Original Article

Ameliorative effect of galantamine on acetic acid-induced colitis in rats

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Abstract

Galantamine (GAL) is a drug for treating Alzheimer's disease which has reasonable and no significant side effects. Studies have shown that GAL possesses antioxidant, anti-inflammatory, and cholinomimetic effects that might be beneficial for inflammatory bowel disease. Therefore, this study was aimed to investigate the anti-inflammatory effect of GAL on acetic acid-induced colitis in rats. GAL at 0.25, 1.25, 2.5 mg/kg/day was administrated orally (p.o.) to different groups of male Wistar rats 2 h before induction of ulcer with acetic acid 3% and continued for 5 consecutive days. Dicyclomine (DIC) was similarly used alone (5 mg/kg/day, p.o.) or together with GAL at doses already mentioned to delineate the impact of muscarinic pathway in probable beneficial effects of GAL on colitis. Control and reference groups received distilled water (5 mL/kg, p.o.), prednisolone (4 mg/kg/day, p.o.), or mesalazine (100 mg/kg/day, p.o.) respectively. At day 6, tissue injuries were assessed for macroscopic, histopathologic, and biochemical indices of myeloperoxidase and MPO activity. Results showed that GAL at 3 applied doses, alone or in combination with DIC diminished ulcer index, total colitis index, and MPO activity as important biomarkers of colitis. DIC alone was not effective on most parameters and its concurrent administration with GAL couldn't reverse its antiulcerative effects. Prednisolone and mesalazine were both effective in this relation. The current research indicated that GAL had anti-inflammatory and antiulcerative activities independent of its muscarinic effects. Thus the antioxidant and anti-inflammatory effects may account for its anti-inflammatory and anti-ulcerative properties. Nevertheless, further detailed studies are warranted for exact elucidation of GAL mechanism on inflammation and colitis.

Keywords: Anticholinergic; Anti-inflammatory; Dicyclomine; Galantamine; Ulcerative colitis; Rats.

INTRODUCTION

One of the well-known gastrointestinal disorders is inflammatory bowel disease (IBD) including ulcerative colitis and Crohn's disease which both are chronic idiopathic inflammatory conditions which affects many peoples (1). Although the absolute cause of IBD is unknown, genetic factors, immune system dysfunction, changes in gut bacterial flora, and other environmental factors might be involved (2). Current treatments of IBD are mainly corticosteroids, 5-aminosalicylates, and immune-suppressive drugs (3). Unpleasant side effects and unsatisfactory control of the disease by current drugs are the main reasons which has led to alternative treatments with less side effects and appropriate efficacy highly attended by therapists (4,5).

Traditional medicines have used herbals with fewer side effects for treating IBDs (2,6). In this direction, several studies have reported that alkaloid and/or flavonoid-enriched extracts of medicinal herbs *e.g. Berberis vulgaris* (7) and *Dracocephalum kotscheyi* (8) or nutriceuticals *e.g. Cydonal oblonga* (9) and *Prunus armeniaca* (10) were beneficial in experimental colitis and it is likely to have benefits in IBD therapy or prevention.

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GAL is an alkaloid extract of *Galanthus woronowii* belongs to Amaryllidaceae family that is currently used for the treatment of Alzheimer's disease (11). The effect of GAL on neurovascular disease and vascular dementia has also attracted attentions (12). Growing medical evidences also has indicated that GAL may improve psychiatric disorders in schizophrenia, major depressive disorder, bipolar disease, and alcohol abuse (13). Though this drug is a selective, reversible, and competitive inhibitor of acetyl cholinesterase (AChE) could selectively stimulate and/or modulate neuronal nicotinic receptors (14). Because GAL has an additional allosteric potentiation at nicotinic receptors both centrally and peripherally, it may affect other neurotransmitters like monoamines, glutamate, and GABA which may be involved in its beneficial effects on IBD (15). Many studies have shown that GAL exerts its anti-inflammatory and even antineoplastic effects on gastrointestinal tract based on its antioxidant, antiapoptotic and likely cholinergic activities (16,17). According to different studies, GAL has no significant side effects and has a reasonable safety profile (18,19). In the current study we aimed to investigate the anti-inflammatory and antiulcerative effects of GAL on acetic acid-induced rat model of colitis and its possible underlying mechanism of action. Besides dicyclomine (DIC) was used as an anticholinergic agent to delineate the role of muscarinic receptors in ameliorative effects of GAL in this model of experimental colitis.

MATERIALS AND METHODS

Materials

GAL and mesalazine powders were prepared from Abu Rayhan Co. (Tehran, I.R. Iran). Prednisolone powder was procured from Iran Hormone Co. (Tehran, I.R. Iran) as a gift. Orto-dianizidin dihydrochloride and hexa-decyl trimethyl ammonium bromide were obtained from Sigma Co. (St. Louis, USA). Formaldehyde solution 37% and glacial acetic acid were purchased from Merck (Darmstadt, Germany).

Animal grouping

Sixty six male Wistar inbred rats weighing 180-220 g with 3 to 4 months old kept in the animal house of School of Pharmacy at Isfahan University of Medical Sciences (I.R. Iran), were used in this study. Rats were fasted for 24 h before starting the experiment having access to tap water *ad libitum*. The animals were randomly assigned in groups of six each and maintained in standard cages under controlled and standard conditions. The study was approved by the Animal Research Ethics Committee of Isfahan University of Medical Science in Iran (ethical approval ID: IR.MUI.RESEARCH.REC.1397.370) and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals. Possible efforts were made to decrease the number of animals used in the study and to lower the experimental distress. The animal's weight was determined using a digital balance at the beginning of experiment and just before sacrificing the animals for evaluating the extent of weight variation. Groupings of rats were as follows:

Group 1 (normal group), received vehicle (distilled water, 5 mL/kg, p.o.), and normal saline (2 mL) rectally; group 2 (control group), received vehicle (distilled water, 5 mL/kg, p.o.) 2 h before induction of colitis (2 mL acetic acid rectally); groups 3 and 4 (reference groups), were given prednisolone (4 mg/kg/day) or mesalazine (100 mg/kg/day, p.o.) 2 h before induction of colitis; groups 5-7, were administered GAL (0.25, 1.25, 2.5 mg/kg/day, p.o.) 2 h before induction of colitis; group 8, received DIC (5 mg/kg/day, p.o.) 2 h before induction of colitis; and groups 9-11 (DIC + GAL groups) received DIC (5 mg/kg, p.o.), 0.5 h before GAL administration (0.25, 1.25, 2.5 mg/kg, p.o.) while GAL was given 2 h before induction of colitis.

All of the treatments were conducted for subsequent 5 days. The rats were sacrificed 24 h after the last dose, their abdomens were opened and 8 cm of the colons, proximal to anus, were excised, washed with normal saline and their wet weight was determined (20).

Induction of colitis

After 24 h fasting, colitis was induced in rats by intra-rectal administration of 2 mL acetic acid 3% under light ether anesthesia. A soft flexible catheter was inserted 8 cm to the anus and acetic acid was carefully applied by a suitable syringe. The rats were then kept in head down position for 1 min to hinder leakage of solution (21).

Evaluation of colon macroscopic damage

Colonic tissue specimens were longitudinally opened, washed with normal saline, and their wet weights were determined. They were then fixed on a flat and white working sheet to observe ulcer scores when a number of suitable photos were taken for subsequent measurement of ulcer areas. The macroscopic damage scores of the tissues were evaluated as fallows (22):

0, no macroscopic damage; 1, mucosal erythema only; 2, mild mucosal edema, slight bleeding or slight erosion; 3, moderate to severe edema, bleeding ulcers or erosions; 4, sever ulceration, erosions, edema and tissue necrosis and/or perforation. Mucosal edema was reflected in tissue thickness and wet weight which was increased due to inflammation and extravasation. Erosion was considered as superficial and flabby ulcer which was limited to mucosal layers. Ulcers were the breach of the continuity of mucosal layer which extended to sub-mucosal and even muscular under layers. Bleeding and perforations were among ulcer complications (23).

Ulcer area was measured by Fiji-win 32 software (NIH Image for the Macintosh, 2004) after opening of each picture and remarking the borders of ulcer by the application. Ulcer index was eventually measured with summing ulcer score and ulcer area using the equation below (22):

Ulcer index = Ulcer score + ulcer area

For histopathology and MPO activity evaluation, the tissue specimens were longitudinally cut into 2 pieces; one piece was kept in 5 mL formalin 10% as fixator for histopathology assessment while the other piece of colon was frozen in liquid nitrogen and then kept in freezer (-70 C°) until the day of analysis of MPO activity.

Histopathology assessment

Formalin-dehydrated tissues were embedded with paraffin, blocked, and sectioned in 4 μm thick slices and then stained with hematoxylin and eosin (H&E). Inflammation severity and extent, edema, congestion, crypt damage, hemorrhage, and leukocyte infiltration were assessed in stained and coded sections utilizing validated scoring system described in previous work (24).

Determination of myeloperoxidase activity

MPO activity was measured according to the method described by Motavallian *et al.* (25). In this case, 0.1 g of tissue was accurately weighted and homogenized in 1 mL solution of 10 mM potassium phosphate buffer containing 0.5% hexa-decyl trimethyl ammonium bromide at 4 C° and then centrifuged at 20,000 rpm for 15 min. MPO activity was determined by adding 0.1 mL of the supernatant to 2.9 mL of 50 μm phosphate buffer (pH 6) containing 1.6 mM orto-dianizidin dihydrochloride and 0.1 mM hydrogen peroxide and measuring the absorbance of the mixture at 450 nm using a UV-Vis spectrophotometer (Kavian Pajouh Co., Tehran, I.R. Iran). The results were reported as units (U) per gram (g) weight of wet colon tissue.

Statistical analysis

Statistical analysis was accomplished using SPSS software (version 22). Data are given as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Turkey's HSD post hoc tests were used to compare differences between the experimental groups while the weight variations were analyzed using student's t-paired test. Scoring data was analyzed by Mann-Whitney U test. P value < 0.05 was considered significant for all results.

RESULTS

Macroscopic evaluation

Normal group did not express any sign of ulcer and/or erosion as expected. Control colitis group showed the highest macroscopic score and even hemorrhage within the colon. Prednisolone and mesalazine as reference agents exerted significant ($P < 0.001$) attenuation in inflammation and ulcerative

features of colitis as shown in Table 1 and Fig. 1. In groups receiving GAL alone, all macroscopic parameters improved with three examined doses (0.25, 1.25, 2.5 mg/kg/day) (at least $P < 0.05$); however, the effects were more prominent for the lowest dose (0.25 mg/kg). On the other hand, the groups treated with GAL + DIC showed similar results suggesting no more additive effects due to DIC usage. The best results achieved with the lowest dose of GAL both alone or together with DIC. DIC alone showed significant ($p < 0.05$) reduction in ulcer area and colonic tissue weight; however, its magnitude was somewhat lower than GAL at applied doses (Table 1 and Fig. 1).

Pathologic assessment

Normal group did not present histological damages. Sever inflammation, edema, and leukocyte infiltration both in mucosal and sub-mucosal layers were observed in control group. Prednisolone- and mesalazine-treated groups had significant reduction (at least $P < 0.01$) in most of histopathological parameters (Table 2 and Fig. 2). Histological assessments also showed that GAL was effective ($P < 0.01$) in reducing inflammation severity and extent, crypt damage, and leukocyte infiltration at lowest dose (0.25 mg/kg/day) with no significant ($P > 0.05$) difference with two other doses.

Table 1. Effect of GAL, alone or in combination with DIC, on the macroscopic parameters of colitis induced with acetic acid in rats. Normal, normal rats received distilled water (5 mL/kg/day); control, rats with colitis received distilled water (5 mL/kg/day). Data are expressed as mean ± SD or median (range) for scoring parameter, n = 6. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ indicate significant differences compared to control group and ### shows significant differences ($P < 0.001$) in comparison with normal group.

| Group (dose, mg/kg/day)	Ulcer score (0-4)	Ulcer area (Cm^2)	Ulcer index (0-12)	Colon weight (g)
Normal	0.0 (0-0)	0.00 ± 0.0	0.00 ± 0.0	0.63 ± 0.11
Control	4.0 (3-4)###	5.48 ± 0.8###	8.48 ± 0.8###	1.56 ± 0.23###
GAL (0.25)	1.5 (1-2)***	2.16 ± 0.4***	3.66 ± 0.9***	0.73 ± 0.1***
GAL (1.25)	2.0 (0-3)***	1.92 ± 0.6***	3.92 ± 0.7***	0.92 ± 0.2**
GAL (2.5)	3.0 (1-4)**	2.91 ± 0.2***	5.95 ± 0.6***	1.07 ± 0.18*
DIC (5) + GAL (0.25)	1.5 (0-3)***	1.54 ± 0.08***	3.04 ± 05***	0.71 ± 0.16***
DIC (5) + GAL (1.25)	1.5 (1-3)***	1.63 ± 0.2***	3.13 ± 0.63***	0.87 ± 0.15**
DIC (5) + GAL (2.5)	2.5 (2-3)**	2.06 ± 0.2***	4.68 ± 0.7***	0.95 ± 0.16*
DIC (5)	3.5 (2-4)	3.31 ± 0.4**	6.81 ± 0.81	1.10 ± 0.13*
Prednisolone (4)	1.0 (0-2)***	1.39 ± 0.3***	2.39 ± 0.4***	0.76 ± 0.1***
Mesalazine (100)	1.5 (1-2)***	1.63 ± 0.5***	3.13 ± 0.92***	0.86 ± 0.2**

GAL, galantamine; DIC, dicyclomine.
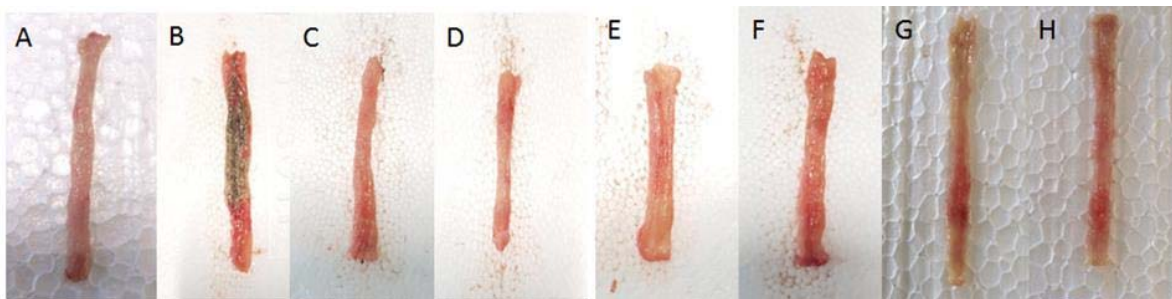
Fig. 1. Photographs of colon tissue in acetic acid-induced colitis in rats. A, Normal colon treated with normal saline; B, control colitis treated with normal saline; edema, erythema, thickness, and necrosis are evident; C, colitis treated with GAL (0.25 mg/kg/day); D, colitis treated with GAL (2.5 mg/kg/day); E, colitis treated with DIC (5 mg/kg/day) + GAL (0.25 mg/kg/day); F, colitis treated with DIC (5mg/kg/day); G, colitis treated with prednisolone (4 mg/kg/day); and H, colitis treated with mesalazine (100 mg/kg/day). GAL, galantamine; DIC, dicyclomine.

Table 2. Effect of GAL, alone or in combination with DIC, on the microscopic parameters of colitis induced by acetic acid in rats. Normal, normal rats received distilled water (5 mL/kg/day); control, rats with colitis received distilled water (5 mL/kg/day). Data are expressed as median, n = 6. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ indicate significant differences compared to control group, ### shows significant differences ($P < 0.001$) in comparison with normal group, and +$P < 0.05$, ++$P < 0.01$ denote significant differences versus related GAL alone group.

| Group (dose, mg/kg/day)	Inflammation severity (0-3)	Inflammation extent (0-3)	Crypt damage (0-4)	Leukocyte infiltration (0-3)	Total colitis Index (0-13)
Normal	0.0 (0-0)	0.0 (0-0)	0.0 (0-1)	0.0 (0-1)	0 (0-0)
Control	3 (3-3)###	2 (2-3)###	3 (2-4)###	3 (2-3)###	11 (9-13)###
GAL (0.25)	0.0 (0-1)***	1 (0-1)***	1 (0-1)***	1.5 (1-2)**	3.5 (1-5)***
GAL (1.25)	1 (1-2)***	2 (1-2)**	2 (1-2)**	1 (1-2)**	6 (4-7)***
GAL (2.5)	2 (2-3)**	2 (1-2)**	2.5 (2-3)**	2 (1-2)**	8.5 (6-10)**
DIC (5) + GAL (0.25)	0 (0-1)***	1 (0-1)***	1 (0-1)***	0.5 (0-1)***	2.5 (0-4)***
DIC (5) + GAL (1.25)	1 (1-2)***	1 (0-1)***	1 (0-1)***	1 (1-1)***	4 (2-5)***,+
DIC (5) + GAL (2.5)	2 (1-2)**	1 (0-1) ***	2 (1-2)**	1 (1-2)***	6 (4-8)***,++
DIC (5)	3 (2-3)	3 (2-3)	2 (2-3)*	3 (2-3)	11 (8-12)
Prednisolone (4)	0 (0-1)***	0 (0-1)***	0.5 (1-2)***	1 (1-2)**	1.5 (1-3)***
Mesalazine (100)	0 (0-1)***	0.5 (0-1)***	1 (0-2)***	1 (1-2)***	2.5 (1-2)***

GAL, galantamine; DIC, dicyclomine.
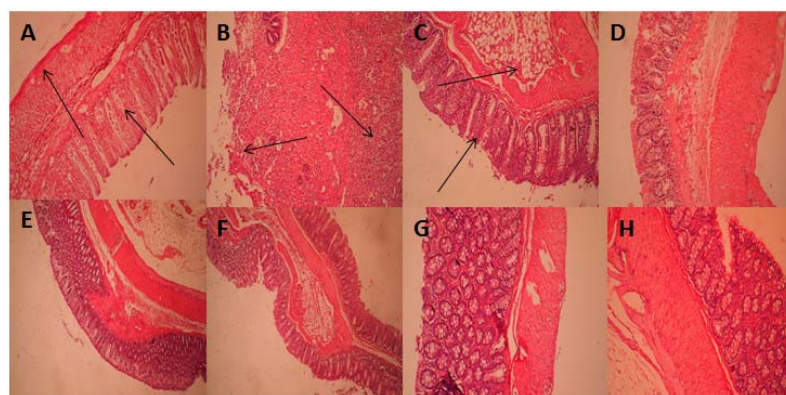
Fig. 2. Microscopic illustration of colonic tissue in acetic acid-induced colitis in rats. A, Normal tissue treated with distilled water (5 mL/kg); B, colitis control group which shows crypt damage, leucocytes infiltration, mucus and sub-mucosal layer edema, and inflammation; C, colitis treated with GAL (0.25 mg/kg/day); D, colitis treated with GAL (2.5 mg/kg/day); E, colitis treated with DIC (5 mg/kg/day) + GAL (0.25 mg/kg/day); F, colitis treated with DIC (5 mg/kg/day); G, colitis treated with prednisolone (4 mg/kg/day); and H, colitis treated with mesalazine (100 mg/kg/day). GAL, galantamine; DIC, dicyclomine.

Myeloperoxidase activity

MPO activity was diminished in all treated groups ($P < 0.001$) with the exception of DIC alone which was not significantly effective ($P > 0.05$) (Fig. 3). DIC + GAL attenuated MPO activity in colonic tissues while represented no more additive effect in comparison with GAL therapy alone (Fig. 3). Prednisolone and mesalazine showed significant diminution in MPO activity and unlike other assessed parameters this effect of mesalazine was better than prednisolone ($P < 0.05$).

Weight variation

Weight variation findings (Table 3) showed that normal group had significant ($P < 0.001$) weight gain at the end of the experiment period while the control colitis group, as expected, meaningfully ($P < 0.01$) lost weight. Weight variation was not significant ($P > 0.05$) in all of GAL and DIC alone as well as GAL + DIC groups indicating that weight loss was prevented by treatments. Prednisolone and mesalazine, on the other hand, were both effective to provide weight gain in this study ($P < 0.05$).
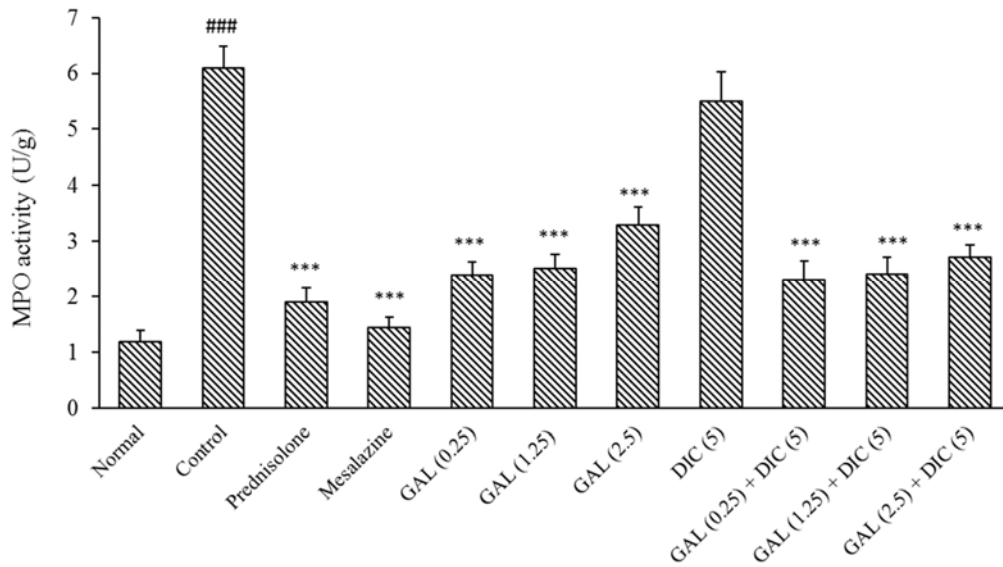
Fig. 3. The activity of MPO in colonic tissue of rats with acetic acid-induced colitis. Normal group received distilled water (5 mL/kg/day), control colitis group received distilled water (5 mL/kg/day), GAL (0.25, 1.25, 2.5 mg/kg/day), DIC (5 mg/kg/day), prednisolone (4 mg/kg/day), and mesalazine (100 mg/kg/day). Data are expressed as mean ± SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ indicate significant differences compared to control group and ### shows significant difference ($P < 0.001$) in comparison with normal group. GAL, galantamine; DIC, dicyclomine; MPO, myeloperoxidase.

Table 3. Weight changes of rats before and after the treatments in different groups. Normal, normal rats received distilled water (5 mL/kg/day); control, rats with colitis received distilled water (5 mL/kg/day). Data are expressed as mean ± SD, n = 6. T-paired test was used for data analysis.

Group (dose, mg/kg/day)	Weight variation (g) Before	After	P value
Normal	192.0 ± 2.9	203.8 ± 1.9	< 0.001
Control	206.2 ± 7.3	192.1 ± 7.2	< 0.01
GAL (0.25)	181.3 ± 11.6	184.8 ± 11.3	NS
GAL (1.25)	202.2 ± 7.7	198.5 ± 7.3	NS
GAL (2.5)	196.0 ± 8.6	187.8 ± 8.6	NS
DIC (5) + GAL (0.25)	180.2 ± 0.7	183.3 ± 0.7	NS
DIC (5) + GAL (1.25)	219.2 ±2.2	218.8 ± 1.8	NS
DIC (5) + GAL (2.5)	205.5±0.9	201.3±0.7	NS
DIC (5)	183.3 ± 3.7	189.2 ± 4.8	NS
Prednisolone (4)	205.0 ± 4.9	215.7 ± 2.0	< 0.01
Mesalazine (100)	211.5 ± 5.1	220.3 ± 7.6	< 0.05

GAL, galantamine; DIC, dicyclomine; NS, non-significant.

DISCUSSION

In this study we investigated the anti-inflammatory effect of GAL on ulcerative colitis and tried to delineate to what extent this effect is mediated through cholinergic pathways. GAL as a natural alkaloid originally extracted from *Galanthus woronowii* encompasses many medical indications for memory and cognition deficits such as Alzheimer's disease and dementia (26). GAL has also several bioactivities which make it a good candidate for investigation on experimental colitis. In recent study, GAL was effective on all colitis indices including ulcer index, total colitis index, and colonic weight gain. Subsequently the animal's weight loss was ceased by GAL treatment representing

beneficial effects of GAL on colitis and probably its associated symptoms like diarrhea and anorexia (27,28). GAL showed remarkable anti-inflammatory effect at nearly all tested doses, however, at greater dose (2.5 mg/kg/day), though not significant, exerted less protection against colitis rather than smaller dose (0.25 mg/kg/day). The dose range used for GAL in the current work was selected based on several previous studies carried out by investigators on different organ systems (29,30). We know that oral bioavailability of GAL is about 90-100% suggesting its remarkable systemic absorption after oral administration (31) indicating that beneficial effects of GAL are due to its systemic activity rather than its local effect within the colon lumen.

Antioxidant (32), anti-inflammatory (33), and antitumor effects (17) of GAL which might be beneficial for colitis alleviation have been previously reported. Melo *et al.* demonstrated that GAL prevented oxidative stress induced by amyloid-beta peptide and other oxidative conditions (32). It has also been reported that GAL can modify tumor necrosis factor alpha and nuclear factor kappa B levels, two critical cytokines in IBD pathology, through stimulation of cholinergic specifically nicotinic related anti-inflammatory pathway (34). Wazea *et al.* demonstrated that GAL anticolitic effect was mediated through alpha-7 nicotinic Ach receptor to suppress pro-inflammatory cytokines (27). Since anti-inflammatory effect of GAL is assumed to be, in part, related to its indirect cholinomimetic activity, and within gastrointestinal tract muscarinic receptors especially upon smooth muscles are dominant (3), we administered DIC, a nonspecific antimuscarinic drug prior to GAL administration, to delineate the underlying mechanism of action in colitis attenuation. We know that GAL is an allosteric modulator of nicotinic Ach receptors in addition to Ach.E inhibition. We hypothesized that if the muscarinic impact of GAL was responsible for its anti-inflammatory effects, DIC would reduce its effect and deteriorate tissue injury and inflammation. The results of current study, however, showed that DIC by itself was not effective in this model of

colitis and when it was administered accompanied with GAL could not reverse or blunt the anticolitic activity of GAL. This is the first study reporting that DIC, as a well-known nonspecific anti-muscarinic drug, could not alleviate and/or aggravate colitis supporting the idea that muscarinic pathway has probably little or no significant impact on colitis manipulation. Additionally this is suggesting that cholinomimetic activity is not supportive for beneficial anti-inflammatory properties of GAL. In support of this hypothesis, there are some studies illustrated that anticholinergic drugs are actually not beneficial for current IBD therapy (3,35) and cholinergic manipulation has not an impact on ulcerative colitis pathology (36). Indeed concerning cholinergic pathway and its impact on IBD, there are controversial findings that necessitate more detailed and mechanistic investigations. Kolgazi *et al.* revealed that both nicotine (1 mg/kg/day) and huperzine A (0.1 mg/kg/day) as AchE inhibitor had anti-inflammatory action by neurologic modulation of cytokine synthesis and resulted in improved acetic acid-induced colitis when assessed by macroscopic, microscopic, and biochemical evaluation (34). Shifrin *et al.* conversely reported that cholinergic anti-inflammatory pathway did not contribute to prevention of ulcerative colitis exerted by two indole carbamates; AN680 (2.5-10 mg/kg) and AN917 (2-5 mg/kg) and even rivastigmine (1 mg/kg) as AchE inhibitor (36). Such controversies are attributed to vagus nerve mediated regulation of immunes system and synthesis of pro-inflammatory cytokines in bowel despite an apparent lack of neural circuit between the vagus nerve and target organs (37). Therefore, further experiments that could better characterize these mechanisms would be critical and highly warranted.

CONCLUSION

In conclusion, GAL exerted beneficial anti-inflammatory and antiulcerative effects on experimental colitis. These effects were surprisingly independent of its muscarinic

activity and so it might be attributed to its other pharmacologic effects like antioxidant, anti-inflammatory, immune-modulatory, and others related to neuronal nicotinic pathways. Further detailed studies are warranted to delineate the absolute mechanisms of GAL action in IBD.

ACKNOWLEGMENTS

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