Original Research Article

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Effect of *Cannabis sativa* on the indomethacin-induced gastric mucosal damage

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Abstract

The effect of *Cannabis sativa* on the development of gastric mucosal lesions induced in rats by indomethacin administration was studied. The extract of *Cannabis sativa* was prepared from the dried flowering tops and leaves of the plant by extraction with chloroform. Rats were treated with indomethacin at a dose of 20 mg/kg s.c. for two consecutive days alone or in combination with the cannabis extract (at doses equivalent to 10 or 20 mg/kg Δ^9-tetrahydrocannabinol, i.p.). Rats were euthanized 24 hr after the last treatment when gastric secretory responses and number and severity of gastric mucosal lesions were determined. Lipid peroxidation (malondialdehyde), nitric oxide, and reduced glutathione in gastric homogenates were measured and gastric mucosal histopathology was done. Results indicated that treatment with *Cannabis sativa* significantly inhibited the development of indomethacin-induced gastric mucosal lesions in a dose-dependent manner. Cannabis decreased gastric acid secretion in rats treated with indomethacin. The increase in gastric lipid peroxidation, nitric oxide and the decrease in reduced glutathione were ameliorated by cannabis extract. Indomethacin resulted in multiple mucosal erosions, necrosis, leukocytic infiltration and vascular congestion. These changes were prevented by Cannabis treatment. In conclusion, the systemic administration of Cannabis exerted protective effect against the indomethacin-induced gastric damage which involved inhibition of gastric acid and oxidative stress. This suggests the involvement of cannabinoid receptors in gastric mucosal protection.

Keywords: *Cannabis sativa*, gastric mucosa, gastric ulcer, indomethacin, non-steroidal antiinflammatory drugs

1 Introduction

Non-steroidal antiinflammatory drugs (NSAIDs) are widely prescribed for the treatment of various inflammatory and painful conditions such as rheumatic arthritis, rheumatoid disease, bursitis, traumatic injuries and fever. The use of these agents is associated with serious gastrointestinal side effects including dyspepsia, gastric and duodenal ulceration, bleeding and even perforation, leading to hospitalisation and endangering life [1, 2]. The prevalence of peptic ulcer disease in patients taking NSAIDs is estimated at 10-30% [3]. The intake of these drugs is also a major cause of peptic ulcer recurrence [4]. NSAIDs compromise gastroduodenal integrity by a number of mechanisms; the most important is inhibition of mucosal prostaglandins synthesis, leading to decreased mucosal blood flow, epithelial mucus/bicarbonate secretion, epithelial proliferation, and thus mucosal resistance to injury [5]. Most NSAIDs are weak acids and have a topical irritant action [1, 6]. Gastric acid is also an important factor in the development of ulcers induced by NSAIDs and administering antisecretory drugs eg., proton pump inhibitors or the synthetic prostaglandin E2 analogue misoprostol is effective in reducing the risk of peptic ulcer disease in those who require chronic treatment with NSAIDs [7-9]. NSAIDs vary in their ability to cause gastroduodenal mucosal damage which is attributable in large part to their action in inhibiting cyclooxygenase (COX), the key enzyme responsible for the synthesis of cytoprotective prostaglandins [10]. There are two forms of the enzyme; COX-1, which is constitutively expressed in most tissues including the stomach and duodenum and have an important role in gastroduodenal mucosal integrity through the supply of prostaglandins which mediate gastrointestinal protection and COX-2 which has low expression but can be induced during inflammatory conditions. It follows that COX-2 inhibitors are less likely to cause serious gastroduodenal mucosal injury compared with the classical NSAIDs [10, 11].

Cannabis (marijuana and hashish) from the female plant *Cannabis sativa* (*C. sativa*) has been used since ancient times for recreational and medicinal purposes [12]. Cannabis is usually smoked in marijuana cigarette or pipe, but can also be eaten, mixed in cakes or cookies or prepared as a liquid infusion or tea [13]. The recent decade has witnessed major advances in the field of cannabis research due to an increasingly growing interest in the medicinal uses of the herb. In this context, cannabis extracts have gained acceptance in treating spasticity in multiple sclerosis [14] and for intractable childhood epilepsy [15]. Synthetic

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preparations of the main psychoactive ingredient i.e., Δ^9-tetrahydrocannabinol (Δ^9-TCH) are in use to overcome nausea and vomiting during chemotherapy [16]. Cannabis is also being prescribed for medical conditions such as chronic pain, arthritis, depression, and neuropathy [17, 18]. Cannabis inhibits gastric acid [19]; the most important aggressive factor in the development of gastric and duodenal ulcers including those caused by NSAIDs [20]. This effect of cannabis on gastric acid secretion has been demonstrated both in humans [21] and animal experiments [19, 22] and could have accounted for the decrease in gastritis among risky alcohol users who also consume cannabis [23] and in the decline in over the counter (OTC) market share of histamine H_2 receptor antagonists and proton pump inhibitors in Colorado after legalization of recreational access to cannabis [24].

In this study, we aimed to investigate the effect of *Cannabis sativa* extract on gastric acid secretion and gastric mucosal lesions induced in rats by indomethacin. The latter is a widely prescribed NSAID and is well known for its propensity to evoke gastric mucosal damage in man and experimental animals.

2 Materials and methods

2.1 Animals

Sprague-Dawley rats of either sex, weighing 180-200 g, provided by the Animal House of the National Research Centre, Cairo were used. Rats were group-housed under temperature- and light-controlled conditions and allowed standard laboratory rodent chow and water *ad libitum*. Animal experiment procedures followed the recommendations of the Ethics Committee of the National Research Centre and the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2 Drugs and chemicals

Indomethacin (Kahira Pharm & Chem. IND Co., Cairo, Egypt) was used and dissolved in a 5% solution of sodium bicarbonate. Other chemicals and reagents were purchased from Sigma (St Louis, MO, USA) and were of analytical grade.

2.3 Plant material and preparation of extract

Cannabis sativa extract was prepared from the dried flowering tops and leaves of the *Cannabis sativa* L plant which was kindly provided by the Ministry of Justice, Egypt. The method of extraction used was that described by Turner and Mahlberg [25] with modification, where 10 g of the dried cannabis material were ground using a mortar and pestle. The plant material was then heated in a glass beaker in boiling water at 100 ºC for a period of two hours for the decarboxylation of the acidic cannabinoids. Chloroform extraction was then carried out for three times and fractions were combined, filtered over filter paper and collected. The filtrate was then evaporated under a gentle stream of nitrogen, kept at 4 ºC and protected from light. The extract was redissolved in 2 mL of 96%v/v ethanol and 100 mL distilled water for the pharmacological experiments. The extract contained ~ 10% Δ^9-THC as determined by gas chromatography–mass spectrometry. The doses of cannabis extract used in the study corresponded to a Δ^9-THC content of 10 or 20 mg/kg, respectively.

2.4 Gastric ulcerogenic studies

Gastric mucosal damage was induced in rats by administration of indomethacin (20 mg/kg, 0.2 mL, s.c., n = 6/group) for two consecutive days. The effect of *C. sativa* extract (10 or 20 mg/kg, i.p.) given at the time of indomethacin injection was studied. Food and water were provided *ad libitum*. Rats were euthanized 24 hr after last treatment. The stomachs were removed, opened along the greater curvature, rinsed with saline, extended on a plastic board, and then examined for the presence of mucosal lesions. The number and severity of mucosal lesions were noted and lesions were scaled as indicated earlier [26].

2.5 Gastric acid secretion studies

Investigations were carried out in the 4 hr pylorus-ligated rat model. Rats were fasted for 18 hr but allowed free access to water. Pylorus ligation was done under light ether anesthesia and care was taken in order not to interfere with the blood supply to the stomach or duodenum. The abdominal wall was closed in layers with silk sutures. Rats then received either saline (0.2 mL/rat, s.c., n = 6) (control), indomethacin (20 mg/kg, s.c.) or indomethacin + *C. sativa* extract (10 or 20 mg/kg, 0.2 mL/rat, i.p.) (n = 6/group). Rats were sacrificed 4 hr later. Gastric acid output was determined by titration to pH 7.0 with 0.01 N NaOH and H^+ output was expressed as µEq/4 hr.

2.6 Determination of oxidative stress markers

2.6.1 Determination of lipid peroxidation

Lipid peroxidation was measured by determining malondialdehyde (MDA) according to Ruiz-Larrea et al. method [27]. In this assay 2-thiobarbituric acid reacts with MDA at 25 °C to yield a red colored complex which is measured with a spectrophotometric detector at 532 nm.

2.6.2 Determination of reduced glutathione

Reduced glutathione was determined according to Ellman et al. [28]. Ellman´s reagent (DTNB; 5, 5'-dithiobis (2-nitrobenzoic acid)) reacts with the free thiol group of GSH to form 2-nitro-s-mercaptobenzoic acid. The chromophore has yellow color and is determined with spectrophotometer at 412 nm.

2.6.3 Determination of nitric oxide

Nitric oxide was determined using Griess reagent. Nitrate is converted to nitrite with by the enzyme nitrate reductase. Nitrite then reacts with the Griess reagent to form a purple azo compound, and its absorbance is measured at 540 nm with spectrophotometer [29].

2.7 Histological studies

Stomachs from all groups were removed rapidly, opened along the greater curvature, and thoroughly rinsed with ice-cold saline. After recording the ulcers produced in the stomach, a longitudinal section of the gastric tissue was taken from the anterior part of the stomach and fixed in a 10% formalin solution. After 24 hr of fixation followed by embedding in a paraffin block, sections of 5 µm were prepared and stained with hematoxylin-eosin for histological assessment of the gastric mucosa.

2.8 Statistical analysis

Results are presented as means ± SEM. Results of ulcer number and severity were analyzed using Kruskal-Wallis non-parametric one-way analysis of variance (ANOVA) followed by Mann Whitney multiple comparisons test. Results of the biochemical analysis were analyzed using one-way ANOVA followed by Duncan's multiple range test for post hoc comparison of group means. Statistical analysis of results was done using SPSS software. Effects with a probability value of $p < 0.05$ were considered to be significant.

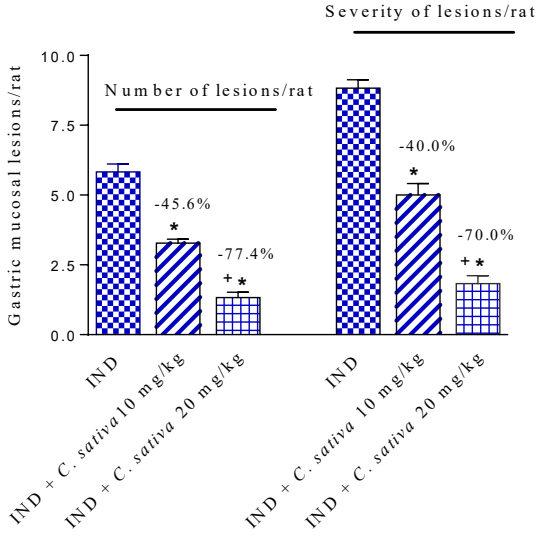

Figure 1. Effect of *C. sativa* extract on the number and severity of gastric lesions induced by indomethacin (IND) in rats. Results are expressed as mean values of 6 observations (± SEM) and percent inhibition (%) compared to the control group. $^{*}p < 0.05$

3 Results

3.1 Effect of Cannabis sativa on gastric mucosal lesions

Cannabis sativa (10 or 20 mg/kg, *i.p.*) given at the time of indomethacin injection prevented the development of gastric lesions caused by the ulcerogen in a dose-dependent manner. The number of lesions caused by indomethacin decreased from a control of 5.83 ± 0.17 to 3.17 ± 0.31 and 1.32 ± 0.21 by *C. sativa* at 10 or 20 mg/kg, respectively. The severity of lesions was decreased by *C. sativa* from a control value of 8.33 ± 0.30 to 5.0 ± 0.41 and 1.83 ± 0.28, respectively by 10 or 20 mg/kg doses (Figure 1 and 2).

Table 1. Effect of *Cannabis sativa* (*C. sativa*) extract on gastric mucosal oxidative stress in rats treated with indomethacin

Group	MDA (nmol/g tissue)	NO (µmol/g tissue)	GSH (µmol/g.tissue)
Saline	281.3 ± 14.6	21.0 ± 1.5	6.20 ± 0.31
IND	495.0 ± 32.0*	36.4 ± 2.7*	2.83 ± 0.11*
IND + *C. sativa* 10 mg/kg	377.2 ± 21.8^{*+} (-23.8%)	29.1 ± 1.3^{*+} (-20.0%)	4.10 ± 0.26^{*+} (44.9%)
IND + *C. sativa* 20 mg/kg	310.0 ± 17.2^{+} (-37.4%)	25.0 ± 1.24^{+} (-31.3%)	5.91 ± 0.37^{+} (108.8%)

Values are means ± SEM (n = 6/group). $^{*}p < 0.05$ vs. saline; $^{+}p < 0.05$ vs. indomethacin (IND) control group. The percentage change from the IND only group is shown in parenthesis.

3.2 Effect of Cannabis sativa on gastric tissue oxidative stress

Table 2. Effect of *Cannabis sativa* (*C. sativa*) extract on gastric acid secretion in rats treated with indomethacin in pylorus-ligated rats.

Group	Gastric volume (mL/4 hr)	Gastric acid secretion (µEq/4 hr)
Saline	3.69 ± 0.17	270.3 ± 14.6
IND	5.3 ± 0.32*	356.5 ± 19.1*
IND + *C. sativa* 10 mg/kg	4.6 ± 0.13* (-13.2%)	301.0 ± 11.2* (-15.6%)
IND + *C. sativa* 20 mg/kg	4.1 ± 0.28^{+} (-22.6%)	270.0 ± 13.5^{+} (-24.3%)

Values are means ± SEM (n = 6/group). $^{*}p < 0.05$ vs. saline; $^{+}p < 0.05$ vs. indomethacin (IND) control group. The percentage change from the IND only group is shown in parenthesis.

The administration of indomethacin caused significant increase in gastric tissue MDA and nitric oxide contents by 76.0% and 73.3%. Meanwhile, there was a significant decrease in reduced glutathione by 54.3%, compared with corresponding control value. Cotreatment with *C. sativa* resulted in significant decrease in MDA by 23.8% and 37.4%. Nitric oxide content was decreased by 20.0% and 31.3% while reduced glutathione content was increased by 44.9% and 108.8% by *C. sativa* at 10 and 20 mg/kg, respectively (Table 1).

3.3 Effect of Cannabis sativa on gastric secretory responses

Compared with the saline control group, gastric secretory volume and acid output were increased by 43.6% and 32.0%, respectively, in rats treated with indomethacin. *C. sativa* given i.p. at 20 mg/kg resulted in significant decrease

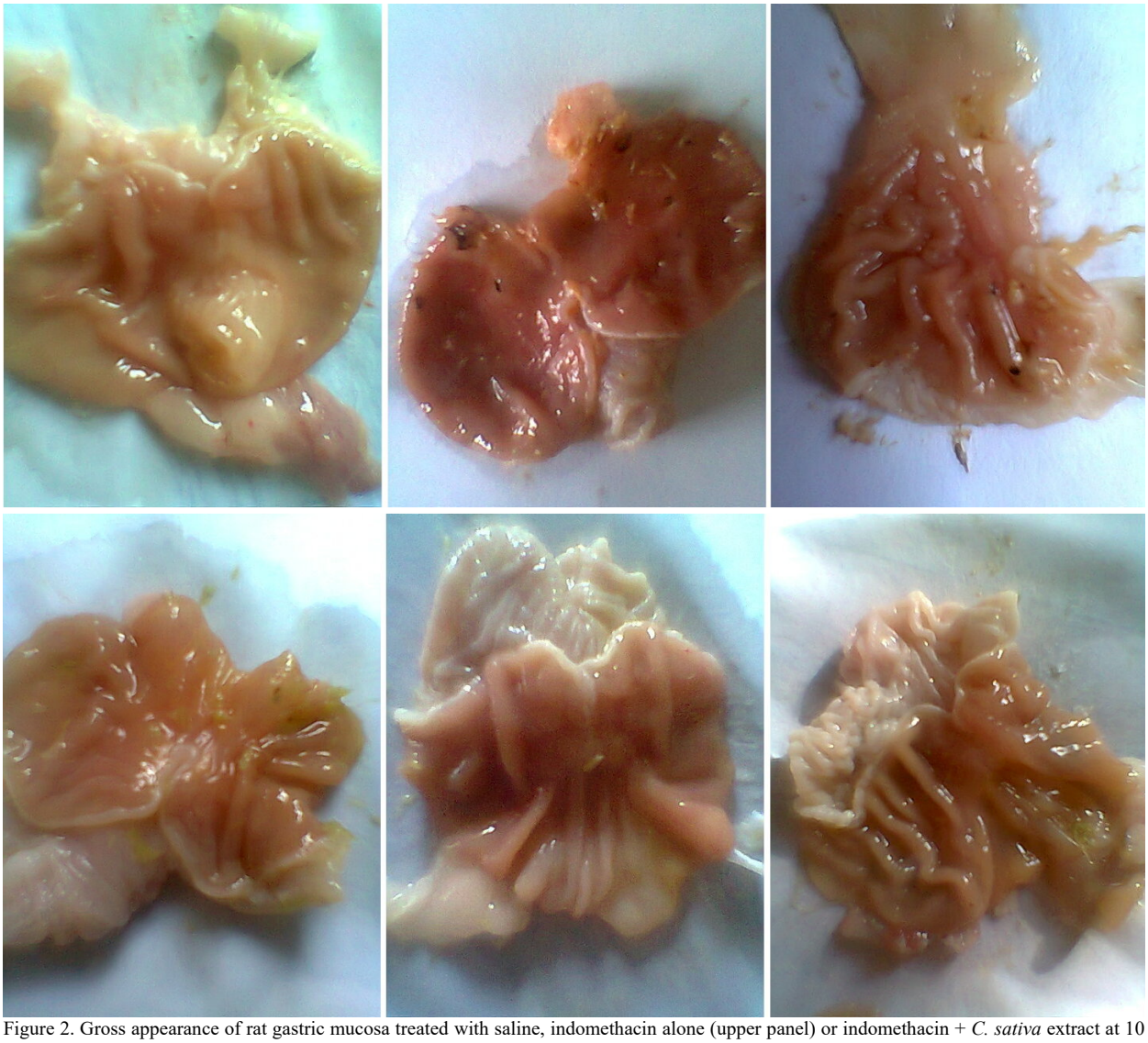

Figure 2. Gross appearance of rat gastric mucosa treated with saline, indomethacin alone (upper panel) or indomethacin + *C. sativa* extract at 10 or 20 mg/kg (lower panel).

in gastric volume and acid output in response to indomethacin by 22.6% and 24.3%, respectively (Table 2).

3.4 Histological results

Results from histological examination are presented in Fig. 3. In control rats treated with saline, sections stained with Hx & E showed normal gastric mucosa studded with many gastric pits (Fig. 3A). In the group treated with indomethacin, examination of stomach sections showed multiple erosions of mucosa with degeneration, necrosis covering of epithelium and discontinuation of gastric pits with cell debris in lumen. Leukocytic cells infiltration in lumina propria, mucosal layer and congestion of blood vessel associated with submucosal edema were seen. Some parietal cells showed necrotic changes and pyknotic nuclei (Fig. 3B & 3C). Rats treated with indomethacin along with *C. sativa* at 10 mg/kg showed the architecture of gastric gland being nearly similar to that of the saline control group although marked vascular congestion and increase in inflammatory cells resulting from indomethacin administration were still present. Thickening of muscularis mucosa could be noticed (Fig. 3D & 3E). Rats given with indomethacin along with *C. sativa* at 20 mg/kg showed regeneration of the gastric mucosal tissue, although, congestion of blood vessels in lumina propria, and submucosal layer were seen (Fig. 3F).

4 Discussion

The results of the present study provide evidence that the administration of *C. sativa* extract rich in Δ^9-THC prevented the development of indomethacin-induced gastric mucosal injury in a dose-dependent manner. The protective effect of *C. sativa* was associated with significant inhibition of gastric acid secretion and oxidative stress and confirmed by histological study of the gastric mucosa. These results are in agreement with previous studies by us and other researchers that indicated gastric protective effects of *C. sativa* [19, 30, 31]. We have shown that *C. sativa* extract (equivalent to 5-20 mg/kg Δ^9-THC) given subcutaneously was able to prevent gastric mucosal damage caused by acidified acetylsalicylic acid (ASA) or 96% ethanol in pylorus-ligated rats in our previous study [19]. Wallace et al. [30] using

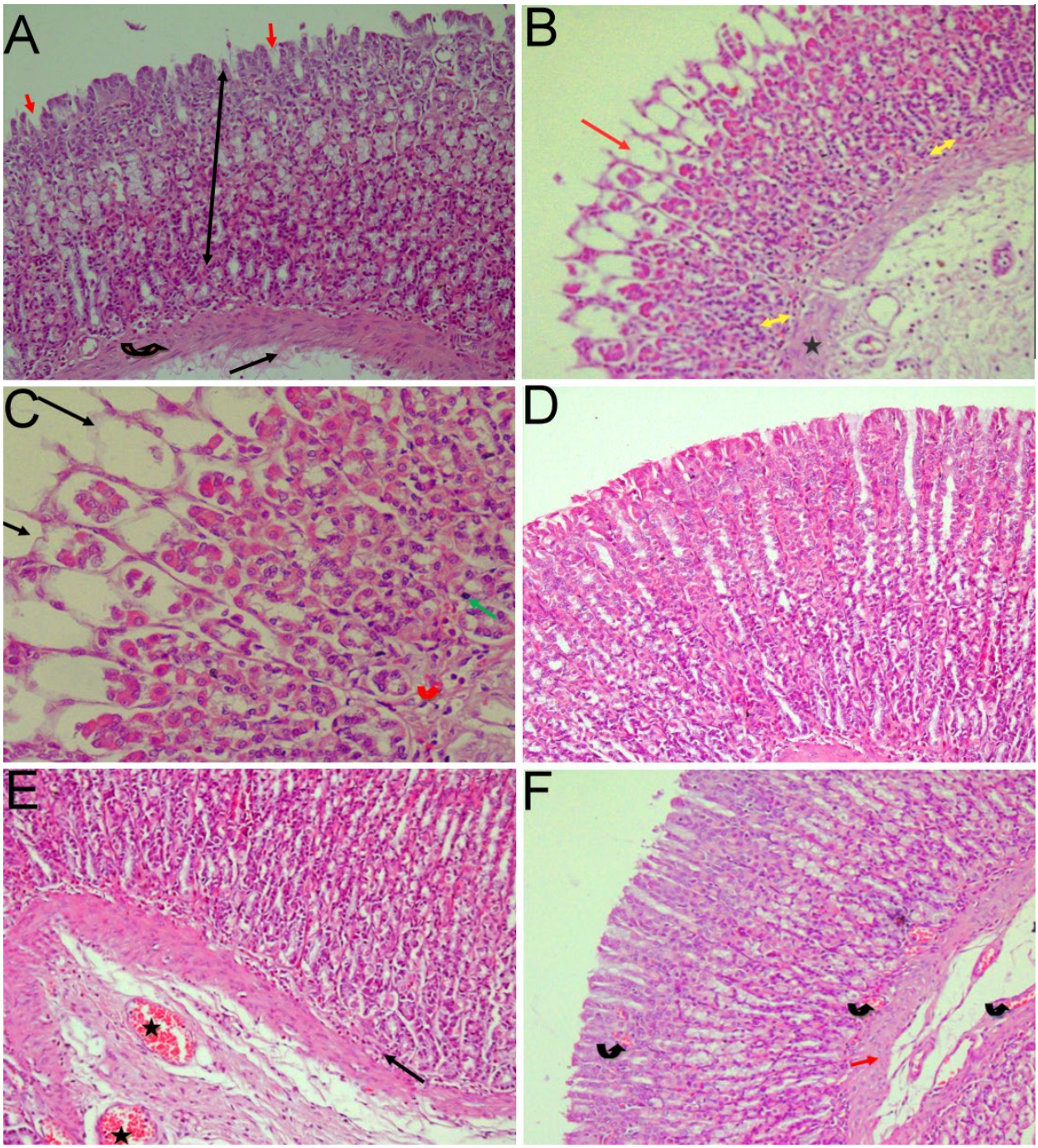

Figure 3. Sections of the rat gastric mucosa treated with: (A) Saline showing the normal architecture of gastric mucosa (long arrow). It shows gastric pits (red arrow) & gastric glands, divided into isthmus, neck & base. The muscularis mucosa (black curved arrow) is also seen (Hx&E x 200). (B) Indomethacin showing acute gastric ulcer (HX&E x 100). (C) High power of the previous section showing multiple erosion of mucosa or superficial eroded with degeneration (black arrow), lumen with cell debris necrosis covering of epithelium and discontinuous gastric pits. Some parietal cells showing necrotic changes, pyknosis (green arrow) and congestion (red arrow) (Hx&E x 400). (D) Indomethacin along with *C. sativa* at 10 mg/kg showing the architecture of gastric gland nearly similar to that of control group (Hx&E x 100). (E) Another field of stomach of rats treated with indomethacin along with *C. sativa* at low dose showing marked vascular congestion in submucosal layer and increase in inflammatory cells resulting from indomethacin administration. Thickening of muscularis mucosa could be observed (red curved arrow) (Hx&E x 100). (F) Indomethacin along with *C. sativa* at 20 mg/kg showing regeneration of the gastric mucosal tissue, although congestion of blood vessel in lumina propria and mucosal layer are seen (red arrow). There is also thickening of muscularis mucosa (black arrow) (Hx&E x 100).

ethanolic extract of *C. sativa* reported that oral (though not *i.p.*) administration of 10 mg/kg of the extract completely protected against gastric mucosal damage caused in rats by the NSAID naproxen. In their study, De Souza et al. [31] found that acute treatment with *C. sativa* extract (40-60 mg/kg, i.p.) protected against restraint-induced ulcers in rats.

The biological effects of *C. sativa* are mediated by cannabinoids acting on two G-protein coupled receptors; CB1 expressed in brain and CB2 expressed predominantly on immune cells in the periphery [32]. CB1 is also expressed on nerve terminals innervating the gastrointestinal tract [33, 34] and on the acid secreting parietal cells of gastric mucosa [35]. Germano et al. [36] found that the CB1 agonist WIN 55,212-2 reduced gastric

ulcer in rats subjected to restraint stress; the effect being blocked by CB1 antagonist. Kinsey and Cole [37] reported that the attenuation by oral or i.p. Δ^9-THC of gastric hemorrhagic lesions caused by the NSAID diclofenac in mice. The above studies suggest that CB1 receptor stimulation mediates gastric protection.

The presence of gastric acid in the lumen of the stomach is a prerequisite for gastric and duodenal ulcers to occur [38]. NSAID-induced gastropathy and ulcers are also dependent on gastric acid and for these heal effectively when administering proton pump inhibitors to patients who require continued therapy with NSAIDs [9]. Gastric acid contributes to the pathogenesis of ulcers, by degradation of mucus, impairing the restitution process, and inactivating several growth factors that are important in the mucosal defense and repair processes [20]. NSAIDs e.g., indomethacin and piroxicam augment the histamine-stimulated gastric acid secretion *in vitro* [39]. In human volunteers, indomethacin given at therapeutic doses enhanced both basal and histamine-stimulated gastric acid secretion [40]. This effect of indomethacin on gastric acid secretion might be due to their inhibitory effect on the antisecretory prostaglandins [39, 40]. Gastric damage caused by indomethacin in the rat could be inhibited by the H_2 receptor antagonist ranitidine [41]. Our present findings indicate that *C. sativa* extract caused significant decrease in gastric acid output in the indomethacin-treated pylorus-ligated rat. Other researchers have also indicated an inhibitory effect for *C. sativa* ethanolic extract (1 g/kg) in the pylorus-ligated rat [22]. *C. sativa* (5, 10 and 20 mg/kg; expressed as Δ^9-THC) given for one month also reduced acid secretion stimulated by carbachol or pentagastrin in pylorus-ligated rats [19]. Synthetic CB1 antagonists given intravenously were reported to inhibit the pentagastrin-stimulated gastric acid secretion in the anaesthetized rat [42]. Moreover, Salama et al. [43] found that the CB1 receptor agonist *N*-arachidonoyl dopamine inhibited gastric acid secretion stimulated by 2 deoxy-D-glucose and carbachol in the pylorus-ligated rat. In humans, Nalin et al. [21] found that self-reported heavy usage of cannabis was associated with low gastric output. The antiulcer effect of *C. sativa* could be mediated by its antisecretory effects as shown in the present study.

Other mechanisms could have accounted for the gastric protective effects of the cannabis extract. Mucin, which is synthesized by the surface epithelial cells, forms a protective mucus layer that covers the apical surface and plays an important role in protecting the gastric mucosa [44]. Mucus forms gel layer that retards the diffusion of luminal acid into the mucosa and both mucus and bicarbonate secreted by the epithelium forms a pH gradient with near-neutral pH at the surface of the gastric mucosa [45]. It is noteworthy to mention that treatment with *C. sativa* extract for one month was found to increase the apical cytoplasmic content of mucus granules in gastric mucosa of saline-treated rats. The decrease in periodic acid Schiff reagent for mucopolysaccharide secretions following acidified ASA or ethanol administration improved by treatment with *C. sativa* extract [19]. Moreover, CB1 receptor stimulation causes reduction of gastric motility [46]. The latter is considered to have a role in the development of indomethacin-induced gastric lesions in rats [47]. Therefore, stimulation of mucus secretion and a decrease in gastric mucosal contractions might represent other important mechanisms underlying the gastroprotective properties of *C. sativa*.

In this study, the administration of indomethacin was associated with significant increase in gastric oxidative stress as evidenced by the increase in the lipid peroxidation marker malondialdehyde and the decrease in the antioxidant and free radical scavenger reduced glutathione, indicative of increased reactive oxygen species with consequent attack on membrane lipids. There was also increased level of nitric oxide. The latter is important vasodilator and is important in gastric mucosal protection by mediating protective vasodilatation [48, 49]. The excessive formation of nitric oxide, however, can be injurious to mucosa via the formation of the strong oxidant peroxynitrite by the reaction of nitric oxide and superoxide [50]. Our findings are therefore in agreement with other studies that have reported increased markers of oxidative stress and decreased antioxidants e.g., reduced glutathione and superoxide dismutase in the gastric mucosa of indomethacin-treated rats [41, 51, 52]. Oxidative stress has been implicated in the development of gastric mucosal damage by NSAIDs [53]. In their study, Vaananen et al. [54] found that perfusion of the rat stomach with indomethacin caused marked increase in gastric blood-to-lumen leakage of 51Cr-EDTA indicative of hemorrhagic mucosal damage. The leakage was significantly reduced by the intravenous infusion of superoxide dismutase, catalase or the iron-chelating agent deferoxamine. NSAIDs damage the gastric mucosa via reactive oxygen species produced by the recruited neutrophils, resulting in apoptosis of the gastric epithelial cells and mucosal injury [55]. The findings in the present study indicate that treatment with *C. sativa* extract was able to decrease gastric oxidative stress induced by indomethacin. *C. sativa* was also found to inhibit oxidative stress in rat gastric mucosa following acidified ASA or ethanol damage [19].

Our histological study confirms the protective effects of *C. sativa* extract. In indomethacin-treated rats, extensive gastric mucosal erosions involving the surface epithelial layer and gastric pits glands, necrosis and apoptosis of parietal cells as well as leukocytic cells infiltration in lumina propria and mucosal layer were seen. C. sativa-treated animals showed nearly normal architecture of gastric glands and regeneration of the gastric mucosal tissue.

5 Conclusion

In summary, the present results show that the systemic administration of *C. sativa* extract rich in Δ^9-THC prevented the development of gastric mucosal lesions induced by the NSAID indomethacin. The gastroprotective action of *C. sativa* might be related to its acid inhibitory and antioxidant effects.

Conflicts of interest

The authors declare that there are no potential conflicts of interest.

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