SAMPLE RESEARCH PROPOSAL

PROTECTIVE EFFICACY OF [redacted] AND [redacted]

AGAINST VARIOUS INFLAMMATORY MODELS

BACKGROUND

Inflammation is the physiological state which occurs during acute biological stress such as pathogenic infection and tissue toxicity. This process is mediated by an array of biological cells and proteins which orchestrates a toxic biological reaction in the affected site. However, when the physiological system fails to control the inflammation homeostasis during the cases of dysregulated inflammation or unchecked systemic inflammation may lead to serious health consequences. Thus, inflammation is a double edged sword and overwhelming inflammatory response may engender fatal complications.

The preclinical models to screen inflammation encompasses carrageenan induced paw edema, streptozocin induced acute pancreatitis, trinitrobenzene sulfonic acid (TNBS) induced colitis, drug induced organ damage, Freund's adjuvant induced arthritis, endotoxin induced inflammation, D-galactosamine and various noxious chemical induced hepatitis, etc. For the present work, the following models have been used to screen the potential anti-inflammatory candidate:

i) Sepsis is a medical condition which occurs during dysregulated systemic inflammatory responses followed by immunosuppression. [redacted] model is considered as the "Gold Standard" model to depict polymicrobial sepsis in human (2).

ii) Neuroinflammation is the serious condition occurs during Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, etc. [redacted] a natural pollutant is one of the best known toxicant for screening anti-neuroinflammatory agents (3).

iii) Acute pancreatitis is a potentially fatal inflammatory condition that may lead to multisystem organ failure. [redacted] is a glucosamine-nitrosourea class of alkylating agent which elicits acute pancreatitis (4).

Meticulous search for novel and safe anti-inflammatory drugs revealed that the antioxidant compounds with multi-faceted anti-inflammatory potential might serve as the benchmark candidates for the screening against anti-inflammatory agents (1). In this milieu, the following compounds with well-documented antioxidant, tissue protective and anti-inflammatory activities might serve as the potential anti-inflammatory agents with assured safety:

1. [redacted] is an phenolic compound and an antioxidant found in many staple foods, such as fruits, vegetables, cereals, coffee and in plant constituent exhibiting a wide range of therapeutic effects such as anticancer, antidiabetic, cardio protective and neuroprotective, anti-inflammatory activity.

2. [redacted] inhibits reactive oxygen species formation, lipid peroxidation, and oxidant-induced apoptosis. It is also proposed for the therapeutic intervention in diabetes, liver injury, kidney diseases, etc.
3. [redacted] an organosulfur compound, is a well known antioxidant that is made by the body and is found in every cell, where it helps turn glucose into energy. It is used as a dietary supplement and in the treatment of various diseases like diabetes, stroke, neurodegenerative and neuroinflammatory diseases, etc.

AIM AND SCOPE OF THE STUDY:

Inflammation is a vital process for the maintenance of normal homeostasis. But, misconception of the immune system may lead to serious or lethal effects. The key objective of this study is to scrutinize the comparative efficacy of few well documented protective molecules by the screening through various standardized anti-inflammatory models in a single study.

PLAN OF WORK:

- Procurement of drugs, chemicals, antibodies and animals.
- Acclimatization of animals
- Conduction of the study
  - Induction of septic shock by [redacted]

- Induction of neuroinflammation by [redacted]
- Induction of acute pancreatitis by [redacted]

❖ Collection of blood and tissue samples

❖ Biochemical Investigation

- Assay of antioxidants
  - Assay of SOD, CAT, GSH, GST, GR, GSH
- Assay of oxidant products
  - Assay of MDA
- Assay of MPO
- Enzymatic assay
  - Assay of blood amylase and lipase

❖ Histopathological Investigation

❖ RT-PCR analysis

- TNF-alpha
- IL-1 beta

RESEARCH METHODOLOGY:

[redacted]

will be purchased from Sigma chemical Company, India. All the other chemicals, reagents and antibodies will be purchased with high quality. The LD_{50} study for determining the extent of surgery/dose in all the animal models used for anti-inflammatory screening will be done according to OECD guidelines.

Treatment Regimen:

i. Assessment of anti-inflammatory activity in [redacted]

Sepsis will be induced by [redacted] method in Wistar albino rats. Animals will be subjected to [redacted] and sham-operated control rats will be given intraperitoneal saline or [redacted] after the operation and lasting for a period of 1 week. One week later, the biochemical changes will be investigated in the liver, kidney, heart, lung, and brain tissues by examining malondialdehyde (MDA) and glutathione (GSH) levels, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR), interleukin (IL)-1 beta, and tumor necrosis factor (TNF)-alpha and myeloperoxidase (MPO) activities.

ii. Assessment of anti-inflammatory activity in [redacted]

Wistar albino rats will be pre-treated with [redacted] for 7 days and will be challenged with a single intraperitonial dose of [redacted] for 24 hours. After 24 hours, rats will be assessed for malondialdehyde (MDA) and glutathione (GSH) levels, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR), interleukin (IL)-1 beta, and tumor necrosis factor (TNF)-

alpha and myeloperoxidase (MPO) activity in the brain as well as histopathological analysis will be done.

iii. Assessment of anti-inflammatory activity in [redacted]

Acute pancreatitis will be induced by single [redacted] . Blood amylase, lipase, interleukin (IL)-1 beta, and tumor necrosis factor (TNF)-alpha levels, pancreas tissue glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR), and malondialdehyde (MDA) levels, as well as myeloperoxidase (MPO) activity will be measured. Pancreatic tissue samples will also be evaluated histopathologically under a light microscope. Besides, gene expression and amplification (RT-PCR) study for TNF-alpha and interleukin-1 beta will be done by using ready assay kits.

All the samples of blood and tissues will be processed and assessed for various biochemical activities according to the instructions given by the manufacturers of the commercial assay kits. The removed tissues that are assigned to histological evaluation will be fixed in 4% formaldehyde solution overnight. The tissues will be dehydrated and processed for embedding in paraffin wax by a standard protocol. Five-µm-thick serial sections will be then cut using a rotary microtome and these will be mounted on 3-amino-propyl-tri-ethoxy-silane-coated slides. The slides will be examined for

various pathological changes by a blind pathologist who is unaware of their assignment to the groups.

PROPOSED OUTCOME:

To the best of my knowledge, this is the first study to compare the anti-inflammatory effects of the antioxidant and tissue protective compounds: [redacted] in various animal models of inflammation. Substantial evidences underscore that [redacted] exhibit good antioxidant, tissue protective and anti-inflammatory activities in relevant models. This is due to the reason that inflammation and oxidative stress cascades are highly interlinked processes and control of oxidant stress by the antioxidants would show beneficial effects against the deleterious effects of inflammation. But, this study would throw light on the lead compound which possesses relatively potent anti-inflammatory activity through the screening in different hi-standard inflammatory models.