PHYTOCHEMICAL CHARACTERIZATION AND NEUROPROTECTIVE ASSESSMENT OF STANDARDIZED EXTRACT OF *PEDALIUM MUREX* LINN. LEAVES IN ENDOTOXEMIA-INDUCED NEURODEGENERATIVE MODEL IN RATS

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NEED OF THE STUDY

NEURODEGENERATIVE WORLD WIDE

- The World Health Organization estimates that 737 million persons worldwide are estimated to be 60 years of age and older in 2009 .
- This is projected to increase to 2 billion in 2050.

Global prevalence of neurodegenerative disease 2005-2030

Disorder	2005	2030	% 2005-2030
Alzheimer's and other dementias	3.79	5.56	46.7
Parkinson's disease	0.81	0.91	12.3
Multiple sclerosis	0.39	0.41	5.13
Migraine	50.64	52.15	2.98
Neurological injuries	26.45	30.66	15.91

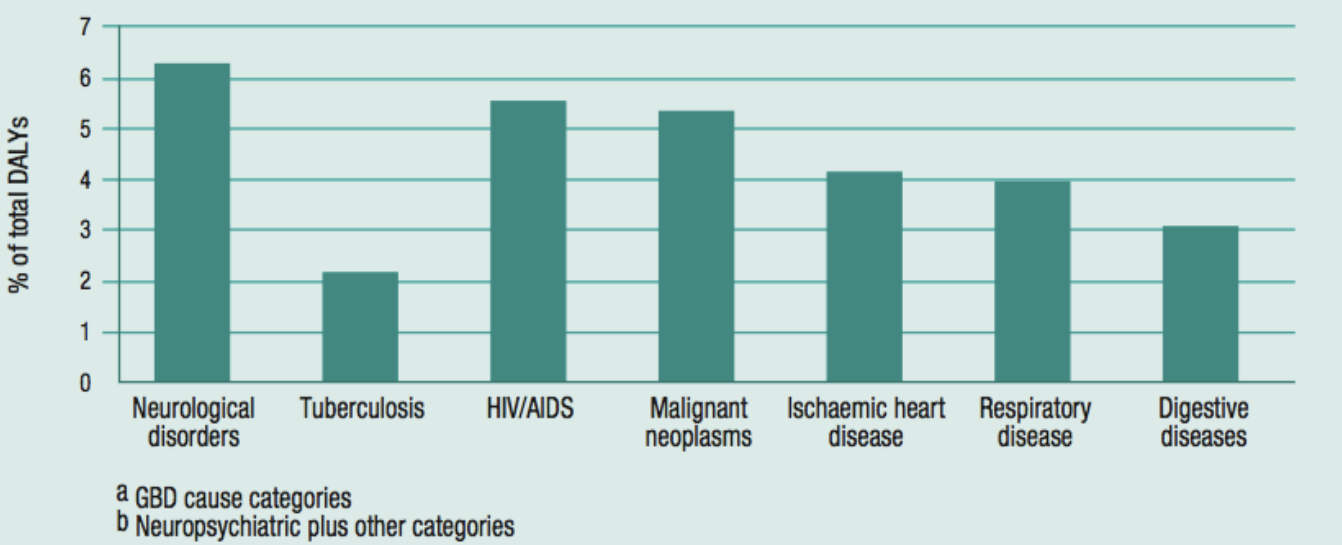
Source: WHO, Neurological disorders: public health challenges. Geneva: WHO; 2006.

Epidemiology of Neurodegenerative disease in India: Prevalence of Neurodegenerative Disease

- Presents data from six of epidemiological studies conducted between 1987-2004 suggests that the prevalence Parkinsonism, peripheral neuropathies and stroke is rising within India.

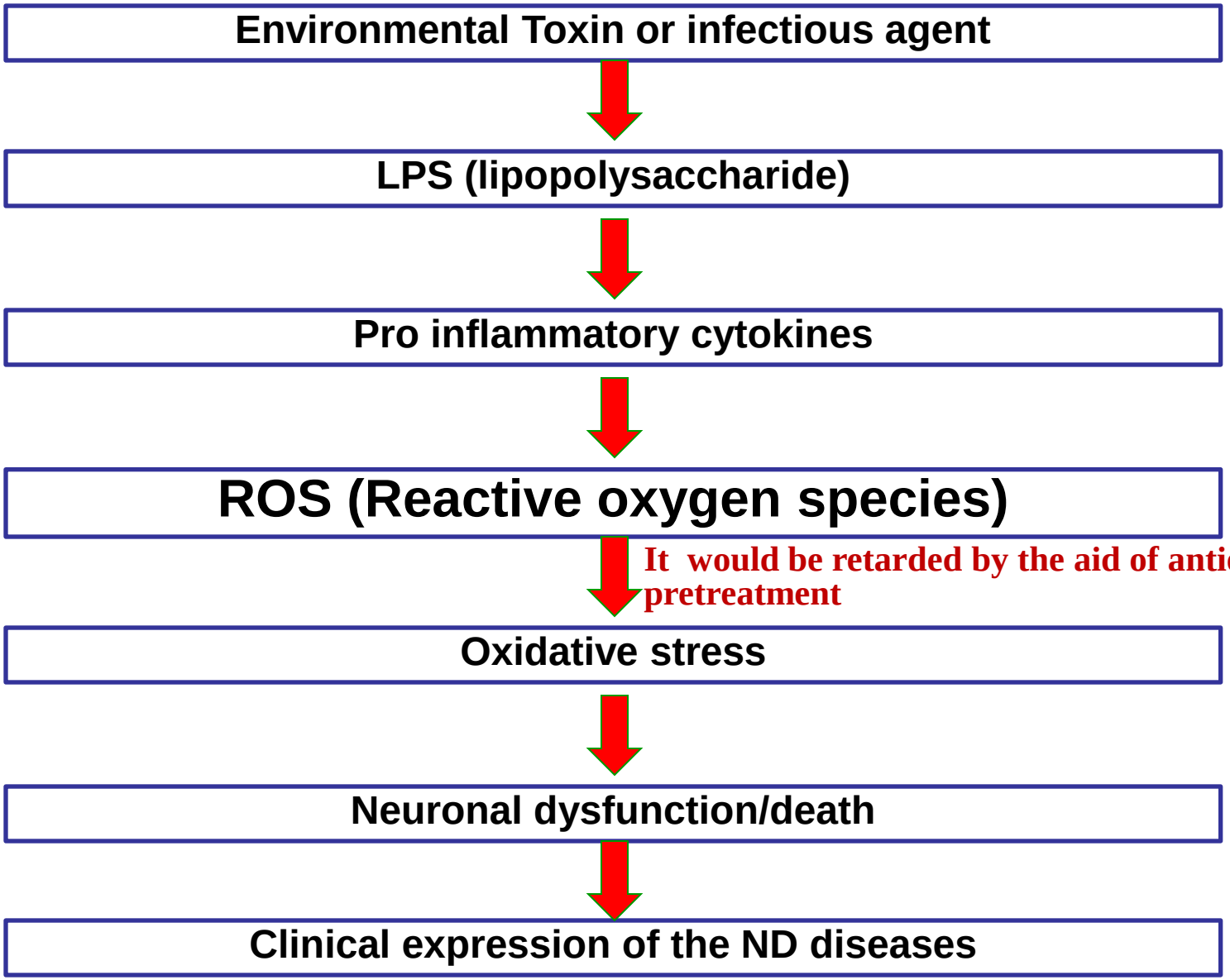
Disability Adjusted Life Years (DALY) for neurological disorders

Source: World Health Organization. Neurological disorders: public health challenges. Geneva: World Health Organization; 2006.



- Due to the prevalence, morbidity and mortality, they represent significant medical, social and financial burden on the society.

LPS induced Endotoxemia



PURPOSE OF THE STUDY

Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the treatment of ND diseases due to their

- ✓ **Potent pharmacological activity**
- ✓ **Low toxicity and less time**
- ✓ **Economic viability and renewable sources**
- ✓ **Long history of use, better patient tolerance, public acceptance**
- ✓ **Cultivation and processing conditions –environmental friendly**
- ✓ **Avoid Environmental pollution by the chemical industry**

- **Similar to many other herbs, its neuroprotective effect in endotoxemia-induced neurodegenerative model is debatable**
- **Neuropharmacological profile has not been investigated before.**
- **To overcome the disadvantage and develop a new therapeutic agents from nature.**

PLANT INTRODUCTION – *PEDALIUM MUREX LINN*

Family : Pedaliaceae

Kingdom : Plantae

Division : Mangoliophyta

Class : Mangnoliopsda

Order : Lamiales

Genus : *Pedalium*

Species : *murex*

Chemical constituents:

- **Fruit : Alkaloids 3.5-5%, stable oil, aromatic oil, resins, glycosides, carbohydrates, saponins and triterpenoids.**
- **Leaves : Flavonoids, alkaloids, steroids, resins, saponins, proteins.**
- **Stem : saponins, phytosterols, tannins, carbohydrates.**
- **Root : reducing sugar, phenolic compounds, saponins, xanthoproteins, alkaloids, triterpenoids and flavonoids.**

Traditional uses:

- **Pain, Inflammation, Piles, Constipation, Heart related problems, Cough, Asthma, Renal Calculi, Dysurea etc.,**

Plan of study

(1) To elucidate the possible mechanism of action of *P.murex* by employing endotoxemia using LPS.

(2) To estimate the quantity of a bioactive phytoconstituent using HPLC technique.

(3) To study the *invitro* antioxidant free radical scavenging and reducing power of *P.murex*.

(4) To observe behavioral parameters following endotoxemia induced neurodegeneration.

(5) To estimate and study the perturbations in the levels of antioxidant defense systems – SOD, CAT, GPx, GR in:

- ✓ **the CA1 hippocampus of the rat brain.**

(6) Histopathological examinations of CA1 cells of the hippocampus and statistical analysis.

METHODOLOGY

Phase-I :Phytochemical studies

- ✓ Collection and extraction(Ethanol).
- ✓ Phytochemical Screening
- ✓ Isolation (TLC & Column chromatography)
- ✓ Characterization (IR, NMR, Mass spectral studies)
- ✓ Quantitative estimation (HPLC)
- ✓ *In vitro* antioxidant and radical scavenging studies

Phase-II :Pharmacological studies

- ✓ Acute toxicity studies
- ✓ Induction of endotoxemia
- ✓ Behavioral studies
- ✓ Biochemical analysis
- ✓ Histopathological studies
- ✓ Statistical analysis

- ➢ *Total antioxidant activity*
- ➢ *Reducing power*
- ➢ *DPPH radical scavenging activity*
- ➢ *Nitric oxide radical scavenging activity*
- ➢ *Superoxide radical scavenging activity*
- ➢ *Hydroxyl radical scavenging activity*

ANIMAL STUDY - PROTOCOL

Animals required: Rats

Species: Sprague- Dawley (SD) Rats weight : 150 - 200 g

Gender: Either sex

Maintained under standard laboratory conditions

ACUTE TOXICITY STUDIES – OECD 423 GUIDELINES

S.NO	GROUPING	NUMBER OF ANIMALS (SD Rats – EITHER SEX)	DOSE OF THE PLANT EXTRACT (mg / kg)
1	Group I	3 No's	5 mg / kg
	Group II	3 No's	50 mg / kg
	Group III	3 No's	300 mg / kg
	Group IV	3 No's	2000 mg / kg
Total no of animals = 12 animals			

Dose will be identified based on the acute oral toxicity studies.

PHARMACOLOGICAL STUDIES

ENDOTOXEMIA-INDUCED NEUROTOXICITY MODEL

Groups	Drugs / extracts	Observation of animals (Days)	Route	No. of Animals	Dose
1.	Control	-	p.o	36	-
2.	LPS only	1 day	LPS:i.p.	36	1 mg/kg
3.	LPS + Extract of *P.murex*	30 days	i.p.+ p.o	36	Low dose: To be determined
4.	LPS + Extract of *P.murex*	30 days	i.p.+ p.o	36	Medium dose: To be determined
5.	LPS + Extract of *P.murex*	30 days	i.p.+ p.o	36	High dose: To be determined
6.	Dexamethasone	30 days	i.p	36	0.5 mg/kg

Total no of animals (228)= Acute toxicity studies (12 animals) + Pharmacological studies(216animals)

IN VIVO STUDIES METHODOLOGY

1. **Acute toxicity studies**
2. **Induction of endotoxemia**
3. **Behavioral studies**

General behavioral studies

- Changes in body weight
- Changes in food and water intake

Tests for anxiety and depression

- Open-field test
- Elevated plus maze test
- Forced swim test

Tests for learning and memory

- Water maze test
- Radial arm maze
- Choice reaction task

4. **Biochemical analysis**

Antioxidant defense elements

- Superoxide dismutase (SOD)
- Catalase (CAT)
- Glutathione reductase (GR)
- Glutathione peroxidase (GPx)

Other enzymes:

- Acetylcholine esterase

Neurotransmitter and others

- Acetylcholine
- Lipid peroxidation
- Nitric oxide
- Protein

5. **Histopathological studies**
6. **Statistical analysis**

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Thank you