CHAPTER II

The results presented in the chapter-I have clearly demonstrated that piroxicam dose-dependently caused damage to rat gastric mucosa and this damage was due to oxidative stress. Crude Tulsi leaf aqueous extract (i.e., aqueous homogenate) [TLE] and the lyophilized material (TLE\textsuperscript{L}) obtained from the crude extract, was found to protect against piroxicam-induced gastric ulceration, also, dose-dependently. The results further indicated that piroxicam induced gastric mucosal damage through oxidative stress and pre-treatment of rats with crude (TLE) and lyophilized aqueous Tulsi leaf extract (TLE\textsuperscript{L}) provided gastro-protection through its antioxidant mechanism(s). From the results of chapter-I, the best effective doses of piroxicam, TLE and TLE\textsuperscript{L} were obtained.

The pineal hormone, melatonin, is a small, highly conserved, amphiphilic indole molecule present virtually in all organisms and known to have no morphophysiologic barrier within the cell. Melatonin has several important physiological functions in mammals including seasonal reproductive regulation, immune enhancement and regulation of light-dark signal transduction along with the capacity to influence some aspects of aging [141, 142]. The role of melatonin and its metabolites as antioxidants is also well documented [142, 119, 23]. The gastro-protective ability of melatonin have also been studied in various models of oxidative stress [32-35].

In this chapter, we provide evidence that the combination of melatonin and TLE is capable of providing protection to rat gastric mucosa against piroxicam induced gastric ulceration at the doses at which neither of them protects the gastric mucosa individually. The results indicate that this combination may have future therapeutic relevance in gastro-protection.
## Results

(I) Dose-response studies with melatonin

Protective effects of melatonin against piroxicam-induced gastric ulceration in rats: Figure 1 documents that pre-treatment of rats with melatonin dose-dependently protected the gastric mucosa from being ulcerated following piroxicam treatment at a dose of 30 mg/kg bw, fed orally. Melatonin was found to be best effective at the dose of 60 mg/kg bw, injected intraperitoneally.

![Figure 1: Protective effect of TLE against piroxicam-induced gastric ulceration (expressed as mean ulcer index). Rats were treated with piroxicam (P) and increasing doses of melatonin. Values are mean ± SEM of 7 rats in each group; *p < 0.001 vs. control (C); **p<0.001 vs. P.](image)

Figure 2A reveals the photographic (i.e., at macroscopic level) and 2B reveals the tissue morphological changes to the rat gastric mucosa due to piroxicam treatment at a dose of 30 mg/kg bw, fed orally, which are dose dependently prevented by melatonin, the best protection being afforded at the dose of 60 mg/kg bw, injected intraperitoneally.

![Figure 2A and B: Histological changes to the rat gastric mucosa due to piroxicam treatment and the protective effect of melatonin.](image)
Figure 2 (A): Representative photographs of rat gastric mucosa (cavity side).
Figure 2 (B): Representative images (400X magnification) of haematoxylin-eosin stained rat gastric tissue sections. Rats were pre-treated with increasing doses of melatonin (M).

**Protective effects of melatonin against piroxicam-induced alterations in the levels of lipid peroxidation and reduced glutathione in rat gastric tissue:**

Figure 3A reveals that pre-treatment of rats with melatonin dose-dependently prevented the piroxicam-induced elevation in the level of LPO of the gastric tissue (p < 0.001 vs. control). A dose-dependant protection of the GSH content by melatonin pre-treatment of the rats is also clearly evident from the data presented in the figure 3B.

![Graph A: Lipid peroxidation level (LPO) vs Melatonin (mg/kg)](image)

![Graph B: nmol GSH/mg protein vs Melatonin (mg/kg)](image)

Figure 3 (A): Protective effect of melatonin against piroxicam-induced increase in lipid peroxidation level.
Figure 3 (B): Protective effect of melatonin against piroxicam-induced decrease in the levels of reduced glutathione of rat stomach tissue. Rats were treated with piroxicam (P) and increasing doses of melatonin (M). Control (C) animals were treated with vehicle only. Values are mean ± SEM of 7 rats in each group; *p < 0.001 vs. C. **p<0.001 vs P.

**Protective effects of melatonin against piroxicam-induced alterations in the activities of the rat gastric antioxidant enzymes:**

Figure 4A reveals that treatment of rats with piroxicam at a dose of 30 mg / kg bw (fed orally) caused significant enhancement in Cu-Zn SOD activity of the gastric tissue compared to control. However, when the rats were pre-treated with melatonin at increasing doses, the activity of this enzyme was completely protected from being altered at the dose of 60 mg / kg bw (i.p.). Similarly, figure...
4B shows a highly significant increase of catalase activity of rat gastric tissue following treatment of the animals with piroxicam at a dose of 30 mg/ kg bw, fed orally. Melatonin dose-dependently protected the catalase activity and this protection was found to be complete at the dose of 60 mg/ kg bw (i.p.).

(III) Studies with the combination of aqueous Tulsi leaf extract [leaf homogenate] (TLE) and melatonin at their otherwise ineffective doses:

**Protective effect of the combination of otherwise ineffective doses of TLE and melatonin against piroxicam-induced gastric ulceration in rats:** The results presented in the figure 5 shows piroxicam-induced gastric ulceration at a dose of 30mg/kg bw as evident from the mean ulcer index (*p < 0.001 versus control). Pre-treatment of rats with TLE (100 mg/kg bw), fed orally and melatonin (20 mg/kg bw bw), injected intraperitoneally, in combination at their otherwise ineffective doses, was found to protect the gastric mucosa from being ulcerated (**p < 0.001 versus P).
Figure 5: Protective effect of TLE and melatonin in combination against piroxicam-induced gastric ulceration as evident from mean ulcer index. The rats were treated with piroxicam (P) at a dose of 30 mg/kg bw, fed orally. TLE (T100) and melatonin (M20) protected rats were treated with 100 mg/kg bw (fed orally), TLE, 1 hr before piroxicam treatment and 20 mg/kg bw melatonin (i.p.) 30 mins before piroxicam treatment (PTM). The control (C) rats were treated with vehicle only. Positive TLE control rats were treated with TLE (T100) only and positive melatonin control rats were treated with melatonin (M20) only. Values are mean ± S.E.M. of seven rats in each group. *p < 0.001 versus C; **p < 0.001 versus C.

Protective effects of TLE and melatonin in combination against piroxicam-induced oxidative stress at their otherwise ineffective doses: Figure 6A shows piroxicam-induced increase in lipid peroxidation level (*p < 0.001 versus control) of the gastric tissue. This elevated LPO levels were significantly protected when the rats were pre-treated with TLE and melatonin in combination (**p < 0.001 versus piroxicam). Figure 6B indicates a significant decrease in the gastric reduced glutathione levels due to piroxicam treatment (*p < 0.001 versus control). This reduction in the level of reduced glutathione was found to completely protected when the rats were pre-treated with the combination of TLE and melatonin at their otherwise ineffective doses (i.e., TLE at 100 mg /kg bw, and, melatonin at 20 mg / kg bw) [**p < 0.001 versus piroxicam].
Figure 6 (A): Protective effect of the combination of TLE and melatonin against piroxicam-induced increase in lipid peroxidation (LPO) level of rat stomach tissue. The rats were treated with piroxicam (P) at a dose of 30 mg/kg bw (fed orally). TLE and melatonin protected rats were treated with 100 mg/kg bw (fed orally), TLE, 1 hr before piroxicam treatment and 20 mg/kg bw (i.p.) melatonin, 30 min before piroxicam treatment (PTM) respectively. The control (C) rats were treated with vehicle only. Positive TLE control rats were treated with TLE (T100) only and positive melatonin control rats were treated with melatonin at the dose of 20 mg/kg (M20) only. Values are mean ± S.E.M. of seven rats in each group; *p < 0.001 versus C; *p < 0.001 versus P.

Figure 6 (B): Protective effect of the combination of TLE and melatonin against piroxicam-induced decrease in reduced glutathione (GSH) level of rat stomach tissue. Values are mean ± S.E.M. of seven rats in each group; *p < 0.001 versus C; **p < 0.001 versus P.

Protective effects of TLE and melatonin in combination at their otherwise ineffective doses against piroxicam-induced alterations in key gastric antioxidant enzymes:

Figure 7A and 7B demonstrate the piroxicam-induced elevation in the activity and the level respectively of Cu-Zn SOD, an important antioxidant enzyme. The activity and the protein levels of the enzyme were found to be completely protected from being increased when the rats were pre-treated with TLE and melatonin in combination at the doses at which none of them were effective individually.

Figure 7 (A): Protective effect of combination of TLE and melatonin against piroxicam-induced increase in Cu-Zn SOD activity of rat gastric tissue in control (C), TLE only (T100), melatonin only (M20), piroxicam-treated (P) and TLE and melatonin combination protected (PTM) rats. Values are mean ± SEM of 7 rats in each group; *p < 0.001 vs. C. **p < 0.001 vs. P.
Figure 7 (B): Representative result of Western blot analysis for determining the level of Cu-Zn SOD (lanes from left) of gastric tissue in control (C), piroxicam-treated (P) and TLE and melatonin combination (PTM) protected rats. The Western blot analysis was repeated at least three times. Actin served as loading control. The pixel density of bands obtained through Western blotting was quantified with imageJ software (NIH, USA) and the values (mean ± SEM) were presented below in the form of a bar graph. *p < 0.001 vs. C; **p < 0.001 vs. P.

Figure 8 reveals a highly significant increase in the activity of gastric Mn-SOD in the rats treated with the present dose of piroxicam. The activity of this enzyme was found to be completely protected from being increased when the rats were pre-treated with TLE and melatonin in combination at their otherwise ineffective doses.

Figure 8: Protective effect of TLE and melatonin combination against piroxicam induced increase in Mn-SOD activity of rat stomach tissue. The rats were treated with piroxicam (P) at a dose of 30 mg/kg bw (fed orally). TLE and melatonin combination protected rats were treated with 100 mg/kg bw (fed orally) TLE, 1 hr before piroxicam treatment and 20 mg/kg bw (i.p.) melatonin, 30mins before piroxicam treatment (PTM) respectively. The control (C) rats were treated with vehicle only. Positive TLE control rats were treated with TLE (T100) [at the dose of 100 mg/kg bw] only and positive melatonin control rats were treated with melatonin at the dose of 20mg/kg (M20) only. Values are mean ± S.E.M. of seven rats in each group; *p < 0.001 versus C; **p < 0.001 versus P.

Figure 9 shows a significant decrease in gastric peroxidase (GPO) activity in the rats treated with the present dose of piroxicam. The GPO activity was found to be completely protected form being decreased when the rats were pre-treated with TLE and melatonin in combination at their otherwise ineffective doses. Surprisingly, the activity of GPO in the TLE and melatonin combination treated rats was found to be little higher when compared to the activity observed in the stomach tissue of the control rats.
Figure 9: Protective effect of TLE and melatonin in combination against piroxicam-induced decrease in GPO activity of rat stomach tissue. The rats were treated with piroxicam (P) at a dose of 30 mg/kg bw (fed orally). TLE and melatonin in combination protected rats were treated with 100 mg/kg bw (fed orally) TLE, 1 hr before piroxicam treatment and 20 mg/kg bw (i.p.) melatonin, 30 mins before piroxicam treatment (PTM) respectively. The control (C) rats were treated with vehicle only. Rats of the positive control groups were treated separately with TLE alone at the dose of 100 mg / kg bw (T100) and melatonin alone at the dose of 20 mg / kg bw (M20). Values are mean ± S.E.M. of seven rats in each group; *p < 0.001 versus C; *p < 0.001 versus P.

Figure 10A demonstrates piroxicam (30 mg / kg bw, fed orally)-induced increase in catalase activity, another important antioxidant enzyme, of rat gastric tissue (33% increase vs. control). When the rats were pre-treated with TLE (100 mg / kg bw; fed orally) and melatonin (20 mg / kg bw; i.p.) in combination, the activity of catalase was found to be completely protected from being increased. This enhanced activity of catalase following piroxicam treatment of rats is concomitant with an increase in the level of this enzyme protein as is evident from our western-blot analysis. The results are presented in figure 10B. The figure further shows that the level of this enzyme protein was found to be completely protected from being increased when the rats were pre-treated with TLE and melatonin in combination at their otherwise ineffective doses.
Figure 10 (A): Protective effect of TLE and melatonin in combination against piroxicam-induced increase in catalase activity of rat gastric tissue. The rats were treated with piroxicam (P), and TLE and melatonin in combination (PTM). The control rats were treated with vehicle only. Positive control rats were treated separately with TLE only and melatonin only respectively. Values are mean ± SEM of 7 rats in each group; *p < 0.001 vs. C. **p < 0.001 vs. P.

Figure 10 (B): Representative result of Western blot analysis for determining the level of catalase (lanes from left) of stomach tissue in control (C), piroxicam-treated (P) and TLE and melatonin in combination (PTM) protected rats. The Western blot analysis was repeated at least three times. Actin served as loading control. The pixel density of bands obtained through Western blotting was quantified with imageJ software (NIH, USA) and the values (mean ± SEM) were presented in the form of a bar graph. *p < 0.001 versus C; **p < 0.001 versus P.

Figure 11 shows that treatment of rats with piroxicam at the present dose significantly reduced the activity of glutathione peroxidase (GPx), an important H₂O₂ scavenging antioxidant enzyme, in the stomach tissue (28% versus control, *p < 0.001). However, the activity of this enzyme was found to be completely protected from being decreased when the rats were pre-treated with TLE and melatonin in combination at their otherwise ineffective doses.

Figure 11. Protective effect of TLE and melatonin in combination against piroxicam-induced decrease in glutathione peroxidase activity of rat gastric tissue. The rats were treated with piroxicam (P) at a dose of 30 mg/kg (fed orally). TLE and melatonin in combination protected rats were treated with 100 mg/kg bw (fed orally) TLE, 1 hr before piroxicam treatment and 20 mg/kg bw (i.p.) melatonin, 30mins before piroxicam treatment (PTM) respectively. The control (C) rats were treated with vehicle only. The rats of the positive control groups were separately treated with TLE only (100 mg / kg bw, fed orally) and melatonin only (20 mg /kg bw, injected ip). Values are mean ± S.E.M. of seven rats in each group; *p < 0.001 versus C; *p < 0.001 versus P.
Protective effect of the TLE and melatonin in combination against piroxicam-induced generation of reactive oxygen species (ROS) in rats:

We have also examined whether piroxicam administration to rats have caused the generation of ROS. The results presented in the figure 12A-E clearly indicate that there was an enhancement in the generation of $\text{O}_2^-$ in vivo following treatment of rats with piroxicam. The activities of xanthine oxidase (XO) and xanthine dehydrogenase (XDH) as well as the total enzyme activity, i.e., XO plus XDH were found to be significantly increased following treatment of rats with piroxicam. Besides, XO-XDH ratio and XO / XO+XDH ratio were also found to be increased significantly. All these parameters were found to be protected from being increased when the rats were pre-treated with TLE and melatonin in combination at the doses at which they are ineffective individually indicating the ability of the combination to neutralize free radicals in vivo. The enzyme activities in the rats treated with TLE only and melatonin only (positive controls) showed no change compared to that of the control rats.
Treatment of rats with piroxicam caused nearly a four-fold increase of endogenous generation of *OH (Figure 13). This increment in hydroxyl radicals in vivo was found to be almost completely ameliorated when the rats were pre-treated with TLE and melatonin in combination at the doses at which none of them are capable of protecting the rat gastric mucosa individually. The TLE alone or melatonin alone was found to have no effect on the basal levels of hydroxyl radicals.

Figure 13: Protective effect of TLE and melatonin in combination against piroxicam-induced increase in hydroxyl radical generation in vivo in rat stomach tissue. The rats were treated with piroxicam (P) at a dose of 30 mg/kg (fed orally). TLE and melatonin in combination protected rats were treated with 100 mg/kg bw (fed orally) TLE, 1 hr before piroxicam treatment and 20 mg/kg bw (i.p.) melatonin, 30 mins before piroxicam treatment (PTM) respectively. The control (C) rats were treated with vehicle only. The rats of the positive control groups were separately treated with TLE only (100 mg/kg bw fed orally) and melatonin only (20 mg/kg, ip). Values are mean ± S.E.M. of seven rats in each group; *p < 0.001 versus C; *p < 0.001 versus P.
Protective effect of the TLE and melatonin in combination against piroxicam-induced changes in rat gastric tissue morphology:

Treatment of rats with piroxicam at the present dose caused ulceration of the stomach. When the rats were pre-treated with TLE at a dose of 100 mg /kg bw (fed orally) or melatonin at a dose of 20 mg / kg bw (injected ip), individually, the rat stomach could not be protected from being ulcerated. However, stomach was found to be completely protected from being ulcerated when the rats were pre-treated with TLE and melatonin in combination at the doses at which they were ineffective individually. The results of macroscopic as well as microscopic examinations are presented in figure 14 A and B.

Figure 14 (A): Representative photographs of the rat gastric mucosa (cavity side) showing ulcers. Figure 14 (B): Representative images (400X magnification) of haematoxylin-eosin stained rat gastric tissue sections of Control (C), TLE (T100), melatonin (M20), piroxicam (P) treated and TLE + melatonin protected (PT100M20) rats.
The diseases associated with inflammation of the tissues are on the rise globally [60]. Use of anti-inflammatory drug of synthetic origin, accordingly, are also rising world-wide [60]. Misuse or over-use of the anti-inflammatory drugs are posing additional problems to clinicians and scientists in the management of patients. The principal problem with these anti-inflammatory drugs is that apart from causing inhibition to cyclooxygenase-2 (COX-2), they also bring about inhibition of cyclooxygenase-1 (COX-1) which produces gastro-friendly prostaglandins. Till date, none of the COX-inhibitors are perfect COX-2 inhibitors. Cyclooxygenase-2 mediates inflammatory prostaglandins at the sites of inflammation. Therefore, while treating a patient with anti-inflammatory drug there always remain a chance of these drugs affecting the gastric physiology with compromised mucosal integrity leading to gastric ulcerations and in many of the cases, deadlier perforating ulcers of the stomach which in many cases are fatal. Use of anti-inflammatory drugs is also associated with the development of oxidative stress in the gastric tissue [60], perhaps in other tissues as well. Therefore, the demand of the time is to look for an anti-inflammatory agent which has both anti-inflammatory as well as antioxidant properties so that the health of the gastric tissue is not compromised while they are in use. This means that there is a need for the development of anti-inflammatory drug(s) that is prepared either synthetically or derived from natural resources which has the capability to maximally inhibit COX-2. The alternative option before us is to use a known antioxidant with anti-inflammatory properties, like melatonin alone or some extract from natural resources like well known medicinal herbs, and, or their combination at the minimum possible doses. This will, on one hand, provide good and safe anti-inflammatory intervention, and on the other hand, also provide gastro-protection. The concept of combinatorial therapeutics is new and needs rigorous investigations [35].
The primary objective of the investigation carried out in this section was to determine whether the combination of TLE and melatonin provides protection against piroxicam-induced damage to gastric mucosa at the doses at which neither of them protects the gastric mucosa individually. The results presented in figures 4-7 of the previous chapter (Chapter-I) and figures 1-4 of the present chapter (Chapter-II) demonstrate clearly that both TLE and melatonin are capable of providing protection to the gastric mucosa against piroxicam-induced gastric injury in a dose-dependent manner. In our experiments, the piroxicam-induced gastric ulceration was maximally protected by melatonin at 60 mg/kg bw (i.p.). Similarly, TLE at the dose of 300 mg/kg bw (fed orally), maximally protected the gastric mucosa against piroxicam-induced gastric injury, indicating clearly an increased requirement of the dose in case of the extract. These biochemical observations have been confirmed by the macroscopic and microscopic observations of the gastric mucosa which indicate gastric tissue damage following piroxicam treatment. This gastric mucosal injury was found to be protected when the rats were pre-treated with different doses of either melatonin or TLE. **Figures 5-13 (Figure 7A & figure 10A) of this chapter (chapter-II) further reveal that melatonin at a dose of 20 mg/kg bw (injected i.p.) or TLE at a dose of 100 mg/kg bw (fed orally) does not protect against piroxicam-induced gastric ulceration in rats.**

This prompted us to investigate whether these two ineffective doses of melatonin and TLE would protect against piroxicam-induced gastric injury when administered in combination. Our experiments revealed that pre-treatment of rats with TLE and melatonin in combination (at the doses at which neither of them was found to be effective) almost completely inhibited the gastric mucosal ulceration (as measured by mean ulcer index), indicating the strong potential of this combination. The individual doses of TLE or melatonin, however, were ineffective against piroxicam-induced gastric injury. Additionally, when the rats were treated singly with melatonin or TLE, they had no effect on the mucosa (positive control). Earlier studies by Bandyopadhyay et al. [35] have demonstrated the co-therapeutic effect of melatonin with ranitidine or omeprazole in providing protection to the
gastric mucosa against cold-restraint stress-induced gastric ulceration in rats at the doses at which none of them were effective individually.

The results of the present investigations indicate clearly that this combination not only inhibited piroxicam-induced ulceration of the gastric mucosa but also decreased the piroxicam-induced oxidative stress as evident from the significantly reduced level of LPO and increased level of gastric GSH which indicates that this combination of a pure compound like melatonin and a crude extract like TLE are highly efficient in mitigating the ill effects of piroxicam administration on gastric mucosa. The combination of TLE and melatonin, at their otherwise ineffective doses, also ameliorates the alterations in the activities of key gastric antioxidant enzymes like Cu-Zn SOD, Mn SOD, gastric peroxidase, glutathione peroxidase and catalase indicating that this combination protects against the changes brought about in the rat gastric tissue antioxidant system following piroxicam treatment.

The alteration in the activities of the gastric antioxidant enzymes, like, Cu-Zn SOD and catalase following piroxicam treatment were further analyzed through western blot analysis. It was revealed that the levels of these two enzymes were increased significantly by piroxicam treatment. However, pre-treatment of rats with TLE and melatonin in combination, at their otherwise ineffective doses, protected the levels of the enzyme proteins from being altered indicating again the superior efficacy of this combination in ameliorating piroxicam-induced alterations in the gastric mucosa. However, neither melatonin nor TLE alone had any effect on the antioxidant levels and the activities of antioxidant enzymes indicating that they do not influence these parameters by themselves (positive control).

It was shown earlier [34] that treatment of rats with piroxicam involves \textit{in vivo} generation of $\cdot$OH and consequential gastric ulceration. Our current experiments again confirmed the generation of $\cdot$OH \textit{in vivo} following treatment of rats with the indicated dose of piroxicam. However, when the rats were pre-treated with the combination of TLE and melatonin, the formation of $\cdot$OH was inhibited to near control levels. However, melatonin or TLE alone at their individual ineffective dose failed to provide any protection
against •OH formation indicating that this combination has strong potential to either inhibit the generation of or scavenge the •OH in vivo.

Treatment of rats with piroxicam was also found to be involved with the generation of $O_2^{•−}$, as is evident from the enhanced activities of xanthine oxidase (XO) and xanthine dehydrogenase (XDH) as well as the increased XO/XDH ratio, the level of total enzyme (XO+XDH) and XO/XO+XDH ratio. These indicate strongly that piroxicam-induced gastric ulceration is the outcome of severe oxidative stress developed within the gastric tissue of rats. However, pre-treatment of rats with the combination of TLE and melatonin, at their otherwise ineffective doses, almost completely prevented the activities of these enzymes from being elevated. This indicates that the combination may have the potential to either inhibit the generation of $O_2^{•−}$ or scavenge this ROS.

Our histological studies further support the biochemical observations as is represented in figure 14 which shows that melatonin at 20 mg/kg bw and TLE at 100mg/kg bw individually are unable to protect the gastric tissue from being damaged following piroxicam treatment. This is evident from our macroscopic as well as microscopic studies of the rat gastric tissue. However, when TLE and melatonin were used in combination, a complete protection of the tissue morphology with no ulcers was observed, indicating again the effectiveness of this combination against piroxicam-induced gastric ulceration in rats. The rat gastric mucosa, however, was not affected when melatonin or TLE were administered alone (positive control).

An advantage of melatonin as an antioxidant lies in the fact that it is amphiphilic [143, 144]; thus, it readily reaches all sub-cellular compartments to scavenge the reactants generated during oxidative stress at their sites of generation. Its potent antioxidant activities and the absence of any demonstrated short or long-term toxicity at even pharmacological concentration [145, 146] may predictably make this small indole an important therapeutic molecule in gastro-protection.

Tulsi (O. sanctum), a plant widely used in Ayurveda, possesses anti-inflammatory and antioxidant properties [147]. Flavonoids isolated from Tulsi scavenged free radicals in vitro and showed anti-liperoxidant activity
*in vivo* at very low concentrations [148]. The free radical scavenging activity of plant flavonoids help in the healing of wounds [148]. Since Tulsi is ubiquitous and abundantly grown, it could be a fairly economical therapeutic agent for wound management as a preventive agent, as well as to control healing.

The number of COX-2 specific NSAIDs is extremely limited [73] and currently misoprostol in combination with anti-inflammatory agents is the only recognized form of protection against gastropathy [149]. Thus, the results of the present studies seem highly important. With this combination of TLE and melatonin, it may be possible to minimize the gastro-toxic effects of piroxicam, when their long-term use is the only choice. Furthermore, these studies establish the versatility in the action of TLE and melatonin in combination as an antioxidant, at an appreciably low dose, through direct scavenging actions as well as indirect antioxidant functions. Besides neutralizing free hydroxyl radicals, a portion of this combination's protective actions may derive from its ability to reduce $O_2^*$ generation at the level of the inner mitochondrial membrane, perhaps, by increasing the efficiency of the electron transport chain.

The protection against piroxicam-induced ulcers in our model by this combination of TLE and melatonin could be used as justification for testing their efficacy in combination for reducing gastric ulcers in humans and oxidative stress in general.