WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 5, 406-421. Research Article ISSN 2277– 7105

ANTI-ULCER ACTIVITY OF MORINGA OLEIFERA LEAVE'S EXTRACT IN SWISS ALBINO MICE AGAINST ASPIRIN INDUCED PEPTIC ULCER

Hamid A. K.[1]*, Shariq B.[2,4], Jiyauddin K.[1], M. Kaleemullah[1], Samer A. D.[1] Sakina R.[3], Rasha S.[1]

[1]School of Pharmacy, Management & Science University, 40100 Shah Alam, Selangor Darul Ehsan, Malaysia.

[2]Faculty of Health and Life Sciences, Management & Science University, 40100 Shah Alam, Selangor Darul Ehsan, Malaysia.

[3]International Medical School, Management & Science University, 40100 Shah Alam, Selangor Darul Ehsan, Malaysia

[4]School of Graduate Studies, Management & Science University, 40100 Shah Alam, Selangor Darul Ehsan, Malaysia.

Article Received on
10 March 2015,

Revised on 01 April 2015,
Accepted on 22 April 2015

***Correspondence for Author**
Dr. Hamid Ali Kazi
School of Pharmacy, Management & Science University, 40100 Shah Alam, Selangor Darul Ehsan, Malaysia.

ABSTRACT

The objective of this study was to evaluate the anti ulcer activity of Moringa Oleifera leaves in swiss albino mice against aspirin induced peptic ulcer. After administration of aqueous extract of Moringa Oleifera, ulcer index, protective index, evaluation and staining of EC cell and determination of mucosal thickness was evaluated. In biochemical parameters, free mucin content, lipid per oxidation, superoxide dismutase was evaluated. HE staining was performed as well. Ranitidine was used as a positive control. All the macroscopic and biochemical parameters showed significant anti ulcer activity of Moringa Oleifera. The anti ulcer activity was almost comparable to the positive control. P value less than 0.05 was considered significant. Mean ± S.E.M and one-way ANOVA followed by multiple comparison t-test were used to analyze the result. Our study shows that Moringa has considerable anti ulcer activity.

KEYWORDS: Moringa Oleifera, Swiss albino mice, Aspirin, Peptic ulcer and Ranitidine etc.

INTRODUCTION

Peptic ulcer is a disease characterized by mucosal damage that usually occurs in the stomach and proximal duodenum.[1] It is a serious injury occurring by spicy food, stress, alcohol, gastric surgery and Helicobacter pylori.[2] Aspirin (ASP), one of the most widely used NSAIDs, damages gastrointestinal mucosa by irritant action, causing alterations in mucosal permeability and/or suppression of prostaglandin synthesis.[3,4] Aspirin induced ulcer has been used as a model for the evaluation of anti-ulcerogenic agents.[5]

Moringa oleifera is rich in fairly unique group of phytochemicals, glucosinolates and isothiocyanates.[6,7] Moringa oleifera leaves contain more vitamin A than carrots, more calcium than milk, more iron thanspinach, more vitamin C than orange and more potassium than bananas[8,9,10] and protein quality is more than milk and eggs. In many cultures through the tropics, different parts of this plant are being used as foods as well as medicinal purpose.[11] Leaves of this plant have been reported to possess hypotensive, antispasmodic, diuretic, abortifacient,[12,13]wound healing,[14] analgesic,[15] hepatoprotective,[16,17] anti-tumor agent[18] and radio-protective effect.[19] Scientific investigations showed that leaves of this plant have depressant effect on heart, reduce stomach pain, exhibit hypocholestermic, antifertility effect and regulate thyroid hormone status.[20,21,22,23]

Moringa leaves possess high quantity of thiamine and riboflavin beside two major bioactive nitrile glycosides namely niaziridin and niazirin in considerable amount along with other vitamins.[24,25] In India and Zimbabwe, young leaves of Moringa are used as cattle fodder to improve milk yields by farmers.[26,27]

The phytochemical benzyl isothiocyanate, isolated from moringa has already been proved as potent antimicrobial agent.[28.29]

Antimicrobial activity of the primary rhamnosylated compound from this plant has been shown.[30,31] Its leaves are also used as nutritional supplement and growth promoters due to the significant presence of protein, Se, P, Ca, β-carotene and ¸α-tocopherol[32,33,34,35,36] and they are not toxic to humans or animals.[37]

MATERIALS AND METHODS

Plant material and drugs: Aqueous extract of Moringa leaf was prepared following the method of Tahiliani and Kar.[23] The extract was stored at 4ºC until further use.[38] In order to

dissolve the extractions later, 4% DMSO was used. Aspirin and ranitidine were obtained from the MSU chemistry Lab.

Experimental Animals: Swiss Albino mice of both sexes weighing 20-30 g were used. The experimental protocol was approved from MSU Animal Ethics Committee. The animals were given standard rodent food and water ad libitum under standard laboratory conditions.

Animals were divided into four groups containing 10 mice each. Control group mice were given water only. Group 2, 3 and 4 were given aspirin (300mg/kg) in a single dose with a trochar needle.[39] While group 3 and 4 were given ranitidine (10 mg/kg),[40] and Moringa leaf extract (200 mg/kg) respectively as well along with aspirin.

Parameters studied

Determination of ulcer index: Ulcer index was determined using magnifying glass as described by Bandyopadhyay *et al*.[41]

Mean ulcer index: The mean ulcer index was calculated following the methods of Szabo *et al*.[42]

Determination of protective index

The protective index was calculated according to the method of El-Abhar et al,[43] while using the following formula

The protective index = mean ulcer index of aspirin – (mean ulcer index of aspirin + MO) x 100

Mean ulcer index of aspirin

Evaluation and staining of EC cell

Stomach tissues were rinsed in 0.9% saline, fixed in Bouin's fluid and stained to study EC cells following the method of Singh.[44]

Determination of mucosal thickness

Mucosal thickness was measured by the method of Sarkar and Guha.[45]

Biochemical estimations

Determination of free mucin content : The free mucin content in the gastric tissues was estimated by method described by Tariq and Al montaeryA.[46]

Measurement of lipid peroxidation (LPO)

The method of Buege and Aust[47]was used to determine Lipid peroxidation quantity.

Determination of gastric superoxide dismutase (SOD)

SOD was analyzed by the methods of McCord et al,[48] and Martin et al methods[49] respectively.

Determination of glutathione reductase (GR) and glutathione peroxidase (GPx)

These parameters were determined as described by Krohne-Ehrich,[50] Paglia and Valentine[51] with some modifications used by Chattopadhyay et al.[52]

Histological studies

Histological studies were performed by Haematoxylin and eosin staining by a routine procedure.[53]

Statistical analysis

All data were expressed as mean ± S.E.M, and analyzed by the one-way analysis of variance (ANOVA) followed by multiple comparison‘t’ test using SSPS software 20 version. Difference below the probability level of less than 0.05 ($P < 0.05$) was considered statistically significant.

RESULTS

Macroscopic study clearly shows that there are no ulcer spots and the ulcer index has been reduced to a minimum of (**$P \leq 0.05$ vs. Aspirin fed group) in 200 mg/kg MO fed group.

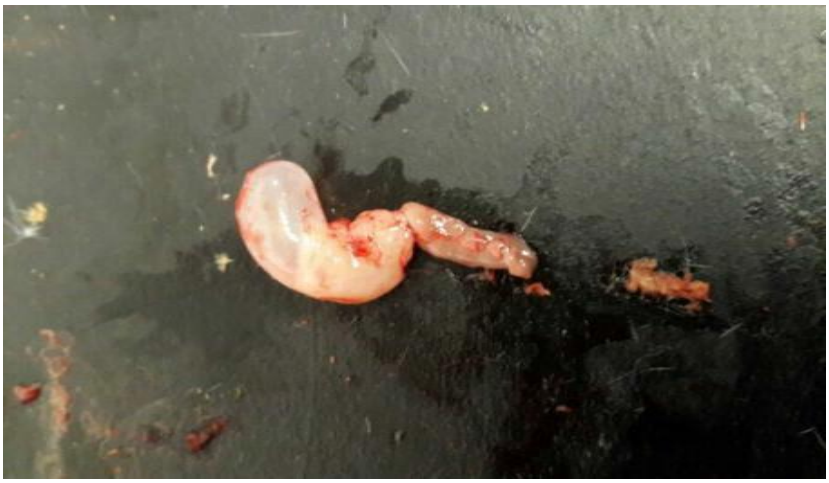

Figure 1 Group 1 (Control)

Figure 2 Group 2 (Aspirin 300mg/kg)

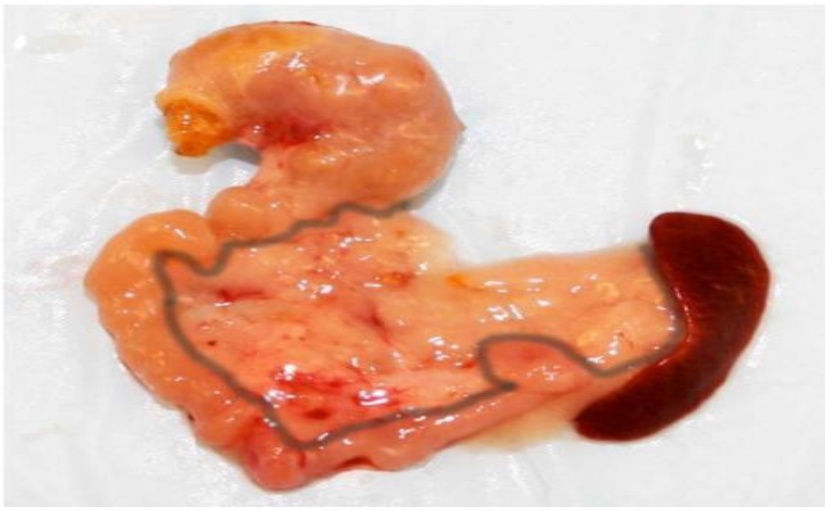

Figure 3 Group 3 (Ranitidine 10mg/kg + Aspirin 300mg/kg)

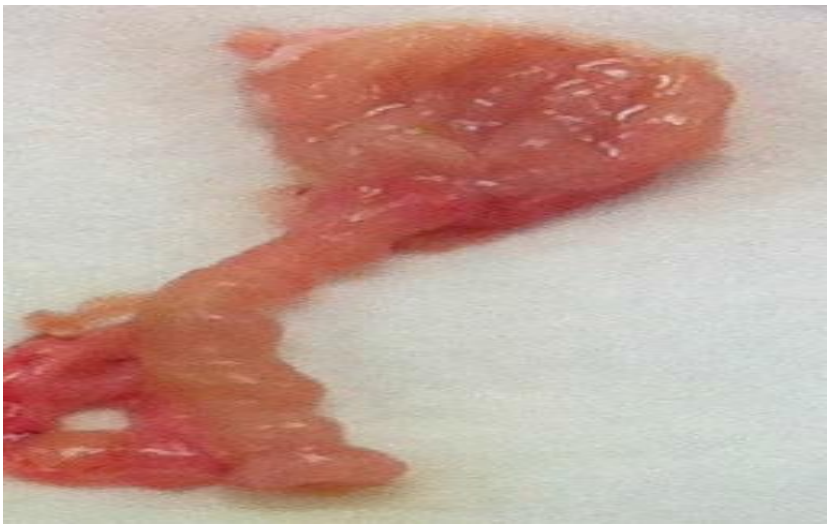

Figure 4 Group 4 (MO leaf extract 200mg/kg + Aspirin)

Mean ulcer index

Treatment with MO leaf extract for 14 consecutive days reduced the severity of ulcer intensity.In this study significant increase in the extent of ulceration mainly in glandular part of gastric mucosa was observed after aspirin treatment, as evidenced by increased mean ulcer index ($p < 0.05$).

Protective index

It was observed that MO group exhibited maximum protection of 93.75% while ranitidine group showed 60.80%.

EC cell count

EC cell density of stomach tissue also decreased after Aspirin treatment ($P < 0.05$). MO treatment increased the number of EC cells as compared to ulcerated group. But ranitidine treatment did not affect the EC cell density (Table 1).

Mucosal thickness

MO treatment for 14 days increased mucosal thickness in group 4 while ranitidine treated group wasnot found to have increasedthe thickness as evidenced by theresults ($P < 0.05$, Table 1).

Table 1. Effect of MO leaf extract on mean ulcer index, % of protection, EC cell density and mucosal thickness of stomach tissues in aspirin induced experimental mice

GROUPS	Ulcer index	%Protection	EC cell (cells/mm3)	Mucosal thickness (µm)
Standard	0.058±0.041	--- --- ---	2200.50 ± 52.50	74.4 ± 3.22
Control (Aspirin) 300mg/kg	1.467±0.089	--- --- ---	1054.5 ± 43.83*	50.5 ± 2.12*
Ranitidine (10mg/kg)	0.575±0.135*	60.80	2172.70 ± 30.50#	66.5 ± 3.15#
MO leaf extract (200mg/kg)	0.092±0.046*	93.75	2183.33 ± 29.59#	80.8 ± 2.55

Values are in mean ± S.E.M. from 10 animals in each group; statistical analyses were done using one-way ANOVA followed by multiple comparison t-test.

* $P < 0.05$ compared to control, # $P < 0.05$ when compared to ulcer.

Biomarkers of oxidative stress altered in aspirin induced gastric ulcer. MO group showed reduction in lipid per-oxidation level by 53.7% (*$P \leq 0.05$ vs. Aspirin fed group).

Table 2. Lipid per oxidation levels and alterations in the activities of key antioxidant enzymes in mice gastric tissue

Antioxidant enzymes (units of activity)	Control	Aspirin treated (Px)	Ranitidine (10mg/kg)	MO leaf extract treated (200mg/kg)
LPO (nmolesTBARS/mg protein)	0.21±0.03	0.92±0.06[a]	0.25±0.02[b]	0.17±0.02
Gastric peroxidase (units/min/mg of tissue protein)	64.41±1.71	26.32±1.11[a]	54.55±1.21[b]	64.98±1.33
Glutathione peroxidase (nmol of NADPH produced/min/mg oftissue protein)	0.18±0.01	0.55±0.02[a]	0.21±0.01[b]	0.17±0.01
Cu–Zn superoxide dismutase (units/min/mg of tissue protein)	1.66±0.21	5.17±0.24[a]	1.94±0.27[b]	1.55±0.24
Mn superoxide dismutase (units/min/mg of tissue protein)	0.82±0.12	2.69±0.21[a]	1.06±0.19[b]	0.87±0.08
Glutathione reductase activity (units/min/mg of tissue protein)	0.19±0.01	0.47±0.02[a]	0.24±0.01[b]	0.20±0.01

[a] $P \leq 0.001$ compared to control values using ANOVA.

[b] $P \leq 0.001$ compared to piroxicam treated values using ANOVA.

H&E stained gastric tissue sections of control group mice and that of MO treated group showed no prominent blood vessels in the mucosa and submucosa. Treatment of mice with aspirin resulted in marked changes in gastric tissue morphology. The mucosa of the gastro-oesophageal junction had few eosinophilic infiltration but sub mucosa showed having both neutrophilic and eosinophilic infiltration in aspirin treated animal group. Tissue sections of aspirin treated animals were discontinuously stained pink along the mucosal border due to degeneration and sloughing of mucosal cells.

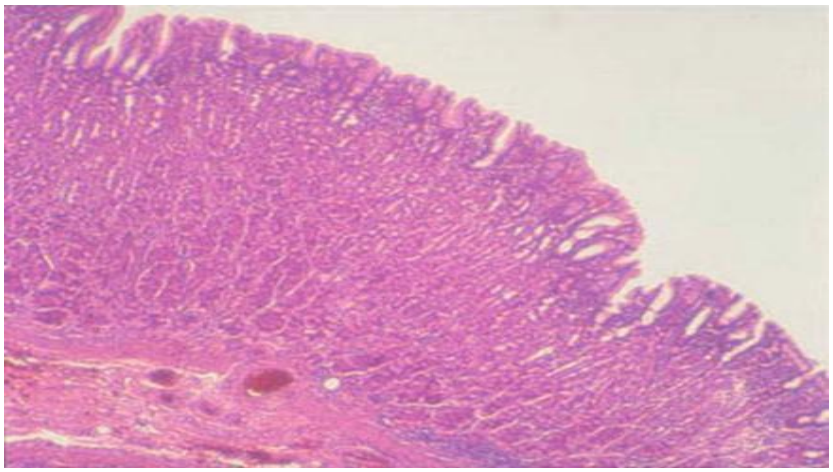

Figure 5 Group 1 (Control)

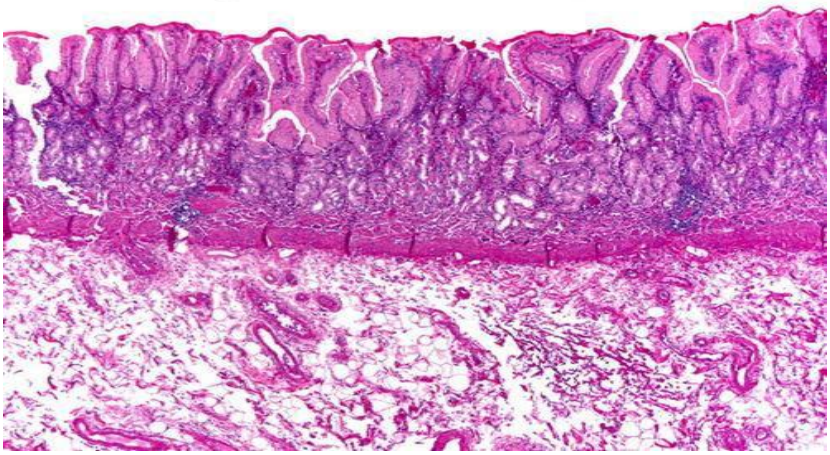

Figure 6 Group 2 (Aspirin)

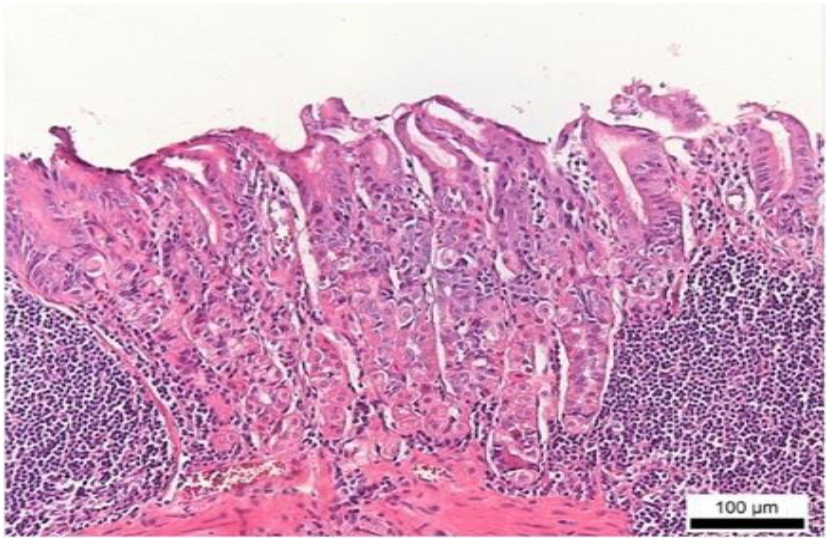

Figure 7 Group 3 (Ranitidine)

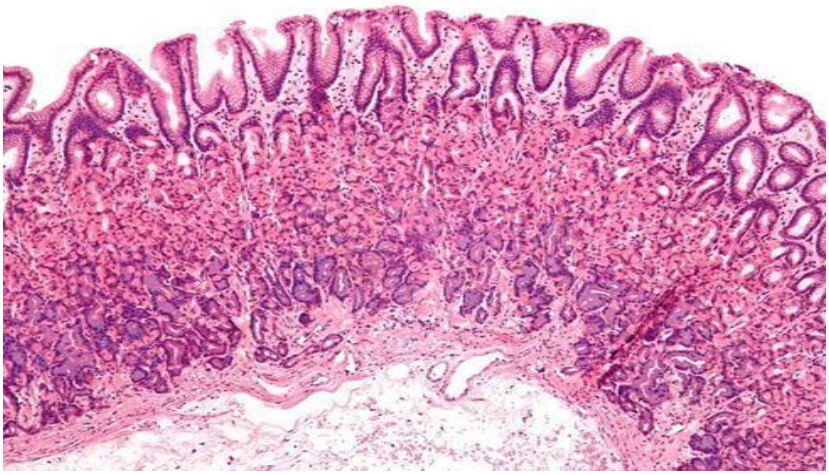

Figure 8 Group 4 (MO leaf extract)

Haematoxylin eosin stained sections reveal that mucosal bleeding occurred due to aspirin feeding, which was protected when doses of the aqueous extract were administered before aspirin feeding. Photographs of the inner surface of stomach show no ulcer spots in the mice fed with 200 mg/kg doses of the MO aqueous extract.

DISCUSSIONS

Gastric ulcers have multiple etiopathogenesis.[54] Gastric mucin is an important factor in protecting gastric mucosa from physical damage and back diffusion of hydrogen ions. Depletion in mucin content in aspirin-administered animals possibly occurs due to the adverse effects of free superoxide anion and hydroxyl radicals. Gastro-mucosal mucin depletion was protected on pre-administration of aqueous MO leaf extract in aspirin-fed animals. Microscopic study of H&E stained gastric sections puts forward the possibility that the leaf extract might have increased or changed the nature of mucous secreted in stomach. Stomach tissues of aspirin fed animals showed increased acid mucin secretion, which was minimized to a great extent in aqueous extract pre-treated aspirin-fed animals.

Considering the rich source of antioxidants in MO leaf extract, we used the dose of 200 mg/kg to study the effect of extract on aspirin induced gastric oxidative stress and ulcer. Our study confirms earlier reported antioxidant activity of MO in vitro and in vivo, against hydroxyl radicals generated by Fenton reaction.[19,30] MO has been reported to contain various antioxidants including Vitamin C.[55] Our macroscopic and histopathological studies showed that almost no ulcerative damage occurred in mice when they were pretreated with the antioxidant rich aqueous leaf extract. Phenol compounds have some antioxidant activity.[56,57] They are able to terminate free radicals and chelate metal ions that are capable of catalyzing formation of ROS that promote lipid per oxidation.[58]

We observed increased accumulation of thio barbituric acid reactive substances (TBARS) and protein carbonyls in gastric tissues of aspirin treated mice indicating involvement of oxidative stress. Administration of aspirin at 300 mg/kg dose further depleted reduced glutathione in gastric tissue.These findings were consistent with earlier reports on piroxicam induced gastric ulcer.[39,59] Increase in lipid peroxidation and protein oxidation by 2.16 folds and 5.57folds from control levels respectively resulted in increased consumption of glutathione. It is also well known that pods and leaves of moringa are rich source of ascorbic acid.[55] Ascorbic acid acts as an antioxidant molecule and its beneficial effects could be

attributed to its ability to form a poorly ionized but soluble complex with toxic metal/metalloid.[60]

Significant decreases in the activities of antioxidant enzymes like gastric peroxidase and increase in the activities of glutathione reductase, glutathione peroxidase, and superoxide dismutases indicate a growing imbalance in oxidants and antioxidants in gastric tissues after aspirin administration. Aqueous moringa leaf extract protected against aspirin induced alterations in activities of antioxidant enzymes. One study has clearly emphasized hydroxyl radical to be the principal causative agent in piroxicam mediated gastric ulcer.[61]

Increased oxidative stress encountered in body due to either environmental hazard, or impairment in the body metabolism due to varying disease conditions including drugs or having insufficient amount of dietary antioxidants, has to be curbed by exogenous supply of antioxidants as a choice of therapy or preventive measure. Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. Natural antioxidants are preferred in allopathic drugs to overcome the side effects. Most of the polar compounds such as phenolic and flavonoid substances are potent inhibitors of ROS attack.[62]

Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers. The phenolic content of moringa is high and the radical scavenging activity is likely to be due to the phenolic, however, phenols may not be solely responsible for the antioxidant activity. In general, extracts with high antioxidant activity show a high phenolic content. Plant extracts with high phenolic contents also show high flavonoids content as reported for other plant species.[63] ROS have been considered to cause harm to living organisms and thus play a significant role in many human diseases such as arthritis, myocardial infarction , atherosclerosis, diabetes mellitus and cancer.[64,65] Phenols are a class of low molecular weight secondary metabolites found in most land plants. Phenol compounds have some antioxidant activity.[56,66]

They are able to terminate free radicals and chelate metal ions that are capable of catalyzing formation of ROS that promote lipid per oxidation.[67]

CONCLUSION

The gastro protective effect of moringa appears to be mediated by decrease in the volume and total acidity of gastric secretion, cell proliferation, reduction of inflammatory process and antioxidant-dependent mechanisms.

AKNOWLEDGEMENTS

The authors are gratefully acknowledged to the Faculty of Health & Life Sciences and the School of Pharmacy, Management & Science University, Shah Alam, Selangor Darul Ehsan, Malaysia for providing the necessary facilities to carry out the research project successfully.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Koffuor G, Ainoonson G, Amponsah K, Addotey J, Asiamah E, Akuffo S, et al. Anti-ulcerant Activity of an Aqueous Fruit Extract of Musa x para-disiaca on Acetic Acid-Induced Gastric Ulceration in ICR Mice. Journal of Medical and Biomedical Sciences 2013; 2(2): 30-39.
2. Bae D, Park D, Lee S, Yang G, Yang Y, Kim T, et al. Different antiulcer activities of pantoprazole in stress, alcohol and pylorus ligation-induced ulcer models. Laboratory animal research,2011; 27(1): 47-52.
3. Simmons DL, Botting RM, Hla T.Cyclooxygenase isoenzymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev, 2004; 56(3): 387-437.
4. Scheon RT and Vender RJ. Mechanisms of NSAID-induced gastric damage. Am J Med, 1989; 86(6): 449-458.
5. Goel RK, Chakrabarti A,SanyalAK. The effect of biological variables on the antiulcerogenic effect of vegetable plantain. Planta Medica1, 985; 51(7): 85-88.
6. Fahey JW. Moringaoleifera:A review of the medical evidence for its nutritional,therapeutic and prophylactic properties, Part 1. Trees for Life Journal, 2005; 10(6): 1-5.

7. Bennett RN, Mellon FA, Foidl N, Pratt JH, Dupont MS, Perkings L. Profilingglucosinolates and phenolics in vegetative and reproductive tissues of the multipurposetrees Moringaoleifera L.(Horseradieh Tree) and Moringastenopetala L. J Agric FoodChem, 2003; 51(8): 3546-3553.
8. Berger MR, Habs M, Jahn SA, Schmahl S. Toxicological assessment of seeds from Moringaoleifera and Moringastenopetala, two highly efficient primary coagulants for domestic water treatment of tropical raw waters. East African Medical Journal, 1984; 61: 712–716.
9. Bennett RN, Mellon FA, Foidl N, Pratt JH, Du Pont MS, Perkins L, et al. Profiling glucosinolates and phenolics in vegetative and reproductive tissues the multipurpose trees Moringaoleifera L. (Horseradish tree) and Moringastenopetala L. Journal of Agriculture and Food Chemistry, 2003; 51: 3546–3553.
10. Gassenschmidt U, Jany KD, Tauscher B,Niebergall H. Isolation and characterization of a flocculating protein from Moringaoleifera Lam. Biochemistry and Biophysics Acta, 1995; 1243: 477–481.
11. Lockett CT, Calvert CC. Energy and micronutrient composition of dietary and medicinal plants consumed during drought. Study of rural Fulani, Northern Nigeria. International Journal of Food Science and Nutrition, 2000; 51(3): 195–208.
12. Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K,Gilani AH. Fully acetylated carbamates and hypotensive thiocarbamate glycosides from Moringaoleifera. Photochemistry, 1995; 38(4): 957–963.
13. Fahey JW. Moringaoleifera:a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for Life Journal, 2005; 80: 1-5.
14. Hukkeri VI, Nagathan CV, Karandi RV, Patil BS. Antipyretic and wound healing activities of Moringaoleifera Lam in rats. Indian J Pharmaceut Sci, 2006; 68(1): 124-126.
15. Rao CV, Ojha SK.Analgeslc effect of Moringaoleifera Lam Leat extract on rats. Second World Congress on Biotechnological Developments of Herbal Medicine. Lucknow: NBRI, 2003; p.42.
16. Selvakumar D, Natarajan P.Hepatoprotective activity of Moringaoleifera Lam Leaves in carbon tetrachloride induced Hepatotoxicity in Albino rats. Phcog mag,2008; 4(13): 97-98.
17. Nadro MS, Arungbemi RM, Dahiru D. Evaluation of Moringaoleifera Leaf extract on Alcohol induced Hepatotoxicity. Trop J Pharm Res2006;5(1):539-544.

18. Murakami A, Kitazonz Y, Jiwajinda S, Koshimizu K,Ohigashi H. Niaziminin, a thiocarbamate from the leaves of Moringaoleifera, holds a strict structural requirement for inhibition of tumorpromotor- induced Epstein-Barr virus activation. Planta Med, 1998; 64: 319–323.
19. Rao AV, Devi PU, Kamath R. In vivo radioprotective effect of Moringaoleifera leaves. Ind J ExpBiol, 2001; 39: 858-863.
20. Dasputra PG, Dharmadikari SO, Bkore A. Pharmacology of Moringaoleifera. Indian Journal of Pharmacology, 1977; 9: 81–89.
21. Shukla S, Mathur R, Prakash AO. Antifertility profile of the aqueous extract of Moringaoleifera roots. Journal of Ethnopharmacology, 1998; 22(1): 51–52.
22. Ghasi S, Nwobodo E,Ofili JO. Hypocholestermic effects of crude extract of leaf of Moringaoleifera Lam in high-fat diet fed Wister rats. Journal of Ethnopharmacology, 2000; 69(1): 21–25.
23. Tahiliani P,Kar A. Role of Moringaoleifera leaf extract in the regulation of thyroid hormone status in adult male and female rats.Pharmacological Research, 2000; 41(3): 319–323.
24. Girija V, Sharada D, Pushpamma P. Bioavailability of thiamine, riboflavin and niacin from commonly consumed green leafy vegetables in the rural areas in Andhra Pradesh in India. International Journal for Vitamin and Nutrition Research, 1982; 52(1): 9–13.
25. Shanker K, Gupta MM, Srivastava SK, Bawankule DU, Pal A, Khanuja SPS. Determination of bioactive nitrile glycoside (s) in drumstick (Moringaoleifera) by reverse phase HPLC. Food Chemistry, 2007; 105: 376–382.
26. Bostock-wood C. Trees in society in rural Karnatica, NRI:Chatham India, 1992; 3(5): 12-17.
27. Clarke J. Building on indigenous natural resource management. Forestry practice inZimbab we's communal Areas, 1994; 5(8): 22-26.
28. Murakami A, Kitazonz Y, Jiwajinda S, Koshimizu K, Ohigashi H,Ziazimin. A thiocarbamate from the leaves of Moringaoleifera holds a strict structural requirement for inhibition of tumorpromoter induced Epstein Barr virus activation. Plant med, 1998; 64: 319-320.
29. Das BR, Kurup PA, Rao PLN. Antibiotic principle from MoringaPterygosperma. Naturwissenschaften,1954; 41-66.
30. Kumar NA,Pari L. Antioxidant action of Moringaoleifera Lam. (Drumstick) against antitubercular drugs induced lipid peroxidation in rats. Journal of Medicinal Food, 2003;

6 (3): 255–259.

31. Kurup PA, Rao PLN. Antibiotic principle from MoringaPterygosperma. Part I. Journal of the Indian Institute of Science, 1952; 34: 219–227.
32. Makkar HPS, Becker K. Nutritional value and antinutritional components of whole and ethanol extracted Moringaoleifera leaves. Animal Feed Science and Technology, 1996; 63: 211–228.
33. Freiberger CE, Vanderjagt DJ, Pastuszyn A, Glew RS, Mounkaila G, Millson M, et al. Nutrient contents of the edible leaves of seven wild plants from Niger. Plant Foods for Human Nutrition, 1998; 53: 57–69.
34. Nambiar VS,Seshadri S. Bioavailability trials of ˇ-carotene from fresh and dehydrated drumstick leaves (Moringaoleifera) in a rat model. Plant Foods for Human Nutrition, 2001; 56: 83–95.
35. Lakshminarayana R, Raju M, Krishnakantha TP,Baskaran V. Determination of major carotenoids in a few Indian leafy vegetables by high-performance liquid chromatography. Journal of Agricultural Food Chemistry, 2005; 53: 2838-2842.
36. Sanchez MDI, Lopez CJ,Vˇazquez NJR. High-performance liquid chromatography method to measure α- and β-tocopherol in leaves, flowers and fresh beans from Moringaoleifera. Journal of Chromatography, 2006; 1105: 111-114.
37. Grabow WOK, Slabert JL, Morgan WSG, Jahn SAA. Toxicity and mutagenicity evaluation of water coagulated with Moringaoleifera seeds preparations using fish, protozoan, bacterial, enzyme and Ames Salmonella assays. J. Am. Water Works Assoc, 1985; 76: 319–321.
38. Okokon J, Nwafor P. Antiulcer and anticonvulsant activity of Croton zambesicus. Pak. J. Pharm.Sci, 2009; 22(4): 384-390.
39. Bose M, Motghare VM, Dakhale GN,Turankar AV. Antiulcer activity of levcromakalim and nicorandil in albino rats: A comparative study. Pol J Pharmacol, 2003; 55(1): 91-95.
40. Kath RK, Gupta RK. Antioxidant activity of hydro alcoholic leaf extract of Ocimum sanctum in animal models of peptic ulcer. Indian Journal of Physiology and Pharmacology, 2006; 50(4): 391–396.
41. Bandyopadhyay D, Ghosh G, Bandyopadhyay A, Reiter RJ.Mela-tonin protects against piroxicam-induced gastric ulceration, J. Pineal.Res, 2004; 36: 195–203.
42. Szabo S, Trier JS, Brown A, Schnoor J, Howan HD, Bradford JC. A quantitative method for assessing the extent of experimental gastric erosions and ulcers. Journal of Pharmacological Method, 1985; 13: 59–66.

43. El-Abhar HS, Abdallah DM, Saleh S. Gastro protective activity of Nigella sativa oil and its component, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats. Journal of Ethnopharmacology, 2003; 84: 251–258.
44. Singh I. (1964) A modification of the Masson-Hamperl method for staining of argentaffin cells. AnatomischeAnzeiger, 2003;115: 81–82.
45. Sarkar S, Guha D. Effect of ripe fruit pulp extract of Cucurbitapepo Linn. In aspirin induced gastric and duodenal ulcer in rats. Indian Journal of Experimental Biology, 2008; 46: 639–645.
46. Tariq M, Al MontaeryA. Menadione protects gastric mucosaagainst ethanol-induced ulcers, Exp. Toxicol. Pathol, 2005; 56: 393–399.
47. Buege JA,Aust SG.Microsomal lipid peroxidation.Methods Enzy-mol, 1978; 52: 302–310.
48. McCord JM, Keele Jr BB,FridovichI.An enzyme based theory ofobligateanaerobiosis: the physiological functions of superoxide dismutase, Proc. Natl. Acad. Sci, 1976; USA. 68: 1024–1027.
49. Martin Jr JP, Daily M,SugarmanE.Negative and positive assaysof superoxide dismutase based on hematoxylinautooxidation, Arch.Biochem. Biophys, 1987; 255: 326–329.
50. Krohne-Ehrich G, Schirmer RH,Untucht-Grau R. Glutathionereductase from human erythrocytes. Isolation of the enzyme andsequence analysis of the redox-active peptide, Eur. J. Biochem. 1977; 80: 65–71.
51. Paglia DE, Valentine WN.Studies on the quantitative and quali-tative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med, 1967; 70: 158–169.
52. Chattopadhyay A, Choudhury TD, Bandyopadhyay D, Datta AG. Protective effect of erythropoietin on the oxidative damage of eryth-rocyte membrane by hydroxyl radical, Biochem. Pharmacol, 2000; 59: 419–425.
53. Basu A, Mitra E, Mukherjee D, Ghosh AK, Firdaus SB, Ghosh D, etal. Aqueous tulsi leaf (Ocimum sanctum L.) extract protects againstpiroxicam-induced gastric ulceration: involvement of antioxidant mechanisms, Int. J. Pharm. Pharm Sci, 2013; 5: 438–447.
54. Bush ES, Mayer SE. 5-Hydroxytryptamine (serotonin): receptor agonists and antagonists. In: Brunton LL, Lazo JS, Parker KL (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics McGraw Hill and Co.USA, 2006; 8: 269–275.
55. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumsticks tree (Moringaoleifera Lam.) leaves. J. Agric. Food Chem. 2003; 51: 44–55.

56. Okpuzor J, Ogbunugafor H, Kareem GK, Igwo-Ezikpe MN. In vitro Investigation of Antioxidant Phenolic Compounds in Extracts of Senna alata. Res J Phytochem, 2009; 3: 68-76.
57. Leamsomrong K, Suttajit M, Chantiratikul P. Flow Injection Analysis System for the Determination of Total Phenolic Compounds by Using Folin-Ciocalteu Assay. Asian J Appl Sci, 2009; 2: 184-190.
58. Muchuweti M, Kativu E, Mupure CH, Chidewe C, Ndhlala AR, Benhura MAN. Phenolic Composition and Antioxidant Properties of Some Spices. American J Food Tech, 2007; 2: 414-420.
59. Das D, Bandyopadhyay D, Bhattacharjee M,Banerjee RK. Hydroxylradical is the major causative factor in stress-induced gastric ulcer-ation.Free Radic.Biol.Med., 1997; 23: 8–18.
60. Flora SJS. Nutritional components modify metal absorption, toxic response and chelation therapy. J. Nutr. Environ. Med, 2002; 12: 53–67.
61. Bandyopadhyay D, Biswas K,Bhattacharyya M. Gastric toxicity andmucosal ulceration induced by oxygen derived reactive species: pro-tection by melatonin, Curr. Mol. Med, 2001; 1: 501–513.
62. Owen RW, Haubner R, Mier W, Giacosa A, Hull WE, Spiegelhalder B et al. Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined oli Food Chem Toxicol, 2003; 41(5): 703-717.
63. Makepeace W, Dobson ET, Scott D. Interference phenomena due to mouse ear and king devil hawkweed. New Zeal J Bot, 1985; 23: 79-90.
64. Elzaawely AA, S Tawata. Antioxidant Activity of Phenolic RichFraction Obtained from Convolvulus arvensis L. Leaves Grown inEgypt. Asian J Crop Sci, 2012; 4: 32-40.
65. Oyedemi SO, Afolayan AJ. Antibacterial and antioxidant activitiesof hydroalcoholic stem bark extract of Schotia latifolia Jacq.Asian Pac J Trop Med, 2011; 4(12): 952-958.
66. Leamsomrong K, Suttajit M, Chantiratikul P. Flow InjectionAnalysis System for the Determination of Total PhenolicCompounds by Using Folin-Ciocalteu Assay. Asian J Appl Sci, 2009; 2: 184-190.
67. Muchuweti M, Kativu E, Mupure CH, Chidewe C, Ndhlala AR,Benhura MAN. Phenolic Composition and Antioxidant Propertiesof Some Spices. American J Food Tech, 2007; 2: 414-420.