PRACTICAL

M.SC. IV

ZOPDLD8: REACTIVE METABOLITES AND DEFENSE SYSTEM IN BIOLOGY

Handling of laboratory animals

1. **Restrainers**

2. **Tail hold**

3. **Body Scoop**

4. **Basic/Four Finger/Roller-Coaster Hold**

5. **Combination/Three Finger Hold**

6. **Alternative Towel Restraint Options**

Demonstration of toxic effects of given xenobiotic using computer simulation programs/virtual labs

1. Select a Xenobiotic
2. Research Toxic Effects
3. Identify Simulation Tools
4. Tox21 Dashboard
    1. VirtualToxLab
    2. OpenTox
    3. Chemicalize
    4. Adverse Outcome Pathway (AOP) Wiki
5. Simulate Toxic Effects
6. Analyze Results
7. Visualize Data
8. Interpretation and Discussion
9. Validation

Assessment of antioxidant potential in given sample

• Catalase

1. Add 1 ml potassium phosphate buffer (50 mM) with 25 µl sample for reaction mixture.
2. Reaction was initiated by addition of and 1 ml H_2O_2.
3. Amount of H_2O_2 consumed was determined by recording absorbance of solution at l240 nm

• Superoxide dismutase

1. In a tube, add 0.5 ml bicarbonate buffer, 50 µl sample, 0.5 ml EDTA and 1 ml distilled water, mixed the sample properly
2. Then incubate the mixture for 5 minutes at room temperature followed by addition of 0.3 ml epinephrine
3. Then read the absorbance at λ480 nm for 3 minutes.

To study the structure and function of metal-ligand complexes

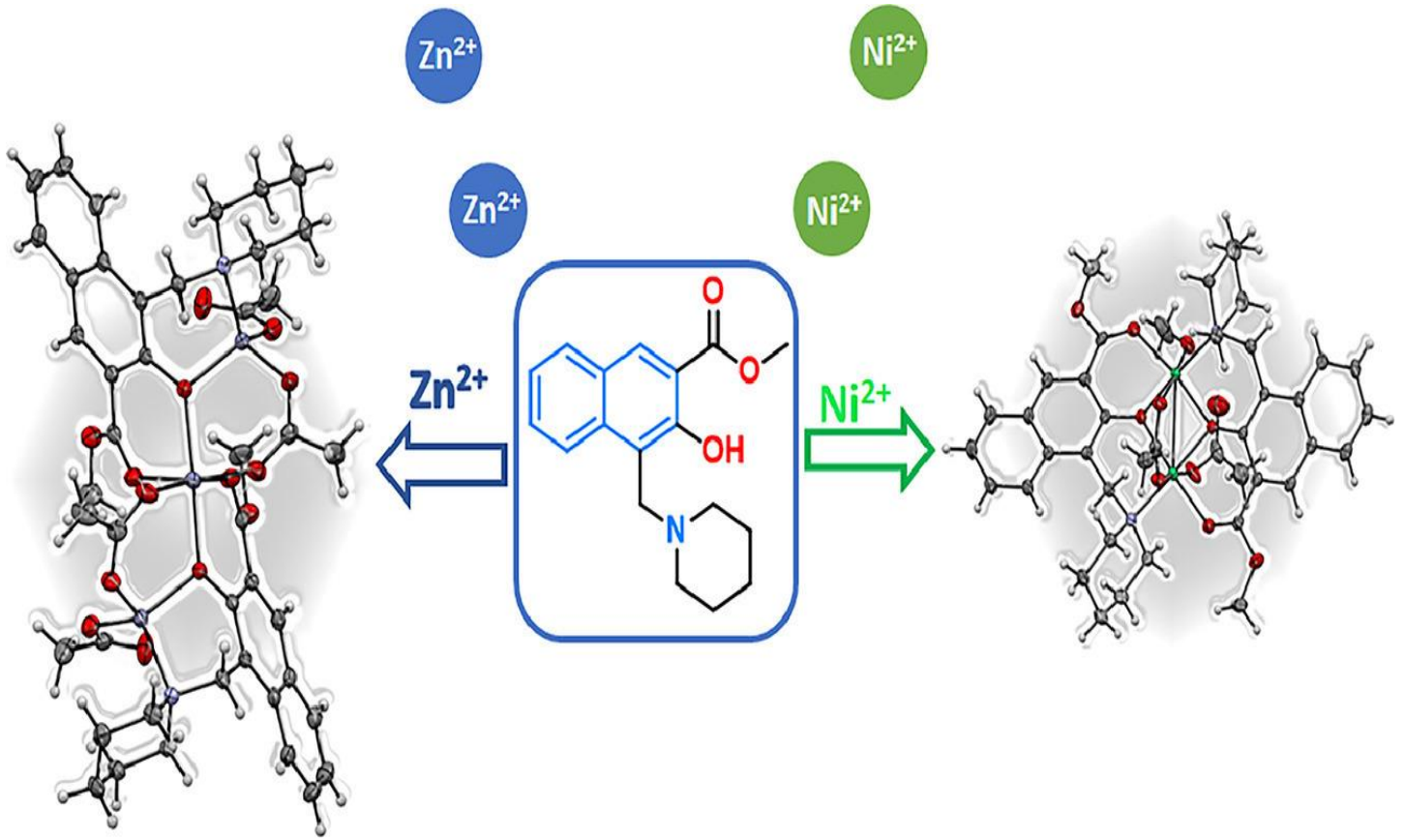
Experimental Techniques:

1.X-ray Crystallography

2.NMR Spectroscopy

3.UV-Vis Spectroscopy

4.Mass Spectrometry



Assessment of chelating effect of molecules against selected metals

Assessing the chelating effect of molecules against selected metals involves experimental techniques and computational methods.

Experimental Techniques:

1.Metal Binding Assays

2.Competitive Metal Binding Assays

3.Chelating Effect on Metal Toxicity

Computational Methods:

1.Molecular Docking:

2.Quantum Chemical Calculations

3.Molecular Dynamics Simulations

Data Analysis and Interpretation:

1.Comparison of Binding Affinities

2.Structure-Activity Relationship (SAR) Analysis

3.Validation and Optimization

Assessment of metal induced haematotoxicity

1. Collect One millilitres of blood into heparinized sample bottles

2. Then using an automatic haematological assay analyser analyse various haematological parameters:

- white blood cells (WBCs)
- red blood cells (RBCs)
- haemoglobin (Hb)
- mean cell volume (MCV)
- mean corpuscular haemoglobin (MCH)
- mean corpuscular haemoglobin concentration (MCHC)
- haematocrit (HCT) and
- total platelets count

Biochemical assessment of glutathione

Reduced glutathione

1. Take 0.1 ml of tissue homogenate in a tube
2. Add 0.9 ml of distilled water and 1.0 ml sulphosalicylic acid in it.
3. Mixed the contents thoroughly and centrifuge it at 5,000 rpm for 10 minutes.
4. Collect the supernatant and draw 0.5 ml of supernatant in a separate test tube.
5. Then add 4.5 ml of tris buffer and 0.5 ml of DTNB solution with the supernatant
6. Allow to stand for 6 minutes and
7. Then read the absorbance at 412 nm wavelength.

Assessment of oxidative stress

Lipid peroxidation

1. Take 1 ml of tissue homogenate in tube.
2. Incubate the sample for 30 minutes at 37°C
3. Then add 1 ml of TCA (10%) in a tube to precipitate proteins
4. Then centrifuge the mixture for 15 minute at 2,000 rpm to collect supernatant
5. In a separate tube, take 1 ml supernatant and add 1 ml of TBA solution to it.
6. Keep the test tubes in boiling water for 10 minutes.
7. Take the reading at 535 nm wavelength.

Histopathological effects of metals on tissues

Tissue Collection and Processing:

1.Tissue Harvesting
2.Tissue Fixation
3.Tissue Processing
4.Tissue Embedding

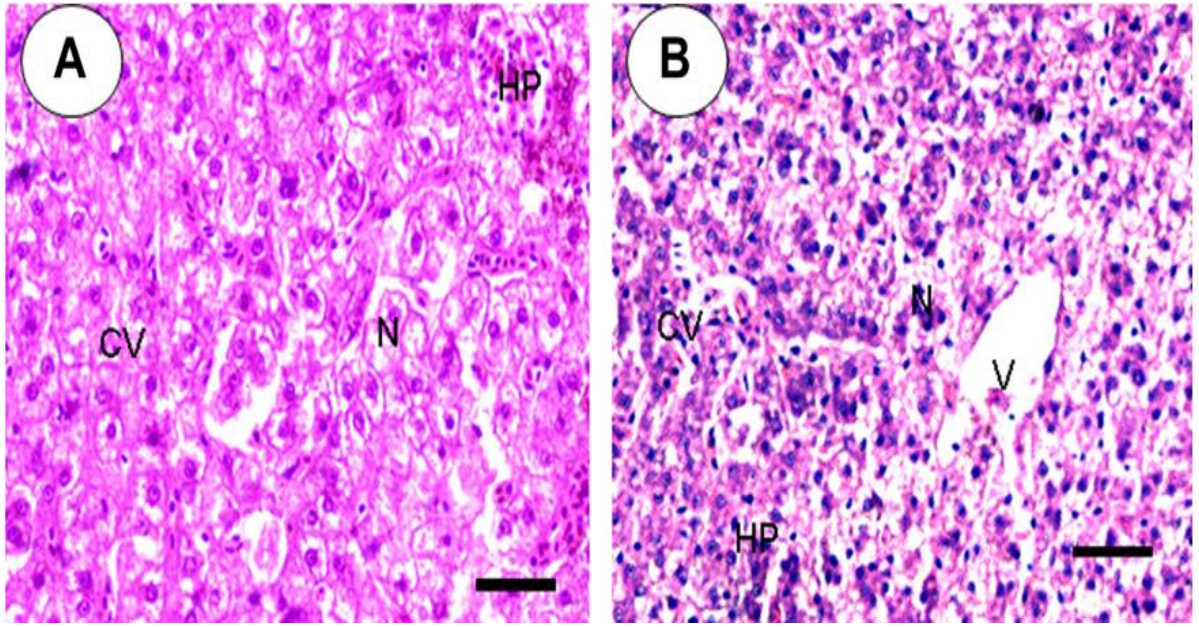
Sectioning and Staining:

5.Tissue Sectioning
6.Histochemical Staining

Examination and Analysis:

7.Microscopic Examination
8.Histopathological Analysis

Reporting and Interpretation



Effects:

- Cellular Damage
- Organ Toxicity
- Carcinogenesis
- Fibrosis and Scarring
- Inflammation and Immune Responses
- Developmental and Reproductive Toxicity
- Metabolic Disruption

To observe toxic effects on cellular level using electron micrographs of tissues

Tissue Sample Preparation

1.Fixation
2.Post-Fixation
3.Dehydration
4.Embedding

Sectioning and Staining

5.Ultramicrotomy
6.Contrast Staining

Electron Microscopy and Imaging:

7.Transmission Electron Microscopy (TEM)
8.Image Capture

Analysis and Interpretation:

9.Ultrastructural Analysis:
10.Quantitative Analysis
11.Interpretation and Discussion