https://doi.org/10.52973/rcfcv-e34423 Revista Científica, FCV-LUZ / Vol. XXXIV, rcfcv-e34423

Assessment of antiulcer activity in crude extract of *Paeonia mascula* subsp. *atlantica* (Coss.) Greuter & Burdet

Actividad antiulcerosa en extracto crudo de *Paeonia mascula* subsp. *atlantica* (Coss.) Greuter & Burdet

***Wafa Nouioua*[1]*, *Sofiane Gaamoune*[2]**

[1]*Ferhat Abbas University Setif 1, Faculty of Natural and Life Sciences, Department of Plant Ecology and Biology, Laboratory of phytotherapy applied to chronic diseases. Setif, Algeria.*
[2]*National Institute of Agriculture Research. Setif, Algeria.*
**Corresponding Author: nouioua.wafa@yahoo.fr*

ABSTRACT

Peptic ulcer disease poses a critical health risk, leading to considerable morbidity and mortality. It arises from an imbalance between aggressive factors that damage the stomach's mucosal lining and protective components that safeguard it. Traditional plant-based remedies have gained traction as potential alternatives to conventional drugs. This experiment aimed to assess the anti-ulcer action of the crude extract of *Paeonia mascula* through two *in vivo* tests using male Wistar rats. The preventive test involved inducing acute gastric lesions, while the therapeutic test involved treating the lesions with crude extract and *in vitro* quantifying stomach parameters such as gastric volume, pH, total acidity, pepsin activity, and ulcer index. The results illustrated a noteworthy reduction in ulcer index in preventive and healing tests. These findings suggest that the extract has an anti-ulcerogenic effect by suppressing acid secretion and reducing gastric injuries.

Key words: Antiulcer; crude extract; *Paeonia mascula;* preventive; curative

RESUMEN

La afección por úlcera péptica representa un riesgo sanitario crítico, que provoca un sufrimiento y una mortalidad considerables. Surge de un desequilibrio entre las variables de fuerza que dañan el revestimiento mucoso del estómago y los componentes protectores que lo aseguran. Las curas convencionales a base de plantas han cobrado fuerza como opciones potenciales a los fármacos ordinarios. Este experimento pretendía evaluar la acción antiulcerosa del extracto crudo de *Paeonia mascula* con dos pruebas *in vivo* utilizando ratas Wistar macho. La prueba preventiva consistió en inducir lesiones gástricas agudas, mientras que la prueba terapéutica consistió en tratar la lesión con extracto crudo y cuantificar *in vitro* parámetros estomacales como el volumen gástrico, el pH, la acidez total, la actividad de la pepsina y el índice de úlcera. Los resultados ilustraron una notable reducción del índice de úlcera en las pruebas preventivas y curativas. Estos descubrimientos recomiendan que el extracto tiene un impacto antiulcerogénico al sofocar la secreción corrosiva y disminuir las lesiones estomacales.

Palabras clave: Antiulceroso; extracto crudo; *Paeonia mascula;* preventivo; curativo

Received: 28/03/2024 Accepted: 27/07/2024 Published: 27/10/2024

INTRODUCTION

Peptic ulcer disease is a group of ulcerative disorders in areas of the upper gastrointestinal tract exposed to acid–pepsin secretions, affecting 10% of the world population [1, 2]. Ulcers that affect the gastrointestinal system are usually aggravated by an imbalance between destructive and defensive factors in the stomach [3]. The major protective factors include adequate blood flow and the secretion of prostaglandins, mucus, and bicarbonate by resident mucosal cells. Aggressive agents include increased secretion of hydrochloric acid and pepsin, inadequate dietary habits, consumption of nonsteroidal anti–inflammatory drugs and alcohol, stressful conditions, and infection by Helicobacter pylori [4].

The treatment of gastric ulcers includes antacids, muscarinic antagonists, histamine receptor antagonists, and proton pump inhibitors. However, long–term use of these drugs can cause side effects on human health, including hypersensitivity, arrhythmia, hematopoietic disorders, impotence, and gynecomastia [5]. This underscores the need for alternative means of control. Currently, plants and plant–based products appear promising in the renewed search for better ulcer treatment. Some medicinal plants have been reported to possess anti–ulcer properties based on studies using experimental animal models [6].

In this study, we aim to scientifically validate the anti–ulcer properties of the methanol extract of *Paeonia mascula*. Peptic ulcer disease is a prevalent gastrointestinal disorder characterised by mucosal disintegration and ulcer formation. Our goal is to enhance the body of knowledge advocating for the use of natural products in treating peptic ulcers by investigating the anti–ulcer effects of the methanol extract of *Paeonia mascula*.

MATERIALS AND METHODS

Plant material

Aerial parts of *Paeonia algeriensis* Chabert = *Paeonia mascula* subsp. *atlantica* (Coss.) Greuter & Burdet were harvested from Kefrida Forest at 36°34'14" N, 5°17'24" E, and dried in the shade for later use.

Methanol extraction

The method of Motamed and Naghibi [7] was employed. Briefly, 10 g of powdered areal parts were macerated in 100 mL of 80% methanol for 24 h at laboratory temperature. The resulting solution was evaporated under a vacuum until dry. The extract was stored at -18 until use.

Animals

Experiments were conducted using adult male Wistar albino rats (*Rattus norvegicus*) (180–240 g). The animals were housed in standard metal cages (33 Wistar albino rats in total, with 15 for testing preventive effects and 18 for testing curative effects), kept at ambient temperature (20–25°C), and illuminated on a 12:12 h dark/light cycle. They were provided with standard food pellets and tap water until the night before the test or sacrifice.

Preventive effects of crude extract against acute gastric lesions

For the determination of anti–ulcerative activity, fifteen male Wistar rats were divided into three groups:

- The first group was orally administered 2.5 mL of water (control).
- The second group was orally administered 2.5 mL of a suspension containing 200 mg of the extract.
- The third group was orally administered 80 mg·kg^{-1} of Omeprazole 30 min before gastric ulcer induction.

The gastric mucosal lesions leading to an acute gastric ulcer were induced by the oral administration of 1.5 mL of a 150 mM HCl/ethanol (40:60, v/v) solution [8]. The animals were sacrificed under anaesthesia 60 min after the HCl/ethanol administration. Stomachs were removed, opened along the greater curvature, rinsed with physiological saline solution, and stretched on polystyrene boards. The degree of gastric mucosal damage was evaluated from digital pictures using a computerised image analysis system (Digimizer version 4.0.0.0). The percentage of the total lesion area (hemorrhagic sites) to the total surface area of the stomach, excluding the forestomach, was defined as the ulcer index.

Curative effects of crude extract against acute gastric lesions

Male Wistar rats weighing about 180–220 g were divided into three groups of at least six rats each. Ulcers were induced in all rats by oral gavage of 1 mL of absolute ethanol. The experimental groups were then treated as follows:

- Group I was the control, treated with 1 mL of water only.
- Group II was treated with 200 mg·kg^{-1} of crude extract.
- Group III was treated with 30 mg·kg^{-1} of Omeprazole for each of 8 successive days [9].

On the 9th day, all animals were sacrificed under anaesthesia. The stomachs were dissected out and opened along the greater curvature. The stomachs were gently rinsed with water to remove the gastric contents and blood clots for subsequent ulcer scoring.

Calculation of ulcer index and percentage ulcer inhibition

The ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach [10]. The score for the ulcer was made as follows:

0: normal–coloured stomach.

0.5: red coloration.

1: spot ulcers.

1.5: hemorrhagic streak.

2: ulcers.

3: perforation

The mean ulcer score for each animal was expressed as an ulcer index. The percentage of ulcer inhibition was determined as follows:

$$Inhibition\ of\ Ulcer\ Index\ (\%) = \frac{(Control\ mean\ index - Test\ mean\ index)}{Control\ mean\ index}$$

Collection of gastric juice

The stomach was excised carefully by keeping the oesophagus closed and opened along the greater curvature, and the luminal content was removed. The samples were collected and centrifuged (Sigma 3–30K, Germany) at 1000 G for 10 min. The volume of the supernatant was

expressed as mL·100 g^{-1} of body weight, and the centrifuged samples were decanted and analysed for gastric volume, pH, and total acidity [11].

Estimation of total acidity

It was measured by the method of Hawk *et al.* [12]. Briefly, 1 mL of the supernatant liquid was pipetted out and diluted to 10 mL with distilled water. The pH of this solution was noted with the help of a pH meter. The solution was titrated against 0.01N sodium hydroxide using a phenolphthalein reagent as an indicator. The endpoint was titrated when the solution turned orange–pink. The volume of NaOH was noted, which corresponds to total acidity. Acidity was expressed as a formula [13]:

$$Acidity = \frac{Volume\ of\ NaOH \times Normality \times 100\ mEq^{-1}}{0.1}$$

Estimation of pepsin activity:

Aliquots of 20 µL of the gastric contents were incubated with 500 µL of albumin solution (5 mg·mL^{-1}, 0.06 N hydrochloric acid) at 37°C for 10 min. The reaction was stopped with 200 µL of 10% trichloroacetic acid, and the samples were centrifuged (Sigma 3–30K, Germany) at 1500 G for 20 min. The supernatant was alkalinized with 2.5 mL of 0.55 M sodium carbonate, and 400 µL of 0.1 N Folin reagent was added to the tubes, which were then incubated for 30 min at room temperature. The absorbance (spectrophotometer UV visible VWR) of the sample was determined at 660 nm. A standard curve of tyrosine was used for the determination of the concentration of pepsin. The pepsin content of the gastric fluid was expressed as µg of tyrosine·mL^{-1} (Eq. tyr) [14].

Statistical analysis

The values are expressed as the mean ± SEM for six rats in each group. All the data were analysed statistically by the *t*–test of the student, followed by the Fisher test. The difference was considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Preventive effect

The anti–ulcerative preventive effect of crude extract of *Paeonia mascula* is shown in TABLE I below

TABLE I
Preventive effect of *Paeonia mascula*, crude extract

	Ulcer Index	Inhibition (%)
Omeprazole	0.15 ± 0.02	83.80 ± 7.20
Crude extract 200 mg·Kg^{-1}	0.15 ± 0.05	70.25 ± 14.24
Control	0.66 ± 0.27	–

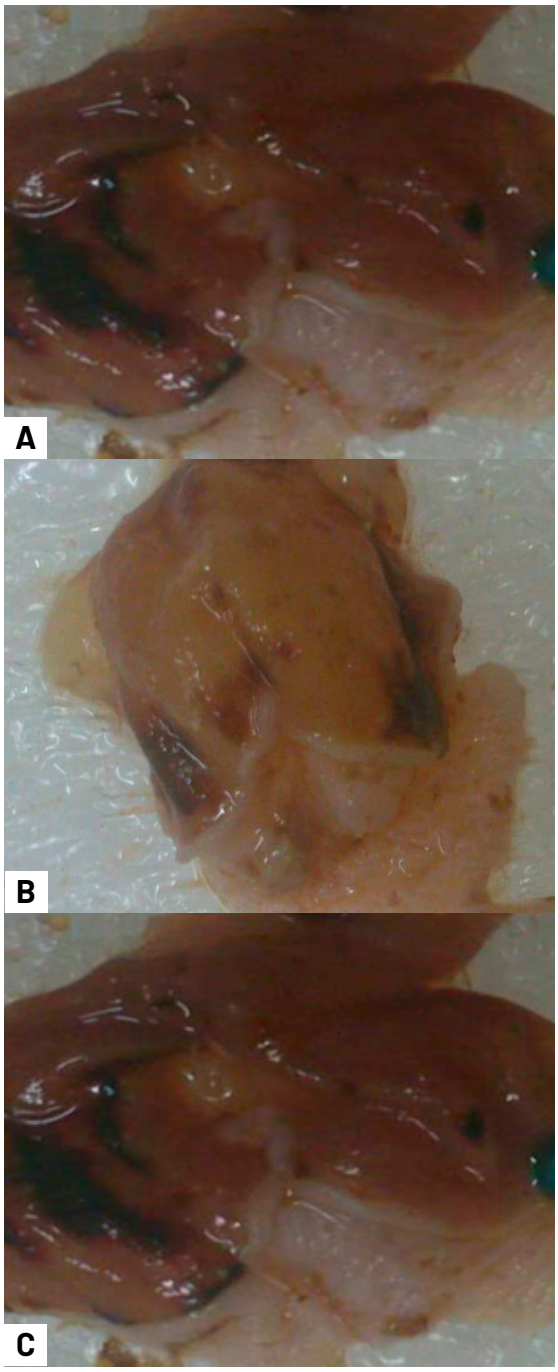

FIGURE 1. A: Gastric lesion observed in crude extract treated group induced by HCl – Ethanol. B: Gastric lesion observed in standard group induced by HCl–Ethanol. C: Gastric lesion observed in control group induced by HCl–Ethanol

Results showed that rats pre–treated with omeprazole or crude extract before being given HCl/ethanol solution had significantly reduced areas of gastric ulcer formation compared with the ulcer control group (FIG. 1).

Pre–treated animals with crude extract (200 mg·kg^{-1}) significantly reduced the formation of ulcers induced by the HCl/ethanol mixture, with a percentage inhibition of 75.30 ± 17.64% in comparison to the standard drug (80 mg·kg^{-1}), which provided 83.80 ± 7.20% protection.

Oxidative stress and mucosal inflammation are the main factors associated with the pathogenesis of the hydrochloric acid (HCl) and ethanol (EtOH)–induced gastric ulcer model [15, 16], a commonly used *in vivo* model that induces gastric mucosal erosion, bleeding,

perforation, and other damage [17, 18]. Driven by oxidative stress, the nuclear factor κB (NF-κB) pathway is regulated to amplify the inflammatory response by increasing the release of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and the expression of the downstream inflammatory mediators cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) [19]. These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H+ and Na+ ions, reduce the transmucosal potential difference, and induce the formation of erosions and ulcers [20]. The crude extract of *Paeonia mascula* was effective in significantly reducing the ulcer index and also increased the mucus content. This model demonstrates the cytoprotective effect of *Paeonia mascula* extract.

Curative effect

The results of the potential curative action against experimentally induced ulcers caused by the administration of HCl-Ethanol are demonstrated in TABLE II.

Oral administration of the crude extract (200 mg·kg^{-1}) for 8 d significantly (P<0.05) reduced the ulcer index in ethanol-induced experimental ulcers in rats, with a percentage of 50.11±3.22%; this result was better than the standard drug, which was 48.77±5.85%.

These results demonstrate that the crude extract of *Paeonia mascula* significantly reduced ethanol-induced ulcers. The extract at a dose of 200 mg·kg-1 significantly decreased the ulcer index, pepsin activity, and total acidity when compared to their respective controls, which indicates the healing of the induced ulcer.

The anti-ulcer activity shown by the crude extract of *Paeonia mascula* in ethanol-induced ulcers suggests that the extract has a cytoprotective effect, meaning that it protects the gastric mucosa through mechanisms other than inhibiting gastric acid secretion. [21]. Such mechanisms include inhibition of leukotrienes [22], pepsinogen [23], and substance P [24], free radical scavenging [25], increasing gastric mucosal blood flow [26], increasing the protective glycoprotein content and thereby strengthening the gastric mucosa, and prevention of oxidation of the mucosal xanthine dehydrogenase.

TABLE II
Curative effect of *Paeonia mascula*, crude extract

Treatment	Dose (mg·kg^{-1}) p.o	Volume of gastric juice (ml·100 g^{-1})	pH	Total acidity (meq·L^{-1})	Ulcer index	Inhibition (%)	Pepsin (µg·ml^{-1}) Eq.tyr
Control 10 ml·kg^{-1}	0	1.84±0.14	2.80±0.14	70.16±0.30	9.00±1.22	–	67.96±1.71
Standard omeprazole	50	1.25±0.05	5.24±0.25	22.5±7.5	4.83±0.20	48.77±5.85	11.15±2.90
Crude extract	200	1.24±1.12	4.50±1.20	24.22±1.12	4.12±0.89	50.11±3.22	9.54±4.14

CONCLUSION

The results of this research show a potential curative effect of the crude extract of *Paeonia mascula*.

The methanol extract of *Paeonia mascula* appears to have a significant anti-ulcer effect, prominently inhibiting acid secretion and the formation of stomach lesions. These findings suggest that the phytoconstituents in the extract may protect against ethanol/HCl^{-} and absolute ethanol-induced gastric mucosal damage by inhibiting gastric acid, pepsin, histamine, and free radicals while promoting mucus secretion. Additionally, the scavenging of reactive oxygen species (ROS) by polyphenols is considered an important mechanism in the healing of gastric ulcers. These multifaceted properties indicate the extract's potential in developing future medications. However, further research is crucial to investigate the extract's toxicity and chemical composition, which are essential for its safe and effective utilization.

Conflict of interests

The authors declare that they have no conflicting interests.

BIBLIOGRAPHICS REFERENCES

[1] Zapata-Colindres JC, Zepeda-Gomez S, Montaño-Loza A, Vasquez-Ballesteros E, Villalobos J, Valdovinos-Andraca F. The association of *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drugs in peptic ulcer disease. Can. J. Gastroenterol. [Internet]. 2006; 20(4):277-280. doi: https://doi.org/g7m9dr

[2] Barazandeh F, Yazdanbod A, Pourfarzi F, Sepanlou SG, Derakhshan MH, Malekzadeh R. Epidemiology of peptic ulcer disease: endoscopic results of a system investigation in Iran. Middle East J. Dig. Dis. [Internet]. 2012 [cited 20 Apr. 2024]; 4(2):90-96. PMID: 24829640. Available in: https://goo.su/V9cOv

[3] Calam J, Baron JH. Pathophysiology of duodenal and gastric ulcer and gastric cancer. BMJ [Internet]. 2001; 323(7319):980-982. doi: https://doi.org/cfrz7s

[4] Klein-Júnior LC, Santin JR, Niero R, Andrade SF, Cechinel-Filho V. The therapeutic lead potential of metabolites obtained from natural sources for the treatment of peptic ulcer. Phytochem. Rev. [Internet]. 2012; 11(4): 567-616. doi: https://doi.org/g7m9ds

[5] Cordeiro KW, Pinto LA, Formagio ASN, Andrade SF, Kassuya CAL, Freitas KC. Antiulcerogenic effect of *Croton urucurana* Baillon bark. J. Ethnopharm. [Internet]. 2012; 143(1):331-337. doi: https://doi.org/gq7gjz

[6] Akomas SC, Ezeifeka GO, Ijioma SN. Justification for the use of *Musa paradisiaca* green fruit extract for GIT mucosa protection and ulcer treatment. Cont. J. Anim. Vet. Res. [Internet]. 2014; 6(1):29-35. doi: https://goo.su/QaczT

[7] Motamed SM, Naghibi F. Antioxidant activity of some edible plants of the Turkmen Sahra region in northern Iran. Food Chem. [Internet] 2010; 119(4):1637-1642. doi: https://doi.org/bcz7fq

[8] Mizui T, Doteuchi M. Effect of polyamines on acidified ethanol-induced gastric lesions in rats. Jpn. J. Pharm. [Internet] 1983; 33(5):939–945. doi: https://doi.org/g7m9dz

[9] Süleyman H, Akçay F, Altinkaynak K. The effect of nimesulide on the indomethacin – and ethanol-induced gastric ulcer in rats. Pharm. Res. [Internet] 2002; 45(2):155–158. doi: https://doi.org/cdhqkm

[10] Devendra K, Surendra P, Kartik CP. Antiulcer activity of ethanolic extract of *Buchanania lanzan* Spreg roots. Ann. Biol. Res. [Internet]. 2010 [cited 19 Feb. 2024]; 1(4):234–239. Available in: https://goo.su/s8WH

[11] Jaikumar S, Ramaswamy S, Asokan BR, Mohan T, Gnanavel M. Anti-ulcer activity of methanolic extract of *Jatropha curcas* (Linn.) on Aspirin-induced gastric lesions in Wistar strain rats. Res. J. Pharm. Biol. Chem. Sci. [Internet]. 2010 [cited 24 Feb 2024]; 1(4):886–897. Available in: https://goo.su/IfvCt

[12] Hawk PB. Hawk's physiological chemistry. 14th ed. New York: McGraw-Hill, Blakiston Division; 1965. 1472 p.

[13] Vinothapooshan G, Sundar K. Anti-ulcer activity of *Mimosa pudica* leaves against gastric ulcer in rats. Res. J. Pharm. Biol. Chem. Sci. [Internet]. 2010 [cited 18 Feb. 2024]; 1(4):606–614. Available in: https://goo.su/dgUBU

[14] Smeeta MM, Subhash LB. Anti-ulcer activity of petroleum ether extract of leaves of *Madhuca indica* J. F. Gmel against pylorus ligation and naproxen-induced gastric mucosal injury in rats. Der. Pharm. Lettre. [Internet]. 2013 [cited 12 Feb. 2024]; 5(2):205–211. Available in: https://goo.su/yDj9i

[15] Arab HH, Salama SA, Eid AH, Kabel AM, Shahin NN. Targeting MAPKs, NF-κB, and PI3K/AKT pathways by methyl palmitate ameliorates ethanol-induced gastric mucosal injury in rats. J. Cell. Physiol. [Internet]. 2019; 234(12): 22424–22438. doi: https://doi.org/gjg7r2

[16] Akanda MR, Park BY. Involvement of MAPK/NF-κB signal transduction pathways: *Camellia japonica* mitigates inflammation and gastric ulcer. Biomed. Pharmacother. [Internet]. 2017; 95:1139–1146. doi: https://doi.org/gcqq9c

[17] Kwiecien S, Jasnos K, Magierowski M, Sliwowski Z, Pajdo R, Brzozowski B, Mach T, Wojcik D, Brzozowski T. Lipid peroxidation, reactive oxygen species and antioxidative factors in the pathogenesis of gastric mucosal lesions and mechanism of protection against oxidative stress-induced gastric injury. J. Physiol. Pharm. [Internet]. 2014 2013 [cited 12 Feb. 2024]; 65(5)613–622. PMID: 25371520. Available in: https://goo.su/fljpitj

[18] Chen S, Zhao X, Sun P, Qian J, Shi Y, Wang R. Preventive effect of *Gardenia jasminoides* on HCl/ethanol induced gastric injury in mice. J. Pharm. Sci. [Internet]. 2017; 133(1):1–8. doi: https://doi.org/f9v32b

[19] Zhang W, Lian Y, Li Q, Sun L, Chen R, Lai X, Lai Z, Yuan E, Sun S. Preventative and Therapeutic Potential of Flavonoids in Peptic Ulcers. Molecules [Internet]. 2020; 25(20):4626. doi: https://doi.org/gsch43

[20] Ashoka MS, Shashidhar CS, Prakash S. Anti Ulcer Activity of *Heliotrpium Indicum* Leaves Extract. Int. J. Pharm. Sci. Res. [Internet]. 2011; 2(5):1288–1292. doi: https://doi.org/g7m9d4

[21] Robert A. Cytoprotection by prostaglandins. Gastroenterology [Internet]. 1979; 77(4):761–767. doi: https://doi.org/g7m9d5

[22] Konda Y, Sakamoto C, Nishisaki H, Nakano O, Matozaki T, Nagao M, Matsuda K, Wada K, Baba S. Ethanol stimulates pepsinogen by opening a Ca^{2+} channels of guinea pig gastric chief cells. Gastroenterology [Internet]. 1991; 100(1):17–24. doi: https://doi.org/g7m9d7

[23] Karmeli F, Eliakim R, Okon E, Rachmilewitz D. Gastric mucosal damage by ethanol is mediated by substance P and prevented by ketotifen, a mast cell stabilizer. Gastroenterology [Internet]. 1991; 100(5 Pt 1):1206–1216. doi: https://doi.org/g7m9d8

[24] Szabo S, Trier JS, Brown A, Schnoor J. Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. Gastroenterology [Internet]. 1985; 88(1 Pt 2):228–236. doi: https://doi.org/gp3wp2

[25] Kauffman Jr. GL. Can the mechanisms of aspirin induced gastric mucosal injury be identified? [Internet]. In: Yoshida H, Hagihara Y, Ebashi S, editors. Toxicology & Experimental Models. Proceedings of the 8th International Congress of Pharmacology; 1981; Tokyo. New York: Pergamon Press; 1981. p. 281–285. doi: https://doi.org/nnck

[26] Horowitz MI, Pigman W. The Glycoconjugates. Vol. 1, Mammalian Glycoproteins and Glycolipids. New York: Academic Press; 1977. Chapter 3, Section 5, Gastrointestinal glycoproteins; p. 189–213.