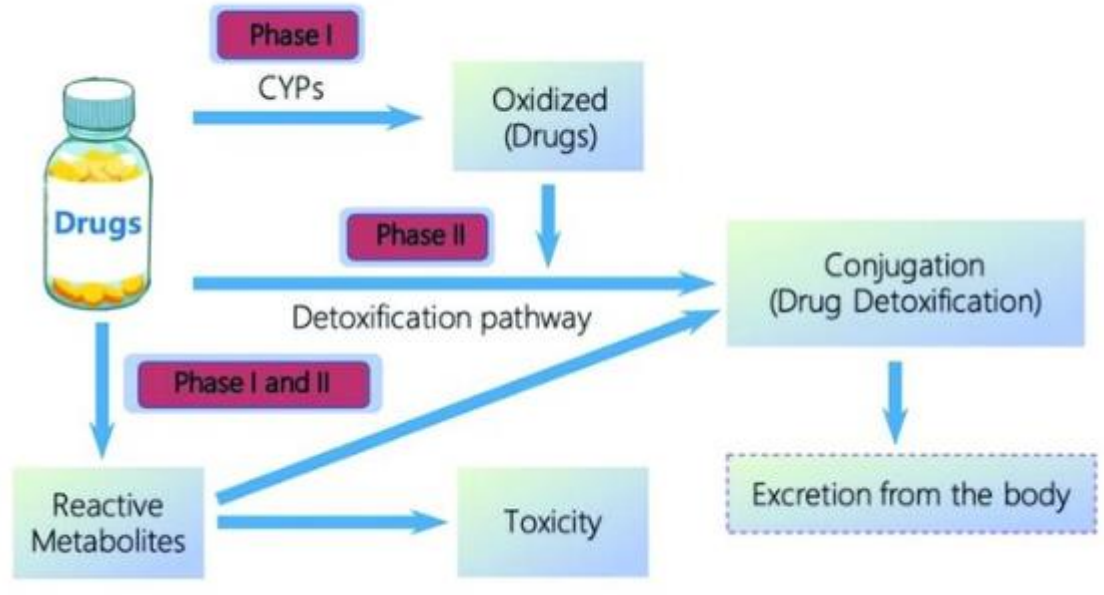
b. Milk Secretion:

- Lipid-soluble herbal compounds may accumulate in milk, impacting nursing offspring or milk safety for human consumption.

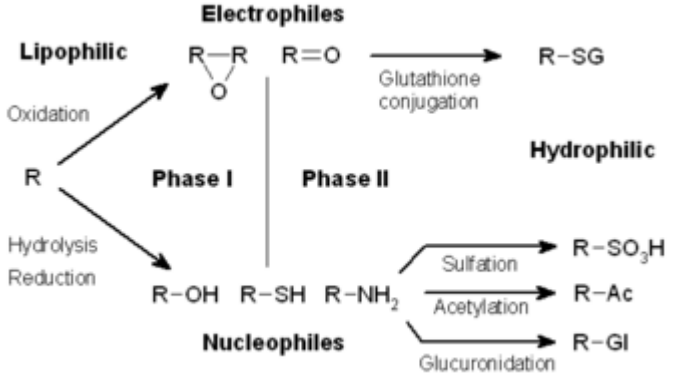
c. Environmental Factors:

- Housing, stress, and activity level in animals can alter blood flow and tissue perfusion, indirectly affecting herbal compound distribution.

Metabolism



Int. J. Mol. Sci. **2021**, *22*, 12808. https://doi.org/10.3390/ijms222312808



- Biological process by which the body chemically alters pharmaceutical substances (drugs) to facilitate their elimination.
- This process primarily occurs in the liver and involves a series of enzymatic reactions that transform drugs into more water-soluble forms, which can then be excreted through urine or bile.

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1. Compound-Related Factors_1

a. Chemical Structure:

- **Complexity**: Herbal compounds such as alkaloids, flavonoids, and terpenoids often have complex structures that influence their metabolic pathways.
  - For example, flavonoids undergo extensive Phase II metabolism (e.g., glucuronidation).
- **Functional Groups**: The presence of hydroxyl, carboxyl, or amine groups can determine the type of metabolic reaction (e.g., oxidation, reduction, conjugation).

b. Stability:

- Herbal compounds susceptible to hydrolysis or oxidation may be metabolized more rapidly. For instance:
  - Catechins in green tea are quickly metabolized due to their instability.

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1. Compound-Related Factors_2

c. Lipophilicity:

- Lipophilic herbal compounds are often metabolized in the liver via oxidation (Phase I) to increase their water solubility for excretion.

d. Synergistic Effects:

- Herbal products often contain multiple bioactive compounds, which may interact synergistically or antagonistically to influence metabolism.
  - For example, polyphenols in a mixture may inhibit or enhance the metabolism of other compounds.

2. Species-Specific Factors_1

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a. Enzyme Variability:

- Different species express varying levels and isoforms of metabolic enzymes, leading to differences in herbal compound metabolism.
  - **Ruminants**: Have unique rumen microbial activity, which can metabolize herbal products before they reach the systemic circulation.
  - **Cats**: Lack certain glucuronidation enzymes, leading to slower metabolism of compounds like flavonoids or essential oils.
  - **Dogs**: Have specific variations in cytochrome P450 (CYP450) enzymes that affect the metabolism of herbal alkaloids or terpenes.

Metabolic Pathways- Enzymes

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a. Phase I Metabolism:

- Involves oxidation, reduction, and hydrolysis, mainly mediated by cytochrome P450 enzymes (CYP450).
  - Example: Terpenes (e.g., menthol) are oxidized in Phase I metabolism.

b. Phase II Metabolism:

- Conjugation reactions like glucuronidation, sulfation, or methylation increase solubility for excretion.
  - Flavonoids undergo glucuronidation and sulfation in most species, but cats are deficient in glucuronidation, leading to accumulation and potential toxicity.

c. Extrahepatic Metabolism:

- Herbal products may also be metabolized in other tissues, such as the kidneys, lungs, or intestines.
  - Example: Lungs may metabolize volatile compounds from essential oils.

Factors Influencing CYP Activity Across Species

- Age: CYP activity generally increases with age and stabilizes in adults.
- Gender: Hormonal differences can influence CYP expression, with some variations observed between males and females.
- Diet: Phytochemicals, such as flavonoids and alkaloids, in the diet can induce or inhibit CYP activity.
- Environment: Exposure to pesticides, pollutants, and other xenobiotics can modulate CYP activity.
- Disease states: Inflammation and liver disease can downregulate CYP activity.
- Drugs: Many veterinary drugs are inducers or inhibitors of CYP enzymes, affecting the metabolism of co-administered compounds.

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Human and Animal CYPs are not orthologs

- Animal and human cytochrome P450 (CYP450) enzymes often differ significantly in their structure, function, and substrate specificity.
- While they belong to the same superfamily of enzymes and may share some evolutionary ancestry, they are not direct orthologs in many cases.

Aspect	Humans	Dogs	Key Differences/Implications
CYP450 Subfamilies	Humans have 57 CYP genes classified into families (e.g., CYP1, CYP2, CYP3).	Dogs have ~50 CYP genes with similar classifications (e.g., CYP1, CYP2, CYP3).	Shared subfamilies but non-orthologous enzymes (e.g., CYP2D6 in humans vs. CYP2D15 in dogs).
Major CYP450 Isoforms	CYP3A4, CYP2D6, CYP1A2, CYP2C9, CYP2C19, CYP2E1	CYP3A12, CYP1A2, CYP2D15, CYP2B11, CYP2C21	Dogs have different dominant isoforms, impacting drug metabolism.
Drug Metabolism	CYP3A4 is the most abundant enzyme, metabolizing ~50% of all drugs.	CYP3A12 and CYP3A26 dominate but metabolize fewer drugs compared to human CYP3A4.	CYP3A differences lead to varied drug clearance rates.
Polymorphisms	Significant genetic variability (e.g., CYP2D6 has poor, intermediate, extensive, and ultra-rapid metabolizers).	Polymorphisms in canine CYPs are less studied; fewer variations reported.	Humans show greater interindividual variability in drug metabolism.

Pharmacokinetics-Metabolism

European Journal of Pharmacology 585 (2008) 502–509

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European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Review

Implications of hepatic cytochrome P450-related biotransformation processes in veterinary sciences

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Cytochrome	Principle reaction	Comparison of the level of activity
CY1A2	7-Ethoxy-4-trifluor-methyl-coumarin-*O*-dealkylation	Dog >> rabbit, monkey > micropig > human > mouse > rat
CYP2A6	Coumarin-7-hydroxylase	Monkey > human > rabbit > micropig > dog > mouse >> rat
CYP2C	Diclofenac-4'-hydroxylase	Human > monkey, rat > rabbit > mouse > micropig > dog
CYP2C19	*S*-mephenytoin-4-hydroxylation	Monkey > human >> dog > rabbit > rat > mouse
CYP2C6	Amphetamine-hydroxylation	Rat >> mouse >> guinea pig, human, rabbit
CYP2E	Chlorzoxazone-6-hydroxylation	Similar in all tested species, despite different k_m values
CYP3A	Testosterone-6β-hydroxylation	Similar in all tested species, with the exception of dogs, having a very low activity

The Veterinary Journal 2003, 165, 33–61
doi:10.1016/S1090-0233(02)00176-6

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Comparative Expression of Liver Cytochrome P450-dependent Monooxygenases in the Horse and in other Agricultural and Laboratory Species

G. NEBBIA, M. DAGASTO, A. ROSSETTO GIACCHERINO, A. GIULIANO ALBO and M. GARLETTI
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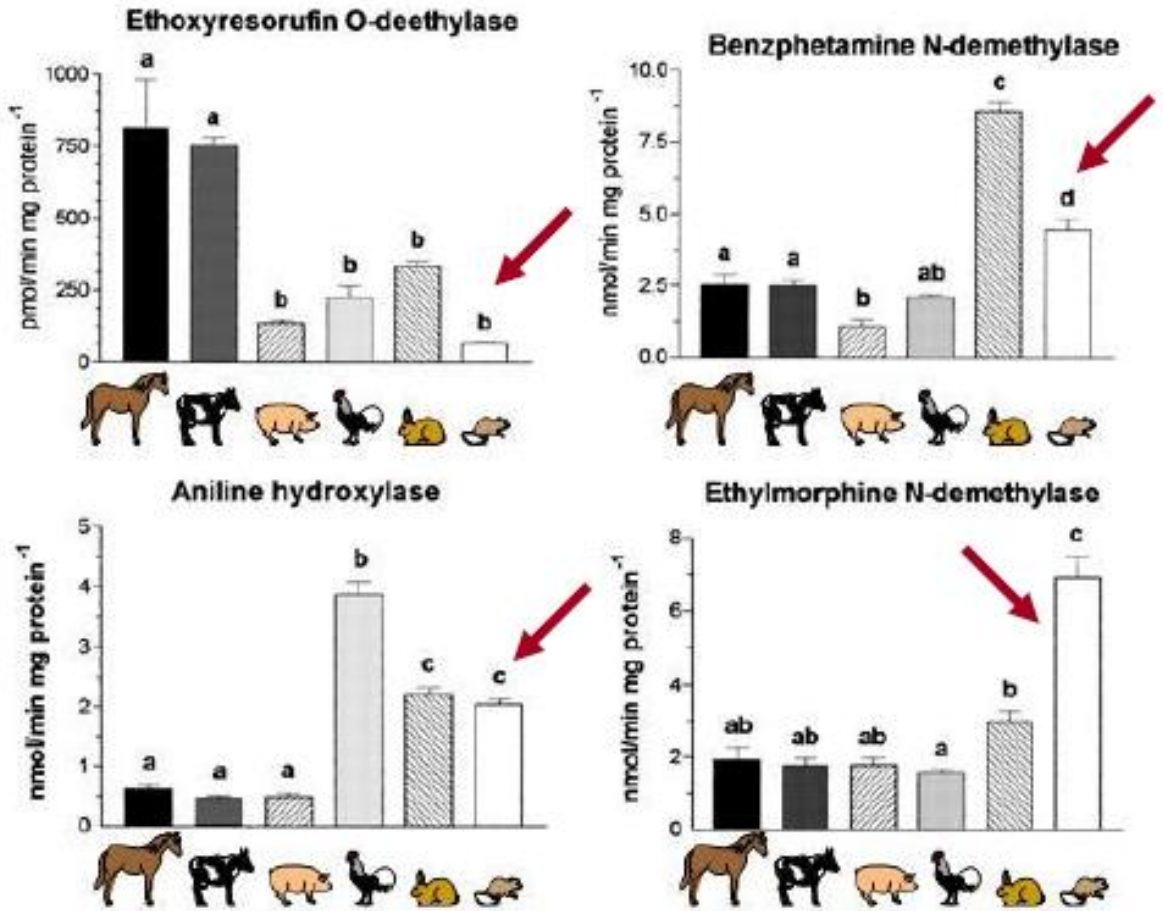
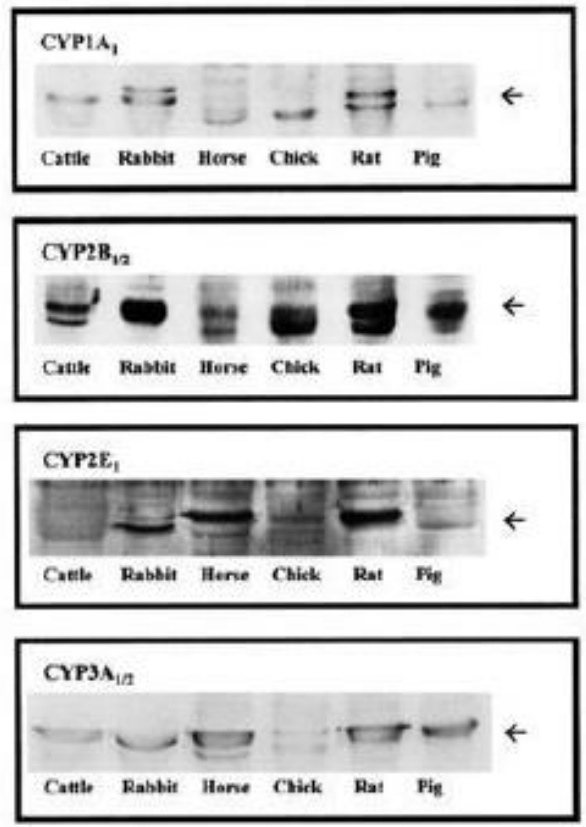




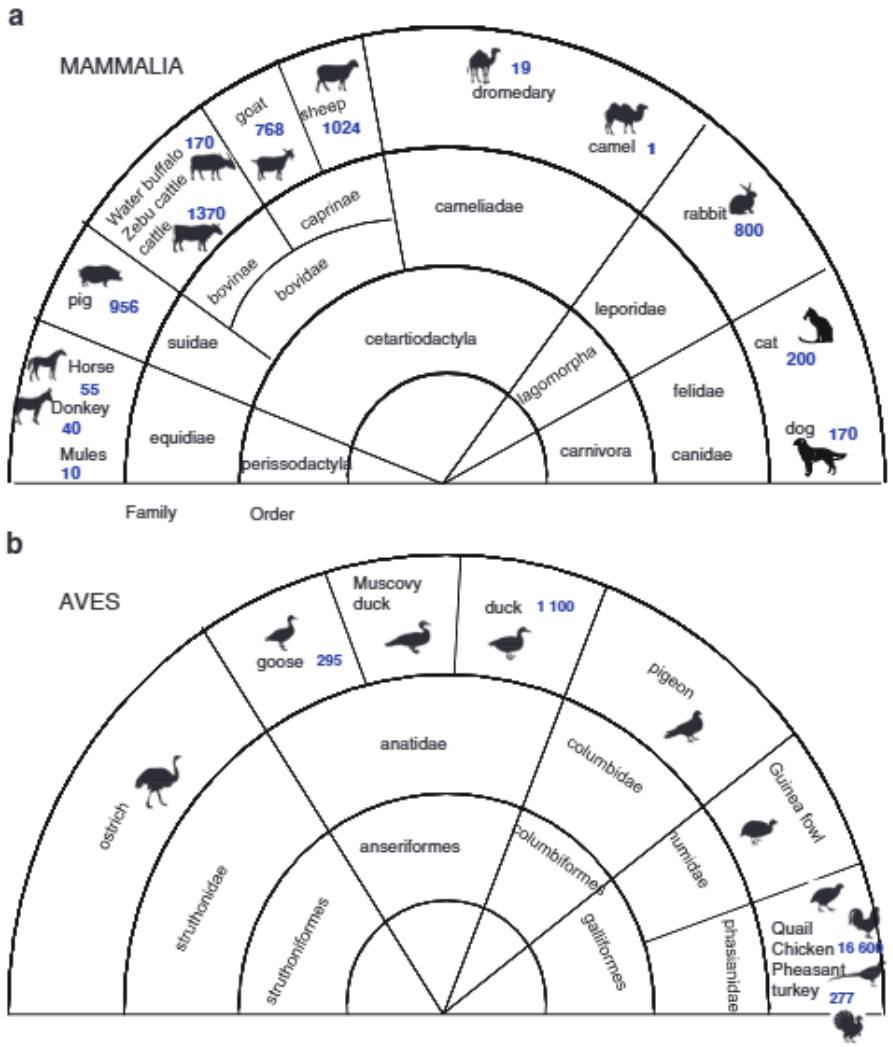
Pharmacokinetics-Metabolism

Polymorphisms in CYPs among dog species

Enzyme	Polymorphism	Impact/Findings	Breeds/Population	Source
CYP2D15	Novel polymorphisms in exon 2	Found in diverse breeds, such as Border Collies; affects variability in drug metabolism.	Border Collies, other breeds	van Hagen et al., 2020
CYP2C41	Gene deletion polymorphism	Variability in presence; high frequency in breeds like Chinese Shar-Pei and Siberian Husky, absent in breeds like Boxers and Bearded Collies.	36 breeds analyzed	Karakus et al., 2021
CYP1A2	SNP causing deficiency	Leads to significant interindividual variability in metabolism; 77% wild-type, 19% heterozygous, and 4% homozygous mutant observed.	Beagle dogs	Whiterock et al., 2007
CYP2B11	POR variants influencing CYP activity	POR-H3 and POR-H4 alleles significantly reduce CYP2B11 catalytic efficiency in Greyhounds and related breeds.	Greyhounds, Scottish Deerhounds	Martinez et al., 2019
CYP2D15	Exon 3 deletion (mRNA variant)	Results in inactive enzyme; significant reduction in enzymatic activity associated with p.Ile109Val and p.Gly186Ser variants.	Beagle dogs, mixed breeds	Jimenez et al., 2023
CYP System	General variability	Significant breed-dependent interindividual variability in CYP abundance, highlighting differences in metabolism and drug response.	Beagle, Greyhound, Chihuahua, and mixed breeds	Martinez et al., 2019

Polymorphisms in CYPs among cattle species

CYP Enzyme	Polymorphism	Impact/Findings	Breed/Population	Source
CYP11B1	Single nucleotide polymorphism (SNP) in 5'-UTR	Mutation (C→T) leads to Ala-Val substitution, potentially useful for developing DNA markers for traits.	Sahiwal Cattle (Pakistan)	Manzoor et al., 2013
CYP3A28	Missense SNPs rs384467435 and rs454167819	Variants reduce enzyme catalytic activity, affecting xenobiotic metabolism such as ivermectin and aflatoxins.	Limousine and Piedmontese breeds	Pauletto et al., 2020
CYP19 (Aromatase)	Polymorphisms in promoter region	Five SNPs identified; differences in haplotype diversity among Holstein, local Iraqi breeds, and crossbreeds.	Holstein, Local Iraqi Breeds	Faraj et al., 2020
CYP2D14	Gene deletions GD1 and GD2	High frequency in Jap Black cattle (58%) but Holstein Friesian (8%); associated with enzym defects.		
Cyt-b	Polymorphism in mitochondrial cytochrome b gene	Six alleles identified in Indonesian breeds; genetic diversity observed among local breeds like Bali cattle, Banteng, and crossbreeds.	Indonesian cattle breeds	Agung et al., 2024
CYP19	AA, AB, and BB genotypes detected using PCR-RFLP	Polymorphisms observed in Rathi cattle with allele A being more frequent; potential genetic markers for production and reproduction traits.	Rathi cattle	Amit et al., 2017



Species Differences in Pharmacokinetics and Pharmacodynamics

Pierre-Louis Toutain, Aude Ferran, and Alain Bousquet-Mélou

Table 1 Major and minor species (EMEA/CVMP 2003) in the EU and the USA

Major food-producing species for MRLs	Cattle (dairy, meat animals)
	Sheep (meat animals)
	Pigs
	Chicken (including laying hens)
	Turkey (USA but not EU)
	Salmonidae
Major non-food-producing animals	Cats
	Dogs
Minor food-producing species for MRLs	Other ruminants (bovidae including caprinae and their milk, deer, reindeer)
	Sheep (dairy)
	Other avian species and their eggs
	Other fish species
	Other mammalian species (horse, rabbit, dromedary)
	Honey bees
Minor non-food-producing species (distinguished from wildlife, exotic species in the USA)	All other species used as pets

Ex. Plant bioactive compounds metabolism by CYP

Plant Compound	CYP Enzyme Subfamily	Metabolic Reaction	Pharmacokinetic Impact	Source
Polyphenols (e.g., flavonoids)	CYP1A2, CYP3A4	Oxidation, hydroxylation, glucuronidation	Alters bioavailability and detoxification, modulates drug-drug interactions by enzyme inhibition.	Ramírez-Gómez et al., 2018
Terpenoids	CYP2C9, CYP3A4	Oxidation	Enhanced water solubility facilitates elimination; potential interaction with co-administered drugs.	Zanger & Schwab, 2013
Alkaloids (e.g., berberine, caffeine)	CYP1A2, CYP3A4	N-demethylation, hydroxylation	Modifies active compounds, influencing systemic exposure, therapeutic efficacy, and potential drug interactions.	Guengerich, 2006; Ramírez-Gómez et al., 2018
Phytoestrogens	CYP1B1, CYP3A4	Hydroxylation, dehydrogenation	Affects estrogen receptor binding and hormonal activity.	Morant et al., 2003
Glycosides	CYP2C8, CYP2D6	Hydrolysis, oxidation	Conversion to active metabolites influences therapeutic outcomes and drug-drug interactions.	Foti & Dalvie, 2016

1. Glucuronidation (UDP-Glucuronosyltransferases, UGTs)

- **Humans**: Highly active glucuronidation plays a significant role in metabolizing plant-derived polyphenols like flavonoids (e.g., quercetin, genistein). Different UGT isoforms process specific plant compounds, aiding detoxification and excretion.
- **Cats**: Limited glucuronidation capacity leads to poor metabolism of plant-derived compounds like phenolic acids and flavonoids. Cats are more prone to toxic accumulation of such compounds.
- **Dogs**: Moderate glucuronidation activity, but they may exhibit limited metabolism for some flavonoids compared to humans.
- **Pigs**: High glucuronidation efficiency for plant-based compounds, enabling robust processing of polyphenols and phenolic acids.
- **Rats and Mice**: Active glucuronidation pathways metabolize plant compounds efficiently, but differences in UGT isoform expression compared to humans can lead to varying pharmacokinetics.

2. Sulfation (Sulfotransferases, SULTs)

- **Humans**: Active sulfation plays a secondary role in the metabolism of plant phenolics like resveratrol and catechins, often complementing glucuronidation.
- **Cats and Dogs**: SULT activity is efficient in cats and dogs, allowing them to process sulfated metabolites of plant-derived polyphenols; however, this pathway alone may not fully compensate for poor glucuronidation in cats.
- **Pigs**: Moderate sulfation capacity, though glucuronidation remains the primary route for most plant-derived active compounds.
- **Rats and Mice**: High sulfation activity efficiently processes plant metabolites such as flavonoids, often resulting in rapid elimination compared to humans.

3. Glutathione Conjugation (Glutathione-S-Transferases, GSTs):

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- **Humans**: GST-mediated metabolism helps detoxify reactive intermediates from plant compounds, such as oxidized polyphenols. Genetic variability in GSTs may influence individual responses to plant antioxidants.
- **Dogs**: Higher GST activity allows for efficient conjugation of oxidative byproducts from plant-derived compounds like tannins and polyphenols.
- **Cats**: Low GST activity makes them more susceptible to oxidative damage from plant metabolites, limiting their detoxification efficiency.
- **Rats and Mice**: Strong GST activity facilitates efficient metabolism of reactive intermediates from plant-based compounds, often leading to faster clearance compared to humans.

4. Acetylation (N-Acetyltransferases, NATs):

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- **Humans**: Acetylation plays a minor role in processing plant-derived compounds but is involved in the metabolism of specific alkaloids and phenolic amines.
- **Dogs**: Lack NAT activity, leading to an inability to acetylate certain plant alkaloids or amines, which may accumulate or require alternative pathways for detoxification.
- **Cats**: Efficient acetylation of some plant-derived metabolites, though overall reliance on this pathway is limited compared to other species.
- **Rats and Mice**: Variable acetylation capacity; rats are moderate acetylators, while mice show lower activity. This may affect the metabolism of specific plant-derived alkaloids.

5. Methylation (Methyltransferases)

- **Humans**: Catechol-O-methyltransferase (COMT) efficiently metabolizes catechol-containing plant compounds (e.g., epicatechin, catecholamines). Methylation is crucial for modulating bioavailability and detoxification.
- **Cats**: Limited methylation activity reduces their capacity to metabolize catechol-containing plant compounds, potentially leading to accumulation.
- **Dogs**: Moderate methylation activity aids in processing plant-derived catechols, though less efficiently than humans.
- **Rats and Mice**: High methylation capacity facilitates the rapid metabolism of plant catechols, often leading to different pharmacokinetic profiles compared to humans.

6. Amino Acid Conjugation

- **Humans**: Glycine and glutamine conjugation metabolizes plant-derived acids, such as benzoic acid from berries. This is a secondary pathway with relatively lower activity.
- **Dogs**: Prefer amino acid conjugation, particularly glycine conjugation, for detoxifying plant-derived aromatic acids. For example, they efficiently conjugate salicylic acid from willow bark.
- **Cats**: Limited amino acid conjugation for plant metabolites, making them more reliant on sulfation or methylation pathways.
- **Rats and Mice**: Active amino acid conjugation pathways process a variety of plant-derived aromatic acids, though this may differ in efficiency from humans.

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Overall species differences

Cats

- **Lack glucuronidation**: Cats have very low levels of UDP-glucuronosyltransferases (UGTs), making them inefficient at metabolizing phenolic compounds and flavonoids found in plants. This leads to toxicity of plant compounds like salicylates.

Dogs

- **Lack acetylation**: Dogs do not express functional N-acetyltransferases (NATs). This makes them unable to metabolize certain plant alkaloids or amines (e.g., some components of herbal remedies) and leads to sensitivity to sulfonamide antibiotics.

Overall species differences_2

Pigs

- **Lack sulfation**: Pigs have reduced sulfotransferase (SULT) activity, which limits their ability to conjugate and metabolize plant-derived compounds that undergo sulfation, such as flavonoids and catechols.

Rats and Mice

- **Excessive sulfation**: Rats and mice are highly efficient at sulfation but may overrepresent this pathway compared to humans, leading to faster clearance of plant metabolites like resveratrol and catechins.

Overall species differences_3

Horses

- **Efficient glucuronidation but poor acetylation**: Horses rely heavily on glucuronidation for detoxification and show deficiencies in NAT activity, similar to dogs.

Ruminants (Cattle, Sheep)

- **Low glucuronidation and sulfation**: Ruminants often rely on other detoxification mechanisms and have reduced capacity for conjugation pathways like glucuronidation and sulfation, which limits their ability to metabolize some plant secondary metabolites.

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Example: n-Propyl disulfide

- The severity of Heinz body anemia varies with the species of animal, rate of ingestion and quantity ingested. In addition to Heinz body anemia, damage to red blood cell membranes can also result in hemolytic anemia.
- There are species differences with regard to susceptibility to intoxication from onions; goats and sheep are much less susceptible than cattle
- **Cats are the most sensitive domestic animal species to hemoglobin oxidation.**
- Interestingly, cats are susceptible to Heinz body formation following the ingestion of foods containing onion powder

Catalase	American foxhound, beagle [55]
Hereditary elliptocytosis	Band 4,1 deficiency [56]
Hereditary spherocytosis	Autosomal recessive trait in chondrodysplastic Alaskan malamute dwarf dogs
Hereditary stomatocytosis	Schnauzers [57,59]
Methaemoglobin redutase	Dogs (toy Alaskan Eskimo, miniature poodle, cocker/poodle cross) and cats – domestic short hair [60,61,62]
Osmotic fragility syndrome	Abyssinian, Somali, Siamese and domestic short hair cats [63–64]
Phosphofructokinase (PFK) deficiency	English springer spaniels, American cocker spaniels, whippets [65–66]
Pyruvate kinase (PK) deficiency	Basenjis, Cairn terrier, West Highland white terriers, beagles, cairn terriers, miniature poodles, dachshunds, Chihuahus, American Es ret sh

DOI: 10.17863/CAM.47497 • Corpus ID: 213913436

Oxidative Stress and Haemolytic Anaemia In Dogs and Cats: A Comparative Approach

J. Gibson, R. Wadud, +2 authors D. Rees • Published in Integrative Journal of... 31 October 2019 • Medicine, Biology, Environmental Science

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Some more examples

- Silymarin (Milk Thistle):Metabolized primarily in the liver through Phase II conjugation. Species differences in glucuronidation affect its metabolism and efficacy.
- Curcumin (Turmeric):Rapidly metabolized by glucuronidation and sulfation in most species. Co-administration with piperine slows its metabolism and improves bioavailability.
- Essential Oils (E.g., Eucalyptus, Peppermint):Metabolized by Phase I enzymes, with species variability in CYP450 activity influencing therapeutic effects.
- Flavonoids:Undergo extensive Phase II metabolism; species like cats with limited glucuronidation pathways are at risk for accumulation and toxicity.
- Isoflavones (Soy):Metabolized by gut microbiota into active metabolites (e.g., equol), but efficiency varies among species, influencing therapeutic outcomes.

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2. Species-Specific Factors_2

- **b. First-Pass Metabolism:**
- In monogastric animals (e.g., dogs, pigs), first-pass metabolism in the liver or gut wall can significantly reduce the bioavailability of orally administered herbal products.
- **c. Gut Microbiota:**
- Herbal products like tannins and saponins are metabolized by the gut microbiota into active or inactive metabolites.
  - For example, isoflavones (from soy) are metabolized into equol by gut bacteria, with varying efficiency across species.

3. Physiological Factors

a. Liver Function:

- The liver is the primary site of metabolism for herbal compounds. Impaired liver function (e.g., due to disease) can slow down metabolism, increasing systemic levels and toxicity risk.
  - Example: Silymarin, a hepatoprotective herb, may be metabolized differently in animals with liver diseases.

b. Age:

- Neonates and young animals have immature metabolic enzyme systems, leading to slower metabolism of herbal products.
- Older animals may experience reduced metabolic capacity due to organ degeneration.

c. Sex:

- Hormonal differences can influence enzyme activity. For example:
  - Female animals may metabolize some flavonoids faster due to hormonal regulation of metabolic pathways.

d. Stress and Disease:

- Conditions like stress or infection can alter liver enzyme activity and gut microbiota composition, indirectly affecting herbal product metabolism.

4- Formulation and Dosage

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a. Dosage Form:

- Formulation methods (e.g., encapsulation, nanoemulsions) can influence metabolism by protecting herbal compounds from first-pass metabolism or enzymatic degradation.
  - Example: Liposomal formulations of curcumin bypass rapid liver metabolism, enhancing systemic availability.

b. Dose:

- High doses of herbal compounds can saturate metabolic enzymes, leading to non-linear pharmacokinetics.

Herb-Drug and Herb-Herb Interactions

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a. Enzyme Induction:

- Some herbal compounds induce metabolic enzymes, increasing the metabolism of co-administered drugs or other herbal compounds.
  - Example: St. John's Wort induces CYP3A4, reducing the efficacy of drugs metabolized by this enzyme.

b. Enzyme Inhibition:

- Other herbal compounds inhibit enzymes, slowing metabolism and increasing the bioavailability or toxicity of co-administered substances.
  - Example: Piperine (from black pepper) inhibits CYP450 enzymes and glucuronidation, enhancing the bioavailability of curcumin.

c. Competition for Enzymes:

- Herbal mixtures may contain multiple compounds competing for the same metabolic enzymes, affecting their metabolism.
  - Example: Polyphenols and alkaloids in a herbal preparation may compete for Phase II conjugation enzymes.

Herbal Drug	Active Compound(s)	CYP Enzyme(s) Affected	Mechanism of Action	Substrate Interaction	Clinical Implication
St. John's Wort	Hyperforin, Hypericin	CYP3A4, CYP2C9, CYP2C19	Induction	Midazolam, Warfarin, Omeprazole	Reduced efficacy of drugs due to enhanced metabolism (e.g., decreased levels of oral contraceptives).
Grapefruit	Furanocoumarins	CYP3A4	Inhibition	Simvastatin, Atorvastatin	Increased drug levels, leading to enhanced side effects or toxicity.
Ginseng	Ginsenosides	CYP2D6, CYP3A4	Inhibition (weak)	Codeine, Midazolam	Possible alteration of drug efficacy, though typically less pronounced.
Garlic	Allicin	CYP2E1, CYP3A4	Induction (CYP3A4), Inhibition (CYP2E1)	Acetaminophen, Saquinavir	Decreased drug levels and potential loss of therapeutic effect, especially for antivirals.
Echinacea	Alkamides, Cichoric acid	CYP3A4, CYP1A2	Inhibition	Caffeine, Midazolam	Altered drug levels, though clinical significance is variable.
Milk Thistle	Silymarin	CYP3A4, CYP2C9	Inhibition	Warfarin, Tacrolimus	Potential for increased drug levels and enhanced pharmacologic effects.
Kava	Kavalactones	CYP2D6, CYP1A2, CYP3A4	Inhibition	Amitriptyline, Diazepam	Increased sedation or drug levels, leading to potential toxicity.
Green Tea	Catechins, EGCG	CYP3A4, CYP1A1	Inhibition	Warfarin, Midazolam	Variable drug interaction depending on dosage; can reduce anticoagulant effect of warfarin.
Peppermint	Menthol	CYP3A4, CYP2C19	Inhibition	Omeprazole, Diazepam	Altered metabolism; potential for enhanced drug levels and effects.
Turmeric (Curcumin)	Curcumin	CYP1A2, CYP3A4	Inhibition	Caffeine, Tacrolimus	Possible increased plasma concentrations of co-administered drugs.

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Excretion

* Process by which the metabolites of plant-derived compounds are removed from the body.

Main Routes

- Renal (urine) and biliary (feces).

Special Considerations:

- Lipophilic herbal compounds may have prolonged half-lives due to enterohepatic recirculation.
- Hydrophilic compounds are excreted faster but may accumulate in renal-impaired animals.

Excretion

- Renal Excretion (via kidneys):
  - The most common pathway for water-soluble herbal bioactive compounds.
  - Includes glomerular filtration, tubular secretion, and tubular reabsorption.
  - Urine pH can influence the elimination of certain drugs (e.g., weak acids or bases).
- Biliary Excretion (via liver):
  - Drugs or metabolites are excreted in bile and may enter the intestines.
  - Some herbal bioactive compounds undergo enterohepatic recirculation, prolonging their effects.
- Other Routes:
  - Lungs (e.g., volatile anesthetics).
  - Milk (important for nursing animals and food safety).
  - Saliva, sweat, or tears (minor routes).

Compound-Related Factors

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a. Physicochemical Properties:

- **Molecular Size**: Smaller herbal molecules are more readily excreted via the kidneys, while larger ones may be eliminated through bile.
- **Solubility**:
  - **Water-Soluble Compounds**: Hydrophilic herbal metabolites (e.g., glucuronides, sulfates) are eliminated more efficiently through the kidneys.
  - **Lipid-Soluble Compounds**: Lipophilic herbal compounds may undergo enterohepatic circulation, delaying their elimination.
- **Ionization**:
  - Ionized compounds are more easily excreted in urine, whereas non-ionized compounds may be reabsorbed in the renal tubules.

b. Stability:

- Herbal compounds prone to degradation (e.g., polyphenols) may be metabolized into water-soluble forms for rapid elimination.

Species specific examples on elimination

- Cats: Reduced glucuronidation capacity slows the elimination of compounds like flavonoids.
- Ruminants: More extensive biliary elimination due to higher bile production.
- Birds: Excrete primarily through feces due to the lack of a separate urinary system.

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Excretion related examples

- **Milk Thistle (Silymarin)**:
  - Eliminated primarily via bile with enterohepatic circulation, prolonging its half-life.
- **Garlic (Allicin)**:
  - Metabolites are excreted via urine, feces, and the lungs (noticeable as garlic breath).
- **Turmeric (Curcumin)**:
  - Rapidly metabolized into water-soluble conjugates and eliminated via bile and urine.
- **Tannins**:
  - Poorly absorbed but metabolized by gut microbiota and excreted in feces or urine.

Pharmacodynamics

- Pharmacodynamics studies how a drug (or herbal product) interacts with the body to produce its effects.

Focus on:

- Mechanisms of action.
- Dose-response relationships.
- Therapeutic and toxic effects.

1. Receptor Binding

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Agonists:

- **Ephedrine** (from *Ephedra* spp.): Acts as a sympathomimetic by stimulating adrenergic receptors, increasing heart rate and bronchodilation.
- **Morphine** (from *Papaver somniferum*): Binds to opioid receptors (μ-receptors) in the central nervous system to provide analgesic effects.

Antagonists:

- **Reserpine** (from *Rauwolfia serpentina*): Depletes neurotransmitters like norepinephrine by inhibiting their storage in presynaptic vesicles, leading to sedative and antihypertensive effects.
- **Curcumin** (from *Curcuma longa*): Acts on various receptors, including antagonism of inflammatory pathways (e.g., TNF-α signaling).

Partial Agonists:

- **Harmine** (from *Peganum harmala*): A partial agonist at serotonin (5-HT) receptors, influencing mood and cognition.

2. Enzyme Modulation

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Inhibitors:

- **Quercetin** (from many plants like *Onion* or *Green tea*): Inhibits enzymes such as tyrosine kinase and xanthine oxidase, offering anti-inflammatory and antioxidant effects.
- **Berberine** (from *Berberis* spp.): Inhibits AMP-activated protein kinase (AMPK), modulating glucose and lipid metabolism.
- **Artemisinin** (from *Artemisia annua*): Inhibits heme detoxification enzymes in *Plasmodium* parasites, making it effective against malaria.

Activators:

- **Silymarin** (from *Silybum marianum*): Activates superoxide dismutase (SOD) and glutathione peroxidase, enhancing antioxidant defenses in hepatocytes.

Prodrugs:

- **Salicin** (from *Willow Bark*): Metabolized to salicylic acid in the body, which inhibits cyclooxygenase (COX) enzymes to reduce inflammation.

3. Ion Channel Modulation

Blockers:

- **Menthol** (from *Mentha* spp.): Blocks calcium channels, resulting in analgesic and cooling effects on sensory neurons.
- **Hyperforin** (from *Hypericum perforatum*): Modulates ion channels, such as sodium and calcium, affecting neurotransmitter reuptake.

Openers:

- **Allicin** (from *Garlic*): Opens potassium ion channels, contributing to vasodilation and antihypertensive effects.

4. Transporter Modulation

Inhibitors:

- **St. John's Wort (Hypericin)**: Inhibits serotonin reuptake transporters, acting as a mild antidepressant.
- **Phlorizin** (from *Apple* and other fruits): Inhibits sodium-glucose co-transporters (SGLTs), reducing glucose reabsorption in the kidneys.

Enhancers:

- **Catechins** (from *Green Tea*): Enhance antioxidant transport mechanisms, such as upregulating glutathione transporters.

Membrane Transporters

ATP-Binding Cassette (ABC) Transporters:

- Function: Active transporters that use ATP to pump molecules across membranes.
- Examples:
  - **P-glycoprotein (P-gp/ABCB1)**: Efflux transporter that pumps drugs out of cells, protecting tissues.
  - **MRP (Multidrug Resistance-associated Proteins)**: Handle organic anions and conjugated metabolites.

Solute Carrier (SLC) Transporters:

- Function: Facilitate the uptake or efflux of substrates like ions, sugars, amino acids, and drugs.
- Examples:
  - **OATs (Organic Anion Transporters)**: Mediate the renal and hepatic uptake of anionic drugs.
  - **OCTs (Organic Cation Transporters)**: Handle cationic drugs and endogenous compounds.
  - **PEPTs (Peptide Transporters)**: Mediate intestinal absorption of peptides and certain drugs

Bile Salt Export Pump (BSEP/ABCB11):

- Function: Transports bile salts from liver cells into bile for excretion.
- Relevance: Variability in BSEP activity affects bile-related drug clearance.

P-gp

Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene

Katrina L. Mealey[a], Steve A. Bentjen[a], John M. Gay[a] and Glenn H. Cantor[b]

A subpopulation of collie dogs is extremely sensitive to neurotoxicity induced by ivermectin. The aim of this study was to determine the mechanistic basis for this phenomenon. The multi-drug-resistance gene (*mdr*1) encodes a large transmembrane protein, P-glycoprotein (P-gp), that is an integral part of the blood–brain barrier. P-gp functions as a drug-transport pump at the blood–brain barrier, transporting a variety of drugs from the brain back into the blood. Since ivermectin is a substrate for P-gp, we hypothesized that ivermectin-sensitive collies had altered *mdr*1 expression compared with unaffected collies. We report a deletion mutation of the *mdr*1 gene that is associated with ivermectin sensitivity. The 4-bp deletion results in a frame shift, generating several stop codons that prematurely terminate P-gp synthesis. Dogs that are homozygous for the deletion mutation display the ivermectin-sensitive phenotype, while those that are homozygous normal or heterozygous do not display increased sensitivity to ivermectin. *Pharmacogenetics* 11:727–733 © 2001 Lippincott Williams & Wilkins

Pharmacogenetics 2001, 11:727–733

Keywords: canine, collie, dog, ivermectin, *mdr*1 gene, P-glycoprotein

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Table: MDR1 Substrates Among Plant Bioactive Compounds

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Compound	Class	Source Plants	Interaction with MDR1	Notes
Quercetin	Flavonoid	Fruits, vegetables (*e.g.*, apples, onions)	Substrate and modulator	May affect drug absorption and efflux.
Kaempferol	Flavonoid	Green tea, broccoli, kale	Substrate	Potential influence on bioavailability.
Berberine	Alkaloid	*Berberis, Coptis chinensis*	Substrate and inhibitor	Alters MDR1 transport and efflux.
Curcumin	Polyphenol	Turmeric (*Curcuma longa*)	Substrate and inhibitor	Modulates MDR1 activity.
Silymarin	Flavonoid	Milk thistle (*Silybum marianum*)	Substrate	Enhances MDR1-mediated efflux.

MDR1 implications

Clinical Implications:

- **Increased Drug Bioavailability**: Inhibition of MDR1 by compounds like curcumin and EGCG can enhance the bioavailability of drugs that are P-gp substrates (e.g., digoxin, paclitaxel, or doxorubicin).
- **Drug Resistance in Cancer**: MDR1 overexpression is a common cause of multidrug resistance in cancer. Plant-derived inhibitors like quercetin and resveratrol have been studied as potential MDR1 modulators to overcome resistance.

Pharmacokinetic Interactions: Co-administration of plant bioactives with drugs that are MDR1 substrates can result in significant changes in drug absorption and efficacy. For example:

- Berberine, an MDR1 substrate, shows enhanced therapeutic effects when co-administered with MDR1 inhibitors.
- Curcumin inhibits MDR1, potentially increasing the effectiveness of chemotherapy drugs.

Concentration-Dependent Effects:

- Some compounds (e.g., quercetin) exhibit concentration-dependent effects on MDR1, acting as substrates at low concentrations and inhibitors at higher doses.

5. DNA and Cellular Interaction

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DNA Intercalators:

- **Camptothecin** (from *Camptotheca acuminata*): Inhibits topoisomerase I, interfering with DNA replication, and is used in cancer treatment.

Epigenetic Modulators:

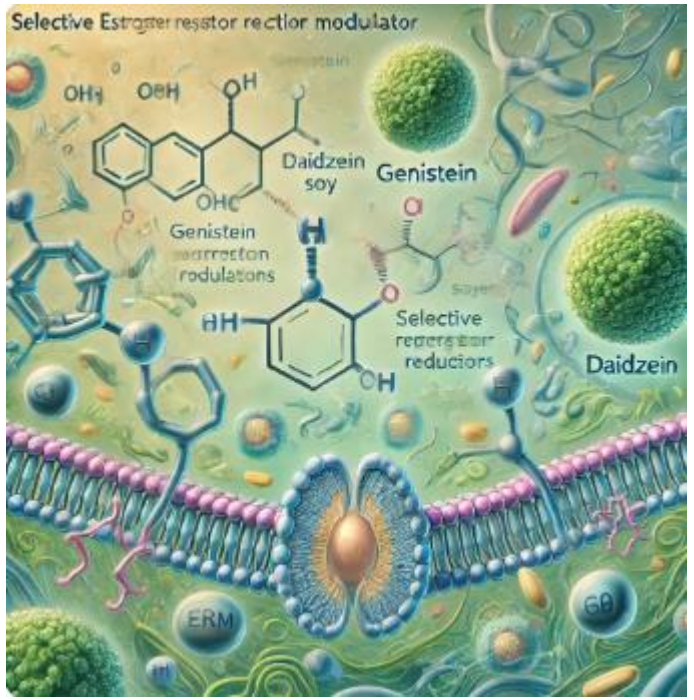
- **Epigallocatechin Gallate (EGCG)** (from *Green Tea*): Modulates DNA methylation and histone acetylation, affecting gene expression involved in cancer and inflammation.

6. Oxidative Stress and Free Radical Scavengin

- **Antioxidants**:
  - **Curcumin** (from *Turmeric*): Neutralizes reactive oxygen species (ROS) and modulates Nrf2 pathways for enhanced antioxidant gene expression.
  - **Rosmarinic Acid** (from *Rosemary*): Scavenges free radicals and protects lipid membranes from peroxidation.

7. Hormonal Modulation

- **Phytoestrogens**:
  - **Ex:**
  - **Genistein** (from *Soy*): Binds to estrogen receptors, acting as a selective estrogen receptor modulator (SERM) with applications in menopausal symptoms and osteoporosis.
  - **Daidzein** (from *Soy*): Mimics estrogen by binding to estrogen receptors, influencing reproductive and bone health.



From Effect to Indication

- Plants have been used in veterinary medicine to treat various conditions in livestock, pets, and other animals.

- The pharmacological activity of medicinal plants in veterinary care arises from their bioactive compounds, which provide therapeutic effects for diverse health issues.

Pharmacological Activity Examples of Herba Compounds in Veterinary Medicine

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- **Antimicrobial Activity**:
  - **Examples**: Garlic (*Allium sativum*), Neem (*Azadirachta indica*), and Tea tree oil (*Melaleuca alternifolia*).
  - **Use**: Treat infections caused by bacteria, fungi, and parasites.
- **Anti-inflammatory and Analgesic Activity**:
  - **Examples**: Turmeric (*Curcuma longa*), Boswellia (*Boswellia serrata*).
  - **Use**: Manage inflammation, arthritis, and pain in animals.
- **Antiparasitic Activity**:
  - **Examples**: Wormwood (*Artemisia absinthium*), Neem (*Azadirachta indica*).
  - **Use**: Control internal and external parasites, such as gastrointestinal worms and ticks.
- **Hepatoprotective Activity**:
  - **Examples**: Milk thistle (*Silybum marianum*), Phyllanthus (*Phyllanthus amarus*).
  - **Use**: Protect the liver from toxins and promote recovery in cases of hepatic damage.
- **Cardiovascular Support**:
  - **Examples**: Hawthorn (*Crataegus spp.*), Garlic (*Allium sativum*).
  - **Use**: Regulate blood pressure, improve heart function.
- **Digestive Health**:
  - **Examples**: Peppermint (*Mentha piperita*), Fennel (*Foeniculum vulgare*), Ginger (*Zingiber officinale*).
  - **Use**: Treat colic, indigestion, and bloating.
- **Immune Modulation**:
  - **Examples**: Echinacea (*Echinacea purpurea*), Astragalus (*Astragalus membranaceus*).
  - **Use**: Boost the immune system and improve disease resistance.
- **Wound Healing**:
  - **Examples**: Aloe vera (*Aloe barbadensis*), Calendula (*Calendula officinalis*).
  - **Use**: Accelerate wound healing and prevent infections.
- **Reproductive Support**:
  - **Examples**: Fenugreek (*Trigonella foenum-graecum*), Ashwagandha (*Withania somnifera*).
  - **Use**: Support fertility and lactation.
- **Respiratory Health**:
  - **Examples**: Eucalyptus (*Eucalyptus globulus*), Licorice (*Glycyrrhiza glabra*).
  - **Use**: Treat coughs, bronchitis, and other respiratory conditions.

Plant secondary metabolites

- Defense Against Herbivores and Pathogens:
  - Alkaloids, tannins, and terpenoids deter predators or inhibit microbial growth.
- Pollinator Attraction:
  - Essential oils and flavonoids contribute to fragrance and color.
- Environmental Adaptation:
  - Phenolics protect against UV radiation and oxidative stress.

From effect to toxicity

- Iatrogenic (Malpractice- species specific considerations, dose)
- Forage/Landscape conditions
- Contaminated feed
- Instinct related (cats vs lily)

What are those bioactive compounds-1

1. Phenolics

- **Definition**: Compounds containing one or more hydroxyl groups attached to an aromatic ring.
- **Subclasses**:
  - **Flavonoids**: E.g., quercetin, catechins, anthocyanins
  - **Phenolic acids**: E.g., caffeic acid, gallic acid
  - **Lignans**: E.g., secoisolariciresinol
  - **Tannins**: E.g., ellagitannins, proanthocyanidins

2. Alkaloids

- **Definition**: Nitrogen-containing compounds derived from amino acids

3. Terpenoids

- **Definition**: Compounds built from isoprene units.
- **Subclasses**:**Monoterpenes**: E.g., menthol, limonene
- **Sesquiterpenes**: E.g., artemisinin
- **Triterpenes**: E.g., ursolic acid
- **Carotenoids**: E.g., beta-carotene, lycopene

4. Glycosides

- **Definition**: Molecules consisting of a sugar bonded to a bioactive compound (aglycone).
- **Examples**:
  - Cardiac glycosides: E.g., digoxin (Digitalis spp.)

What are those bioactive compounds-2

5. Saponins

- **Definition**: Amphipathic glycosides with a steroidal or triterpenoid aglycone.
- **Sources**: Legumes, quinoa, ginseng, fenugreek.

6. Glucosinolates

- **Definition**: Sulfur-containing compounds derived from amino acids.
- **Sources**: Cruciferous vegetables (broccoli, kale, Brussels sprouts).

7. Polyacetylenes

- **Definition**: Compounds with alternating single and triple carbon bonds.
- **Sources**: Carrots, celery, parsley, and ginseng.

8. Sterols and Phytosterols

- **Definition**: Plant-derived sterols structurally similar to cholesterol.
- **Sources**: Vegetable oils, nuts, seeds, whole grains.

9. Coumarins

- **Definition**: Aromatic compounds derived from cinnamic acid.
- **Sources**: Cinnamon, citrus fruits, tonka beans.

10. Polysaccharides

- **Definition**: Long chains of monosaccharides with structural and functional roles.
- **Sources**: Medicinal mushrooms (e.g., Reishi, Shiitake), seaweed, aloe vera.

Class	Type	Examples	Source Plants	Biological Activities
True Alkaloids	Pyrrolidine Alkaloids	Nicotine	Tobacco (*Nicotiana tabacum*)	Stimulant, insecticide
	Pyridine Alkaloids	Arecoline	Betel nut (*Areca catechu*)	Anthelmintic, stimulant
	Tropane Alkaloids	Atropine, Hyoscyamine, Cocaine	Deadly nightshade (*Atropa belladonna*), Coca (*Erythroxylum coca*)	Anticholinergic, anesthetic
	Quinoline Alkaloids	Quinine, Cinchonine	Cinchona (*Cinchona spp.*)	Antimalarial, antipyretic
	Isoquinoline Alkaloids	Morphine, Codeine, Berberine	Opium poppy (*Papaver somniferum*), Barberry (*Berberis spp.*)	Analgesic, antimicrobial
	Indole Alkaloids	Reserpine, Vinblastine	Snakeroot (*Rauwolfia serpentina*), Periwinkle (*Catharanthus roseus*)	Antihypertensive, anticancer
	Imidazole Alkaloids	Pilocarpine	Jaborandi (*Pilocarpus spp.*)	Miotic, treatment for glaucoma

Proto-Alkaloids	Phenylethylamine Alkaloids	Ephedrine	Ephedra (*Ephedra sinica*)	Bronchodilator, stimulant
	Tryptamine Alkaloids	Serotonin, Psilocybin	Mushrooms (*Psilocybe spp.*), Plants	Psychoactive, neurotransmitter
Pseudo-Alkaloids	Steroidal Alkaloids	Solanine, Tomatine	Potato (*Solanum tuberosum*), Tomato (*Solanum lycopersicum*)	Antimicrobial, insecticidal
	Terpenoid Alkaloids	Taxol, Aconitine	Yew tree (*Taxus brevifolia*), Aconite (*Aconitum spp.*)	Anticancer, neurotoxic
	Purine Alkaloids	Caffeine, Theobromine	Coffee (*Coffea spp.*), Cocoa (*Theobroma cacao*)	CNS stimulant, bronchodilator

Pharmacological Applications of Alkaloids in Veterinary Medicine

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Alkaloid	Indication in Animals	Toxicity in Animals
Morphine	Pain management in dogs and horses	Respiratory depression, gastrointestinal effects
Atropine	Pre-anesthetic agent; treats bradycardia and spasms	Dry mouth, tachycardia, CNS disturbances
Quinine	Treatment of babesiosis and malaria-like infections in dogs	Gastrointestinal upset, neurotoxicity at high doses
Theophylline	Management of COPD in horses and asthma in dogs/cats	Nausea, vomiting, seizures, arrhythmias in overdose
Caffeine	Respiratory stimulation in neonatal animals	Hyperexcitability, tremors, tachycardia
Colchicine	Treatment of gout-like conditions in birds; chronic laminitis in horses	Severe gastrointestinal distress, renal toxicity
Reserpine	Sedative for horses with behavioral issues	Hypotension, sedation, bradycardia
Vincristine	Chemotherapy for canine lymphomas and mast cell tumors	Bone marrow suppression, gastrointestinal issues
Apomorphine	Induction of vomiting in dogs after toxin ingestion	Prolonged vomiting, lethargy, CNS depression

Example of morphine-1

PK

- Morphine undergoes **Phase I (oxidation)** and **Phase II (glucuronidation)** reactions in the liver.
- **Glucuronidation Pathways**:
  - **Morphine-3-glucuronide (M3G)**: Inactive, potentially neurotoxic at high concentrations.
  - **Morphine-6-glucuronide (M6G)**: Active metabolite with potent analgesic properties.
- **Species Differences**:
  - **Cats**: Limited glucuronidation capacity; rely on sulfate conjugation, leading to slower metabolism and prolonged drug effects.
  - **Dogs**: Predominantly produce M3G, with less M6G, resulting in reduced analgesic potency compared to humans.

Example of morphine-2

PD

Receptor Interaction:

- Morphine primarily acts on **mu-opioid receptors (MOR)** and, to a lesser extent, **kappa (KOR)** and **delta (DOR)** receptors.

Species-Specific Receptor Sensitivity:

- **Dogs**: Mu-opioid receptors mediate sedation and mild analgesia. Higher doses can cause dysphoria.
- **Cats**: Exhibit excitatory effects (e.g., hyperactivity, mydriasis) at high doses due to kappa-receptor involvement.
- **Horses**: Activation of kappa receptors leads to excitation and potential colic-like symptoms at high doses.

Example of morphine-3
Clinical manifestations

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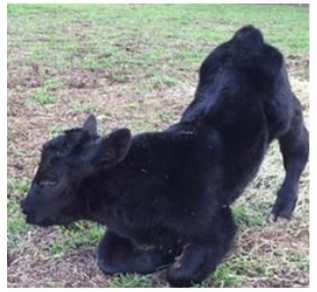
Species	Effects	Notes
Dogs	Sedation, analgesia, vomiting, dysphoria	Vomiting is common due to stimulation of the chemoreceptor trigger zone (CRTZ).
Cats	Excitement, hyperactivity, mydriasis	Reduced glucuronidation prolongs drug effects; careful dosing is required to avoid toxicity.
Horses	Excitement, reduced gut motility	May lead to ileus and colic symptoms; not preferred for equine pain management.
Ruminants	Sedation, mild analgesia	Sensitive to respiratory depression at higher doses.
Birds	Variable analgesia	Poor CNS penetration; higher doses often required.

Teratogenic Alkaloids

Alkaloid	Source Plants	Mechanism of Teratogenicity	Affected Species	Teratogenic Effects
Pyrrolizidine Alkaloids	*Senecio spp., Crotalaria spp., Heliotropium spp.*	DNA crosslinking and inhibition of cell division	Livestock (cattle, sheep, horses)	Skeletal malformations, liver damage in offspring
Cyclopamine	*Veratrum californicum*	Inhibition of the Hedgehog signaling pathway	Sheep, cattle	Cyclopia (single eye), craniofacial defects
Coniine	Poison hemlock (*Conium maculatum*)	Blocks nicotinic acetylcholine receptors	Cattle, pigs, goats	Limb deformities, cleft palate
Anabasine	Tree tobacco (*Nicotiana glauca*)	Nicotinic acetylcholine receptor agonism	Sheep, cattle	Arthrogryposis (joint contractures)
Ergot Alkaloids	*Claviceps purpurea* (ergot fungus)	Vasoconstriction leading to reduced placental blood flow	Livestock (cattle, horses)	Reduced fetal growth, abortion, limb malformations
Lupinine and Related Alkaloids	Lupines (*Lupinus spp.*)	Neuromuscular inhibition	Cattle, sheep	Arthrogryposis, crooked calf syndrome



Glycosides

- Organic compounds in which a sugar molecule is bonded to a non-sugar moiety (aglycone) through a glycosidic bond, often found in plants with various medicinal and biological activities.

Type of Glycoside	Examples	Source Plants	Mechanism of Toxicity	Toxic Effects	Affected Species
Cardiac Glycosides	Digoxin, Oleandrin	Foxglove (*Digitalis spp.*), Oleander (*Nerium oleander*), Milkweed (*Asclepias spp.*)	Inhibit Na^+/K^+-ATPase in cardiac myocytes	Cardiac arrhythmias, bradycardia, vomiting	Livestock, dogs, horses
Cyanogenic Glycosides	Amygdalin, Linamarin	Cherry (*Prunus spp.*), Sorghum (*Sorghum spp.*), Cassava (*Manihot esculenta*)	Release of cyanide upon hydrolysis	Cellular hypoxia, respiratory distress	Ruminants, horses, pigs

Saponin Glycosides	Diosgenin, Aescin	Soybeans (*Glycine max*), Horse chestnut (*Aesculus hippocastanum*)	Disrupts cell membranes, leading to hemolysis	Gastrointestinal irritation, hemolytic anemia	Ruminants, horses
Anthraquinone Glycosides	Aloin, Emodin	Aloe (*Aloe vera*), Senna (*Senna spp.*)	Irritation of gastrointestinal mucosa	Diarrhea, dehydration	Livestock, small animals
Steroidal Glycosides	Solanine, Tomatine	Potato (*Solanum tuberosum*), Tomato (*Solanum lycopersicum*)	Disrupts nervous and gastrointestinal systems	Neurological signs, GI upset	Cattle, pigs, dogs
Coumarin Glycosides	Melilotin	Sweet clover (*Melilotus spp.*)	Converts to dicoumarol, inhibiting vitamin K recycling	Hemorrhage due to coagulopathy	Cattle, sheep
Flavonoid Glycosides	Rutin, Hesperidin	Citrus fruits (*Citrus spp.*), Buckwheat (*Fagopyrum esculentum*)	Antioxidant effects at low doses; oxidative stress at high doses	Photosensitization, skin lesions	Horses, sheep

Phytosterols

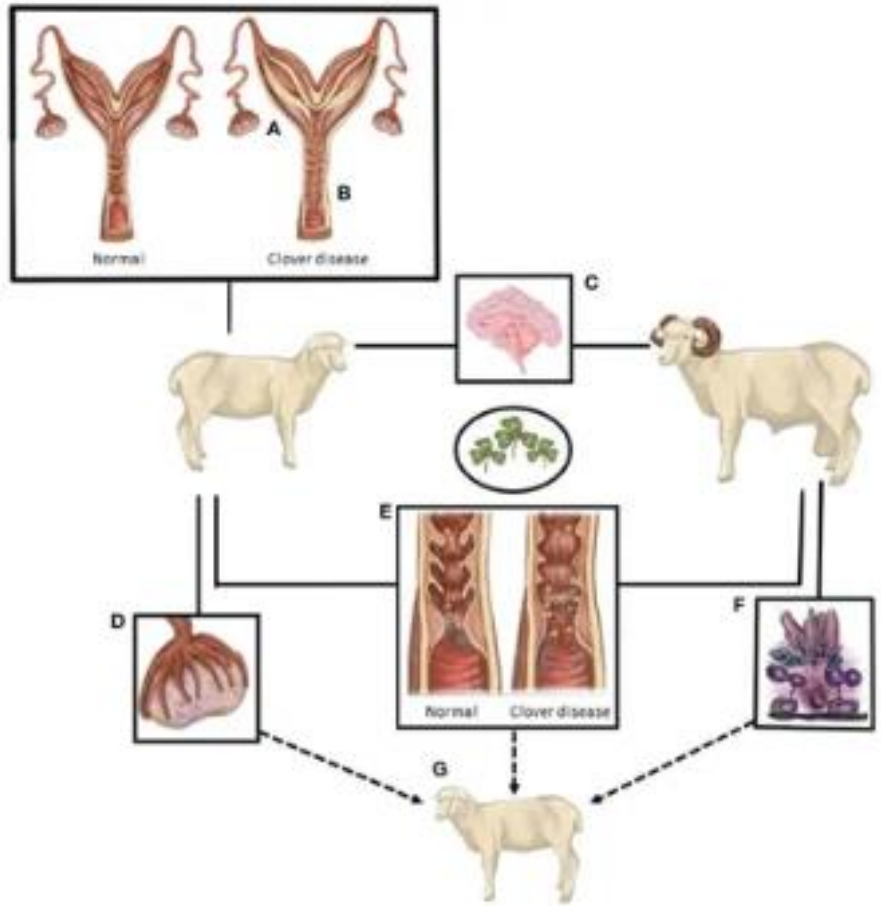
- Phytosterols, also known as plant sterols, are naturally occurring compounds structurally similar to cholesterol. They are widely present in plants and commonly found in oils, nuts, seeds, and grains.
- Phytosterols compete with cholesterol for absorption in the gastrointestinal tract.
- Excessive phytosterols can disrupt lipid metabolism and membrane integrity in cells.

Species	Toxic Effects	Mechanism/Pathophysiology
Cattle	Fatty liver, growth retardation	Excessive phytosterols impair lipid metabolism.
Sheep	Hepatotoxicity, poor weight gain	Oxidized phytosterols induce oxidative stress and liver damage.
Poultry	Reduced egg production, weight loss	Disruption of lipid metabolism affects growth and reproduction.
Horses	Gastrointestinal distress, colic	High levels of phytosterols interfere with gut health.
Dogs	Lipid metabolism disturbances, hepatotoxicity	Limited detoxification pathways for oxidized phytosterols.

Phytoestrogens

Class	Examples	Source Plants	Mechanism of Action
Isoflavones	Genistein, Daidzein	Soybeans (*Glycine max*), Clover (*Trifolium spp.*)	Bind to estrogen receptors (ERα and ERβ), modulating estrogenic effects.
Coumestans	Coumestrol	Alfalfa (*Medicago sativa*), Clover (*Trifolium spp.*)	High estrogen receptor affinity, potent estrogenic activity.
Lignans	Secoisolariciresinol	Flaxseed (*Linum usitatissimum*), Sesame (*Sesamum indicum*)	Converted by gut microbiota into estrogenic metabolites (e.g., enterolactone).
Flavones	Apigenin, Luteolin	Parsley (*Petroselinum crispum*), Celery (*Apium graveolens*)	Weak estrogenic activity through receptor binding.

Figure 1



REVIEW article

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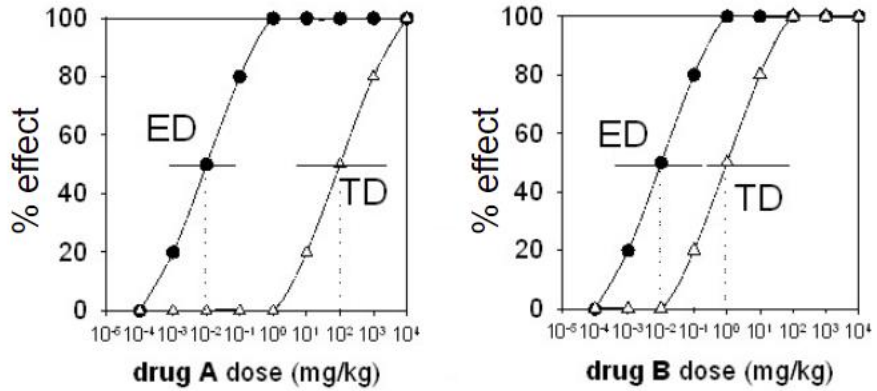
Figure 1 A summary of the known and postulated sites of action of compounds from oestrogenic pasture that lead to compromised sheep reproduction. **(A)** Endometrial thickening/edema. **(B)** Loss of cervical folds. **(C)** Excessive oestrogen-like actions in the neuroendocrine control of reproduction. **(D)** Follicle development, quality and potentially ovulatory ability are reduced. **(E)** The interaction between spermatozoa and the female tract is altered. Loss of cervical crypts, changes in mucus composition, and consistency and changes in the female immune response hinder sperm navigation of the female tract. **(F)** Sperm production and quality is potentially reduced. **(G)** Exposure of both male and female gametes, and the ovine embryo, may cause differential developmental programming of the subsequent generation.

Therapeutic Index (TI)

- Therapeutic Index (TI)

$$TI = LD_{50}/ED_{50}$$

$$\text{or} \quad TI = TD_{50}/ED_{50}$$



- Comparative toxicity- relatively safe
- Larger ratio- greater the relative safety
- Meanwhile- TI does not take into account of the slope of DR curve
- Margin of safety= LD1/ED99 or TD1/ED99

Acute Toxicity

- Occurs after a single exposure or short-term ingestion of a toxic dose of a bioactive compound.

Key Features	Examples
Onset: Rapid (minutes to hours).	**Alkaloids**: Atropine from *Atropa belladonna* causing seizures or respiratory distress.
Mechanism: Immediate disruption of physiological systems, often due to high doses.	**Essential Oils**: Tea tree oil (*Melaleuca alternifolia*) causing tremors and ataxia in cats.
Symptoms: Vomiting, diarrhea, seizures, respiratory failure.	**Cyanogenic Glycosides**: *Prunus spp.* releasing cyanide, causing hypoxia in ruminants.

Subacute Toxicity

- Results from repeated exposure to moderate doses over days to weeks.

Key Features	Examples
Onset: Gradual (days to weeks).	**Tannins:** From oak (*Quercus spp.*) causing kidney damage in cattle.
Mechanism: Cumulative effects impair organ function over time.	**Pyrrolizidine Alkaloids:** *Senecio spp.* causing progressive liver damage.
Symptoms: Weight loss, lethargy, mild organ dysfunction.	**Saponins:** From alfalfa (*Medicago sativa*) leading to gastrointestinal irritation.

Chronic Toxicity

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- Develops from prolonged exposure to low doses over weeks to months.

Key Features	Examples
Onset: Delayed (months to years).	**Phenolic Compounds**: Chronic exposure to flavonoids causing hepatotoxicity in dogs.
Mechanism: Long-term accumulation or metabolic interference causing irreversible damage.	**Pyrrolizidine Alkaloids**: Progressive liver cirrhosis in horses and cattle.
Symptoms: Organ failure, reproductive issues, carcinogenesis.	**Coumarins**: From spoiled sweet clover (*Melilotus spp.*) leading to coagulopathy.

Residues of Plant Bioactive Compounds in Animal-Origin Food

- Residues of plant bioactive compounds in animal-origin food, such as milk, meat, and eggs, pose potential risks to human health.
- These residues can result from the direct consumption of herbal feed additives, contaminated forage, or therapeutic use of plant-based products in livestock.
- The risks depend on the compound's pharmacokinetics, persistence, and potential to exert pharmacological or toxic effects in humans.

Risks to Human Health

- **a. Pharmacological Effects**
- **Estrogenic and Hormonal Effects**: Phytoestrogens (e.g., genistein, daidzein) from soy or clover residues in milk or meat may interfere with hormonal balance, potentially affecting reproductive health in humans.
- **Cardiac Effects**: Residues of cardiac glycosides from plants like *Digitalis* spp. may affect the human cardiovascular system, especially in sensitive individuals.
- **Neurotoxicity**: Alkaloid residues (e.g., atropine, scopolamine) from certain plants may cause neurological disturbances if consumed in significant quantities.

Risks to Human Health_2

b. Toxicity

- **Hepatotoxicity**: Pyrrolizidine alkaloids from plants like ragwort (*Senecio* spp.) can accumulate in animal tissues and pose a risk of liver toxicity in humans.
- **Carcinogenicity**: Certain plant bioactive compounds, such as aristolochic acid, found in residues, are linked to genotoxic and carcinogenic effects.
- **Kidney Damage**: Oxalates or nephrotoxic compounds from plant residues in animal products can impair renal function.
- **c. Allergic Reactions**
- Plant-derived bioactive residues may act as allergens, triggering hypersensitivity or anaphylactic reactions in susceptible individuals.
- **d. Antimicrobial Resistance**
- Herbal compounds with antimicrobial properties (e.g., allicin, berberine) could alter the gut microbiota of humans and contribute to the development of AMR.

Factors Influencing Residue Risks

- **Lipophilicity**: Highly lipophilic compounds (e.g., certain flavonoids, terpenoids) may accumulate in fatty tissues, leading to prolonged persistence in meat and milk.
- **Metabolic Stability**: Compounds resistant to enzymatic degradation (e.g., alkaloids and phenolic acids) are more likely to persist as residues.
- **Withdrawal Period**: Insufficient withdrawal periods after herbal treatment can result in residues in edible animal products.

1. Regulation on Feed Additives

- **Regulation (EC) No 1831/2003**
- **Scope**: Governs the use of feed additives, including plant-based supplements.
- **Relevance**:
  - Phytoestrogens and other bioactive compounds must be approved for use in animal feeds.
  - Only authorized additives with proven safety and efficacy are permitted.
- **Example**: Red clover or soy-derived isoflavones must demonstrate that residues in milk or meat pose no risk to human health before authorization.

2. Regulation on Undesirable Substances in Feed

- **Directive 2002/32/EC**
- **Scope**: Regulates undesirable substances, including natural plant toxins, in feed.
- **Relevance**:
  - Limits are set for toxic compounds like pyrrolizidine alkaloids (PAs) to prevent residues in animal-origin food.
- **Example**: Monitoring of PA levels in animal feeds derived from contaminated plants like *Senecio spp.* or *Crotalaria spp.*

3. Regulation on Maximum Residue Limits (MRLs)

- **Regulation (EC) No 470/2009**
- **Scope**: Establishes MRLs for pharmacologically active substances in food-producing animals.
- **Relevance**:
  - Although primarily aimed at synthetic drugs, herbal veterinary medicines are also assessed for residue risks.
- **Example**: Herbal remedies containing alkaloids (e.g., atropine) used in livestock require MRL assessments to ensure consumer safety.

4. Organic Certification Standards

- **Regulation (EU) 2018/848**
- **Scope**: Governs organic production and prohibits the use of synthetic additives while promoting natural alternatives.
- **Relevance**:
  - Use of herbal supplements is encouraged, but contamination from toxic plants must be strictly avoided.
- **Example**: Livestock grazing on organic pastures must not ingest toxic plants like *Digitalis purpurea* (foxglove) to avoid cardiac glycoside residues in milk or meat.

5. Regulation on Contaminants in Food

- **Regulation (EC) No 1881/2006**
- **Scope**: Sets maximum levels for contaminants in foodstuffs, including plant-derived toxins.
- **Relevance**:
  - Ensures that harmful residues like cyanogenic glycosides or coumarins in animal-origin food are below safety thresholds.
- **Example**: Milk or meat contaminated by animals consuming cyanogenic plants like *Prunus spp.* must comply with established cyanide residue limits.

6. Herbal Veterinary Medicine Regulation

- **Directive 2001/82/EC (Veterinary Medicinal Products Directive)**
- **Scope**: Regulates the use of herbal veterinary medicines in food-producing animals.
- **Relevance**:
  - Mandates safety evaluations for residues in animal-origin food.
  - Requires withdrawal periods for herbal treatments used in livestock.
- **Example**: Products containing essential oils (e.g., thymol or eugenol) must ensure residues in milk and eggs are within safe levels.

7. Pesticide Residues in Feed and Food

- **Regulation (EC) No 396/2005**
- **Scope**: Sets limits for pesticide residues, which can include plant-derived pesticides like neem oil (azadirachtin).
- **Relevance**:
  - Ensures that residues from plant-based pesticides used in livestock production are safe for human consumption.
- **Example**: Residues of neem-derived azadirachtin in animal products are monitored under this regulation.

Risks from Mycotoxins in Veterinary Herbal Products

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- Mycotoxins are toxic metabolites produced by fungi such as *Aspergillus*, *Penicillium*, and *Fusarium*.
- They can contaminate herbal products during cultivation, harvest, or storage.

Mycotoxin	Source in Herbal Products	Toxic Effects	Species Affected
Aflatoxins	Poorly stored herbal powders or seeds	Hepatotoxicity, carcinogenicity	Livestock, poultry, pets
Ochratoxins	Contaminated herbal grains or roots	Nephrotoxicity, immunosuppression	Livestock, dogs, poultry
Zearalenone	Moldy herbal feed additives (e.g., corn, soy)	Estrogenic effects, infertility	Livestock, particularly swine
Fumonisins	Herbal products derived from corn-based plants	Neurotoxicity, hepatotoxicity, pulmonary issues	Horses (equine leukoencephalomalacia), swine
Trichothecenes	Moldy herbal supplements or feeds	Gastrointestinal irritation, immunosuppression	Poultry, ruminants, pigs

Risks from Environmental Pollutants in Veterinary Herbal Products

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- Environmental pollutants include heavy metals, pesticides, and persistent organic pollutants/plasticizers (PCB, PBDE, PAH, PFAS, phthalates/adipates, bisphenols etc) that contaminate plants through soil, water, and air.

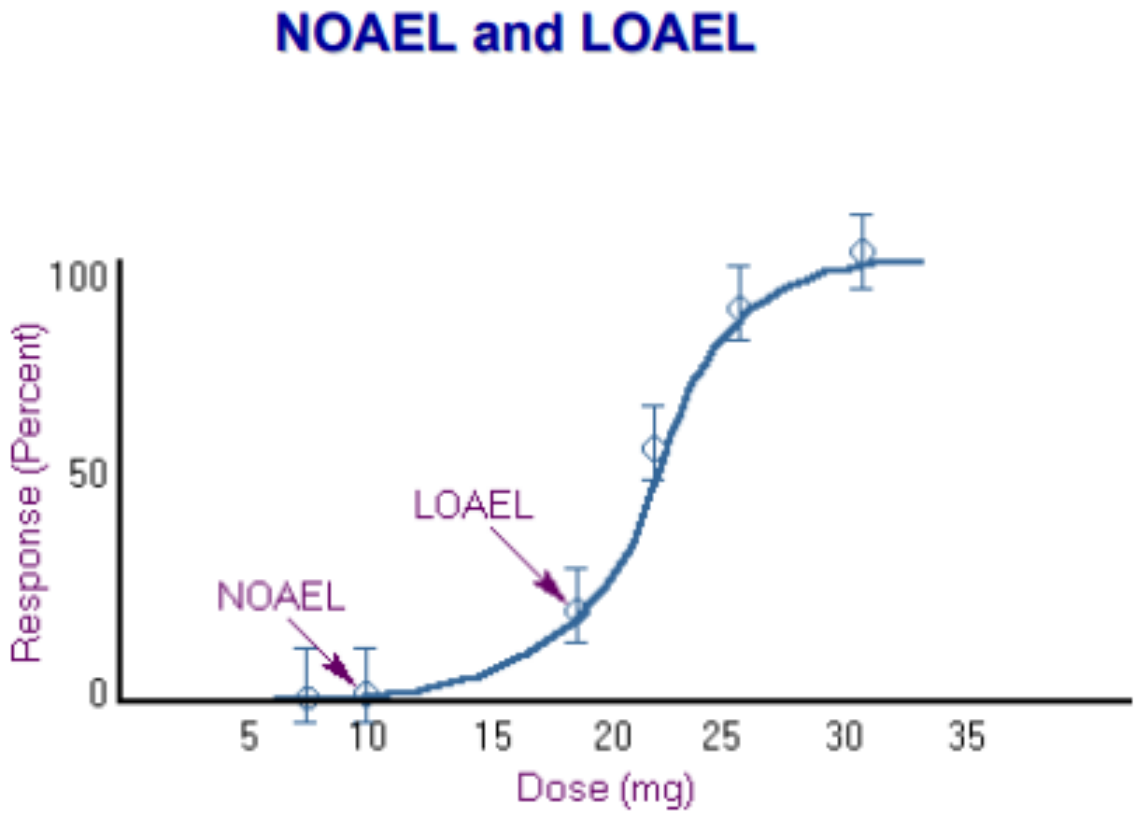
Pollutant	Source	Toxic Effects	Species Affected
Heavy Metals	Contaminated soil or water used for crops	Lead: Neurotoxicity; Cadmium: Nephrotoxicity	Livestock, pets, poultry
Pesticides	Residues from sprayed crops	Endocrine disruption, immunotoxicity	All species, particularly grazing animals
Dioxins and PCBs	Industrial pollution, contaminated herbs	Carcinogenicity, reproductive toxicity	Livestock, poultry
Herbicide Residues	Glyphosate-contaminated herbal products	Gastrointestinal and liver toxicity	Livestock, dogs

Risks from Environmental Pollutants in Veterinary Herbal Products_2

Risk Type	Description
Toxicity in Animals	Impaired health, reduced productivity, organ damage, immunosuppression, and reproductive failure.
Food Safety Concerns	Residues of mycotoxins and pollutants in milk, meat, and eggs entering the human food chain.
Drug Interactions	Contaminants may interact with other veterinary treatments, altering efficacy and safety.
Efficacy Loss	Herbal products contaminated with mycotoxins or pollutants may lose their intended therapeutic effects.

- Regulation (EC) No 396/2005: Sets limits for pesticide residues in herbal products and animal feeds.
- Directive 2002/32/EC: Regulates undesirable substances like mycotoxins in animal feed.

How are those limits set



- No Observed Adverse Effect Level (NOAEL) and Low Observed Adverse Effect Level (LOAEL)
- Actual data points
- NOAEL, LOAEL, NOEL, and LOEL have great importance in the conduct of risk assessments.

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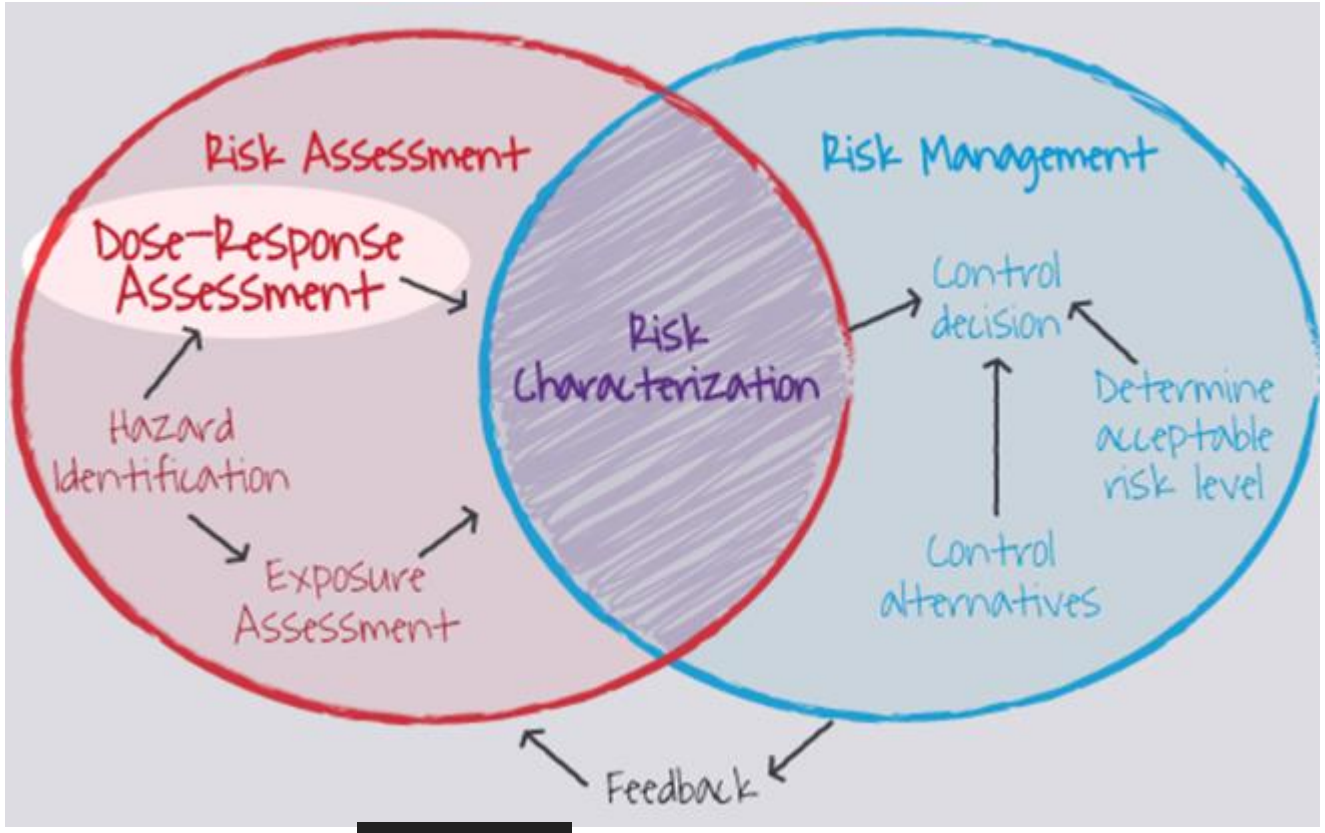
RISK ASSESSMENT

- Hazard identification
- Dose Response Assessment
- Exposure Assessment
- Risk Characterization



ToxTutor - NIH

Chemical Analysis

- Chemical analysis focuses on identifying and quantifying the active ingredients, contaminants, and potential toxic compounds in herbal products.

Method	Purpose	Examples
High-Performance Liquid Chromatography (HPLC)	Quantifies bioactive compounds.	Measurement of alkaloids, flavonoids, or glycosides.
Gas Chromatography-Mass Spectrometry (GC-MS)	Detects volatile compounds and contaminants.	Identification of essential oils, pesticides, or toxic residues.
Atomic Absorption Spectroscopy (AAS)	Measures heavy metals.	Testing for lead, cadmium, mercury, and arsenic contamination.
Fourier Transform Infrared Spectroscopy (FTIR)	Identifies functional groups in compounds.	Verification of compound purity and chemical structure.
Mass Spectrometry (MS)	Identifies and quantifies bioactive compounds based on their mass-to-charge (m/z) ratio.	Detection of alkaloids, flavonoids, or toxins in complex herbal matrices.
Tandem Mass Spectrometry (MS/MS)	Provides detailed structural information by fragmenting parent ions and analyzing daughter ions.	Identification of unknown metabolites or degradation products in herbal formulations.
Liquid Chromatography-Mass Spectrometry (LC-MS)	Combines separation power of LC with MS for identifying compounds in mixtures.	Analysis of secondary metabolites, pesticides, or contaminants in herbal products.
Gas Chromatography-Mass Spectrometry (GC-MS)	Used for volatile or semi-volatile compounds.	Identification of essential oils, terpenoids, or solvent residues in herbal products.
MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight)	Detects high molecular weight compounds such as proteins, peptides, and polymers.	Authentication of herbal species by profiling unique biomolecules (e.g., proteins, metabolites).
High-Resolution Mass Spectrometry (HRMS)	Provides accurate mass measurements to identify complex molecules.	Used for exact molecular weight determination of novel bioactive compounds.
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	Quantifies trace elements and heavy meta herbal samples.	Detection of contaminants like lead, cadmium, and arsenic in plant-based veterinary products.

Toxicological testing

- Toxicological testing evaluates the potential adverse effects of herbal products on animal health, reproduction, and the environment.

Testing Type	Purpose	Examples and Applications
Acute Toxicity Tests	Assess effects of single high doses.	LD50 determination in target species to identify lethal doses.
Subacute and Chronic Toxicity Tests	Evaluate repeated or long-term exposure to assess organ-specific toxicity.	Monitoring liver and kidney function after prolonged use.
Mutagenicity and Genotoxicity Tests	Evaluate the risk of DNA damage or mutation caused by herbal products.	Ames test, micronucleus assay for DNA strand breakage.
Carcinogenicity Testing	Determine the potential of herbal products to cause cancer.	Long-term studies in rodents to assess tumor formation.
Reproductive Toxicity (Reprotoxicity)	Assess impact on fertility, fetal development, and lactation.	Testing herbal compounds like phytoestrogens for teratogenicity or impaired fertility.
Developmental Toxicity Testing	Evaluate effects on embryonic and fetal development.	Detection of skeletal deformities caused by toxic alkaloids (e.g., from *Veratrum spp.*).
Endocrine Disruption Testing	Assess impact on hormonal systems.	Evaluating phytoestrogens for effects on estrogen receptor activity.
Environmental Toxicity Tests	Assess the impact of herbal residues on the environment.	Monitoring effects of herbal runoff on aquatic species or soil microbes.
Ecotoxicological Studies	Evaluate toxicity to non-target organisms in ecosystems.	Testing essential oils or plant-based pesticides for toxicity to fish, bees, and birds.

Pharmacokinetic Studies

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- Pharmacokinetic testing examines how herbal compounds are absorbed, distributed, metabolized, and excreted (ADME) in animals.

Parameter	Purpose	Examples
Absorption Testing	Assess bioavailability of active compounds.	Measuring blood levels after administration.
Distribution Testing	Determine how compounds are distributed in tissues.	Tissue residue analysis in edible parts of livestock.
Metabolism Studies	Study metabolic pathways and identify toxic metabolites.	Identification of glucuronidation pathways in species like dogs or cats.
Excretion Testing	Evaluate elimination routes and half-life.	Urine and fecal sample analysis.

Microbiological Testing

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- Microbiological tests ensure that herbal products are free from microbial contamination, which can affect their safety.

Method	Purpose	Examples
Total Viable Count (TVC)	Measure bacterial load.	Ensure product compliance with microbial standards.
Pathogen Testing	Identify harmful bacteria or fungi.	Detection of *E. coli*, *Salmonella spp.*, or *Aspergillus spp.*.
Endotoxin Testing	Evaluate bacterial endotoxin levels.	Limulus Amebocyte Lysate (LAL) assay for endotoxins in injectable herbal products.

Efficacy Testing

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- Efficacy tests ensure the herbal product delivers the intended therapeutic effects.

Testing Type	Purpose	Examples
In Vitro Assays	Evaluate biological activity of compounds.	Antimicrobial tests, antioxidant assays (e.g., DPPH radical scavenging).
In Vivo Studies	Confirm therapeutic effects in animals.	Pain relief, anti-inflammatory effects in target species.
Comparative Testing	Compare herbal products with synthetic alternatives.	Assess equivalent or superior therapeutic outcomes.

Other tests

- Risk assessment evaluates the presence of contaminants such as pesticides, heavy metals, mycotoxins, and residual solvents.
- Regulatory testing ensures herbal products comply with safety and efficacy standards for veterinary use.
- Quality control testing ensures consistency and quality of veterinary herbal products.

Testing Focus	Purpose	Examples
Identity Testing	Verify the plant species and origin.	DNA barcoding, macroscopic and microscopic analysis.
Stability Testing	Ensure product remains safe and effective over time.	Shelf-life determination under storage conditions.
Batch-to-Batch Consistency	Maintain uniform quality across batches.	HPLC fingerprinting for active compounds.

Key Takeaways

- **Growing Demand**:
  - Herbal veterinary products are widely used due to their natural origin, cost-effectiveness, and perceived safety.
- **Safety Concerns**:
  - Risks include contamination, variable potency, toxicity, and residues in animal-origin food.
- **Pharmacokinetic and Pharmacodynamic Variability**:
  - Significant differences exist in how herbal compounds are absorbed, distributed, metabolized, and excreted among species and even within a species (e.g., age, health status).
- **Comprehensive Assessment**:
  - Safety and risk assessment require a multi-pronged approach involving chemical analysis, toxicology, pharmacokinetics, and environmental evaluation.

Challenges in Risk Assessment

- **Lack of Standardization**:
  - Variability in plant composition, dose, and preparation methods.
- **Toxicity Risks**:
  - Acute, subacute, and chronic effects on target and non-target species.
- **Environmental Concerns**:
  - Potential ecological impacts from residues of herbal products.
- **Regulatory Gaps**:
  - Need for harmonized standards for herbal veterinary products (We are working☺

Pharmacokinetic and Pharmacodynamic Considerations

MedPlants 4Vet

- **Species Differences**:
  - Cats: Poor glucuronidation capacity leads to prolonged herb metabolism (e.g., essential oils).
  - Ruminants: Microbial fermentation in the rumen can activate or deactivate herbal compounds.
  - Horses: Rapid gastrointestinal transit may reduce herb absorption.
- **Intraspecies Variability**:
  - Young animals: Immature liver enzymes reduce herb metabolism.
  - Diseased animals: Impaired renal or hepatic function alters elimination rates.
  - Genetic variation: Different metabolic rates or receptor sensitivities within the same species (Especially dogs).
- **Impact**:
  - These differences require tailored dosing, careful monitoring, and species-specific safety studies.

Strategies for Ensuring Safety

- **Stringent Quality Control**:
  - Use advanced analytical techniques like MS/MS, MALDI-TOF, and HPLC.
- **Toxicological Testing**:
  - Assess for acute, chronic, reproductive, and environmental toxicity.
- **Regulatory Compliance**:
  - Adhere to EU, FDA, and other global regulations for herbal feed and medicines.
- **Education and Training**:
  - Equip farmers, veterinarians, and manufacturers with knowledge about safe herbal practices.

- Let’s discuss

frontiers in Veterinary Science

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Herbal Bioenhancers in Veterinary Phytomedicine

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Herbal bioenhancers are active phytomolecules that increase the bioavailability, bioefficacy and biological activity of various drugs when coadministered at low concentrations. These valuable compounds reduce the dose, increase the treatment rate, decrease the treatment duration, drug resistance or related adverse reactions which have economical implications in livestock and pet medicine. Eventhough the concept of herbal bioenhancers are known for years through Ayurvedic medicine, the underlying mechanisms remains unclear. The main mechanisms involved are related to drug absorption (effect on solubility, drug efflux and transport proteins, increased permeability in gastrointestinal system) and drug metabolism (inhibition/induction of drug metabolysing enzymes, thermogenic effect). Due to species specific differences in these mechanisms, corresponding data on human and laboratory animal could not be attributed. As multidrug resistance is a major treat to both human and animal health, within “One Health” concept, efficient therapeutical strategies are encouraged by authorities, where focus on herbal supplements as a vast unexploited field remains to be researched within “Bioenhancement Concept.” This review brings insight to mechanims involved in bioenhancing effect, examples of herbal extracts and phytoactive compounds and their potential in the veterinary medicine including different classes of drugs such as antibiotics, anticancerous, antiviral, and antituberculosis.

Keywords: herbal bioenhancers, veterinary medicine, bioenhancing mechanisms, herb-drug interactions, phytomedicine

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