Open Access

Original Article

Evaluation of Nitric Oxide Activity of Torilis Leptophyllain Indomethacin Induced Gastric Ulcer in Mice

Saba Batool, Saima Noreen*, Tooba Malik, Javaria Fatima***, Bushra Shaheen****, Sheikh Maria Qammar*****

Department of Pharmacology, Combined Military Hospital, Multan/National University of Medical Sciences (NUMS) Pakistan, *Department of Pharmacology, Lahore Medical and Dental College, Lahore Pakistan, **Department of Pharmacology, Services Institute of Medical Sciences, Lahore Pakistan, ***Department of Pharmacology, Fatima Jinnah Medical University, Lahore Pakistan, ****Department of Pharmacology, Bakhtawar Ameen Medical and Dental College, Multan Pakistan, *****Department of Pharmacology, Rashid Lateef Medical and Dental College, Lahore Pakistan

ABSTRACT

Objective: To evaluate the Nitric Oxide activity of Torilis leptophylla in Indomethacin-induced gastric ulcers in mice.
Study Design: Laboratory-based experimental study
Place and Duration of Study: Pharmacology Department, University of Health Sciences, Lahore Pakistan, from Jan to Dec 2016.
Methodology: Thirty-six adult healthy male BALB/C mice with a weight range of 25-35 g were equally divided into six groups and designated as Group-I (Control), Group-II (Positive Control), Groups III to V (Torilis leptophylla extract trial Groups) and Group-VI (Omeprazole). Gastric ulceration was induced by a single dose of Indomethacin (20mg/kg) in Groups II–VI. The plant extract was administered in Groups III-V (100, 200, 300 mg/kg) whereas Omeprazole (3mg/kg) in Group-VI by gavage daily for three days as treatment of gastric ulceration. Nitric Oxide content was measured in gastric juice and serum of all groups by Nitric Oxide assay kit.
Results: The positive Control-Group showed little Nitric Oxide in gastric juice and serum. Torilis leptophylla treated Groups (100 mg/kg, 200mg/kg and 300 mg/kg) showed significantly raised Nitric Oxide content in both gastric juice and serum than the Control Group (p<0.001).
Conclusion: The study has determined that Torilis leptophylla possesses antiulcer and mucosal protective effects owing to the antioxidant activity of nitric oxide. This supports the use of the plant for the treatment of gastric ulcers.

Keywords: Gastric ulcer, Indomthacin, Nitric oxide, Torilis leptophylla.

How to Cite This Article: *Batool S, Noreen S, Malik T, Fatima J, Shaheen B, Qammar SM. Evaluation of Nitric Oxide Activity of Torilis Leptophyllain Indomethacin Induced Gastric Ulcer in Mice. Pak Armed Forces Med J 2023; 73(6): 1590-1593. DOI: https://doi.org/10.51253/pafmj.v73i6.6904*

INTRODUCTION

Peptic ulcer (PU) is one of the major gastro-intestinal diseases, affecting 5-15% of the global population.[1] Multiple gastro-protective factors include mucus, mucosal blood flow, bicarbonates, pro-staglandins (PGs) and nitric oxide. PU is caused by various aggressive factors like gastric acid, pe-psin, Helicobacter pylori, alcoholic drinks, stress, and ischemia followed by reperfusion.[2] Drugs, particularly non-steroidal anti-inflammatory drugs (NSAIDs), produce inflammation and hemorrhagic erosions in the mucosa. Indomethacin (IND) inhibits the synthesis of cytoprotective PGs, which leads to increased gastric acid secretion diminished bicarbonate and mucus secretion.[3]

Pathogenesis of gastric ulcers also contributes to reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide and hydroxyl radicals.[4] Antioxidants normally neutralize these radicals.[5,6] Nitric Oxide acts as an antioxidant in gastric mucosa. It regulates mucosal preservation by maintaining the formation of free radicals and facilitates mucus secretion and blood flow.[7]

Torilis leptophylla,commonly known as "Bristle fruit Hedge parsley", is found in Margalla, Hazara and Kashmir mountain areas.[8] Preceding phytochemical screening of T. leptophylla methanolic extract (TLM) proved the presence of Tannins, Phlorotannins, Alkaloids and Terpenoids.[9] Literature has reported its protective effect in CCl4-induced hepatotoxicity owing to its antioxidant properties.[10] Fruit extract of plants showed significant inhibition of the growth of microorganisms.[8] We, therefore, hypothesized that TLM could restore the gastric mucosa and help combat the inflammation of gastric tissue injury by raising Nitric Oxide levels. In the present study, we attempted to investigate the effects of T. leptophylla related to Nitric Oxide antioxidant activity.

METHODOLOGY

The laboratory-based experimental study was conducted at the Department of Pharmacology,

Correspondence: Dr Saba Batool, Department of Pharmacology, Combined Military Hospital, Multan Pakistan
Received: 16 Jun 2021; revision received: 11 Apr 2022; accepted: 12 Apr 2022

University of Health Sciences, Lahore, Pakistan from January to December 2016. The study was performed per standard animal handling guidelines issued by the Ethical Review Committee for Medical and Biomedical Research, UHS, Lahore.

Inclusion Criteria: Adult healthy male BALB/c mice with the weight range of 25-35 g were included.

Exclusion Criteria: Mice with any obvious injury and disease were excluded.

Pharmaceutical-grade drugs, chemicals and Nitric Oxide assay kits were purchased from authorized dealers in Pakistan. The flowering shoots of T. leptophylla were collected in April 2016 from the Botanical Garden, Quaid-e-Azam University, Islamabad. The plant specimen was submitted to the Director of the National Agriculture Research Centre (NARC), Islamabad, for identification and authentica-tion. Five hundred grams of dry flowering shoots were crushed into small pieces. The sample was soaked in 95% methanol at 25 C for three days. A rotary evaporator filtered and concentrated the resultant mixture under reduced pressure at 40C. The concentrate was freeze-dried by lyophilizer and stored at -4C for further pharmacological evaluation. Thirty-six adult healthy male BALB/c mice (25-35 g) were randomly divided into six Groups (n=6). The animals were kept at controlled room temperature (22-24C), humidity (45-65%) and natural 12/12h light and dark cycle. All animals were fed on rodent chow and water ad libitum. They were kept for one week in the Experimental Research Laboratory, UHS, Lahore, to acclimatize to the environment. Gastric ulceration in mice was induced by a single oral dose of Indomethacin (20 mg/kg) .[11,12] The mice were fasted but had free access to water 24 hours prior to the drug administration.

Antiulcer therapy was commenced 6 hours after induction of the ulcer in treatment Groups (III-VI). Group-I served as a negative control, and Group II, as a positive control (ulcer induced), was given distilled water by gavage once daily for three days. Ulcerinduced mice of treatment Groups (III-VI) were given T.leptophylla extract in 100, 200, and 300 mg /kg and Omeprazole 3 mg/kg by gavage once daily for three days, respectively. The blood samples were collected through cardiac puncture four hours after the last dose administration. Then centrifuged at 4000 r.p.m for 5 min at 10C, clear serum samples were separated and stored at -80C until further analysis. The samples of gastric contents were collected through pylorous ligation and centrifuged at 3500 rpm for ten min. Nitric Oxide concentrations in gastric juice and serum of all Groups were measured by Nitric Oxide assay kit, colourimetric.

Statistical Package for Social Sciences (SPSS) version 20.0 was used for the data analysis Quantitative data was described in the form of Mean±S.D. One-way ANOVA was applied, followed by post hoc Tukey's test. The *p*-value of ≤0.05 was considered statistically significant.

RESULTS

The Nitric Oxide concentration was measured in the serum and gastric juice of all the Experimental Groups on Day 3. Group-I showed 135.53 14.14 µmol/L in gastric juice and 71.51±11.61 in serum. The Positive Control-Group depicted significantly decreased Nitric Oxide concentration in gastric juice, a mean of 86.1±15.3, thus demonstrating that Indomethacin has significantly induced gastric ulceration. Treatment Groups III-VI showed 124.30±13.2, 119.26±14.9, 129.31±9.86, & 120.13±11.13, respectively, at day 3. Similarly, Group-II represented significantly reduced Nitric Oxide concentration in serum, a mean of 45.80±8.0 compared to Group-I. Serum Nitric Oxide concentration in Groups III-VI showed 56.82±8.59 µmol/L, 64.24±9.83 µmol/L, 66.174±9.725 µmol/L and 52.78±7.68 µmol/L respectively and was as compared to Group II (Table-I) Treatment with T. leptophylla for three days raised the Nitric Oxide concentration in gastric juice and serum. InterGroup comparison of all six Groups for both parameters depicted a statistically significant *p*-value<0.001, as shown in Table-II.

Table-I: Effect of T. leptophylla on Nitric Oxide Concentration in serum and Gastric Juice of Indomethacin Induced Gastric Ulcer in Mice (n=36)

Parameters	Group-I (n=6)	Group-II (n=6)	Group-III (n=6)	Group-IV (n=6)	Group-V (n=6)	Group–VI (n=6)	*p*-value Anova
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Nitric Oxide in serum (µmol/L)	71.51±11.61	45.80±8.0	56.82±8.59	64.24±9.83	66.17±9.72	52.78±7.68	<0.001
Nitric Oxide in gastric juice (µmol/L)	135.53±14.14	86.1±15.3	124.30±13.2	119.26±14.9	129.31±9.86	120.13±11.13	<0.001

Table-II: Intergroup comparison of Nitric Oxide Concentration in serum and Gastric Juice of Indomethacin Induced Gastric Ulcer in Mice(n=36)

Group Comparison	Group-I Vs. Group-II	Group-II Vs. Group-III	Group-I Vs. Group-III	Group-II Vs. Group-IV	Group-I Vs. Group-IV	Group- III Vs. Group-IV	Group-I Vs. Group-V	Group-II Vs. Group-V	Group-I Vs. Group-VI	Group-II Vs. Group-VI
Nitric Oxide in serum (µmol/L)	0.001	0.343	0.100	0.021	0.756	0.740	0.917	0.008	0.018	0.786
Nitric Oxide in gastric juice (µmol/L)	<0.001	<0.001	0.687	0.002	0.303	0.985	0.963	<0.001	0.360	0.001

DISCUSSION

The concentration of Nitric Oxide measurement is difficult because of its brief half-life. Consequently, nitrate and nitrite levels, which are stable end products of Nitric Oxide metabolism, were used as markers.[13,14] Nitric Oxide shields the mucosa through the secretion of mucus and electrolytes via guanylate cyclase activation.[15] It serves as a potent vasodilator and thus improves the mucosal blood flow. Impaired Nitric Oxide generation can lead to inflammation, vasoconstriction and tissue injury.[16]

In our study, concentration of Nitric Oxide in gastric juice of Group-II (Positive Control) displayed a decline in concentration by 37 % as compared to the Control Group. Compared to the diseased Group, T. leptophylla treatment Groups demonstrated an increase in Nitric Oxide concentration in gastric juice by 31%, 28 % and 34% in minimum, median and maximum dose groups. Standard drug Omeprazole also demonstrated a significant increment of Nitric Oxide concentration by 29% as compared to the Positive Control Group. Groups III and V showed raised Nitric Oxide content in stomach juice by 4% and 8%, respectively, compared with Group-VI. Nitric Oxide concentration in gastric juice was almost the same in Groups IV and V. Pan *et al*. demonstrated a 21% increase in Nitric Oxide concentration in gastric juice in mice by the treated Group. The result of berberine resembles that of the plant under study.[17]

Similarly, reduced Nitric Oxide concentration in serum was observed in the Indomethacin-induced Group by 36% compared to the control Group. Plant treatment showed dose-related increments in serum Nitric Oxide concentration by 20%, 31% and 32% with minimum, median and maximum doses of plant extract, respectively, compared to the positive control Group. Omeprazole showed a 13% increase in serum Nitric Oxide compared to diseased mice. T. leptophylla demonstrated a better serum Nitric Oxide concentration increment than the standard drug. Similarly, an increased concentration of Nitric Oxide in serum was observed by 4% with the standard drug (ranitidine) and 34% with Piptadeniastrum africanum.[14] The current study supported the notion that Indomethacin reduces Nitric Oxide levels. Nitric Oxide contributes to restoring the gastric mucosa damaged by Indomethacin.[18,19] Better results observed in the present study may be attributed to the better protection of T. leptophylla against oxidative stress induced by GU.

ACKNOWLEDGEMENT

The authors would like to thank Prof. Dr Naseem Saud Ahmed, Head of the Pharmacology Department, UHS, Lahore, for his supervision. Special gratitude to UHS, Lahore, for facilitating us throughout this project.

CONCLUSION

Torilis leptophylla extract showed effectiveness against Indomethacin-induced gastric ulcer in mice. The Nitric Oxide concentrations in gastric juice and serum were raised, probably due to its antioxidant properties. Besides the results obtained in this experimental study, it is recommended that further extractions and isolation from T. leptophylla could be used for the development of new phytotherapeutic drugs to treat gastric ulcers.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

SB & SN: Conception, study design, drafting the manuscript, approval of the final version to be published.

TM & JF: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

BS & SMQ: Data acquisition, drafting the manuscript, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Katzung BG, ed. Basic and clinical pharmacology. 14th Edition. New York: McGraw-Hill Education; 2018.

2. Scally B, Emberson JR, Spata E, Reith C, Davies K, Halls H, et al. Effects of gastroprotectant drugs for the prevention and treatment of peptic ulcer disease and its complications: a meta-analysis of randomised trials. Lancet Gastroenterol Hepatol 2018; 3(4): 231-241. https://doi.org/10.1016/s2468-1253(18)30037-2.
3. Katary MA, Salahuddin A. Gastroprotective effect of vanillin on indomethacin-induced gastric ulcer in rats: protective pathways and anti-Secretory mechanism. Clin Exp Pharmacol 2017; 7(2): 2161. http://dx.doi.org/10.4172/2161-1459.1000232.
4. Vona R, Pallotta L, Cappelletti M, Severi C, Matarrese P. The Impact of Oxidative Stress in Human Pathology: Focus on Gastrointestinal Disorders. Antioxidants 2021; 10(2): 201-205. https://doi.org/10.3390/antiox10020201.
5. Huang H, Ullah F, Zhou DX, Yi M, Zhao Y. Mechanisms of ROS regulation of plant development and stress responses. Front Plant Sci 2019; 10: 1800. https://doi.org/10.3389/fpls.2019.00800.
6. Sun G, Lian T, Yang B, Gu Y, Li X. Ameliorative effect of Sargassum fusiforme polysaccharides on oxidative stress and inflammation in ethanol-induced gastric ulcer. Pharmacogn Mag 2019; 15(61): 244. https://dx.doi.org/10.4103/pm.pm_457_18.
7. Abdelfattah MS, Elmallah MI, Ebrahim HY, Almeer RS, Eltanany RM, Abdel Moneim AE, et al. Prodigiosins from a marine sponge-associated actinomycete attenuate HCl/ethanol-induced gastric lesion via antioxidant and anti-inflammatory mechanisms. PloS One 2019; 14(6): e0216737. https://doi.org/10.1371/journal.pone.0216737.
8. Maleki S, Seyyednejad SM, Damabi NM, Motamedi H. Antibacterial activity of the fruits of Iranian Torilis leptophylla against some clinical pathogens. Pak J Biol Sci 2008; 11(9): 1286-1289. https://doi.org/10.3923/pjbs.2008.1286.1289.
9. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complement Altern Med 2012; 12(1): 1-2. https://doi.org/10.1186/1472-6882-12-221.
10. Shah NA, Khan MR, Nadhman A. Antileishmanial, toxicity, and phytochemical evaluation of medicinal plants collected from Pakistan. BioMed Res Int 2014; 2014: 1-7. https://doi.org/10.1155/2014/384204.
11. Jiang GL, Im WB, Donde Y, Wheeler LA. EP4 agonist alleviates indomethacin-induced gastric lesions and promotes chronic gastric ulcer healing. World J Gastroenterol 2009; 15(41): 5149. https://doi.org/10.3748%2Fwjg.15.5149.
12. Banerjee D, Bauri AK, Guha RK, Bandyopadhyay SK, Chattopadhyay S. Healing properties of malabaricone B and malabaricone C, against indomethacin-induced gastric ulceration and mechanism of action. Eur J Pharmacol 2008; 578(2-3): 300-312. https://doi.org/10.1016/j.ejphar.2007.09.041.
13. Ateufack G, Mokam EC, Mbiantcha M, Feudjio RB, David N, Kamanyi A, et al. Gastroprotective and ulcer healing effects of piptadeniastrum Africanum on experimentally induced gastric ulcers in rats. BMC Complement Altern Med 2015; 15(1). https://doi.org/10.1186/s12906-015-0713-5.
14. Albayrak A, Alp HH, Suleyman H. Investigation of antiulcer and antioxidant activity of moclobemide in rats. Eurasian J Med 2015; 47(1): 32. https://doi.org/10.5152%2Feajm.2014.0034.
15. Sayed DA, Fahmy SR, Soliman AM, Hussein NS. Antiulcerogenic efficacy of ethanolic extract of Vitis vinifera leaves in rats. Int J Pharm 2016; 8(9): 163-172. https://doi.org/10.22159/ijpps.2016v8i9.12846.
16. Wallace JL. Nitric oxide in the gastrointestinal tract: opportunities for drug development. Br J Pharmacol 2019; 176(2): 147-154. https://doi.org/10.1111/bph.14527.
17. Pan LR, Tang Q, Fu Q, Hu BR, Xiang JZ, Qian JQ, et al. Role of nitric oxide in protective effect of berberine in ethanol-induced gastric ulcer mice. Acta Pharmacol Sin 2005; 26(11): 1334-1338. https://doi.org/10.1111/j.1745-7254.2005.00186.x.
18. Kumar S, Prahalathan P, Saravanakumar M, Raja B. Vanillic acid prevents the deregulation of lipid metabolism, endothelin 1 and up regulation of endothelial nitric oxide synthase in nitric oxide deficient hypertensive rats. Eur J Pharmacol 2014; 743: 117-125. https://doi.org/10.1016/j.ejphar.2014.09.010.
19. Majouli K, Hamdi A, Abdelhamid A, Bouraoui A, Kenani A. Anti-inflammatory activity and gastroprotective effect of Hertia cheirifolia L. roots extract. J Ethnopharmacol 2018; 217: 7-10. https://doi.org/10.1016/j.jep.2018.02.010.