Anti-Ulcerogenic Impact of Cannabis Extract On Experimental Induced Gastric Ulcer

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Cannabis sativa is one of the most recreational drugs used across the globe for its psychoactive effects. It has been found to possess many pharmacological effects on gastrointestinal functions. The goal of this study was to investigate the gastro-protective effect of Cannabis extract on indomethacin-induced gastric ulcer in male rats and the underlying biochemical pathways that are related to oxidative stress, inflammation and cyto-protection in the gut. Cannabis extract significantly attenuated ulcer index and increased percentage of ulcer inhibition as compared to indomethacin treated rats. These results were similar to those achieved by omeprazole treatment. Gastric mucosal total oxidant status, transforming growth factor beta 1, interleukin 1 beta and tumor necrosis factor alpha were significantly depressed, whilst total antioxidant capacity, prostaglandin 2 and vascular endothelial growth factor were significantly elevated in cannabis groups relative to indomethacin group p<0.05. Histopathological investigation revealed that cannabis treated rats showed amelioration of epithelial erosion and necrosis induced by indomethacin administration associated with regeneration of enterocytes. Cannabis extract exhibited anti ulcerogenic, anti-inflammatory, antioxidant effects and notable cyto protective activity against indomethacin induced gastric ulcer.

Keywords: Cannabis extract; Indomethacin; gastric ulcer; Growth factors; inflammatory cytokines.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are used globally as analgesic, anti-inflammatory and antipyretic agents. Unfortunately their use is frequently associated with gastric erosions and ulcerations (Lee et al., 2016). Indomethacin is an important member of the NSAIDs family. It is used in the treatment of arthritis, joint stiffness, gout and tendonitis. It is also used in patent ductus arteriosus closure in the neonatal unit and in obstetrics to delay uterine contractions (Simon, 1993). However, indomethacin provokes aggressive gastric ulceration in both humans and animals. This gastrointestinal toxicity is mediated through several mechanisms including elevation gastric acidic secretions, inhibition of mucosal regeneration via inhibiting prostaglandins synthesis (Tibble et al., 2001). Moreover, Indomethacin stimulates free radicals production, suppresses gastric nitric oxide level, increases leucocyte infiltration in gastric mucosa (Abdel Raheem, 2010) and induces gastric cells apoptosis (Matsui, 2011). The prevention of gastric ulcer pathogenesis or its recurrence is a desired goal in the present era.

Cannabis sativa is an annual flowering herbal plant (family Cannabidaceae). Although cannabis is one of the most recreational drugs used across the global (Khadrawy et al., 2017) due to its psychotropic properties, it has been used as a medicine for centuries. It possess many profound
Cannabis extract alleviates gastric ulcer.

Therapeutic effects as treatment of epilepsy (Reddy and Golub, 2016) inflammation (Naftali et al., 2013), Parkinson’s disease, cancer, neuropathic pain, fibromyalgia and psychoactive disorders (Madras, 2015). Also, cannabis is an effective analgesic, reduces anxiety and improves sleep (Robson, 2001). In gastroenterology, cannabis sativa has been used to treat inflammatory bowel diseases, nausea, and emesis due to chemotherapy, anorexia, abdominal pain, diarrhea, diabetic gastroparesis and gastroenteritis (Naftali et al., 2013). Also, cannabis is used to improve appetite in people with HIV/AIDS and to treat muscle spasms and chronic pain (Massa and Monory, 2006). Cannabis produces a group of chemicals named cannabinoids. The most important cannabinoids are Δ9-tetrahydrocannabinol (Δ9-THC), cannabidiol, cannabidiol-acid, cannabiol and cannabichromene (Borgelt., 2013). Cannabinoid receptors (CB1 which are mostly in enteric and central neurons and CB2 in immune cells) are G-protein coupled receptors (Izzo and Sharkey, 2016) and their endogenous ligands (anandamide and 2-arachidonoyl glycerol) have been identified in the gastrointestinal tract and are involved in mediation of several gastrointestinal functions as relaxation of the lower esophageal sphincter, gastric acid secretion, gastric emptying, gastrointestinal motility and fluid secretion (Borgelt, 2013). Endogenous cannabinoids may inhibit the release of many proinflammatory mediators, as TNF α, interleukin-1 beta (IL-1β) and nitric oxide (Burstein and Zurier, 2009) thus controlling the inflammation that is characterized by massive release of cytokines (Abdel Salam et al., 2015). Moreover, Cannabis has been shown to curb oxidative stress markers and to boost antioxidants activity in gastric mucosa (Massa and Monory, 2006). These important pharmacological actions may help in the spread of legalization of cannabis use across the world.

Therefore, the current study was conducted to highlight the role of cannabis extract as a gastro-protective agent against INDO-induced gastric ulcer in male rats. In addition, the study also examined underlying biochemical pathways that were related to oxidative stress, inflammation and cyto-protection in the gut.

**MATERIALS AND METHODS**

**Drugs and chemicals**

Indomethacin was obtained from Sigma Chemical Co. (St. Louis, MO, USA) and suspended in 1% aqueous solution of Tween 80. A single dose (30 mg/kg) was administered orally (gavage) with a met al orogastrictube.

**Omeprazole**

Napizole (20mg) was bought from Global Napi Pharmaceuticals (GNP) – Egypt

**Cannabis saliva**

The plant was obtained from Ministry of Justice. All other chemicals were of analytical grades

**Cannabis extract preparation:**

Cannabis sativa resin extract was prepared from the dried flowering tops and leaves of the plant. The method of extraction followed was described by Turner and Mahlberg (1984). Semi-quantitative analysis of cannabis resin extract was done using GC/MS technique by computerized matching of the obtained mass spectral data with those in the computer library and referring each compound relative abundance to the whole peaks of the sample.

The presence of tetrahydrocannabinol THC was confirmed at retention time 28.20 min with relative abundance of 20% and revealed other cannabinoid constituents in minor quantities, such as cannabidiol CBD at 27.3 min (2.5%) and cannabiol CBN at 28.96 min (2 %) among the cannabis resin extract constituents.

**Animals:**

Fifty adult male albino rats weighing 150 ± 10 g were used in the present study. The animals were obtained from the animal house colony of National Research Centre. The animals were maintained in wire bottomed cages at room temperature (25 ± 2 °C) with controlled lightening (12 h light & 12 h darkness cycle). All animal were accommodated with laboratory conditions for one week before treatment and maintained under the same conditions all over the experimental period. Rodents chow diet and clean water were allowed ad libitum. All animals received human care in compliance with the guidelines of the Ethical Committee of Medical Research of National Research Centre, Egypt. which conform to the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised1996).

**Experimental design**

Animals were randomly assigned into five groups. They were deprived of food but had free access to water 24 h prior to ulcer induction.
Group (1): (control group) rats received oral saline
Group (2): (Indo group) rats received a single oral dose of indomethacin (30 mg/kg.bwt) (Sayanti et al., 2007)
Group (3): (Indo+ Omepr. group) rats received a single oral dose of indomethacin + oral omeprazole 5 mg/kg/day (Izzettin et al., 2012) omepr. Serves as reference drug
Group (4): (Indo+ Cannabis 10) rats received a single oral dose of indomethacin + oral cannabis extract 10 mg/kg/day
Group (5): (Indo+ Cannabis 20) rats received a single oral dose of indomethacin + oral cannabis extract 20 mg/kg/day
All Treatments were administered four hours after indomethacin administration and lasted for three consecutive days
At the end of treatment period, animals were fasted overnight, anaesthetized using urethane (1.4 ml/kg), rapidly sacrificed and the stomach of each animal was removed by dissection

Macroscopic evaluation and Ulcer scoring
Stomachs obtained were opened along the greater curvature and rinsed with saline. Degrees of ulceration in all groups were determined according to the method of Szabo and Hollander (1985). The ulcer score for each group was expressed as a percentage of the total surface area of the stomach measured in square millimeters. Mean ulcer score for each animal was expressed as ulcer index (U.I) and the percentage of inhibition against ulceration was determined using the expressions:

\[ \text{U.I.} = \left( \frac{\text{Ulcercated area}}{\text{total stomach area}} \right) \times 100 \%
\]
\[ \text{Ulcercation inhibition} = \left( \frac{\text{U.I. in control} - \text{U.I. in test}}{\text{U.I. in control}} \right) \times 100 \%
\]

Gastric tissues preparation for analysis
The gastric mucosa of the dissected stomachs was rapidly scraped using two glass slides from the gastric wall of each rat, weighed, homogenized with 0.1 M phosphate buffer saline at pH7.4, to give a final concentration of 10 % w/v and centrifuged at 3000xg for 15 min at -4°C. The obtained supernatant was used for subsequent biochemical investigations

Determination of oxidants and antioxidants markers
Gastric mucosal total oxidant status and total antioxidant capacity were measured by the methods developed by Erel (2004, 2005) respectively using spectrophotometer.

Determination of inflammatory markers
 Levels of tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and prostaglandin 2 (PGE2) in gastric mucosa were detected using ELISA kits obtained from Cusabio Biotech Co., Ltd (Wuhan, China), Koma Biotech Co., Ltd (Seoul, Korea) and Glory Science Co., Ltd. (Hangzhou, China), respectively, according to manufacturer protocols.

Determination of growth factors
Vascular endothelial growth factor (VEGF) and transforming growth factor (TGF-β1) concentrations in gastric mucosa were estimated using a commercial ELISA kit obtained from R&D Systems, Inc. (Rochester, USA) according to instructions of (R&D Systems).

Histopathological investigation
Stomach specimens were fixed in 10% formol-saline, embedded in paraffin then sectioned (5-μm thick) by microtome (Leica, Berlin, Germany), mounted on glass slides and then stained using hematoxylin and eosin solution and examined under light microscope.

Statistical Analysis
Results were expressed as mean ± SE of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program version 11, followed by Duncan post hoc test to compare significance between groups. Difference was considered significant when P< 0.05.

RESULTS
Antiulcer activity of cannabis extract against Indo induced gastric ulcer:
Administration of cannabis extract 10mg/kg to Indo treated rats significantly attenuated ulcer index by 26% as compared to Indo treated group. The reduction in ulcer index reached 62% by increasing the dose of cannabis extract to 20 mg/kg. However, Omeprazole treatment significantly decreased ulcer index by 64% in comparison with Indo treated rats (Fig.1A). Based on the calculated ulcer inhibition, cannabis 20 appeared to have imparted a greater gastro-protective effect in comparison with rats in cannabis 10 regimen (Fig.1B).
Cannabis extract alleviates gastric ulcer.

Figure (1): Effect of Cannabis extract on Ulcer index (A) and percentage of inhibition (B) in Indomethacin induced gastric injury in rats. (n = 10, X ± SEM). Bars with different superscripts a,b,c, for the parameter are significantly different (p < 0.05). Indo: indomethacin (30 mg/kg b.w.), Omep: Omeprazole (5 mg/kg b.w.), cannabis 10: cannabis extract 10 mg/kg b.w, cannabis 20: cannabis extract 20 mg/kg b.w.

Figure (2): Macroscopical appearance of the gastric mucosa in rats. The negative control group (A) has no injury in gastric mucosa. The ulcer control group (B) displays sever injuries in the gastric mucosa. The reference control group (omeprazole, 5 mg/kg) (C) illustrations slight injuries in the gastric mucosa comparing to the injuries seen in ulcer control group. Rats fed with cannabis extract 10mg/kg (D) has moderate injuries in the gastric mucosa. Rats received cannabis 20mg/kg (E) showing healthy gastric mucosal surfaces that resemble the normal to a great extent with marked improvement than (D).

Macroscopic evaluation of gastric lesions
Gross evaluation of the dissected stomachs revealed that control group showed no injury in gastric mucosa (Fig.2A). While Indo treated group displayed severe injuries in the gastric mucosa (Fig.2B). Gastric mucosa of the group administered the reference drug (omeprazole, 5 mg/kg) illustrated slight injuries comparing to the injuries seen in indo treated group (Fig.2C). Interestingly, rats fed with cannabis extract 10 mg/kg showed moderate injuries in the gastric mucosa (Fig.2D). However, rats received cannabis 20mg/kg indicated healthy gastric mucosal surfaces that resemble the normal to a great extent with marked improvement (Fig.2E).

Antioxidant effect of cannabis extract
Indomethacin treatment induced significant elevation in total oxidant status (TOS) by 96% as compared to control group.
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Figure (3): Effect of cannabis extract on total oxidant status and total antioxidant capacity in indomethacin induced gastric injury in rats. (n = 10, X ± SEM). Bars with different superscripts a,b,c, for the parameter are significantly different (p < 0.05). Indomethacin (30 mg/kg b.w.), Omeprazole (5 mg/kg b.w.), cannabis10: cannabis extract 10 mg/kg b.w, cannabis 20: cannabis extract 20 mg/kg b.w.
Figure (4): Effect of cannabis extract on inflammatory markers in indomethacin induced gastric injury in rats. Tumor necrosis factor alpha (TNFα) (A), Interleukin 1 beta (IL-1 β) (B) and prostaglandin 2 (PGE2) (C). (n = 10, X ± SEM). Bars with different superscripts ab,bc for the parameter are significantly different (p < 0.05). Indomet: indomethacin (30 mg/kg b.w.), Omepr: Omeprazole (5 mg/kg b.w.) ,cannabis10: cannabis extract 10 mg/kg b.w, cannabis 20: cannabis extract 20 mg/kg b.w

Fig.(5): Effect of cannabis extract on growth factors in indomethacin induced gastric injury in rats. Vascular endothelial growth factor (VEGF) and Transforming growth factor beta (TGF-β1) (n = 10, X ± SEM). Bars with different superscripts ab,bc for the parameter are significantly different (p < 0.05). Indomet: indomethacin (30 mg/kg b.w.), Omepr: Omeprazole (5 mg/kg b.w.) ,cannabis10: cannabis extract 10 mg/kg b.w, cannabis 20: cannabis extract 20 mg/kg b.w

Administration of cannabis 10mg/kg significantly attenuated TOS by 19% in comparison with Indo treated rats. However, this reduction reached 45% by increasing the dose of cannabis extract to 20 mg. The same effect was observed with omeprazole treatment. Total antioxidant concentrations (TAC) were significantly reduced after indomethacin ingestion by 52% as compared to control rats. Administration of cannabis 10 or 20 mg/kg significantly increased TAC by 50% and 100 % respectively as compared to indo treated rats (Fig.3).
Figure (6): Histopathological investigation of hematoxylin and eosin staining of the gastric mucosa of normal control rats (A): showing normal histological structures. Sections of gastric mucosa of indomethacin-treated rats (B): indicating areas of necrotic surface, loss of the epithelial layer and gastric pits with decrease in mucosal thickness. Sections of gastric mucosa of omeprazole-treated rats (C): showing normal tips enterocytes with normal villus tall and width (arrow). Sections of gastric mucosa of cannabis 10 mg/kg -treated rats (D): showing regenerative attempts of enterocytes with still present desquamated sheet (arrow) while their sub muscular layers were mild hyperplastic and dilated blood vessels (star). Sections of gastric mucosa of cannabis 20 mg/kg -treated rats (E): showing amelioration of epithelial erosion, regeneration of enterocytes (arrow) while their muscular layers have hyalinization (star).
**Anti-inflammatory effect of cannabis extract:**

Upsurge of TNFα and IL-1β gastric mucosal level was observed after indo treatment by 194% and 264% respectively. While, PGEs level was significantly depressed by 68% as compared to control group. Rats treated with cannabis extract 10mg/kg showed a significant reduction in TNFα and IL-1β levels by 43% and 34% in the same respect, while PGE2 level was significantly increased by 111% as compared to indo treated rats. However the amelioration of the aforementioned inflammatory markers was more pronounced after cannabis 20mg/kg administration (Fig. 4A, 4B&4C).

**Effect of cannabis extracts administration on growth factors**

Administration of indomethacin significantly reduced mucosal vascular endothelial growth factor (VEGF) by 53% while elevated Transforming growth factor beta1 (TGF-β1) by 45% of their control value. On the other hand, cannabis extract given at 20 mg/kg significantly increased mucosal VEGF and suppressed TGF-β1 by 86% and 24% respectively as compared to indo treated group (Fig.5).

**Histopathological investigation**

Gastric mucosa of normal control rats showed normal histological structures (Fig.6A). However, those of indomethacin-treated rats indicated areas of necrotic surface, loss of the epithelial layer and gastric pits with decrease in mucosal thickness (Fig.6B). Rats treated with omeprazole showed normal tips enterocytes with normal villus tall and width (Fig.6C). Rats fed with cannabis extract 10mg/kg revealed regenerative attempts of enterocytes with still present desquamated sheet while their sub muscular layers were mild hyperplastic and dilated blood vessels (Fig 6D). Gastric mucosa of cannabis 20 treated rats illustrated amelioration of epithelial erosion and necrosis, regeneration of enterocytes while their muscular layers have hyalinization (Fig.6E).

**DISCUSSION**

Gastric ulcers are one of the major health hazards that affect a considerable number of the population across the global in terms of morbidity and mortality (Chaturvedi et al., 2007). The present study was designed to estimate the anti-ulcerative effect of Cannabis sativa extract against indomethacin -induced gastric ulcer in rats and the possible mechanisms implicated in it. Gastric ulcers are formed due to aggressive pepsin activity and increased gastric secretions accompanied with decrement in the maintenance of the mucosal membrane by endogenous defense mechanisms (Abdel Salam et al., 2015). NSAIDS are known to increase gastric acidity and secretions causing mucosal damage and depletion of gastric wall (Sayant et al., 2007). The ulceration induced by NSAIDS may be attributed mainly to their Inhibitory action on prostaglandin (PGE) synthesis coupled with free radicals formation and inflammatory responses (Lichtenberger, 2005). These observations are consistent with the results obtained in the present study where intragastric administration of NSAIDS triggered ulcer index, oxidative stress and inflammatory responses as indicated by elevated levels of serum total oxidant status , mucosal TNFα and IL-1β, associated with evoked depletion in serum total antioxidant capacity and mucosal PGE2. NSAIDs inhibit cyclooxygenases (cox-1 and cox-2) which are responsible for the production of the prostaglandins. The reduced level of PGs induces ulcer formation and exacerbates the pre-existing ulcers in rodents and humans (Inas et al., 2011, Ajani et al., 2014). PGs induce angiogenesis, stimulate the secretion of mucus to protect the gastric mucosa, increase blood flow and secretion of bicarbonate by gastric mucosa (Erel, 2004) and at the same time decrease gastric acid and pepsin formation (aggressive factors) and elevate mucosal resistance to accelerate ulcer healing (Chaturvedi et al., 2007). Back-diffusion of acid and pepsin into the tissues stimulate further acid and pepsin secretion to cause more damage, release histamine causing inflammation, lower in the mucosal blood flow and decrease gastric motility and inflicts necrotic gastric damage (Ajani et al., 2014). Earlier stages of damage induced by indomethacin in the mucosa, parietal and endothelial cells are due to inhibitions of PG synthesis (Inas et al., 2011).Production of free radicles plays an essential role in the pathogenesis of indomethacin induced gastric ulcer (Esfandyari et al., 2007). Indomethacin has been reported to induce stomach ulceration by attracting polymorph nuclear (PMN) cells and triggering production of reactive oxygen species (ROS) in gastric tissue causing damage to key biomolecules such as lipids and stimulation of lipid oxidation leading to an increase in the accumulation of MDA as well as reduction in the gastric activity of antioxidants (Lancaster-Smith et al., 1991). Also, inflammation and neutrophil infiltration are tightly linked to gastric damage
induced by NSAIDs. The pro-inflammatory cytokine (TNF-α) is a critical mediator in gastric injury induced by NSAIDs (Ajani et al., 2014) as it stimulates the synthesis of adhesion molecules and their expression on neutrophils and endothelial cells (Chaturvedi et al., 2007), production of IL-1β and augments the generation of neutrophil-derived superoxide causing neutrophil adherence and accumulation (Wallace., 2002).

The present data also indicated that indomethacin ingestion significantly interferes with the action of growth factors evidenced by reduction in mucosal VEGF and an elevation in TGF-β1 levels. Previous studies revealed that NSAIDs slow the gastro-duodenal ulcer healings by affecting the growth factors action, delaying maturation of the granulation tissues and decreasing various stages of angiogenesis (Sorbye and Svanes, 1994). Many growth factors as VEGF accelerate wound healing via triggering endothelial proliferation and migration and promoting angiogenesis (Morris et al., 1998). The mechanism by which NSAIDs inhibit VEGF could be due to blockage of COXs which are responsible for the production of the PGs and cell proliferation. Gallo et al. (2002) reported a significant correlation between COX2 activity and VEGF expression where the increase in VEGF production following COX2 over expression is associated with increased PGE2 levels and these effects can be reversed by inhibition of COX2 following treatment with COX inhibitor indomethacin (Russo., 2011).

On the other hand, our data revealed a significant elevation in TGF-β1 following indomethacin administration. This could be explained on the basis that acute exposure of stomach membrane to ulcerogenic drugs as aspirin and indomethacin causes local injury in the gastric mucosa accompanied with disruptions in the superficial epithelial and deeper mucosal layers (Santucci et al., 1994). For mucosal regeneration immune cells like monocytes, lymphocytes and leukocytes produce certain cytokines as TGF-β1 to promote epithelial apoptosis by arresting the cell cycle at the G1 stage (Yoshikawa et al., 1992). Activation of the TGF-β1 death-signaling pathway contributes to the induction of apoptosis, which in turn restricts the dis-semination of the damaged area by tissue remodeling surrounding the ulcer (Kokura et al., 2000).

Although cannabis sativa is mostly used as a recreational drug, it has been used as a medicine for centuries. Cannabinoids extracted from (Cannabis sativa) have many effects on gastrointestinal tract (GIT) (Jones et al., 1999). CB1 receptors are found on the enteric nervous system (myenteric and submucosal nerves) which is embedded in the lining of the GIT(Halter et al., 2001). Delta 9-tetrahydrocannabinol is CB1 receptor agonists which bind to the cannabinoid-1-receptor decrease stomach motility (Wallace.,1997), gastric acid secretions (Hornby and Prouty, 2004) and inflammation in animals (Krowickiet al., 1999). In 1993, CB2 receptor was identified in rat spleen macrophages. It has relevance for anti-inflammatory and immunosuppressive activity (Cabral and Griffin-Thomas, 2009). Cannabis extract exerted significant protection against indomethacin induced gastric insult mainly through suppression of proinflammatory cytokines and combating reactive oxygen species in a dose dependent manner. The gastroprotective effects of cannabis extract (10mg/kg) were analogous to the reference drug omeperazole .While cannabis extract (20mg/kg) surpassed those effects.

Cannabis extract in both doses reduced the values of lesion index and elevated the ulcer inhibition percentage as compared to control group suggesting its potent gastroprotective effect against indomethacin challenge. Previous studies have documented that cannabis gastroprotective effect has been accompanied with the maintenance of blood flow and DNA synthesis in the gastric mucosa. Gastric blood flow has a significant importance in the protection and curing of gastric mucosa as it provides oxygen and bicarbonates to the mucosa and eliminates carbon dioxide, hydrogen ions and toxic agents diffusing from gastric lumen (Folkman and Shin, 1992).

Data of the current study demonstrated that Cannabis extract combated oxidative stress and enhanced antioxidant capacity in rats with indomethacin gastritis as revealed by significant reduction in TOS in addition to upregulation in TAC concentrations. These findings are in agreement with previous studies (Hampson et al., 1998). Cannabinoids were found to prevent dihydrorhodamine oxidation (Fenton reaction) and attenuate lipid hydroperoxides induced neurotoxicity. These observations indicate that cannabinoids possess considerable protective antioxidant potentials thereby sparing endogenous antioxidants (Cabral and Griffin-Thomas, 2009). Moreover Abdel salam et al., (2015) reported that Cannabis exerts antioxidant...
activities that is evidenced only upon exposure of gastric mucosa to an ulcerogenic challenge.

Administration of cannabis extract attenuated mucosal levels of the pro-inflammatory cytokine TNF-α and IL-1β and elevated PGE2. These findings are consistent with (Esposito et al., 2011). Jenny et al. (2010) reported that cannabinoids exhibit potent anti-inflammatory impact manifested by suppression of mitogen-stimulated proliferation, T cell-dependent antibody responses and the production of inflammatory cytokines IL-2 and TNF-α. These anti-inflammatory effects may be explained on the basis that cannabinoids activate peroxisome proliferator-activated receptor gamma (PPARγ). Which in turn massively inhibits the activity of pro-inflammatory transcription factors such as nuclear factor kappa (Esposito et al., 2011). Inhibition of nuclear factor kappa is a chief mechanism for suppression of gastric TNFα and IL-1β since their expression are regulated by the transcription of nuclear factor kappa (Szelenyi et al., 1983). The elevation in mucosal PGE2 level after cannabis extract ingestion may be due to the ability of cannabinoids especially Δ9-THC to induce COX-2 mediated via CB1 receptor- βγ subunits. COX-2 is an inducible enzyme that its expression correlated with basal and arachidonic acid (AA) stimulated PGE2 production (Chen et al., 2013).

The increase in PGE2 level stimulates VEGF expression via transactivation of epidermal factor growth receptor (EGFR) and triggering mitogenic signaling in culture gastric epithelial cells as well as rat gastric mucosa (Ben-Av et al., 1995). These findings coincide with the current data, which revealed a significant increase in mucosal VEGF following cannabis extract administration.

VEGF protects and repairs the mucosa by improving mucosal resistance by increasing vascular permeability that dilutes gastrotoxic agents and reduces hemorrhagic areas. It also contributes in the progression of angiogenic response with other growth factors. So, VEGF repairs gastric ulcers by maintaining the viability of endothelial cells (Baker et al., 2003) and inhibiting their apoptosis via inducing an increase of anti-apoptotic proteins Bcl-2 (Blobe et al., 2000). Meanwhile, the present study demonstrated that Cannabis extract ingestion significantly suppressed mucosal TGF-β1. Takahashi et al.,(1998) reported that transforming growth factor-β1 (TGF-β1) mRNAs, TNF-α and IL-1β were highly expressed in the ulcerated tissue. Prevention of TGF-β1 action promoted the production of PGE2 and COX-2 mRNA expression. Moreover, the increment in VEGF level by cannabis extract administration may be associated with down-regulation of mucosal TGF-β1. Li et al., (2013) demonstrated that VEGF indirectly stimulates smooth muscle cell proliferation and migration through stimulation of the expression of FGF-2 and inhibition of TGF-β1 released by endothelial cells. After ischemic injury TGF-β1 expression at low levels may help in repairing epithelium cells (Blobe et al., 2000). On the other hand, upregulation in TGF-β1 expression is associated with activation of fibroblasts, macrophages and deposition of collagen and fibrosis (Li et al., 2013).

CONCLUSION

Our results indicate that cannabis extract exerts a potent anti-ulcerogenic and cyto protective effects by widely affecting several pathways involved in this process. Its pleiotropic effects on oxidative stress, inflammation and growth factor regulation further suggests that Cannabis could represent a potential effective agent in gastric ulcer therapy.

CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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AUTHOR CONTRIBUTIONS

NS designed and performed the experiments and also wrote the manuscript. MS performed animal treatments, tissue collection, and data analysis. NS reviewed the manuscript. All authors read and approved the final version.

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