INTRODUCTION
Methotrexate (MTX) (4-amino-N10-methylpteroyl glutamic acid) is an analogue of folic acid. It is used as antifolate and antimetabolite in the treatment of various malignancies. It was developed by Y. Subba Rao, a professor of biochemistry at Harvard Medical School. He had originally developed folic acid and later developed several folic acid analogues. Inspired by the observations in mid 1940s about the role of folic acid in the inhibition of sarcomas, Sydney Farber – a pathologist at the Boston Children’s Hospital had worked on folic acid antagonists in wide variety of malignancies. Among the folic acid analogues, aminopterin was the first drug used to treat children suffering with acute lymphoblastic leukemia (ALL). But, the use of this drug is limited by its side effects seen chiefly in the mouth and in the intestinal tract where hemorrhages occurred frequently. Later, another less toxic antifolate called methotrexate (amethopterin), developed by Y. Subba Rao was tried and found to be safer and more effective (1). Since then, MTX is the most preferred drug of choice in the treatment of malignancies (2). Methotrexate is a widely used drug in the treatment of various carcinomas (3), lymphomas (4) and sarcomas (5). It is also used in the treatment of rheumatoid arthritis (6), psoriasis (7), ectopic pregnancy (8) and inflammatory bowel disease (9).

The use of MTX is limited by the enhanced toxicity including the gastrointestinal, renal, hepatic and bone marrow toxicity. The cytotoxic effects of MTX can be antagonized by activated folic acid (leucovorin) and by using combination therapy (10). Folate supplementation had reduced the incidence of elevated hepatic enzyme levels, resulting in decrease in the frequency of MTX discontinuation, but had no effect on the incidence, severity, and duration of other adverse events, including gastrointestinal and mucosal side effects (11). But, the optimal dose, the duration of drug infusion and the folic acid rescue remain controversial (12-17). Though these
strategies decreased the extent of side effects, the root cause of the side effects was not properly understood.

Being a competitive inhibitor of dihydrofolate reductase, MTX acts readily on rapidly dividing cells of bone marrow and intestinal epithelium. The use of the drug is known to be associated with gastrointestinal toxicity such as diarrhea, nausea and decreased nutrient absorption (18). Enteritis is a common side effect of MTX treatment. It is characterized histologically by villus atrophy or crypt loss (19, 20). MTX treatment induces severe loss of villi, decreased number of crypt cells, cellular edema and bleb formation (21). The barrier function of mucosa against intravascular bacteria was found to be deteriorated, resulting in bacterial translocation and occurrence of inflammation, with cellular infiltrates (21). Methotrexate had been shown to cause increased epithelial apoptosis, increased villus damage and ulceration (2, 22). Changes in the absorptive function of the intestine have been reported (23). Increased lysozyme expression, decreased sucrase – isomaltase and decreased sodium/glucose transport 1 expression (24) have also been reported.

The precise mechanisms by which such changes occur are not well known. Since the mechanism of gastrointestinal toxicity of MTX is not completely known, cancer chemotherapy has to be accompanied by symptomatic therapy such as antibiotics and anti-diarrheal drugs. It is important to unravel the mechanism by which MTX induces intestinal damage in order to perform effective cancer chemotherapy by preventing the side effects.

Mitochondrial damage to enterocytes has been shown to occur upon administration of MTX (25, 26). As damaged mitochondria are a good sources of reactive oxygen species and reactive nitrogen species (27, 28), we hypothesized that oxidative stress, nitrosative stress, and
mitochondrial damage may play an important role in methotrexate induced small intestinal damage.

Melatonin (N-acetyl-5-methoxytryptamine) is a natural occurring compound with well known antioxidant properties. Melatonin is ubiquitously distributed and because of its small size and amphiphilic nature, it is able to reach easily all cellular and sub cellular compartments. Melatonin has been shown to interact with lipid bilayers (29) and stabilize the mitochondrial inner membranes that may improve electron transport chain (ETC) activity (30). Melatonin is known to neutralize singlet oxygen, peroxynitrite anion, and nitric oxide (31). Melatonin does not undergo redox cycling, thus acting as suicidal or terminal antioxidant (32).

**PURPOSE OF THE STUDY**

Methotrexate is the most commonly used drug in the treatment of different malignancies and inflammatory disorders that include rheumatoid arthritis, inflammatory bowel disease, ankylosing spondylitis, and wegener’s granulomatosis. The present study was designed to elucidate the possible mechanism by which MTX causes small intestinal damage.

Based on the already existing information on the mechanisms of MTX induced small intestinal damage, the following mechanism is hypothesized for methotrexate induced small intestinal damage.
Methotrexate

Increased production of ROS (including super oxide) and nitric oxide

Damage to mitochondria of enterocytes

$O_2^{.\cdot} + NO$

Protein tyrosine nitration

Peroxynitrite

Increased lipid peroxidation, and decreased glutathione

Inhibition of electron transport chain complexes

PARP activation

Increased oxidative stress

Decreased ATP synthesis

NAD$^+$ and ATP depletion

Depletion of antioxidant enzymes

Necrotic Cell death

Villus damage and ulceration

Fig: 1.1

Mechanism hypothesized for methotrexate induced small intestinal damage.