Methotrexate (MTX) is widely used in the treatment of different malignancies, psoriasis, rheumatoid arthritis, ectopic pregnancy and eczema. The use of MTX is limited by its side effects on rapidly dividing cells of the gastrointestinal epithelium causing diarrhea, nausea, malabsorption, impaired mucosal function, enteropathy, mucositis, and jejunal crypt cell abnormalities. Mechanisms such as ROS – induced oxidative stress; fluid retention and mitochondrial damage have been suggested in MTX – induced small intestine damage. Although there are some preliminary clinical studies and reports, the precise mechanisms of these MTX – induced small intestine damage has not been clarified completely.

MTX is shown to damage the mitochondria and there are reports of MTX – induced ROS production in the small intestine. Many studies show that mitochondria are good source of nitric oxide (298-300). Mitochondrial superoxide can react with nitric oxide forming the potent oxidant peroxynitrite (164, 165). Nitric oxide (NO) and peroxynitrite (ONOO⁻) were shown to inhibit mitochondrial complexes. Nitric oxide can reversibly inhibit enzymes containing transition metals. Thus damaged mitochondria are very good sources of reactive oxygen species and reactive nitrogen species. Peroxynitrite can trigger cytotoxic processes including lipid peroxidation and DNA damage. It is not known whether such events occur in the pathogenesis of MTX – induced small intestinal damage. Hence, it was proposed to study these events in a rat model.

Different antioxidants have been used to reduce the MTX – induced toxicity in the small intestine that include vitamin – A (24,112,301-305), aged garlic extract (306-310), prostaglandins and its derivatives (21, 311-313), polyphenols (314), ozone (315), beta carotenes (316), curcumin (317), N – acetyl cysteine (318), and L – carnitine (319). Melatonin was shown
to be a very good scavenger of both reactive oxygen species and reactive nitrogen species. Melatonin is produced endogenously in the pineal gland as well as in the gastrointestinal tract, retina, skin, hematopoietic cells and is also easily available for use and hence was chosen for the studies done. The ability of melatonin to decrease the MTX – induced small intestine damage was assessed.

Hence, the hypothesis of the study is that MTX causes increased reactive oxygen species and reactive nitrogen species by direct effect on the enterocytes or by damaging the mitochondria of enterocytes. This leads to nitration and/or oxidation of proteins (including the mitochondria), thus effecting the oxidative phosphorylation and depleting the antioxidant enzymes leading to mitochondrial damage and cell death. The damaged mitochondria further generate reactive species that propagate the cell injury. It is also proposed that melatonin would ameliorate the oxidative stress and nitrosative stress produced by MTX and hence be protective against small intestinal damage.

Adult male Wistar rats were chosen for the study. MTX was administered at a dose of 7 mg/ kg body weight intraperitoneally for three consecutive days. The rats were sacrificed 12 hours/ 24 hours after the final dose of MTX. The intestines were taken for light microscopic analysis. The mucosa of the small intestine was scraped off and the parameters of oxidative stress and nitrosative stress were measured. The different enterocyte population were isolated and assessed for the mitochondrial function. Finally, the rats were pretreated with 20mg/kg and 40mg/kg body weight of melatonin one hour prior to the administration of MTX to assess whether melatonin is effective in ameliorating MTX induced oxidative stress, nitrosative stress, and small intestinal damage.