Research Article

Gastroprotective effect of febuxostat on indomethacin-induced peptic ulcer in rats

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Abstract
Febuxostat one of the anti-hyperuricemic drugs which is commonly used with NSAIDs which commonly cause peptic ulcer. We aimed to evaluate the gastroprotective effect of febuxostat in indomethacin-induced peptic ulcer in rats. Adult male albino rats were divided to four groups; control, indomethacin (40 mg/kg), febuxostat-treated (10 mg/kg), LNNA (10 mg/kg) co-administered with febuxostat. The effects of the previous treatments on the ulcer index, gastric mucosal malondialdehyde (MDA), total nitrites (NOx) and superoxide dismutase (SOD).Febuxostat reduced ulcer index, which increased with indomethacin administration. at the same time, it reduced MDA, increased SOD and NOx. All these effects abolished with LNNA co-administration. In conclusion, febuxostat showed gastroprotective effect in indomethacin induced peptic ulcer in rats mostly through anti-oxidant effect.
Key words; febuxostat, indomethacin, peptic ulcer.

Introduction
Peptic ulcer is a gastrointestinal disorder resulting from the loss of the balance between aggressive and defensive factors of the gastric mucosa[1]. It is known that an increase in oxidative stress is linked to the aggressive factor-induced gastric mucosal damage[2].

Indomethacin is one the non-steroidal anti-inflammatory drugs (NSAIDs) with anti-inflammatory, anti-pyretic, and pain-relieving properties, and is known to produce erosions, ulcerative lesions and petechial bleeding in the stomach as its serious side effects[3]. Furthermore, generation of oxygen free radicals and depletion of endogenous prostaglandins via inhibition of the cyclooxygenase enzyme mainly mediate development of the gastric mucosal lesions induced by indomethacin[4].

Hyperuricemia is a pre-disposing factor of gout, affecting adult population in the developed as well as developing countries. Either over production or under-excretion of urate can lead to hyperuricemia. So that, treatment of hyperuricemia are by excretion of excessive uric acid or blocking the uric acid production. The later strategy appears to be safer since it involves the inhibition of Xanthine oxidase (XO), the key enzyme responsible for the production of uric acid. Febuxostat have been clinically approved as XO inhibitors for the treatment of hyperuricemia and gout. Pain associated with hyperuricemia, is usually treated with NSAIDs [5].

In the current study, we aimed to evaluate the possible gastroprotective effect of febuxostat in indomethacin-induced peptic ulcer.

Material and methods
Animals
Male wistar rats weighing 150-200 gm were used throughout the present study. Rats were purchased from the National Research Center, Cairo, Egypt. Rats were housed in stainless steel cages offering individual housing. Each rat had a tag number. They were left freely wandering in their cages for two weeks with normal hour's dark: light cycle for acclimatization before starting the experiment. They were allowed free access to tap water and normal rats' diet (El-Nile Company, Egypt). All experimental protocols were approved by the animal care committee of Minia University and coincide with international guidelines.

Chemicals

Febuxostat was purchased from Eva pharma, Egypt, indomethacin was purchased from Nile co. for pharmaceuticals, glibenclamide was purchased from sanofi-aventis, Egypt and omega N-nitro-L-arginine (LNNA) was purchased from Sigma Aldrich, Germany. All other chemicals were of analytical grade and were obtained from commercial sources.

Experimental design

Experimental Procedures

Rats were fed a standard diet of commercial rat chow and tap water and left to accommodate to the environment for at least one week before the start of the experiments. Rats were fasted for 24 hours prior to the study. All experiments were performed at the same time of the day to avoid variations due to diurnal rhythms of putative regulators of gastric functions.

The animals were randomly divided into 4 experimental groups of 6 animals each. Group 1; *control group*, group 2; received indomethacin (40 mg/kg; i.p.)[6], group 3; received indomethacin and febuxostat (10 mg/kg; i.p.)[7], group 4; received indomethacin, febuxostat (10 mg/kg; i.p.) and LNNA (25 mg/kg; i.p.)[8].

The stomach was washed with ice-cold saline and scored for macroscopic gross mucosal lesions and stored at -80°C until used for assessment of gastric mucosal lipid peroxides, superoxide dismutase and nitric oxide.

Assessment of gastric mucosal lesions:

Gastric mucosal lesions were expressed in terms of the ulcer index (U.I.). The severity factor is graded as follows; 0 for no lesions; 1 for petechae; 2 for erosions less than 1 mm; 3 for erosions of 1-2 mm; 4 for erosions of 2-4 mm and 5 for erosions greater than 4 mm in length. The partial scores were then summated to obtain the ulcer index of the animal examined. The U.I. for each group taken as the mean lesion score of all the rats in that group[9].

Preparation of Tissue Homogenates

Gastric mucosa was scraped and homogenized separately in potassium phosphate buffer 10 mM pH (7.4). The ratio of tissue weight to homogenization buffer was 1:5. The homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for determination of Malondialdehyde (MDA) level, total nitrite/nitrate (NOx) and superoxide dismutase (SOD).

Malondialdehyde, a measure of lipid peroxidation, was evaluated by a method that depends on the reaction between MDA with thiobarbituric and the color developed was measured spectrophotometrically at 535 nm against a blank. Standard curve by 1,1,3,3-tetramethoxypropane was prepared. From this curve, the MDA concentration was expressed as nmol/g tissue then multiplied in the tissue dilution factor[10].

Brain NOx, the stable oxidation end products of nitric oxide, served as an index of nitric oxide level and was measured by reduction of nitrate into nitrite using activated cadmium granules, followed by color development with Griess reagent in acidic medium[11]. Reduced glutathione was measured by using commercial kit (Biodiagnostic, Egypt).

Superoxide dismutase activity was measured by method of Marklund and Marklund[12] with a slight modification. This method is based on inhibition of the autoxidation of pyrogallol by SOD. The percentage of inhibition for the samples was calculated by the aid of running a control with no sample under the same conditions. SOD enzyme activity was expressed as U/mg protein, where one unit was defined as the amount of the enzyme that inhibited the rate of pyrogallol autoxidation by 50%.

Statistical analysis

Results were expressed as means ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by the Tukey post analysis test was used to analyze the

results for statistically significant difference. p values less than 0.05 were considered significant. Graph Pad Prism was used for statistical calculations (version 5.03 for Windows, Graphpad Software, San Diego California USA, www.graphpad.com).

Results

Effect of febuxostat and co-administered drugs on ulcer index.

In the group treated with febuxostat, there was a significant reduction in ulcer index, as compared to indomethacin treated group. Meanwhile, co-administration of febuxostat with LNNA showed ulcer index insignificant from indomethacin-treated group [figure1].

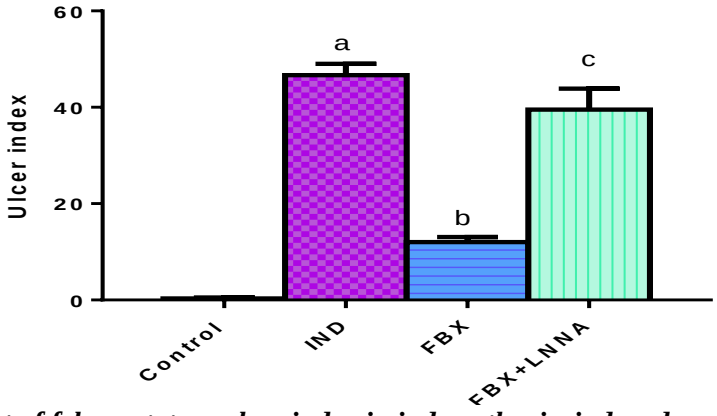
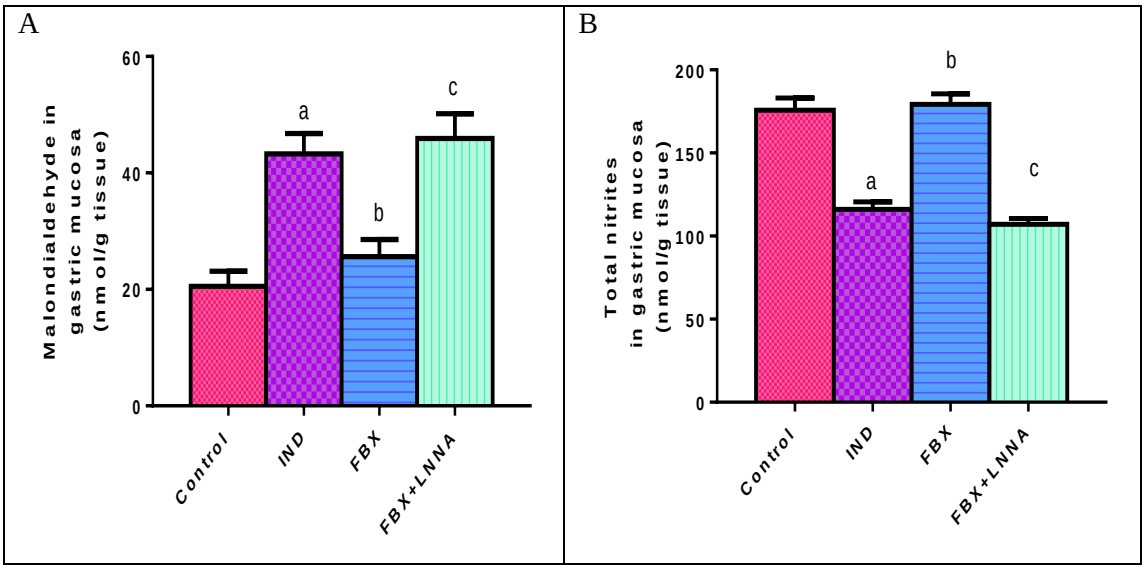


Figure (1): Effect of febuxostat on ulcer index in indomethacin-induced peptic ulcer.
Data represent mean ± SEM for 8 rats. IND; indomethacin-treated group; FBX; febuxostat-treated group; FBX+LNNA; febuxostat and LNNA-treated group.

Effect of febuxostat and co-administered drugs on Malondialdehyde, Total Nitrites Level and superoxide dismutase in gastric mucosa.

Febuxostat significant reduced MDA, as compared to indomethacin treated group, however, there was a significant increase in SOD and NOx, as compared to indomethacin treated group.

Coadministration of LNNA with febuxostat resulted in MDA, NOx and SOD levels insignificant from indomethacin treated group [figure2].



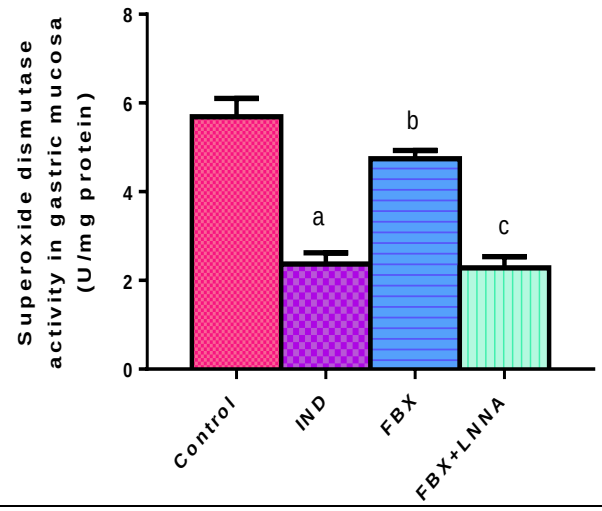


Figure (2): Effect of febuxostat on malondialdehyde (a), total nitrites (b) and superoxide dismutase (c) in gastric mucosa in indomethacin-induced peptic ulcer.
Data represent mean ± SEM for 8 rats. IND; indomethacin-treated group; FBX; febuxostat-treated group; FBX+LNNA; febuxostat and LNNA-treated group.

Discussion

Peptic ulcer may be induced by one of the most commonly prescribed drugs in the world; NSAIDs. Because of their anti-inflammatory and analgesic effects, NSAIDs used in clinical practice for treatment and prevention of gouty arthritis[13]. At the same time, febuxostat, one of the commonly used drugs in treatment hyperuricemia[5].

Indomethacin is known to induce gastric ulcer by inhibition of the gastric cytoprotective mediators, prostaglandins, particularly due to the inhibition of COX pathway of arachidonic acid metabolism resulting in excessive production of leukotrienes and other products of 5-lipoxygenase pathway together with induction of oxidative stress[14].

The results of this study indicate that febuxostat displays an antiulcerogenic effect related to its gastroprotective activity; it significantly reduced indomethacin-induced gastric ulcers. Febuxostat antagonized oxidative stress induced by indomethacin, which evidenced by significant reduction in MDA level together with significant increase in SOD and NOx in gastric mucosa. This finding is in agreement with previous studies, which reported the anti-oxidative stress action of febuxostat in different models of cell injury[15,16].

Surprisingly, co-administration of LNNA, nitric oxide synthase (NOS) inhibitor, with febuxostat abolished its gastroprotective effect, which shown by significant increase in ulcer index. This effect explained partly by ameliorating the anti-oxidative stress action of memantine shown by increase in MDA together with reduction in NOx and SOD in gastric mucosa.

In conclusion, febuxostat showed a gastroprotective effect mostly through anti-oxidant effect evidenced by reduction in MDA, increase in both SOD and NOx. NOS inhibitor, LNNA, abolished gastro-protective effect of febuxostat indicating the role of nitric oxide in such effect indicating the possible role of NOS in gastroprotective effect of febuxostat.

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