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Morin protects gastric mucosa from nonsteroidal anti-inflammatory drug, indomethacin induced inflammatory damage and apoptosis by modulating NF-κB pathway

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ABSTRACT

Background: Deregulation in prostaglandin (PG) biosynthesis, severe oxidative stress, inflammation and apoptosis contribute to the pathogenesis of nonsteroidal anti-inflammatory drug (NSAID)-induced gastropathy. Unfortunately, most of the prescribed anti-ulcer drugs generate various side effects. In this scenario, we could consider morin as a safe herbal potential agent against IND-gastropathy and rationalize its action systematically.
Methods: Rats were pretreated with morin for 30 min followed by IND (48 mg kg^{-1}) administration for 4 h. The anti-ulcerogenic nature of morin was assessed by morphological and histological analysis. Its effects on the inflammatory (MPO, cytokines, adhesion molecules), ulcer-healing (COXs, PGE_2), and signaling parameters (NF-κB and apoptotic signaling) were assessed by biochemical, RP-HPLC, immunoblots, IHC, RT-PCR, and ELISA at the time points of their maximal changes due to IND administration.
Results: IND induced NF-κB and apoptotic signaling in rat's gastric mucosa. These increased proinflammatory responses, but reduced the antioxidant enzymes and other protective factors. Morin reversed all the adverse effects to prevent IND-induced gastric ulceration in a PGE_2 independent manner. Also, it did not affect the absorption and/or primary pharmacological activity of IND.
Conclusions: The gastroprotective action of morin is primarily attributed to its potent antioxidant nature that also helps in controlling several IND-induced inflammatory responses.
General significance: For the first time, the study reveals a mechanistic basis of morin mediated protective action against IND-induced gastropathy. As morin is a naturally abundant safe antioxidant, future detailed pharmacokinetic and pharmacodynamic studies are expected to establish it as a gastroprotective agent.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely prescribed to recover clinical cases of pain and inflammation in rheumatic disorders and osteoarthritis [1]. These drugs are also used as antineoplastic agents and for the prevention and treatment of ischemic heart disease [2]. However, they also contribute to severe gastrointestinal complications like gastric mucosal bleeding, decreased gastric mucosal blood flow and induced mucosal cell apoptosis [1,3–7]. Among arthritis patients alone, around 16,500 NSAID related deaths occur in the United States annually [8]. Inhibition of the cyclooxygenases (COXs), subsequent reduction in prostaglandin (PG) synthesis along with the NSAID-induced production of reactive oxygen species (ROS) and associated gastric mucosal apoptosis are thought to be the most important reason of gastropathy [1]. Indomethacin (IND), a potent NSAID, was found to bind to a site near complex I and ubiquinone of mitochondrial electron transport chain to generate ROS [9]. ROS can also generate hydroxyl radical (•OH) by inactivating mitochondrial aconitase [10]. This inactivated aconitase produces free iron which subsequently generates more mitochondrial •OH [1,4,6]. The oxidative stress is associated with the uncoupling of mitochondrial respiration, formation of the mitochondrial permeability transition pore, mitochondrial dysfunction and generation of mitochondrial oxidative stress, which is associated with proinflammatory cytokine production and inflammation [1,3]. Inflammation plays a significant role in the pathogenesis of gastric mucosal injury [5,11]. The attraction and subsequent involvement of leukocytes to the specific tissue site are a hallmark incident in the initiation of inflammation and pathogenesis of gastric mucosa [12–15]. It is also becoming increasingly evident that various adhesion molecules mediated leukocyte–endothelial cell (EC) interaction which is an early and significant episode in the NSAID-induced gastropathy [16]. Activated neutrophils can induce injury by physically occluding micro-vessels via the production of many proinflammatory and pro-oxidative enzymes, for instance, myeloperoxidase (MPO) or through production of superoxide and other reactive oxidants [5,9,17]. These dramatically

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increase the oxidative burden of gastric mucosa and also damage endothelial lining [11,18–20]. Therefore antioxidant, anti-inflammatory and antiapoptotic therapy would be a rational approach in preventing NSAID-induced gastropathy.

In spite of medicinal advances, many of the currently prescribed anti-ulcer drugs (such as omeprazole, lansoprazole etc.) confer various side effects, and are expensive particularly for the rural population [21–26]. Considering these limitations, the development of affordable and safe anti-ulcer formulations is an important goal in medicinal research. From this perspective, herbal antioxidants may provide the desired anti-ulcer medications.

Morin (2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one) (Fig. 1a) is a kind of flavonoid belonging to the group of flavonoids found in almond (*Psidium guajava*) [27], mill (*Prunus dulcis*), old fustic (*Chlorophora tinctoria*), osage orange (*Maclura pomifera*), fig (*Chlorophora tinctoria*) and other family members of Moraceae [28]. These are used as food and also as herbal medicines [29]. It has been shown to be acting as a potent antioxidant [30] and possessing various biological and biochemical effects including anti-inflammation [31], xanthine oxidase inhibition activity [32] etc. Most importantly morin also shows intestinal anti-inflammatory activity on chronic experimental colitis in the rat [33]. However, the protective role of morin against IND-induced gastric injury has not been investigated yet.

In this study, we investigated the protective effect of morin against IND-induced gastric injury in rats. For detailed study on the mechanism we have used rats and AGS cells as models. Here we report that morin corrects NSAID-induced inflammatory gastric mucosal damage and apoptosis by inhibiting ROS generation, inducible nitric oxide synthase (iNOS) activation, NF-κB activation and neutrophil infiltration. The study for the first time, reveals a mechanistic basis of morin mediated protection against IND-induced inflammatory gastric pathophysiology.

2. Materials and methods

2.1. Materials and reagents

Indomethacin (IND), DPPH (2, 2-diphenyl-1-picrylhydrazyl), collagenase type I, hyaluronidase, Rhodamine123 (Rh123) and 2′, 7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) were obtained from Sigma (St Louis, MO, USA). Morin ($C_{15}H_{10}O_7$) (CAS number: 654055-01-3), methylthiazolyldiphenyl-tetrazolium bromide (MTT), and bovine serum albumin (BSA) were purchased from Sisco Research Laboratory (Mumbai, India). Formalin and dimethyl sulfoxide (DMSO) were obtained from Merck (Worli, Mumbai). Fetal bovine serum (FBS) was obtained from Gibco, Invitrogen (Carlsbad, CA, USA). Ham's F-12 medium was purchased from HIMEDIA (Mumbai, India). Halt Protease and Phosphatase Inhibitor Cocktail was obtained from Thermo Fisher Scientific Inc., USA. Primary antibodies against phospho-NF-κB p65 (p-NF-κB p65) (#3033), phospho-IKK (p-IKK) (#2859), phospho-IκBα (p-IκBα) (#2859), caspase-3 (#9662), PARP (#9532) and β-actin (#4970) were purchased from Cell Signaling (Cell Signaling Technology Inc., Danvers, MA). Anti-HSP70 (ab79852), anti-PGE_2 (ab2318), anti-IκB (ab32518), and HRP (ab97051) and FITC-tagged secondary (ab6717) antibodies were obtained from Abcam (Cambridge, UK). Anti-γ-actin

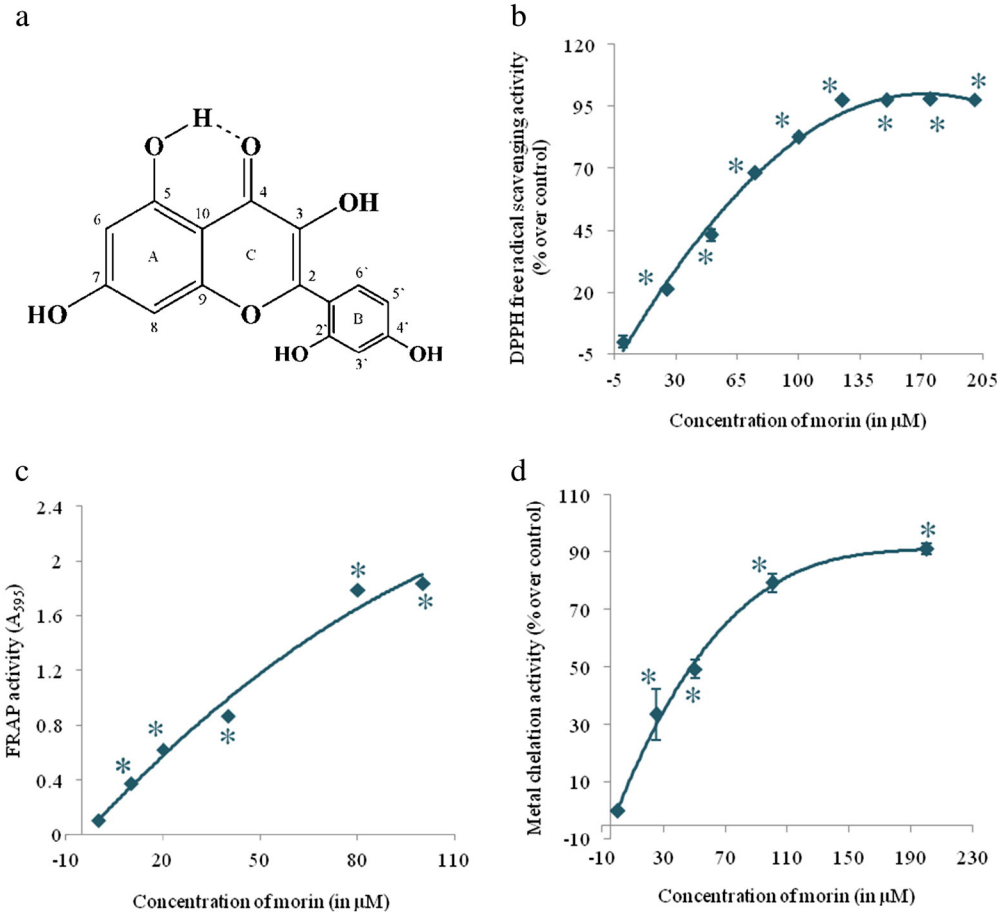

Fig. 1. Structure and biochemical properties of morin in vitro. (a) Structure of morin. (b)Morin showed dose dependent DPPH free radical scavenging activity expressed as % over control. (c). Change of absorbance at 595 nm wave length at various doses of morin in the FRAP activity assay. (d) Metal (iron) chelation activity of morin at various concentrations expressed as % over control. Data are represented as the mean ± SEM of three independent experiments. *P < 0.05 vs. Control.