Objectives and Plan of work

The present work was undertaken with an objective to lay down the pharmacognostic standardization, phytochemical investigation with chromatographic profiles and to give scientific validation for the traditional claim of the Corms of the *Amorphophallus paeioniifolius* (Dennst.) Nicolson plant in various human ailments.

The present work was planned with the following objectives –

- To establish pharmacognostical standardization of plant *Amorphophallus paeioniifolius* (Dennst.) Nicolson.
  - To study the morphological and microscopical characters of Aerial and underground parts of plant for authentication that aid in their identification.
  - Pharmacognostical standardization of corms of the study plant.

- To establish phytochemical profile of the corms of the plant
  - To prepare the various successive extracts, Methanolic and 70% ethanolic (Hydro-alcoholic) extracts and analyze them for presence of various phytoconstituents.
  - To establish various chromatographic profiles by qualitative TLC, HPTLC finger print analysis and Mass spectroscopic analysis of extracts.
  - Bioactively guided isolation and characterization of phytoantioxidants viz., polyphenolics and their fractionation by chromatographic and spectral techniques.
2. Objectives and plan of work

- To establish pharmacological profile of the corms of study plant
  - To assess acute toxicity of the corms of study plant
  - *In vitro* estimation of Total phenolic and Flavonoidal content of Methanolic and 70% Hydro-alcoholic extracts.
  - To assess antioxidant property of the Methanolic and 70% Hydro-alcoholic extracts of corms.
  - To assess hepatoprotective activity against experimentally induced hepatotoxicity (CCl₄, Paracetamol and Thioacetamide induced) in albino rats.
  - To assess nephroprotective role against experimentally induced nephrotoxicity (Cisplatin, Gentamicin and Paracetamol induced) in albino rats.
  - To assess gastroprotective activity against experimentally induced gastric ulceration (Ethanol, Indomethacin and Pyloric ligated induced) in albino rats.
PLAN OF WORK

The plant material corm of *Amorphophallus paeoniifolius* (Dennst.) Nicolson for the present study are collected and subjected for Pharmacognostical, Phytochemical and *in vitro* and *in vivo* Organ protective potentiality in three phases.

**Phase I**: As a measure of authentication of selected plant material, the morphological and microscopical characters of its leaf, petiole, corm and roots, and quantitative microscopy will be studied. It was scheduled to assess physicochemical constants viz., Total ash, Water soluble ash, Acid insoluble ash, Sulphonated ash, pH, Specific gravity and Loss on drying for the corm powder as per WHO guideline to standardize the plant material.

**Phase II**: In this phase, the various prepared extracts of corm will be analyzed to identify the presence of phytoprinciples. Further qualitative TLC, HPTLC fingerprint analysis and Mass spectroscopic analysis of selected extract will be carried out to establish the chromatographic profiles. An attempt is made to isolate and characterize the polyphenolics by using column chromatography and characterize the same.

**Phase III**: In the final phase of study the total phenolic, flavonoid and antioxidant capacity, the free radical scavenging potential through DPPH, Hydroxyl, Nitric oxide radical scavenging activity and reducing power of the Hydro-alcoholic and methanolic extracts are to be assessed to select the extract for further pharmacological screening.
Later the selected extract (methanolic extract) after toxicity studies was planned to screen for Hepatoprotective, Nephroprotective and Gastroprotective activities.

**Rationale for selection of experimental models**

The hepatic damage caused by CCl$_4$, Paracetamol and Thioacetamide involve oxidative stress. Similarly for nephroprotective screening Cisplatin, Gentamicin and Paracetamol induced were also used because they cause renal necrosis by involving oxidative stress. Even in case of screening of ulceration properties, the models used in study also involve oxidative stress in inducing ulcers.

Since study plant possesses antioxidant principles, the toxicants causing organ toxicity by oxidative stress have been selected.
Amorphophallus Paeoniifolius

Three phases of study

I. Pharmacognostic profile
   A) Authentication of plant material
      i) T.S. of leaf, petiole, corm, root.
      ii) Quantitative microscopy
          - Stomatal No. and Index
          - Vein islet and termination No.
          - Palisade ratio
          - Dimension of starch grains.
   B) Standardization of corm
      i) Proximate values
      ii) Reaction of powder with different reagents

II. Phytochemical Profile
   Preparation of extracts
      i) Successive extraction
      ii) Extraction with Hydro-alcohol
      iii) Extraction with Methanol
   Preliminary phytochemical screening of extracts
   Fractionation of selected extract
   TLC, HPTLC and Mass spectroscopic analysis
   Isolation and characterization of phytoantioxidants

III. Pharmacological Profile
   A) In vitro estimations
      i) Total Phenolic Content
      ii) Total Flavonoidal Content
      iii) Total Antioxidant Capacity
   B) In vitro antioxidant activity
      iv) DPPH radical
      v) Hydroxyl radical
      vi) Nitric oxide radical scavenging
      vii) Reducing power assay
   C) Antimicrobial activity
   D) Acute toxicity studies
   E) Organ protective activities
      i) Hepatoprotective activity
         (against CCl₄, PCM and THA challenges)
      ii) Nephroprotective activity
         (against Cisplatin, PCM and Getamicin challenges)
      iii) Gastroprotective activity
         (against Indomethacin, Ethanol and Pyloric ligation induced ulceration)