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EVALUATION OF MELATONIN AND COENZYME Q10 FOR GASTROPROTECTIVE EFFECT IN ASPIRIN AND IBUPROFEN INDUCED GASTRIC ULCERS IN RATS

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ABSTRACT: The present study was carried out to evaluate the gastroprotective effect of melatonin, coenzyme Q10 (CoQ10) and its combinations with rabeprazole in aspirin (400 mg/kg) and ibuprofen (300 mg/kg) induced gastric ulcers in albino Wistar rats. In aspirin-induced gastric ulcer model, the treatment of melatonin (20 mg/kg), CoQ10 (100 mg/kg) and rabeprazole (20 mg/kg) for 10 days significantly reduced the ulcer index with the values of 1.333 ± 0.30, 1.250 ± 0.2 and 0.916 ± 0.18 respectively whereas the ulcer index of combination treatment of melatonin with rabeprazole and CoQ10 with rabeprazole for 10 days was found to 0.416 ± 0.12 and 0.250 ± 0.11 respectively compared with control wherein the ulcer index was 2.88 ± 0.16. In ibuprofen induced gastric ulcer model, the treatment of melatonin (20 mg/kg), CoQ10 (100 mg/kg) and rabeprazole (20 mg/kg) for 10 days significantly reduced the ulcer index with the values of 1.500 ± 0.21, 1.417 ± 0.15 and 0.833 ± 0.27 respectively whereas the ulcer index of combination treatment of melatonin with rabeprazole and CoQ10 with rabeprazole for 10 days was found to 0.583 ± 0.20 and 0.333 ± 0.10 respectively compared with control wherein the ulcer index was 2.500 ± 0.34. The combination treatment of rabeprazole with melatonin and rabeprazole with CoQ10 has shown better gastroprotective effect compared to rabeprazole alone. Treatment of CoQ10 with rabeprazole showed more gastroprotection than the treatment of melatonin with rabeprazole in both aspirin and ibuprofen induced gastric ulcers.

INTRODUCTION: Gastric ulcer is still the most prevalent cause of gastrointestinal diseases around the world. It is estimated that about 14.5 million people worldwide develop ulcers in some stage of life with a mortality rate of more than 4 million people annually. A peptic ulcer is a disease characterized by the disruption of the mucosal integrity of the esophagus, stomach and duodenum.

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Stress, nutritional disorders, alcohol consumption, prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs), and glucocorticoids are followed by gastric complications, including stomach ulcers [1]. The pathophysiology involves an imbalance between offensive (acid-pepsin secretion, alcoholic beverages, NSAIDs use and *Helicobacter pylori* infection) and defensive factors (mucus secretion, blood flow, prostaglandin, bicarbonate, nitric oxide, sulfhydryl compounds and epidermal growth factors). Furthermore, reactive oxygen species (ROS) and lipid peroxidation are involved in the etiology of gastric mucosal lesions [2].

Different medications among which are antacids, antibiotics, proton pump inhibitors, other

antisecretory, and cytoprotective agents are employed for pain relief, healing of ulcers and delaying ulcer recurrence, clinical evaluation of most of these drugs have shown incidences of relapses, side effects and drug interactions. In view of this, there is a need to search for drugs with greater or equal therapeutic benefits but with reduced or minimal side effects [3].

Melatonin is an endogenous hormone synthesized and released by the pineal gland. This hormone is known to be a powerful direct scavenger of free radicals. Melatonin was reported to be more effective than some other antioxidants in protecting against oxidative damage as well as showing potent anti-ulcer effect and protecting against lipid peroxidation. Melatonin and even its precursor, L-tryptophan has been shown to be highly gastroprotective against various models of mucosal injury. It was revealed that total amounts of melatonin in the digestive system maybe about 400 times larger than in the pineal gland and particularly in the stomach, ileum and colon of all species of animals tested including humans with a high concentration in the gallbladder concentrated bile. Assuming gastrointestinal melatonin is involved in the local protection, it is expected that, exogenous melatonin and its precursor should be protective against the mucosal lesions even after pinealectomy. Pinealectomy was found to greatly reduce the basal plasma levels of melatonin and enhanced gastric ulcerogenic of stress but failed to prevent the gastroprotective activity of melatonin and its precursor, tryptophan [4].

CoQ10 is a producing center of the cell known as the mitochondria and has an effect on electron transport and energy effect on electron transport and energy production (ATP). CoQ10 has also an antioxidant effect on mitochondria and cell membranes and protects lipids from oxidation and thereby stabilizes biological membranes, so it is essential for the health of all human tissue and organs. CoQ10 is a potent free radical scavenger.

This effect related to its electron-donating property that neutralizes free radicals and its ability to replenish endogenous antioxidants such as GSH-PX and superoxide dismutase (SOD). The protective effect of CoQ10 against cardiovascular and neurodegenerative diseases is well established.

It has also been shown that CoQ10 acts against gastric ulceration and healing of chronic acetic acid gastric ulcer and acts against an indomethacin-induced acute gastric ulcer in rats. The oxidized and reduced forms of CoQ10 called ubiquinone and ubiquinole respectively. Ubiquinole is a biologically active form of CoQ10 and has received more interest due to its higher bioavailability and its rapid and better effects [5].

In this study, we have investigated the possible gastroprotective effect by melatonin, CoQ10 and its combination with rabeprazole in gastric ulcers caused by aspirin and ibuprofen in Albino Wistar rats.

MATERIALS AND METHODS:

Material: Aspirin, ibuprofen, and rabeprazole were gift samples from Shilpa Medicare Ltd, Raichur. Melatonin and CoQ10 were gift samples from Pharma Assure USA and Anthem Cellutions Pvt. Ltd., Bangalore respectively.

Animals: Albino Wistar rats of either sex weighing between 200 to 250 g were used for the study. They were procured from the central animal house, M. R. Medical College, Kalaburagi. The animals were acclimatized for seven days and housed under standard conditions of temperature (25 ± 2 °C) and relative humidity (35-50%) with a 12:12 light-dark cycle. The rats were fed on standard food pellets and water *ad libitum*. Prior approval (approval no. HKECOP/IAEC/68/2014-2015) of the Institutional Animal Ethics Committee of HKES MTRIPS, Kalaburagi was taken for conducting the experiment and the animal studies were performed in accordance to guidelines of CPCSEA.

Aspirin Induced Gastric Ulcer: Animals were divided into 7 groups with 6 animals in each.

Group I (Normal): Carboxymethylcellulose (p.o. 0.5%)

Group II (Control): Aspirin (p.o. 400 mg/kg) [6]

Group III (Standard): Rabeprazole (p.o. 20 mg/kg) [7]

Group IV: Melatonin (i.p. 20 mg/kg) [8]

Group V: CoQ10 (p.o. 100 mg/kg) [9]

Group VI: Melatonin (i.p. 20 mg/kg) and rabeprazole (p.o.20 mg/kg)

Group VII: CoQ10 (p.o. 100 mg/kg) and rabeprazole (p.o. 20 mg/kg).

The standard drug rabeprazole, melatonin and CoQ10 were administered daily for 10 days. On the 10th day after 1 h of treatment, all the groups except normal group received aspirin (p.o. 400 mg/kg) after 24 h fasting to induce ulcers, 4 h after the administration of aspirin, the animals were sacrificed with an excess of ether. The stomach of the rats was removed and opened along the greater curvature and washed with normal saline. Gastric mucosal were examined for evaluation of the degree of ulceration which was expressed in terms of ulcer index according to Peskar *et al*., [10] which depends on the calculation of a lesion index by using a 0 - 3 scoring system based on the severity of each lesion.

The severity factor was defined according to the length of the lesions. Severity factor 0= no lesions; 1= lesions < 1 mm length; 2 =lesions 2–4 mm length and 3= lesions > 4 mm length.

The lesions/ulcer score for each rat was calculated as the number of lesions in the rat multiplied by their respective severity factor. The mean ulcer index was calculated by the method of Raji *et al*. [11]

Ulcer index (U.I.) = Mean degree of ulceration X% of a group of ulceration / 100

The percentage protection of a given drug was calculated by using the following equation.

% Protection = [(UI control – UI treated) /UI control] × 100

Ibuprofen Induced Gastric Ulcer: Animals were divided into 7 groups with 6 animals in each.

Group I (Normal): Carboxymethylcellulose (p.o. 0.5%)

Group II (Control): Ibuprofen (p.o. 300 mg/kg) [12]

Group III (Standard): Rabeprazole (p.o. 20 mg/kg)

Group IV: Melatonin (i.p. 20 mg/kg)

Group V: CoQ10 (p.o. 100 mg/kg)

Group VI: Melatonin (i.p. 20 mg/kg) and rabeprazole (p.o. 20 mg/kg)

Group VII: CoQ10 (p.o. 100 mg/kg) and rabeprazole (p.o. 20 mg/kg)

The standard drug rabeprozole, melatonin and CoQ10 were administered daily for 10 days.

After treatment, the animals were kept on fast for 48 h with free access to drinking water. Ibuprofen at a dose of 300 mg/kg was orally administered twice at 15 h intervals, and they were sacrificed 6 h after the second dose of ibuprofen with an excess of ether. The rest of the procedure is the same as described in aspirin-induced gastric ulcer model.

Statistical Analysis: All the results are expressed as mean ± SEM. All the data were analyzed by using Graphpad Prism 3.0 software. Data were analyzed using One-way Analysis of Variance (ANOVA) followed by Dunnett's test. P-value <0.05 was considered to be statistically significant.

RESULTS:

Macroscopic Evaluation of Gastric Ulcers: In the present study, a macroscopic evaluation was done for determining the size of gastric lesions. Oral treatment of aspirin at a dose of 400 mg/kg and Ibuprofen at a dose of 300 mg/kg markedly induced gastric ulcer on the stomach mucosa **Fig. 1**. Treatment of rabeprazole with melatonin and rabeprazole with CoQ10 combinations for 10 days succeeded in protecting the aspirin and ibuprofen induced gastric ulcers, as indicated by the smaller size of gastric lesions in comparison to the controls **Fig. 1**. The protective effect of combination therapy of rabeprazole and antioxidants (melatonin and CoQ10) was investigated by the ulcer index and% protection. The results indicated a synergistic effect in the combination therapy of rabeprazole with antioxidants in aspirin and ibuprofen induced gastric ulcers.

In Aspirin-induced gastric ulcer model **Table 1** and **Fig. 2**, the ulcer index in rabeprazole, melatonin and CoQ10 treated group was decreased significantly to 0.916 ± 0.18, 1.333 ± 0.30 and 1.250 ± 0.21 respectively compared to control group in which the ulcer index value was 2.833 ± 0.16. The percentage protection of rabeprazole, melatonin and CoQ10 treated group was 85.1%, 78.3% and 79.7% respectively. In groups treated rabeprazole with melatonin and rabeprazole with CoQ10, the ulcer index was reduced to 0.416 ± 0.12 and 0.250 ± 0.11 and the percentage protection was 93.2% and 95.9% respectively.

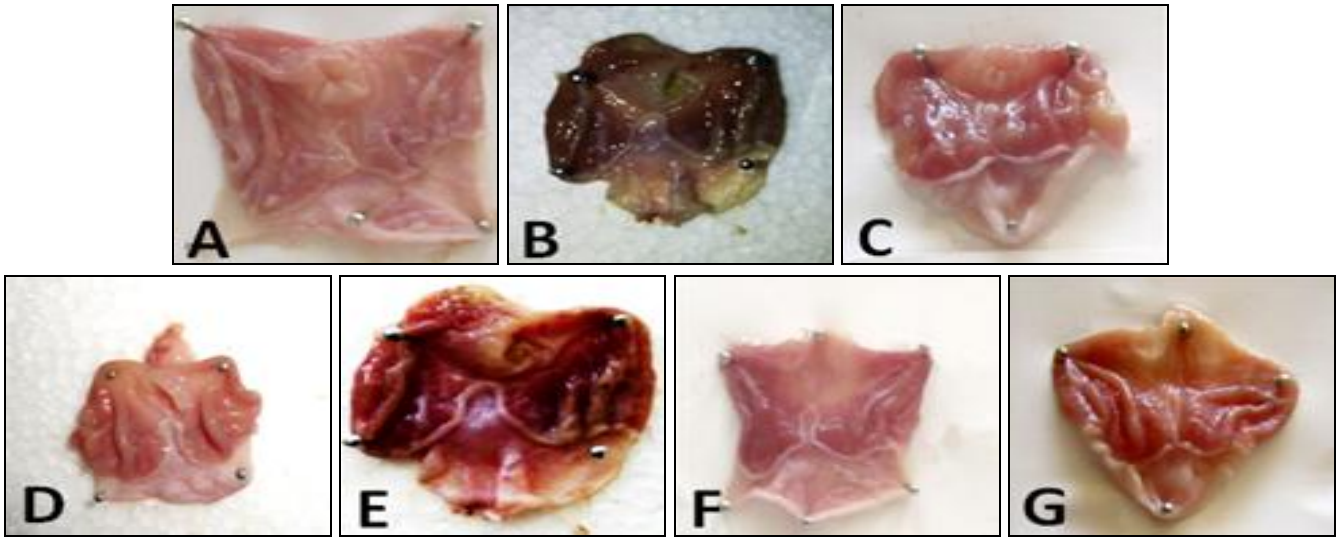

FIG. 1: MACROSCOPIC OBSERVATIONS OF GASTRIC MUCOSA. ASPIRIN INDUCED GASTRIC ULCERS: A-NORMAL GROUP (0.5% CMC), B- ASPIRIN (400 mg/kg), C- RABEPRAZOLE (20 mg/kg) WITH MELATONIN (20 mg/kg), D- RABEPRAZOLE (20 mg/kg) WITH CoQ10 (100 mg/kg). IBUPROFEN INDUCED GASTRIC ULCERS: E-IBUPROFEN (300 mg/kg), F- RABEPRAZOLE (20 mg/kg) WITH MELATONIN (20 mg/kg), G-RABEPRAZOLE (20 mg/kg) WITH CoQ10 (100 mg/kg)

TABLE 1: EFFECT OF MELATONIN, CoQ10 AND ITS COMBINATION WITH RABEPRAZOLE ON GASTRO-PROTECTIVE EFFECT AGAINST ASPIRIN INDUCED GASTRIC ULCERS

Groups	Ulcer Index	% Protection
Normal	0	100 %
Control	2.833 ± 0.16	54.0%
Rabeprazole	0.916 ± 0.18**	85.1%
Melatonin	1.333 ± 0.30**	78.3%
$CoenzymeQ_{10}$	1.250 ± 0.21**	79.7%
Rab + Melatonin	0.416 ± 0.12**	93.2%
Rab +CoQ10	0.250 ± 0.11**	95.9%

All the values were expressed as mean ± SEM. Data were analyzed using one-way ANOVA followed by Dunnett's test. *P< 0.05, **P< 0.01 compared to control

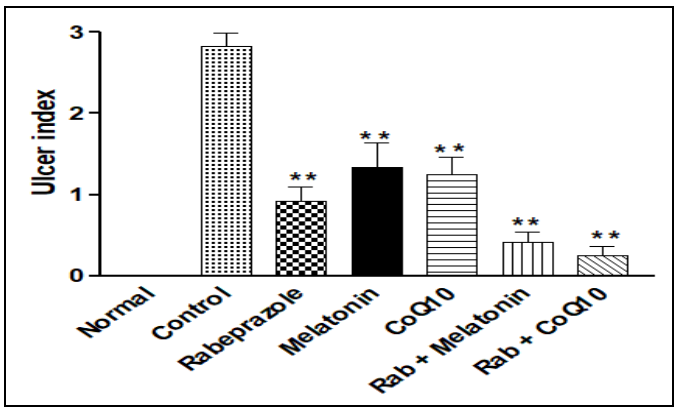

FIG. 2: EFFECT OF MELATONIN, CoQ10 AND ITS COMBINATION WITH RABEPRAZOLE ON GASTRO-PROTECTIVE EFFECT AGAINST ASPIRIN INDUCED GASTRIC ULCERS. NORMAL- CMC (0.5%), CONTROL-ASPIRIN (400mg/kg), RABEPRAZOLE (20 mg/kg), MELATONIN (20 mg/kg), CoQ10 (100 mg/kg), RABEPRAZOLE (20 mg/kg) WITH MELATONIN (20 mg/kg), D- RABEPRAZOLE (20 mg/kg) WITH CoQ10 (100 mg/kg). *P< 0.05, **P< 0.01 compared to control

In Ibuprofen induced gastric ulcer model **Table 2** and **Fig. 3**, the ulcer index in rabeprazole, melatonin and CoQ10 treated group was decreased significantly to 0.833 ± 0.27, 1.500 ± 0.21 and 1.417 ± 0.15 respectively compared to control group in which the value was 2.500 ± 0.34. The percentage protection of rabeprazole, melatonin and CoQ10 treated group was 86.4%, 75.6% and 77.0% respectively. In groups treated rabeprazole with melatonin and rabeprazole with CoQ10, the ulcer index was reduced to 0.583 ± 0.20 and 0.333 ± 0.10 and the percentage protection was 90.5% and 94.5% respectively.

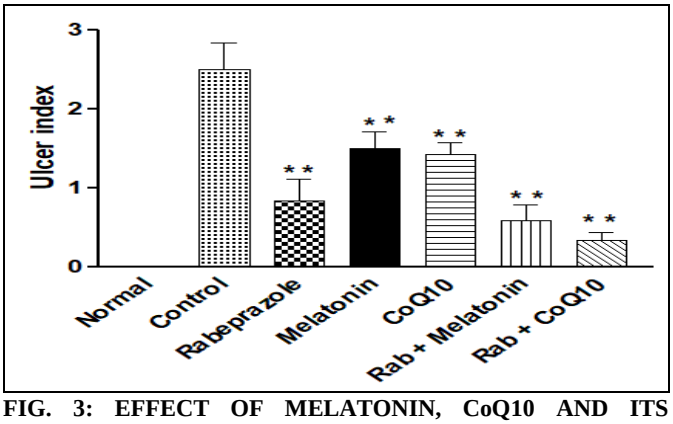

FIG. 3: EFFECT OF MELATONIN, CoQ10 AND ITS COMBINATION WITH RABEPRAZOLE ON GASTRO-PROTECTIVE EFFECT AGAINST IBUPROFEN INDUCED GASTRIC ULCERS. NORMAL- CMC (0.5%), CONTROL-IBUPROFEN (300mg/kg), RABEPRAZOLE (20 mg/kg), MELATONIN (20 mg/kg), CoQ10 (100 mg/kg), RABEPRAZOLE (20 mg/kg) WITH MELATONIN (20 mg/kg), D- RABEPRAZOLE (20 mg/kg) WITH CoQ10 (100 mg/kg). *P< 0.05, **P< 0.01 compared to control

TABLE 2: EFFECT OF MELATONIN, CoQ10 AND ITS COMBINATION WITH RABEPRAZOLE ON GASTRO-PROTECTIVE EFFECT AGAINST IBUPROFEN INDUCED GASTRIC ULCERS

Groups	Ulcer Index	% Protection
Normal	0	100 %
Control	2.500 ± 0.34	59.4%
Rabeprazole	0.833 ± 0.27**	86.4%
Melatonin	1.500 ± 0.21**	75.6%
$CoenzymeQ_{10}$	1.417 ± 0.15**	77.0%
Rab + Melatonin	0.583 ± 0.20**	90.5%
Rab +CoQ10	0.333 ± 0.10**	94.5%

All the values were expressed as mean ± SEM. Data were analyzed using one-way ANOVA followed by Dunnett's test. *P< 0.05, **P< 0.01 compared to control

DISCUSSION: Analgesics like NSAIDs especially ibuprofen, indomethacin, and aspirin for the treatment of various acute and chronic inflammatory diseases are generally associated with severe adverse effects in the upper GI tract leading to esophagitis, peptic ulcers and peptic ulcer complications. For example, aspirin, used for the treatment of inflammatory disorders also destroys the protective measures of GI tract like mucus and bicarbonate secretion, surface epithelial hydrophobicity, and mucosal blood flow by inhibiting the eicosanoid (prostaglandin) synthesis.

It also leads to acid reflux through the ruptured surface and destroys the cells, capillaries, and veins to induce hemorrhagic ulcers and reduces mucosal ATP synthesis and cell turnover process [13]. There is a need for some bioactive alternative to subside the adverse effects of NSAIDs.

In the present study, the administration of aspirin (400 mg/kg) and ibuprofen (300 mg/kg) to fasting animals produced severe gastric mucosal damage as evidenced by ulcer index with the values of 2.833 ± 0.16 and 2.500 ± 0.34 respectively.

Pre-treatment with rabeprazole, melatonin, and CoQ10 at 20, 20 and 100 mg/kg respectively and the combination treatment of rabeprazole with melatonin and rabeprazole with CoQ10 prior to the administration of aspirin and ibuprofen resulted in a significant decrease in gastric mucosal lesions as evidenced by the minimal ulcer index values compared to control. The combination of rabeprazole with melatonin and rabeprazole with CoQ10 has shown better gastroprotective effect compared to rabeprazole alone in aspirin and ibuprofen induced gastric ulcers.

Previous studies implicated melatonin, the chief secretory product of the pineal gland in the mechanism of gastric mucosal integrity and in gastroprotection against various irritant because pretreatment with melatonin or its precursor, l-tryptophan, applied exogenously, prevented the formation of acute gastric lesions induced by ethanol, acetylsalicylic acid and ischemia-reperfusion [14]. Hanan *et al*. reported that the gastroprotective effect CoQ10, which may be mediated by maintaining the function of mitochondria, increasing prostaglandin level and magnifying mucosal nitric oxide, besides its well documented antioxidant properties [9].

The present study shows the protection of gastric mucosa by melatonin and CoQ10 alone as well as in combination with rabeprazole against aspirin and ibuprofen induced gastric ulcers. The treatment of rabeprazole with melatonin and CoQ10 was found to be more effective than rabeprazole alone as evidenced by less ulcer index with more gastroprotection.

CONCLUSION: The results of these studies indicate that the effect of the combination of rabeprazole with melatonin and CoQ10 was found to be synergistic and more effective in treating gastric ulcers. The combination treatment of rabeprazole with melatonin and rabeprazole with CoQ10 has shown better effect compared to rabeprazole alone. Treatment of CoQ10 with rabeprazole showed more gastroprotection than the treatment of melatonin with rabeprazole. It may be possible to minimize the adverse effects of NSAIDs if co-administered with melatonin and CoQ10. However, further studies are required to explore the exact mechanism of action of these antioxidants for gastroprotective effect.

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CONFLICTS OF INTEREST: All the authors declare no conflict of interest.

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