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Evaluation of Anti-oxidant and Anti-ulcerogenic activity of *Cannabis Sativa* extract against aspirin-induced gastric ulcer in Rabbits

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Abstract

Cannabis sativa is an herbaceous perineal plant which has been used in traditional medicine for its anti-inflammatory, analgesic and sedative properties. In this study, the anti-oxidant and gastroprotective properties of *C. sativa* ethanolic extract were evaluated against aspirin-induced gastric lesions. For this purpose, thirty healthy rabbits were selected and divided into five groups (n=6) as follows: (I) normal saline, (II) aspirin alone (150mg/kg*day), (III) omeprazole (20mg/kg*day) plus aspirin, (IV and V) *C. sativa extract* (10mg/kg*day and 20mg/kg*day) with aspirin. All treatments were given orally for 14 days. Gastric ulcer parameters including ulcer index, ulcer score, gastric acid volume, total acid output and pH were assessed. Blood samples were collected to determine oxidative stress parameters (TOS, TAC, MDA and CAT). *C. sativa* exhibited significant ($p \leq 0.01$) antiulcer effect in comparison with aspirin alone treatment. Histopathological analysis revealed normalization of gastric mucosa in *C. sativa* treated animals. *C. sativa* administration showed a reduction in oxidative stress parameters (TOS and MDA) and increased anti-oxidants (TAC and CAT) level. Moreover, *C. sativa,* particularly at 20mg/kg, showed 70.66% ulcer protection which was comparable to 72.85% with omeprazole. Collectively, this study suggested that *C. sativa* has antioxidant and antiulcer properties.

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Introduction

Gastric ulcers, manifested as damage to gastric mucosa due to various factors, are common disorders of the gastrointestinal tract. Despite clinical and pharmaceutical advancements, foremost clinical needs such as symptom control, rapid healing and tolerance avoidance against drugs remain unmet. Pathogenesis of gastric ulcers includes physiological alterations such as mucus barrier disruption, excessive gastric acid secretion, free radical generation, lipid peroxidation and inflammatory mediator's release (Kavitt *et al.*, 2019; Lanas and Francis, 2017).

Prevalence of gastric ulcers is associated with various factors such as age, gender and geographical location. An imbalance between gastro aggressive and protective factors is linked to loss of normal gastric mucosa maintenance (Hooi *et al.*, 2017; Schubert, 2017). Gastric acid, pepsin, abnormal motility, bile salts, alcohol abuse, microbial infections (*H. pylori*) and NSAIDs overuse exert damaging effects on the gastric mucosa. While, gastroprotective prostaglandins, proper tissue microcirculation, mucus secretion, antioxidants and bicarbonate salts exhibit preventive role (Wallace, 2013).

Overburden of reactive oxygen species (ROS) increases oxidative stress in body tissues. Mechanisms such as lipid peroxidation involve ROS generation (Baigent *et al.*, 2009). NSAIDs considered a secondary underlying cause of PUD. Previous studies showed that 20% of people receiving NSAIDs develop a peptic ulcer. Long term NSAIDs use causes an increase in gastric acid and enzyme secretion which reduces gastric mucus and bicarbonate ion secretion (Longo *et al.*, 2012; Mukherjee *et al.*, 2010). Aspirin (ASA) is NSAIDs, used as an analgesic and antiplatelet in cardiovascular patients. It diminishes gastro-protective prostaglandins synthesis by reducing nonselective cyclooxygenase enzymes, results in poor blood supply, mucosal damage and gastric erosions (Burke *et al.*, 2006).

Synthetic drugs including antibiotics, proton pump inhibitors and histamine receptor blockers are being used in peptic ulcer treatment. These synthetic drugs are associated with side effects including headache, dizziness, sleep deprivation, nausea and abdominal discomfort (Jaikumar *et al.*, 2010; Kaner and Lapidot, 2001). Therefore, the development of plant-derived drugs as an alternative strategy for PUD treatment is focused on nowadays. Medicinal plants have been studied for anti-ulcer activity. Herbal remedies are getting more popular as compared to synthetic drugs due to efficacy, fewer side effects and cost-effectiveness (Andre *et al.*, 2016).

Cannabis sativa, belongs to family Cannabaceae, has pharmacological importance due to its analgesic, antiemetic, diuretic, anti-inflammatory as antiepileptic activities. *C. sativa* contains bioactive compounds such as cannabinoids (Δ9- Tetrahydro-cannabinoids) and non-psychoactive cannabinoids (cannabigerol, cannabichromene and cannabidiol). *C. sativa* is potentially used in depression, glaucoma, Alzheimer's, multiple sclerosis and neuralgia (Frassinetti *et al.*, 2018; Zhou *et al.*, 2018). Antioxidant activity of *C. sativa* also has been reported (Abdel-Salam *et al.*, 2018). Therefore in the current study, the antiulcer activity of *C. sativa* against aspirin-induced ulcer was evaluated.

Materials and methods

Plant collection and extract preparation

C. sativa leaves and aerial parts were collected and identified by taxonomist Dr. Mansoor Hameed, Department of Botany, University of Agriculture, Faisalabad. The plant was shade dried for one month and then grounded to make coarse powdered. For extract preparation, 100g of powder was used to prepare ethanolic extract by using soxhlet apparatus. The prepared extract was filtered through Whatman no. 1 paper, then lyophilized and concentrated using rotary evaporator (Heizbad Hei-VAP, Heidolph, Germany) at 40°C and reduced pressure.

Experimental animals

Thirty healthy rabbits (1000-1500g) were purchased and placed in the animal room of Institute of Microbiology, University of Agriculture Faisalabad.

All animals were kept according to International guidelines for care and use of animals. The study was approved by the Directorate of Graduate Studies (No. DGS. 3920-24).

Experimental design

The experiment was conducted for 14 days. Aspirin (150mg/kg*day) was administered for gastric ulcer induction. Rabbits were grouped as; (I) normal control (normal saline 5ml/kg*day), (II) ulcerated control was given aspirin alone, (III) reference group received omeprazole (20mg/kg*day) plus aspirin. *C. sativa* extract was administered at two different dose rates of 10mg/kg and 20mg/kg along with aspirin in groups (IV) and (V), respectively. All treatments were given orally.

Oxidative status determination

For determination of oxidative status, blood samples were collected in clot activator tubes. Blood samples were kept in the refrigerator for 20 min for clotting. Serum was separated by centrifuging blood at 3000rpm for 10 min. Separated serum was collected and stored at -4°C temp. The parameters analysed included total oxidant status (TOS), total antioxidant capacity (TAC), malondialdehyde (MDA) and catalase (CAT) activity (Erel, 2004; Erel, 2005; Goth, 1991; Ohkawa *et al.*, 1979).

Antiulcer evaluation studies

At the end of treatment, animals were kept hungry, with free access to water for 24 hours. Then, animals were slaughtered, the organs were collected and cut open on the greater curvature. Gastric contents were transferred in falcon tubes and centrifuged for 10 min at 3000rpm. Serum was separated and used to analyze parameters included gastric acid volume, total acid output and pH. Gastric tissue samples were used to analyze ulcer index, ulcer score and curative ratio (%).

pH

pH meter was used to check the pH of all supernatants of gastric contents.

Gastric volume

The graduated cylinder was used to measure the gastric volume of the supernatant obtained from gastric content.

Total acid output

Total acid output was assessed by treating the supernatant (diluted) of gastric content against NaOH (0.01N) using an indicator (Topfer's reagent). The volume of NaOH consumed to titrate supernatant was used to calculate the total acid output. Total acid output was calculated by the prescribed formula (Raju *et al.*, 2009).

$$A = \frac{\text{NaOH (ml)} \times \text{N}}{0.1} \times 100$$

Where, A= Acid output, N= Normality of NaOH

Ulcer score and Ulcer index

Ulcer score was assessed to check number and severity gastric ulcer (Kulkarni and Vallabh, 2002). Ulcer index was measured according to the below-mentioned formula (Vogel, 2002).

$$\text{UI=US+ UN+ UP}\times 10^{-1}$$

Where, UN: ulcers average number per animal, US: ulcer scores mean severity, UP: ulcerated animal percentage.

Curative ratio (%)

The curative ratio was determined by using the given formula (Akhtar and Kamal, 1995).

$$\text{CR} = \frac{\text{LUC} - \text{LUT}}{\text{LUC}} \times 100$$

Where, LUT: Ulcer length in the treated group, LUC: Ulcer length in the control group.

Histopathological studies

Gastric tissues of all animals were isolated and preserved in formalin solution. After proper fixing, tissues were processed and sectioned were made at a thickness of 5µm. Hematoxylin and eosin (H&E) dyes were used for staining (Bancroft and Gamble, 2002). Prepared slides were examined under a light microscope (PM-10ADS, Olympus Optical Co., Tokyo, Japan).

Statistical Analysis

All data were statistically analysed by using SPSS (version 23) and presented as Mean±SD. Data were

subjected to one-way ANOVA and post-hoc Duncan's multiple range test, with ($p \leq 0.05$) difference between groups considered as significant (Steel *et al*., 1997).

Results

Antiulcer activity of C. sativa

The gastroprotective effect of *C. sativa* is presented in Table 1 and Table 2. Aspirin administration significantly ($p \leq 0.05$) raised gastric acidic pH, acid volume and total acid output. Omeprazole treatment prevented an increase in gastric acidic pH, acid volume and total acid output on aspirin co-administration. *C. sativa* showed significant ($p \leq 0.05$) dose-dependent prevention of increase in gastric acidic pH, acid volume and total acid output as compared to the untreated ulcerative group.

Ulcer score and ulcer index were significantly ($p \leq 0.05$) increased with aspirin administration (Table 2). While non-significant ($p \leq 0.05$) difference between omeprazole and *C. sativa* treatments on the ulcer index and ulcer score was observed as compared to aspirin alone treated group. *C. sativa* has shown the dose-dependent antiulcer effect as the curative ratio was observed 53% at 10mg/kg and 70% at 20mg/kg, while 72% was observed with omeprazole treatment.

Table 1. Effect of *C. sativa* treatment on gastric acid secretion in aspirin induced-ulcerated rabbits.

Group	Treatment	Gastric Volume (ml)	Total Acid Output (mEq/L)	pH
I	NC	16.434 ± 0.369^{c}	71.98 ± 2.181^{b}	2.481 ± 0.119^{b}
II	ASA	20.768 ± 0.274^{d}	96.634 ± 4.173^{c}	1.623 ± 0.126^{a}
III	OMEP+ASA	11.368 ± 0.274^{a}	49.378 ± 3.614^{a}	4.074 ± 0.079^{d}
IV	CS 10+ASA	13.468 ± 0.296^{b}	65.828 ± 1.848^{b}	3.133 ± 0.065^{c}
V	CS 20+ASA	11.518 ± 0.296^{a}	50.621 ± 1.773^{a}	3.843 ± 0.111^{d}

NC-Normal control, ASA-aspirin treated, OMEP+ASA -Omeprazole plus aspirin-treated, CS 10+ASA-*C. sativa* (10mg/kg) plus aspirin-treated, CS 20+ASA-*C. sativa* (20mg/kg) plus aspirin-treated; variable letters express a significant ($p \leq 0.05$) difference between the groups.

Table 2. Effect of *C. sativa* treatment on gastric mucosal integrity in aspirin induced-ulcerated rabbits.

Group	Treatment	Ulcer score	Ulcer index	Curative Ratio
I	NC	_____	_____	_____
II	ASA	2.251±0.251c	14.624±0.336d	_____
III	OMEP+ASA	0.918±0.202b	3.974±0.118b	72.85%
IV	CS 10+ASA	1.501±0.225b	6.846±0.346c	53.15%
V	CS 20+ASA	1.001±0.184b	4.291±0.289b	70.66%

NC-Normal control, ASA-aspirin treated, OMEP+ASA -Omeprazole plus aspirin-treated, CS 10+ASA-*C. sativa* (10mg/kg) plus aspirin-treated, CS 20+ASA-*C. sativa* (20mg/kg) plus aspirin-treated; variable letters express a significant ($p \leq 0.05$) difference between the groups.

Antioxidant activity of C. sativa

The values of oxidative status parameters are presented in Table 3. Results showed significant ($p \leq 0.05$) increase in serum TOS and MDA level with aspirin. However, omeprazole administration significantly ($p \leq 0.05$) reduced the TOS and MDA level. *C. sativa* caused a reduction in TOS and MDA level comparable to omeprazole. Serum TAC and CAT activity significantly ($p \leq 0.05$) reduced with aspirin administration. While omeprazole treatment showed an increase in TAC and CAT activity. *C. sativa* exhibited a dose-dependent effect on TAC and CAT.

Histopathological examination of gastric tissues

Normal gastric epithelial surface of a normal control group (Fig. 1A). In Aspirin treated group, gastric epithelium indicated severe degenerative changes in the glandular region, necrosis, epithelial sloughing, edema and leukocytes infiltration (Fig. 1B) in comparison with the control group. Omeprazole administration showed protection against aspirin. Absence of hemorrhagic erosion, edema, epithelial degenerative changes were observed (Fig. 1C). *C. sativa* (10mg/kg and 20mg/kg) treatment showed dose-dependent protective effect. At 10mg/kg less necrotic changes were observed while 20mg/kg showed ulcer protection nearly to omeprazole (Fig. 1D&1E).

Table 3. Antioxidant effect of *C. sativa* in aspirin induced-ulcerated rabbits.

Group	Treatment	TOS (umol/L)	TAC (umol/L)	MDA (nmol/L)	CAT (ku/L)
I	NC	3.541±0.229^{a}	1.533±0.026^{c}	4.103±0.247^{a}	7.778±0.212^{c}
II	ASA	9.648±0.234^{d}	0.799±0.084^{a}	9.446±0.195^{c}	3.711±0.191^{a}
III	OMEP+ASA	4.773±0.179^{b}	1.423 0.044^{c}	4.516±0.278^{a}	7.618±0.225^{c}
IV	CS 10+ASA	6.663±0.343^{c}	1.166±0.087^{b}	7.111±0.259^{b}	5.756±0.189^{b}
V	CS 20+ASA	4.989±0.187^{b}	1.379±0.042^{c}	4.719±0.211^{a}	7.666±0.182^{c}

NC-Normal control, ASA-aspirin treated, OMEP+ASA -Omeprazole plus aspirin-treated, CS 10+ASA-*C. sativa* (10mg/kg) plus aspirin-treated, CS 20+ASA-*C. sativa* (20mg/kg) plus aspirin-treated; variable letters express a significant ($p \leq 0.05$) difference between the groups.

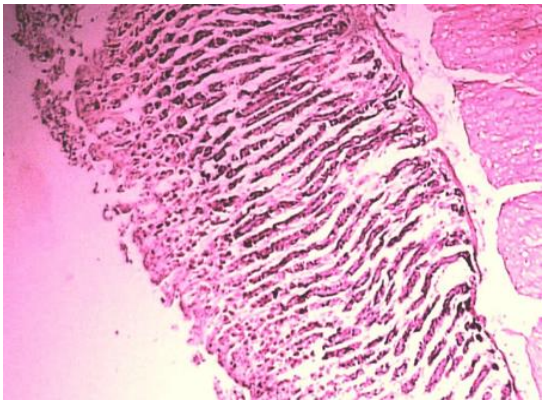

Fig. 1A. Normal control.

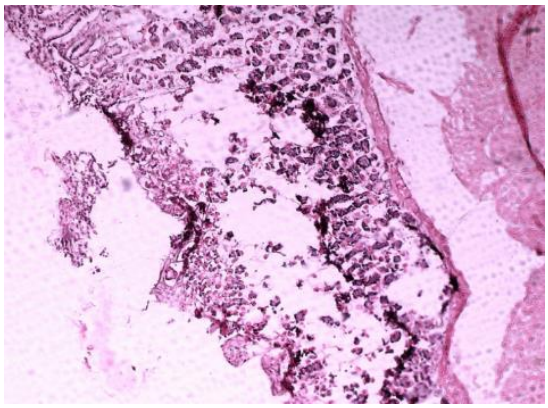

Fig. 1B. Aspirin (150mg/kg*day) alone treated group.

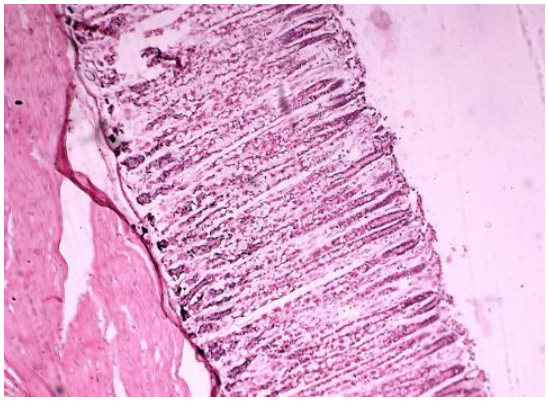

Fig. 1C. Reference group; Omeprazole (20mg/kg*day) plus aspirin (150mg/kg*day).

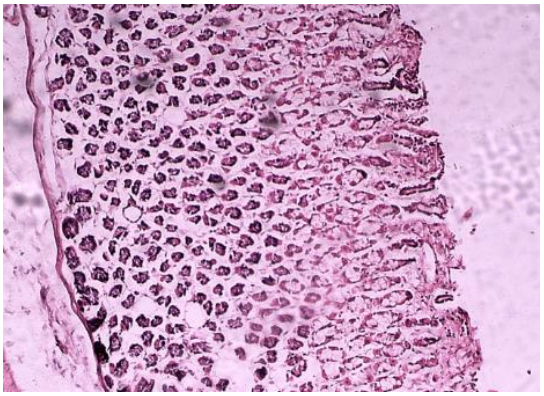

Fig. 1D. Treated group-I; *C. sativa* (10mg/kg*day) plus aspirin (150mg/kg*day).

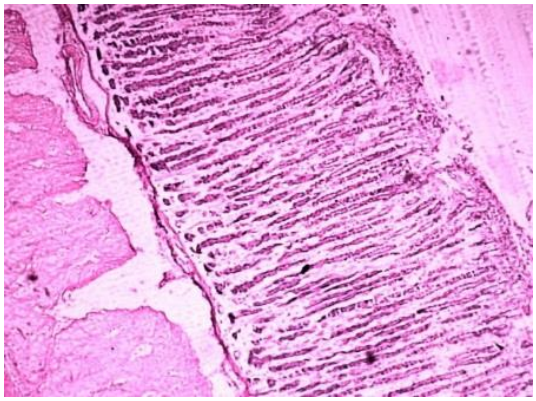

Fig. 1E. Treated group-II; *C. sativa* (20mg/kg*day) plus aspirin (150mg/kg*day).

Fig. 1(A-E). Histopathological examination has shown the effect of different treatments on aspirin-induced gastric mucosal lesions in rabbits (40X).

Discussion

Gastric ulcer is one of the most prevalent diseases of the gastrointestinal tract manifested by gastric mucosal lesions. Various factors included microbial infections (*H. Pylori*), NSAIDs, ROS and alcohol abuse induces gastric ulcer. NSAIDs are the most common drugs used for the treatment of osteoarthritis, rheumatoid arthritis, cardiovascular

diseases (CVD) and many others. Almost 30% of the population experienced NSAIDs induced gastric ulcer which may result in hospitalization and high mortality rate (Kavitt *et al*., 2019; Lanas and Francis, 2017; García-Rayado *et al*., 2018).

Prostaglandins (PG) enhances secretion of gastric mucus and bicarbonate ion. PG regulates of blood circulation towards the stomach. Aspirin causes gastric ulcer as it inhibits gastroprotective prostaglandins synthesis by non-selectively inhibiting cyclo-oxygenase enzymes. Overuse and long-term use of aspirin are linked to gastric ulcer induction. Enhanced leukocyte infiltration further exacerbates gastric mucosal damage by aspirin (Sarri *et al*., 2019; Silva *et al*., 2018).

Recently, the trend of herbal drugs is increased due to less ADRs associated with their use. According to the World Health Organization (WHO), to cure diseases 80% population of the world is now taking herbal drugs (Asnaashari *et al*., 2018). Traditional medicines have an advantage over synthetic drugs as these contain a combination of constituents which have multiple properties including anti-oxidant, analgesic, anti-inflammatory and antiulcer. Therefore, the present study was conducted for evaluation of the anti-ulcer effect of *C. sativa* leaves ethanolic extract in rabbits.

In this study, aspirin significantly ($p \leq 0.05$) increased the gastric acid volume and total acid output due to its acidic nature. Omeprazole significantly ($p \leq 0.05$) reduced the acid volume and total acid output. *C. sativa* at both doses along with aspirin reduced the acid volume and total acid output significantly ($p \leq 0.05$). However, *C. sativa* 20mg/kg produced comparable results to the synthetic antiulcer drug (El-Ghffar *et al*., 2018).

Results have been shown that aspirin administration causes ulcerative changes, ulcer score and ulcer index significantly ($p \leq 0.05$) increased as compared to control animals. Reduction in ulcer score and ulcer index were observed in omeprazole along with aspirin. *C. sativa* at 20mg/kg significantly ($p \leq 0.05$) reduced ulcer score, provide protection against aspirin. Ulcer score and ulcer index of *C. sativa* (20mg/kg) were not significantly different from omeprazole (Saha *et al*., 2018).

Aspirin use significantly ($p \leq 0.05$) increased gastric acidic pH. It exacerbated gastric ulcer by increasing gastric acidity which damages the gastric mucosa. Gastric acidic pH was significantly ($p \leq 0.05$) decreased with omeprazole and exhibited the preventive effect. Concomitant use of *C. sativa* (20mg/kg) and aspirin significantly ($p \leq 0.05$) reduced gastric acidic pH. *C. sativa* at 20mg/kg produced a similar increase in pH as omeprazole (Suheryani *et al*., 2017). Ulcer protection of *C. sativa* at 10mg/kg and 20mg/kg was observed in this study. *C. sativa* 10mg/kg showed 53.15% ulcer protection while *C. sativa* 20mg/kg showed ulcer protection 70.66% that was nearly similar to omeprazole 72.85% (Miranda *et al*., 2015).

Antioxidant effect of *C. sativa* was observed in this study by determining the serum levels of TOS, TAC, MDA and CAT activity. Antioxidants provide defensive mechanisms to prevent cellular damage. Increased oxidative stress is responsible for lipid peroxidation which results in impaired ion balance, membrane fluidity and integrity. Results of this study showed reactive oxygen species generation enhanced the oxidative stress in ulcerated groups. Aspirin administration increased the TOS and MDA while significant ($p \leq 0.05$) reduction in TAC and CAT enzyme activity was detected. Preventive effect of omeprazole was suggested by significant ($p \leq 0.05$) reduction in TOS and MDA, increase in TAC and CAT levels. *C. sativa* showed decrease generation of reactive oxygen species and revealed the anti-ulcer potential of *C. sativa* leaves extract by the anti-oxidative mechanism (Gregory *et al*., 2013; Mirje and Zaman, 2014).

Histopathological examination showed the gastroprotective effect of *C. sativa*. It observed that *C. sativa* administration prevented gastric epithelial damaging effects of aspirin. *C. sativa* leaves extract prevented the inflammation, gastric lesions, edema and leukocyte infiltration (Aslam *et al*., 2013; Omar, 2016).

Conclusion

This study suggested the anti-oxidative and anti-inflammatory potential of *C. sativa* extract for the prevention of gastric ulcer formation. Despite the large number of studies on *C. sativa,* supporting its gastroprotective effect, still, there is need of detailed clinical studies at the molecular level by using advanced novel technologies to evaluate the exact mechanism of action by which *C. sativa* shows gastro-protective effect.

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Novelty statement

Cannabis (C.) sativa is famous due to its psycho-active nature. In the current study, it is observed that *C. sativa* showed anti-oxidant and gastroprotective activities at low doses along with less psycho-active effects. Therefore, suggesting *C. sativa* as a potential antiulcer plant, requiring further clinical studies.

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Author contributions

SN and BA designed this study. SN and AH prepared the manuscript. MM helped in performing the biochemical analysis. SN and ZS guided in histopathological studies. SN and AH interpreted the results. FA statistically analysed the data. RS was involved in extract preparation, animal handling and sampling.

Conflict of interest

All authors are agreed and have no competing interest.

Abbreviation

CAT: catalase, MDA: malondialdehyde, TOS: total oxidant status, TAC: total antioxidant capacity,

References

Abdel-Salam OME, Amany AS, Eman RY, Fatma AM. 2018. Preventive effects of cannabis on neurotoxic and hepatotoxic activities of malathion in the rat. Asian Pacific Journal of Tropical Medicine **11(4),** 272-279. https://doi.org/10.4103/1995-7645

Akhtar AH, Kamal UA. 1995. Anti-ulcerogenic evaluation of the methanolic extracts of some indigenous medicinal plants in aspirin-ulcerated rats. Journal of Ethnopharmacology **46(1),** 1-6. https://doi.org/10.1016/0378-8741(94)01220-T.

Andre CM, Jean-Francois H, Gea G. 2016. *Cannabis sativa*: The Plant of the Thousand and One Molecule. Frontiers in Plant Science **7,** 7-19. https://doi.org/10.3389/fpls.2016.00019.

Aslam B, Wafa M, Ijaz J, Faqir M, Tanweer K, Junaid AK, *et al.* 2013. Gastroprotective effect of *Berberis vulgaris* seeds against gastric ulcer induced by aspirin in male adult albino mice. Indo American Journal of Pharmaceutical Research **3(6),** 4518-4527.

Asnaashari S, Siavash D, Yousef J. 2018. Gastroprotective effects of herbal medicines (roots). International Journal of Food Properties **21(1),** 902-920. https://doi.org/10.1080/10942912.2018.1473876.

Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, *et al.* 2009. Aspirin in the primary and secondary prevention of vascular disease: Collaborative meta-analysis of individual participant data from randomized trials. Lancet **373,** 1849-1860. https://doi.org/10.1016/S0140-6736.

Bancroft JD, Gamble M. 2002. Theory and Practice of Histological Techniques, 5th ed. Edinburgh; Churchill Livingstone.

Burke A, Smyth E, Fitzgerald GA. 2006. Analgesic-Antipyretic Agents. In: Goodman and Gilman, Pharmacological Bases of Therapeutics. 11th ed. New York; McGraw-Hill. p. 671-715.

El-Ghffar EAA, Eman AS, Safia MS, Omayma AE, Thomas E. 2018. The protective role of *Ocimum basilicum* L. (Basil) against aspirin-induced gastric ulcer in mice: Impact on oxidative stress, inflammation, motor deficits and anxiety-like behavior. Food and Function **9(8),** 4457-4468. https://doi.org/10.1039/c8fo00538a.

Erel O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry **37(4),** 277-285. https://doi.org/10.1016/j.clinbiochem.2003.11.015.

Erel O. 2005. A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry **38(12),** 1103-1111. https://doi.org/10.1016/j.clinbiochem.2005.08.008.

Frassinetti S, Eleonora M, Leonardo C, Morena G, Vincenzo L, Lorenza B, *et al.* 2018. Nutraceutical potential of hemp (*Cannabis sativa* L.) seeds and sprouts. Food Chemistry **262,** 56-66. https://doi.org/10.1016/j.foodchem.2018.04.078.

García-Rayado G, Mercedes N, Angel L. 2018. NSAID induced gastrointestinal damage and designing GI-sparing NSAIDs. Expert Review of Clinical Pharmacology **11(10),** 1031-1043. https://doi.org/10.1080/17512433.2018.1516143.

Goth L. 1991. A simple method for determination of serum catalase and reversion of reference range. Clinica Chimica Acta **2,** 143-151. https://doi.org/10.1016/0009-8981(91)90067-M.

Gregory M, Divya B, Revina AM, Viji MMH, Kalaichelvan VK, Palanivel V. 2013. Anti-ulcer activity of *Ficus religiosa* leaf ethanolic extract. Asian Pacific Journal of Tropical Biomedicine **3(7),** 554-556. https://doi.org/10.1016/S2221-1691(13)60112-4.

Guide for the Care and Use of Laboratory Animals. 2011. The National Academies Press. 8th ed. Washington (DC) USA.

Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D. *et al.* 2017. Global prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology **153(2),** 420429. https://doi.org/10.1053/j.gastro.2017.04.022.

Jaikumar S, Ramaswamy S, Asokan BR, Mohan T, Gnanavel M. 2010. Anti-ulcer activity of methanolic extract of *Jatro phacurcas* (Linn.) on aspirin-induced gastric lesions in wistar strain rats. Research Journal of Pharmaceutical Biological and Chemical Sciences **1,** 886-897.

Kaner J, Lapidot T. 2001. The stomach as a bioreactor, Dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. Free Radical Biology and Medicine **31(11),** 1388-1395. https://doi.org/10.1016/S0891-5849(01)0071.

Kavitt RT, Anna ML, Adjoa AY, Ian MG. 2019. Diagnosis and Treatment of Peptic Ulcer Disease. The American Journal of Medicine **132(4),** 447-456. https://doi.org/10.1016/j.amjmed.2018.12.009.

Kulkarni SK, Vallabh P. 2002. Hand Book of Experimental Pharmacology. New Delhi. p. 149-150.

Lanas A, Francis KLC. 2017. Peptic ulcer disease. Lancet **390(10094),** 613-624. https://doi.org/10.

Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J. 2012. Harrison's Principles of Internal Medicine. In: Valle JD, Ed. Peptic ulcer disease and related disorders. New York; McGraw-Hill. p. 683-688.

Miranda MA, Lemos M, Cowart KA, Rodenburg D, McChesney JD, Radwan MM, *et al.* 2015. Gastroprotective activity of the hydroethanolic extract and isolated compounds from the leaves of *Solanum cernuum* Vell. Journal of Ethnopharmacology **172,** 421-429. https://doi.org.

Mirje MM, Zaman SU. 2014. Evaluation of the antiulcer activity of *Ocimum sanctum L.* in indomethacin-induced gastric ulcers in albino rats. International Journal of Life Sciences Biotechnology and Pharma Research **3(1),** 274-279. https://doi.org/10.20959/wjpr20179-9196.

Mukherjee M, Bhaskaran N, Srinath R. 2010. Anti-ulcer and antioxidant activity of Gut Gard. Indian Journal of Experimental Biology **48,** 269-274.

Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxide in animal tissue by the thiobarbituric acid reaction. Analytical Biochemistry **95,** 351-358. *https://doi.org/*10.1016/0003-2697(79)90738-3.

Omar AS. 2016. Gastric acid inhibitory and gastric protective effects of *cannabis* and cannabinoids. Asian Pacific Journal of Tropical Medicine **9(5),** 413-419. *http://dx.doi.org/*10.1016/j.apjtm.2016.04.021.

Raju D, Ilango K, Chitra VI, Ashish K. 2009. Evaluation of the anti-ulcer activity of methanolic extract of *Terminalia chebula* fruits in experimental rats. Journal of Pharmaceutical Sciences and Research **1,** 101-107.

Saha L, Alka B, Amitava C. 2016. Gastroprotective effect of bezafibrate, a peroxisome proliferator-activated receptor α agonist and its mechanism in a rat model of aspirin-induced gastric ulcer. Advances in Digestive Medicine **3(3),** 101-110. https://doi.org/10.1016/j.aidm.2016.04.001.

Sarri GL, Grigg SE, Yeomans ND. 2019. Helicobacter pylori and low-dose aspirin ulcer risk: A meta-analysis. Journal of Gastroenterology and Hepatology **34(3),** 517-525. *https://doi.org/10.1111.*

Schubert ML. 2017. Physiologic, pathophysiologic, and pharmacologic regulation of gastric acid secretion. Current Opinion in Gastroenterology **33(6),** 430-438. https://doi.org/10.1097/MOG.00.

Silva DM, José LRM, Danillo RO, Iziara FF, Daiany PBS, Fernanda CAS. 2018. Effect of allantoin on experimentally induced gastric ulcers: Pathways of gastroprotection. European Journal of Pharmacology **821,** 68-78. *https://doi.org/10.1016.*

Steel RGD, Torrie GH, Dickey DA. 1997. Principles and procedures of statistics. New York; McGraw-Hill. p. 1746-1762.

Suheryani I, Yujuan L, Rongji D, Xiujie L, Shoaib A, Song J, 2017. Gastroprotective effects of Dregea sinensis Hemsl. (Daibaijie) against aspirin-induced gastric ulcers in rats. International Journal of Pharmacology **13(8),** 1047-1054. https://doi.org/10.3923/ijp.2017.1047.1054.

Vogel HG. 2002. Drug Discovery and Evaluation of Pharmacological Assay. 2nd ed. Germany; Springer. p. 759-767.

Wallace JL. 2013. Mechanisms, prevention and clinical implications of nonsteroidal anti-inflammatory drug-enteropathy. World Journal of Gastroenterology **19(12),** 1861-1867. *https://doi.org.*

Zhou Y, Shanshan W, Hongxiang L, Peihong F. 2018. Chemical constituents of hemp (Cannabis sativa L.) seed with potential anti-neuroinflammatory activity. Phytochemistry Letters **23,** 57-61. https://doi.org/10.1016/j.phytol.2017.11.013.