BJMHR
British Journal of Medical and Health Research
Journal home page: www.bjmhr.com

Investigation of *Calendula officinalis* whole plant as a gastroprotective and antioxidant in peptic ulcer

Anil K.Yadav[1], Pushpesh K.Mishra[1], P.K.Jain[1], Chandana V. Rao[2], Shikha Tiwari[1*],Vikas Singh[1]

1. Faculty of Pharmacy, Naraina VidyaPeeth Group of Institutions, Kanpur 208020, India.
2.Ethnopharmacology and Pharmacognosy Division, National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, India

ABSTRACT

Peptic ulcer is a most common ulcer of the gastrointestinal tract that is usually acidic and thus extremely painful. This pathological condition is caused by chronic inflammation due to *Helicobacter pylori*, excessive use of of NSAIDs like aspirin and smoking. This disorder also results in release of massive amount of toxic free radicals which results in oxidative stress. Ethnobotanically, the whole plant of *Calendula officinalis* has been reported to be used in the treatment of various disorders including stomach and other diseases. Antiulcer-activity of the 50% ethanolic extracts in order to validate ethnobotanical claims regarding the plant, used in the above disorders. Four groups of six albino rats in each group were used. They were pretreated with (0.25% w/v) carboxymethyl cellulose (negative control, 10 ml/kg), 50 mg/kg ranitidine (positive control), whole plant extract of *Calendula officinalis* (250 and 450 mg/kg/body weight) and their effect was studied on aspirin induced ulcer, cold-resistant stress-induced ulcers, pylorus ligation and ethanol-induced ulcers. The results of the present study showed that the whole plant extract of *Calendula officinalis* possessed gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by physical and chemical agents with a maximum of 87.15 % therapeutic efficiency (450 mg/kg b.w.) in cold resistant stress-induced ulcers. The present study was also aimed to investigate the effect of this extract on oxidative stress by measuring the level of various oxidative markers. The result of enzyme assay and lipid peroxidation clearly indicates the whole plant of *Calendula officinalis* extract have significant antioxidant effect on ulcer pathology. Whole plant extract have decreased LPO ($p< 0.001$) and SOD ($p<0.01$) with concomitant increase in catalyse activity in cold resistant stress-induced ulcers.

Keywords: Calendula officinalis, Acidity, CRS induced ulcer, Ulcer index.

*Corresponding Author Email: anu7364@gmail.com
Received 2 June 2016, Accepted 26 June 2016

Please cite this article as: Yadav AK *et al.*, Investigation of Calendula officinalis whole plant as a gastroprotective and antioxidant in peptic ulcer. British Journal of Medical and Health Research 2016.

INTRODUCTION

Peptic ulcer is a lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to aggressive factors. In spite of the vast amount of research on ulcer, the cause of chronic peptic ulceration is still not clear. Although in most of the cases the etiology of the ulcers is unknown, it is generally accepted that they result from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defence mechanisms[1].To regain the balance, different therapeutic agents have used. Traditional plant remedies have been used for very long time in the treatment of ulcer, but only a few of them have been significantly evaluated. Therefore, the present work aimed to evaluate the effect of purified standard extract of *Calendula officinalis* whole plant on ulcer induced by different method and its efficacy in oxidative stress[2]. *Calendula officinalis* a medicinal plant is referred as a wonder drug in Siddha and Ayurvedic formoulation used for gastrointestinal ailments[3]. Pharmacological and clinical studies suggest that *Calendula officinalis* possess anti-inflammatory, anti-viral, hepatoprotactive, and cardio protective activites. In the present study the gastroprotective and antioxidant activities of ethanolic extract of *Calendula officinalis* whole plant was studied in Asprin induced ulcer, cold resistant stress induced ulcer, Pylorus ligated induced ulcers and ethanol induced ulcer.

MATERIALS AND METHOD

Collection of plant material

The whole plant of the *Calendula officinalis (Asteraceae)* was collected from Botanical Garden of N.B.R.I (National Botanical Research Institute), Lucknow, India in month of Sept. 2015. The plant materials were authenticated by Dr. Sayeeda Khatoon, chemotaxonomic at National Botanical Research Institute, Lucknow and voucher specimens were deposited in the departmental herbarium of National Botanical Research Institute, Lucknow, India for future reference.

Extraction of *Calendula officinalis*

The whole plant of *Calendula officinalis* was dried and powdered homogenously. The powdered material was kept in 50% aq.ethanol for 24 hours. Ethanol extract was filtered and concentrated under vaccume in rotary evaporator (R110 Buchi, Switzerland) at 60 °C and dried to a constant weight in an oven set at 40 °C. The dried extract gave a yield of 29.15% (w/w) and was stored in an air-tight container at about 4 °C until required. The extracts obtained was further subjected pharmacological investigation to dryness to one third of original volume and stored overnight at 35^0C filtrate was lyophilized and the drug material obtain was used for study.

Animals

Swiss albino rats weighing (165-200 gm) and were procured from National Botanical Research Institute (Lucknow). They were housed in the departmental animal house under standard conditions (26 ± 2°C and relative humidity 30-35%) in 12 hours light and 12 hours dark cycle respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10% protein, 4% arachis oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D.[4]

Experimental Procedure

In the experiment, the rats were divided into four groups (n = 6). Group 1 was the control group which received suspension of 1% carboxy-methyl cellulose in distilled water (10 ml/kg). Groups 3 and 4 received COE in doses of 250 and 450 mg/kg b.w. Group 2 received ranitidine in the dose of 50 mg/kg body weight. These were administered orally twice daily at 10:00 and 16:00 h, respectively, for five days for acute ulcer protective studies.

Aspirin (ASP)-induced ulcers

ASP in dose of 250 mg/kg (20 mg/ml) was administered to the animals on the day of the experiment and ulcers were scored after 4hour[5]. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The pooled group ulcer score was then calculated according to the method[6].

Cold-resistant stress (CRS)-induced ulcer

Rats were deprived of food, but not water, for about 18 h before the experiment. On day six, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of 4–6 ◦C[7].Two hours later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs as described above. Extent of lipid peroxidation (LPO) was also estimated under the stress condition using the standard method[8]. The activity of superoxide dismutase (SOD) was determined by monitoring the inhibition of the autoxidation of pyrogallol[9]. CAT activity was determined by monitoring the enzyme catalyzed decomposition of hydrogen peroxide by potassium permanganate[10] .

Pylorus ligated induced ulcers

Drugs were administered for a period of 5 days as described above and the rats were kept for 18 h fasting. Animals were anaesthetized using pentobarbitone (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply.

The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period[11]. After 4 h, stomachs were dissected out and cut open along the greater curvature and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach.

Ethanol - induced ulcer

The gastric ulcers were induced in rats by administrating ethanol (1 ml/200 g, 1 h)[12] and the animals were sacrificed by cervical dislocation and the stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm2/rat).

Statistical analysis

The statistical analysis of all the pharmacological analyses was carried out using SPSS 13.0 for Windows. The values are represented as mean±S.E.M. (n=6). Paired *t*-test (Newman-keuls multiple comparison test).was used for reporting the *p* value and significance with respect to the control group and ANOVA for the comparison between more than two groups.

RESULTS AND DISCUSSION

Table 1: Effect of ethanolic whole plant extract of *Calendula officinalis* on ulcer index in Asprin induced ulcer:

Group	Treatment	Dose(mg/kg)	Ulcer index (mm^2/rat)	Protective ratio (%)
I	Vehicle	PL	19.4±0.07	_
II	COE	250	8.5±0.08[b]	64.4±0.15
III	COE	450	7.3±0.09[d]	68.5±0.13
IV	Ranitidine	50	7.4±0.13[b]	61.3±0.13

Values are mean±S.E.M. (n=6)

a$P < 0.001$ compared to respective control group.

b$P < 0.01$ compared to respective stress group.

c$P < 0.05$ compared to respective stress group.

d$P < 0.001$ compared to respective stress group.

Table-2 Effect of ethanolic whole plant extract of *Calendula officinalis* on ulcer index in cold resistant stress induced ulcer:

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	Protective ratio (%)
I	CR stress	_	25.3±0.09	58.2±0.08
II	COE	250	15.2±0.04[c]	
III	COE	450	6.3±0.13[d]	89.2±0.07
IV	Ranitidine	50	7.2±0.11[d]	82.4±0.13

Values are mean±S.E.M. (n=6)

a$P < 0.001$ compared to respective control group.

b*P* < 0.01 compared to respective stress group.

c*P* < 0.05 compared to respective stress group.

d*P* < 0.001 compared to respective stress group.

Table 3: Effect of ethanolic whole plant extract of *Calendula officinalis* on ulcer index in Pylorus ligation induced ulcer:

Group	Treatment	Dose mg/kg)	Ulcer index mm^2/rat	Protective ratio (%)
I	Vehicle	_	15.4±0.16	_
II	COE	250	9.6±0.1	49.4±0.12
III	COE	450	8.5±0.13	68.8±0.09
IV	Ranitidine	50	7.4±0.1	63.5±0.13

Values are mean±S.E.M. (n=6)

a*P* < 0.001 compared to respective control group.

b*P* < 0.01 compared to respective stress group.

c*P* < 0.02 compared to respective stress group.

d*P* < 0.001 compared to respective stress group

Table 4: Effect of ethanolic whole plant extract of *Calendula officinalis* on ulcer index in Ethanol induced ulcer:

Group	Treatment	Dose mg/kg	Ulcer index mm^2/rat	Protective ratio (%)	Gastric wall mucus (µg/g wet glandular tissue)
I	Ethanol	1ml/200g	26.4±0.01	_	179.3±0.12
II	COE	250	21.3±0.11	18.2±0.07	175.3±0.12
III	COE	450	17.3±0.14	39.4±0.12	184.3±0.13
IV	Ranitidine	50	13.5±0.12	57.3±0.11	267.3±0.11

Values are mean±S.E.M. (n=6)

a*P* < 0.001 compared to respective control group.

b*P* < 0.01 compared to respective stress group.

c*P* < 0.02 compared to respective stress group.

d*P* < 0.001 compared to respective stress group.

Table 5.Effect of whole plant extract of *Calendula officinalis*on cold-restraint stress-induced gastric ulcers and LPO, SOD and CAT activity in rat gastric mucosa.

Group	Treatment	Dose (mg/kg)	LPO (nmol of MDA formed/h/100mg protein)	SOD(nmol/gtissue)	CAT(unit/mg protein)
I	CR stress	_	54±0.07[b]	248±8.2[b]	25.3±1.3[b]
II	COE	250	30±0.01[a]	213±4.3[a]	34.1±4.2[a]
III	COE	450	14±0.01[b]	169±5.7[d]	42.51±2.5[d]
IV	Ranitidine	50	45±0.02[b]	125±1.5[d]	41.31±1.8[d]

Values are mean±S.E.M. (n=6).

a*P* < 0.001 compared to respective control group.

b$P < 0.001$ compared to respective cold-resistant stress group.

c$P < 0.05$ compared to respective cold-resistant stress group.

d$P < 0.01$ compared to respective cold-resistant stress group.

It has been established that the causative agent for peptic ulcer is use of excessive NSAID's like aspirin, H.coli bacteria and smoking, alcoholism etc. These factors after prolonged duration results into ulcer in gastrointestinal track. These also aggravate oxidative stress in body which may also results into other complication like diabetes, cardiovascular diseases. As we know that there are many synthetic therapeutic agents are available which efficiently controls it, but they further precipitates side effects and creates complication. So there is need arises for the use of herbal medicines which are devoid of side effects. In our present work ulcer have been introduced in albino rat by means of various agent like aspirin, cold, pylorus ligation and ethanol. In case aspirin induced ulcer it has been found that by using dose of 450 mg /b.w. of COE have improved the condition statistically significantly as compared to ranitidine, as shown in Table 1. Also we know that stress plays an important role in etiopathology of gastroduodenal ulceration. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucous production. Increase in gastric motility, vagal overactivity[13] mast cell degranulation[14] decreased gastric mucosal blood flow[15] and decreased prostaglandin syntheses[16] is involved in genesis of stress-induced ulcers. In cold stress induced ulcer, the protective ratio of COE at dose of 450 mg/b.w. was also found much more significant as compared to ranitidine, as shown in Table 2, which confirmed the histamine antagonistic, anticholinergic and antisecretory effects of extract. Also in case of pylorus ligation induced ulcer the use of extract have showed significantly promising therapeutic action. (Table 3).Alcoholism along with social abuse also proved as a prime reason for the ulceration. Ethanol elevates the pepsin secretion which leads to degradation of endogenous protein in the mucous layer of gastric wall and causes the inflammation. Ethanol also elevates the hypersecreation of acid which further elevates the severity of inflammation. From the results as shown in (Table 4), it is evident that the COE reduced the ulcer index significantly. This effect achieved due to blockage of H^+K^+ATPase, the enzyme involved in gastric acid secretion. The pepsin reducing effect of COE was found to be significant in ethanol-induced ulcer model[17]. Thus, from these results it is revealed that COE contains flavonoids such as quercitin, formononetin and biochin, which inhibits H^+K^+ATPase activity.

It has been established that oxidative stress lies at the root of a number of pathological processes and diseases such as cancer, atherosclerosis, rheumatic arthritis, hematological and neurodegenerative disorders are not exempt with more making the list among which is peptic

ulcer. The experimental data revealed that the cold-resistant stress aggravated the ulcer severity and induced oxidative stress as compared to normal rats. Lipid peroxidation is a free radical-induced process leading to oxidative deterioration of polyunsaturated fatty acids under physiological condition, low concentration of lipid peroxides are found in tissue[18]. Elevated level of peroxides is a characteristic feature of chronic ulcer. Superoxide dismutase (SOD) protects tissue against reactive oxygen species (ROS) by catalyzing the removal of superoxide radical (O2.−) which damages the membrane and biological structure. Catalase has been shown to be responsible for detoxification of scavenging enzymes that remove the toxic free radicals in vivo. Reduced activities of SOD and catalase resulted in number of deleterious effects due to accumulation of superoxide radicles (O2.−) and hydrogen peroxide. This effect was significantly reversed by prior administration of whole plant extract of *Calendula officinalis* providing a close relationship between free radical scavenging activity and gastroprotective effect as Shown in (Table 5).

The results of the present study showed for the first time that the whole plant extract possessed gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by physical and chemical agents. These findings have justified, the inclusion of this plant in the management of gastric disorders in ethnomedicine[19]. Also it is now clear from above study that oxidative stress[20] act as a root cause of spread of peptic ulcer, so there is need arises for the study of those plants which posses anti ulcer activity along with the antioxidant potential[21]. These studies have created a new ray of hope for the use of whole plant of *Calendula officinalis* treatment of ulcer.

CONCLUSION

Recently, the use of herbal medicines for the treatment of peptic ulcer is increasing and most patients consider herbal medicines are safer as they are of natural origin. Since these herbal medicines have a tremendous potential to combat the oxidative free radical generated during disease with least potential to cause any adverse effects as compared to allopathic drugs. To date the literature describing these herbal drugs with their oxidative potential is limited and most are in preclinical studies. Hence, more research is required to explore their beneficial therapeutics effect. *Calendula officinalis* found to possess an efficient antiulcer and antioxidant property which have now increased their demand in market. In present study *Calendula officinalis* have reduced the ulcer index and also reversed oxidative stress induced in disease. Therefore, the present study concluded that *Calendula officinalis* whole plant is useful in ameliorating the oxidative stress induced in peptic ulcer.

However, more mechanism based research work is required to seek out the effect of this herbal medicine on other enzymes which involves in the pathophysiology of peptic ulcer. In

future more will be focused on evaluating the efficacy of this amazing drug against bacterial growth and chronic peptic ulcer.

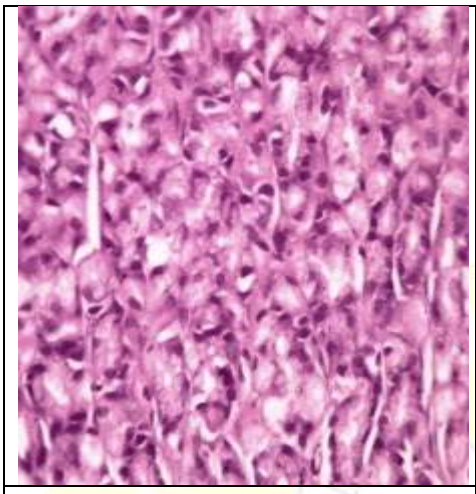

Figure 1: Control, Histopathology of stomach Showing effect of 1% carboxy-methyl cellulose in distilled water.

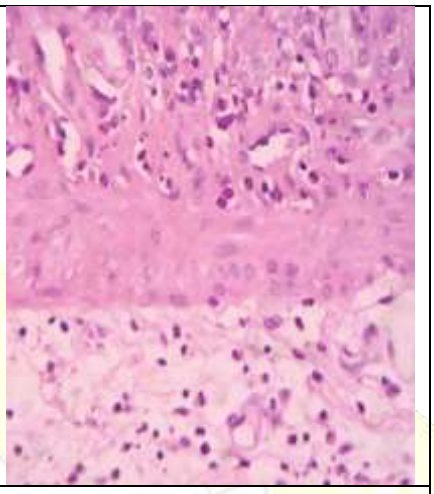

Figure 2: Standard, Histopathology of stomach showing effect of ranitidine (50 mg/kg) on ulcer Sore.

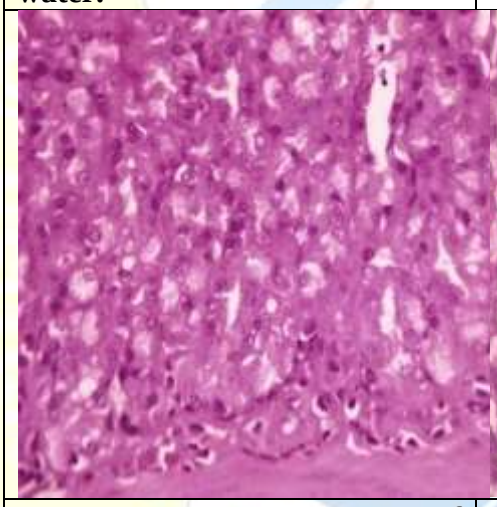

Figure 3: Histopathology of stomac showing effect of *Calendula officinalis* (250 mg/kg) on ulcer sore.

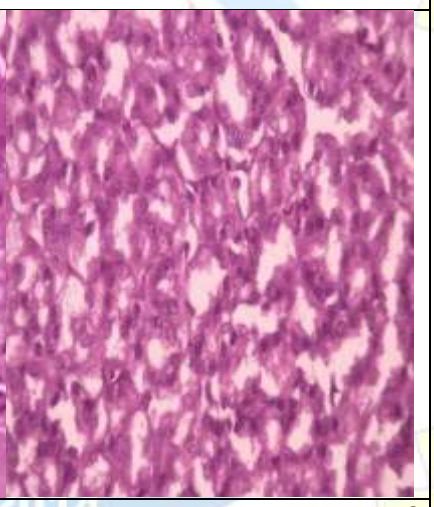

Figure 4: Histopathology of stomach showing effect of *Calendula officinalis*(450 mg/kg) on ulcer sore.

REFERENCE

1. Piper, D.W., Stiel, D.D.,.Pathogenesis of chronic peptic ulcer, current thinking and clinical implications. Medical Progress, 1986, 2:7–10.
2. Bhattacharya, M., Bhattacharya, S., Gupta, A., Banerjee, R.K., Critical role of endogenous gastric peroxidase in controlling oxidative damage in *H. pylori* mediated and nonmediated gastric ulcer. Free Radicals in Biology and Medicine, 2000,32:731–743.

3. Govindarajan, R., Vijayakumar, M., Pushpangadan, P., Antioxidant approach to disease management and the role of Rasayana herbs of Ayurveda. Journal of Ethnopharmacology, 2005,99:165–178.
4. Zimmerman, M.,.Ethical guidelines for investigations of experimental pain in conscious animals. Pain, 1983,16: 109–110.
5. Goel, R.K., Das, G.D., Sanyal, A.K., Effect of vegetable banana powder on changes induced by ulcerogenic agents on dissolved muco substances in gastric juice. Indian Journal of Gastroenterology 1985,4:249–251.
6. Sanyal, A.K., Pandey, B.L., Goel, R.K.,The effect of a traditional preparation of copper, tamrabhasma, on experimental ulcers and gastric secretion. Journal of Ethnopharmacology, 1982, 5: 79–89.
7. Gupta, M.B., Nath, R., Gupta, G.P., Bhargava, K.P., A study of the antiulcer activity of diazepam and other tranquillosedatives in albino rats. Clinical and Experimental Pharmacology, 1985, 12: 61–63.
8. Okhawa, H., Ohishi, N., Yagi, K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 1979, 95:351–355.
9. Marklund, S., Marklund, G.,.Involvement of superoxide anion radical and a convenient assay of superoxide dismutase. European Journal of Biochemistry, 1974, 47:469–474.(7
10. Cohen, G., Dembiec, D., Marcus, J., Measurement of catalase activity in tissue extracts. Analytical Biochemistry, 1970,34: 30–38.
11. Shay, H., Komarov, S.A., Fels, S.S., Meranze, D., Gruenstein, M., Siplet, H., A simple method for the uniform production of gastric ulceration. Gastroenterology, 1945, 5: 43–61.
12. Hollander, D., Tarnawski, A., Krause, W.J., and Gerely, H,Protective effect of sucralfate against alcoholinduced gastric mucosal injury in the rat. Gastroenterology, 1985, 88, pp. 366-374.
13. Cho, C.H., Ogle, C.W., Dai, S., Acute gastric ulcer formation in response to electrical vagal stimulation in rats. European Journal of Pharmacology, 1976, 35:215–219.
14. Cho, C.H., Ogle, C.W., Cholinergic-mediated gastric mast cell degranulation with subsequent histamine H1 and H2-receptor activation in stress ulceration in rats. European Journal of Pharmacology, 1979, 55, 23–33.
15. Hase, T., Moss, B.J., Microvascular changes of gastric mucosa in development of stress ulcers in rats. Gastroenterology, 1973, 65: 224– 226.

16. Rao, Ch.V. Sairam, K., Goel, R.K., Experimental evaluation of *Bacopamonniera*on rat gastric ulceration and secretion. Indian Journal of Physiologyand Pharmacology, 2000, 44: 35–41.
17. Peskar, B.M., Lange, K., Hoppe, U., Peskar, B.A., Ethanol stimulates formation of leukotriene C4 in rat gastric mucosa. Prostaglandins, 1986, 31:283–293.
18. Phull, P.S., Green, C.J., Jacyna, M.R., A radical view of stomach: the role of oxygen-derived free radicals in gastroduodenal disease. American Journal of Gastroenterology and Hepatology , 1995,7:265–274.
19. Rao, Ch.V, Maiti, R.N., Goel, R.K., Effect of mild irritant on gastric mucosal offensive and defensive factors. Indian Journal of Physiology and Pharmacology, 1999, 44:185–191.
20. Das, D., Bandyopadhyay, D., Bhattacharrya, M., Banerjee, R.K., Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. Free Radicals in Biology and Medicine, 1997, 23: 8–18.
21. Corne, S.J., Morrissey, S.M., Woods, R.J., A method for the quantitative estimation of gastric barrier mucus. Journal of Physiology, 1974, 42:116–117.