Gastroprotective effect of 8-alkylberberine derivatives on ethanol-induced gastric ulcer in rats

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Article

Keywords:

Posted Date: April 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1567588/v1

Abstract

Berberine (BBR) is an isoquinoline alkaloid which protects the acute gastroenteritis and inhibits gastric ulcers, and 8-Alkylberberine (8-BBR-Cn, n=4, 8, 12 and 16) with a long aliphatic chain are synthesized have been reported. This paper investigated the possible mechanisms underlying the gastroprotective effect of 8-BBR-Cn derivatives at dose of 25, 50 and 100 mg/kg body weight on enthanol-induced gastric mucosal injury in rats. The ulcer index and percentage inhibition were evaluated. The stomachs tissues were chosen for determination of nitric oxide synthase (NOS) activity and prostaglandin E2 (PEG2) levels, and assay kit were used for the above two assays. Compared with model control group, the ulcer indexes of 8-BBR-Cn (25, 50 and 100 mg/kg, i.g.) treatment groups decreased significantly. Additionally, 8-BBR-Cn could enhance PEG2 concentration in mucosal tissue of gastric ulcer rats. Moreover, in mucosal tissue, activities of total NOS (TNOS) and constitutive NOS (cNOS) were increased, but inducible NOS (iNOS) was decreased by 8-BBR-Cn. 8-BBR-Cn derivatives had protective effect on gastric mucous membrane, and 8-BBR-C_{16} displayed stronger gastroprotective effect as carbon chain at C8 position lengthens, which was related to prostaglandin E2 and nitric oxide synthase pathways.

Introduction

Gastric ulcer is becoming one of the most common diseases of the digestive system and is of global economic importance due to its higher and higher morbidity and mortality[1]. Gastric lesions are results of mucosal damages caused by cellular influx and free radical generation[2, 3]. Gastric ulcer pathophysiology has been centered on the imbalance between aggressive factors (chloridric acid and pepsin) and protective ones (mucus, bicarbonate, prostaglandins, nitric oxide and dopamine, sulphydryl compounds, polyamines) [4-6].

The continuous generation of prostaglandins by cyclooxygenase isoenzymes in the gastric mucosa helps to maintain an adequate mucosal blood flow and stimulate the generation of mucus[7] and low level of prostaglandin impairs gastric mucosa[8]. Besides prostaglandins, the second major defensive system in the gastric mucosa is the L-arginine/nitric oxide (NO) pathway[9, 10]. NO is generated by L-arginine and the rate-limiting enzymes is NOS. The protective properties of the NO derived from cNOS have already been well established, but less clear is the role assigned to iNOS[11]. NO dilates blood vessels, increases gastric blood flow, and stimulates angiogenesis during the ulcer healing processes[12-14].

8-BBR-Cn (n=4, 8, 12 and 16) with a long aliphatic chain were synthesized in our laboratory including the compounds 8-butyl-, 8-octyl-, 8-dodecyl- and 8-hexadecylberberine chloride[15]. The difference among 8-BBR-Cn derivatives is the aliphatic chain at C8 position. Moreover, BBR had pronounced anti-ulcer activity of in gastric ulcer[16, 17]. However, the relation between 8-BBR-Cn derivatives and gastric mucosal injury has not been investigated. In this study, the gastric ulcer in rat was induced by ethanol, the prostaglandin concentration and NOS activity were used to evaluate the protective effect of 8-BBR-Cn on ethanol-induced gastric ulcer in rats. This is the first report regarding the gastroprotective effect of 8-BBR-Cn

derivatives, and the results may contribute to increase the knowledge on herbal-derived substances and can provide a reference for the research on treatment of gastric ulcer.

Materials And Methods

Animals

Female Sprague-Dawley rats (180-200g) were purchased from Experimental Animals Co. LTD of Chongqing Tengxin Bier (Grade，SYXK-(YU), 2009-0002). Rats were housed under constant environmental conditions with temperature (25 ± 1℃) and humidity (50 % ± 10 %) for a week before the experiment. They were kept under a 12 h light/12 h dark cycle (08:00–20:00, light) and free access to food and water. All rats were fasted for 48 h before experiments with free access to water. Coprophagy was prevented by keeping the animals in cages with gratings as the floors. The distribution of animals in the groups and the treatment allotted to each group with ten animals (seventeen groups) were randomized.

17 groups were treated as follows: Group 1: the rats in normal control were fed with 0.8 ml normal saline (0.9% NaCl) by gavage, rats in Group 2 to Group 17 were fed with 0.8 ml anhydrous alcohol by gavage. Group 2 served as model control, Groups 3 to Groups 17 were administered 8-BBR-Cn (suspended in distilled water) at dose of 25, 50 and 100 mg/kg body weight respectively. Groups 3 to Groups 17 were (25, 50 and 100 mg/kg) BBR group, (25, 50 and 100 mg/kg) 8-BBR-C_4 group, (25, 50 and 100 mg/kg) 8-BBR-C_8 group, (25, 50 and 100 mg/kg) 8-BBR-C_{12} group and (25, 50 and 100 mg/kg) 8-BBR-C_{16} group respectively.

Ethics statement

The study was designed in accordance with ARRIVE guidelines. All experiments approved by the Ethical Committee of Animal Care of the Third Military Medical University under the bioethical allowance number SYXK-(Yu) 2007-0005 and complied with the Guide for Care and Use of Laboratory Animals.

Reagents and chemicals

PGE2 assay kit was obtained from Shanghai Baiwo technology co. LTD (Shanghai, China). NOS assay kit was obtained from Nanjing Jiancheng bioengineering institute (Jiangsu, China), other reagents and chemicals were of analytical reagent grade. Berberine (purity > 98%) was obtained from Xi'an Realin Biotechnology Co., Ltd. (Xi'an, China). 8-BBR-Cn (n=4, 8, 12 and 16, purity > 98%) were synthesized and provided by our laboratory [15].

Ethanol-induced gastric lesions

The rat model with gastric ulcer was established according to literatures [18, 19]. The rats were fed with 8-BBR-Cn (n=0, 4, 8, 12 and 16) by gavage and two hours later the rats were fed with 0.8 ml anhydrous

ethanol. One hour after the administration of ethanol, the animals were sacrificed and examined for lesions in the stomachs.

Stomach was incised along the greater curvature and examined for ulcers in the glandular region. Usually, circular lesions consisted of long, dark red bands running vertically down the corpus of the stomach were observed but, sometimes, linear lesions were also seen. The lesions were scored on a 0-10 scale according to the estimated percent of corpus mucosa covered with lesions. Stomachs of rats were subjected to visual macroscopic examination (Fig. 1) and ulcer score was calculated. The gastric tissues were stored at -80 ℃ before biochemical analysis.

Determination of ulcer index (UI) and percentage inhibition

UI and percentage inhibition in ethanol-induced rats were calculated. The stomach was cut open along the greater curvature and the inner surface was examined by dissecting microscope. The UI was calculated according to the scoring method of Tan *et al*[20]. Percentage ulcerated surface (US), was calculated as: US (mm^2) = (total area covered by ulcers ÷ total corpus mucosal surface)×100. The following score was used in order to calculate ulcer indices: 0, no ulcer; 1, US≤0.5; 2, 0.5≤2.5; 3, 2.5≤5; 4, 5≤10; 5, 10≤15; 6, 15≤20; 7, 20≤25; 8,25≤30; 9, 30≤35; 10, US>35. The UI for each animal was then calculated as the mean ulcer score. Percentage inhibition was calculated according to the formula method of Hariprasath *et al*[21]. The percentage of inhibition was calculated by the following formula: [($UI_{control} - UI_{treated}$)÷$UI_{control}$]×100.

PGE2 estimation

The mucosa was scrapped and rapidly rinsed with ice-cold saline. The tissue was weighed and homogenized in 10 volumes of phosphate buffer (0.1 M, pH-7.4). The homogenate was centrifuged (3000×g, 15 min, 4 ℃) and the supernatant was transferred into a new test tube. The supernatant was processed for PGE2 estimation using the enzyme linked immunosorbent assay kit, following the manufacturer's instructions. Results were expressed as pg PGE2/ml.

Determination of NOS activity in mucosal tissue

The stomach was removed and gently rinsed with 0.9 % NaCl. Mucosa was scrapped and rapidly rinsed with ice-cold saline. The tissue was weighed and homogenized in 10 volumes of phosphate buffer (0.1 M, pH-7.4). The homogenate was centrifuged (3000×g, 15 min, 4 ℃) and the supernatant was used for NOS activity determination by NOS assay kit, following the manufacturer's instructions. The activites of iNOS and TNOS were measured directly and the cNOS activity was caculated by subtracting iNOS activity from TNOS activity. The OD value was determined by a spectrophotometer (U-3010, Hitachi, Japan) at 530 nm. Results were expressed as U NOS/mg prot (A unit of NOS was defined as 1 nmol NO produced by reacting 1 min in 1 mg of tissue protein at 37℃).

Protein concentration of the supernatants in tissue sample was determined by spectrophotometry using a commercial assay kit by Coomassie blue method.

Statistics analysis

Data from this study were analyzed by the version 17.0 of the SPSS programme and reported as mean ± SEM of three separate experiments for each sample. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test.

Results

Anti-ulcer effect of 8-BBR-Cn derivatives

The anti-ulcer effects of 8-BBR-Cn 25, 50 and 100 mg/kg derivatives on ethanol-induced ulcer model in rats were shown in Table 1. The UI in model control rats was 9.77±0.41 (P<0.001) compared with the blank control group, which explained the successful establishment of experimental model. The UI of 8-BBR-Cn derivatives (n=0, 4, 8, 12 and 16) (25, 50 and 100 mg/kg) was reduced significantly (P<0.001) compared with the model control group. The ulcer inhibition rates of 8-BBR-C_{16} (25, 50 and 100 mg/kg) were 73.18%, 82.01% and 91.30% respectively, which were higher than other treated group (40~60 %).

Effect of 8-BBR-Cn derivatives on PGE2 level of mucosal tissue

It showed that ethanol reduced the content of PGE2 in mucosal tissue significantly from 154.78±5.04 to 95.18±5.05 pg/ml by comparing blank control group (P<0.001) (Fig. 2). Administration of 8-BBR-C_{12} (50 and 100 mg/kg) enhanced PGE2 concentration in mucosal tissue (P<0.05). Moreover, 8-BBR-C_{16} (25 mg/kg) significantly increased PGE2 concentration (P<0.01), and 8-BBR-C_{16} (50, 100 mg/kg) significantly increased PGE2 concentration (P<0.001) respectively.

Effect of 8-BBR-Cn derivatives on NOS activity in mucosal tissue

Fig.3 showed that administration of ethanol very significantly (comparing blank control group) reduced the activities of TNOS in mucosal tissue from 51.41±0.63 to 35.19±1.50 U/mg prot (P<0.001), and reduced also cNOS from 33.09±0.47 to 10.73±3.06 U/mg prot (P<0.001), while iNOS activity was enhanced from 18.32±0.66 to 24.46±1.56 U/mg prot (P<0.05). As for the TNOS, BBR, 8-BBR-C_4 and 8-BBR-C_8 enhanced TNOS activity (P<0.05). Meanwhile, administration of 8-BBR-C_{12} (25, 50 and 100 mg/kg) were significantly enhance TNOS activity (P<0.01), reaching 44.32±1.55, 45.29±1.79 and 46.22±1.68 U/mg prot respectively. On the other hand, 8-BBR-C_{16} (25, 50 and 100 mg/kg) were significantly enhance TNOS activity (P<0.001), reaching 50.88 ±0.89, 51.77±1.60 and 52.24±1.10 U/mg prot respectively, and 8-BBR-C_{16} showed the strongest effect. As for the iNOS, 8-BBR-C_{16} reduced iNOS activity significantly (P<0.05). 8-BBR-Cn (25, 50 and 100 mg/kg) enhanced the activities of TNOS in mucosal tissue. As for the cNOS, 8-BBR-C_{12} and 8-BBR-C_{16} enhanced cNOS activity significantly (P<0.01, P<0.001) respectively.

Discussion

The present study showed that 8-BBR-Cn (n=0, 4, 8, 12 and 16) given overall provided dose-dependent gastric protection against the effects of ethanol in rats, and 8-BBR-Cn reduced more significantly as indicated by ulcer score with increasing carbon chain at C8 position. It found that 8-BBR-Cn derivatives adhered to the gastric mucosa in the experiment, and were increasingly the attached objects that would form the physical protective properties of surface layer along with increasing carbon chain of the derivatives. This finding demonstrated that BBR with a long aliphatic chain increased lipophilic (hydrophobic) properties to exerting Lipid-modulating effect in vivo and combined firmly together with membrane proteins of the gastric mucosa. So, 8-BBR-Cn showed a significant gastroprotective effect.

Prostaglandins play important roles in modulating the mucosal integrity and various functions of gastrointestinal tract[22]. Decreased prostaglandin level impairs the gastric mucosa and aggravates the ulcer[8]. This study showed that increasing carbon chain at C8 position in the molecular structure to 8-BBR-Cn would enhance PGE2 level of mucosal tissue from ethanol-induced gastric mucosal injury especially when carbon chain number (Cn) exceeded twelve in 8-BBR-Cn derivatives. Since, 8-BBR-C_{16} would have stronger mediating the gastroprotective effect in the role of PGE2 with increasing carbon chain at C8 position in 8-BBR-Cn derivatives.

NO plays an important role in the mechanism of gastric mucosal protection[16, 23, 24]. NO concentration is affected by NOS directly. Protective effects are mediated by cNOS/NO and proulcerogenic effects are mediated by iNOS/NO in the gastrointestinal tract[25]. In this study, 8-BBR-Cn enhanced the activities of TNOS and cNOS in mucosal tissue, while reduced iNOS activity in mucosal tissue in gastric ulcer rats. These results suggested that 8-BBR-Cn protected gastric mucosa from ethanol-induced gastric ulceration. Moreover, 8-BBR-C_{16} showed the stronger effect on NOS activity in mucosal tissue compared with other 8-BBR-Cn (n=0, 4, 8 and 12). The result showed that 8-BBR-C_{16} showed stronger gastroprotective effect as the carbon chain at C8 position lengthened.

This study examined the possible mutual interaction of NOS and PGE2 in the mechanisms responsible for the anti-ulcer activity of 8-BBR-Cn in ethanol-induced gastric mucosal injury. Thus, it is plausible to envision the use of compounds with structure modification or herbal-derived substances as gastric protective agents for the gastric ulcer development.

Conclusions

The gastroprotective effects of 8-alkylberberine derivatives were first reported. Results in this study showed that 8-BBR-Cn protected gastric mucosa from ethanol-induced gastric ulceration. The mechanisms for anti-ulcer activity can be attributed to enhance PGE2 concentration and the activities of TNOS and cNOS, and reduce iNOS activity. Moreover, NOS and PGE2 pathways play an essential role in the gastroprotective effects of 8-BBR-Cn. From molecular solid structure perspective, increasing carbon chain at C8 position in 8-BBR-Cn would mediate the gastroprotective effect from ethanol-induced gastric

ulceration. Since, structure modification of BBR would change its biological activities. This study was conducted in rat, its relevance to human gastric ulcers is not known and warrants further study.

Declarations

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by the Department of Science and Technology of Sichuan Province (2021YFN0101). The authors thank Prof. Xuegang Li (Southwest University, China) for enlightening discussions and experimental supports.

Author contributions

Y.H.S. and J.F.T. contributed to do the experiment, and to write the manuscript and analyze the data.

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Table

Table 1 Effects of 8-BBR-Cn derivatives on ethanol-induced gastric mucosal ulcers in rats.

Groups	Treatments (ig)	Dose (mg/kg)	Mean ulcer index	Inhibition (%)
Group 1	blank control	-	0	-
Group 2	model control	-	$9.77\pm0.41^{\#\#\#}$	-
Group 3	BBR	25	$5.61\pm0.29^{***}$	42.61
Group 4	BBR	50	$5.28\pm0.26^{***}$	45.96
Group 5	BBR	100	$5.26\pm0.59^{***}$	46.03
Group 6	8-BBR-C_4	25	$5.84\pm0.17^{***}$	40.23
Group 7	8-BBR-C_4	50	$5.47\pm0.38^{***}$	43.98
Group 8	8-BBR-C_4	100	$5.55\pm0.23^{***}$	43.19
Group 9	8-BBR-C_8	25	$5.53\pm0.20^{**}$	43.43
Group 10	8-BBR-C_8	50	$5.14\pm0.42^{***}$	47.42
Group 11	8-BBR-C_8	100	$5.08\pm0.39^{***}$	48.00
Group 12	8-BBR-C_{12}	25	$4.72\pm0.40^{***}$	51.69
Group 13	8-BBR-C_{12}	50	$3.88\pm0.15^{***}$	60.21
Group 14	8-BBR-C_{12}	100	$3.84\pm0.19^{***}$	60.25
Group 15	8-BBR-C_{16}	25	$2.62\pm0.23^{***}$	73.18
Group 16	8-BBR-C_{16}	50	$1.76\pm0.49^{***}$	82.01
Group 17	8-BBR-C_{16}	100	$0.85\pm0.45^{***}$	91.30

Data represent the mean ± SEM of observations from 10 rats.

a: Group 1 compared with Groups 2, $^{\#}P<0.05$ $^{\#\#}P<0.01$ $^{\#\#\#}P<0.001$,

b: Group 2 compared with Groups 3–17, $^{*}P<0.05$ $^{**}P<0.01$ $^{***}P<0.001$.

Figures

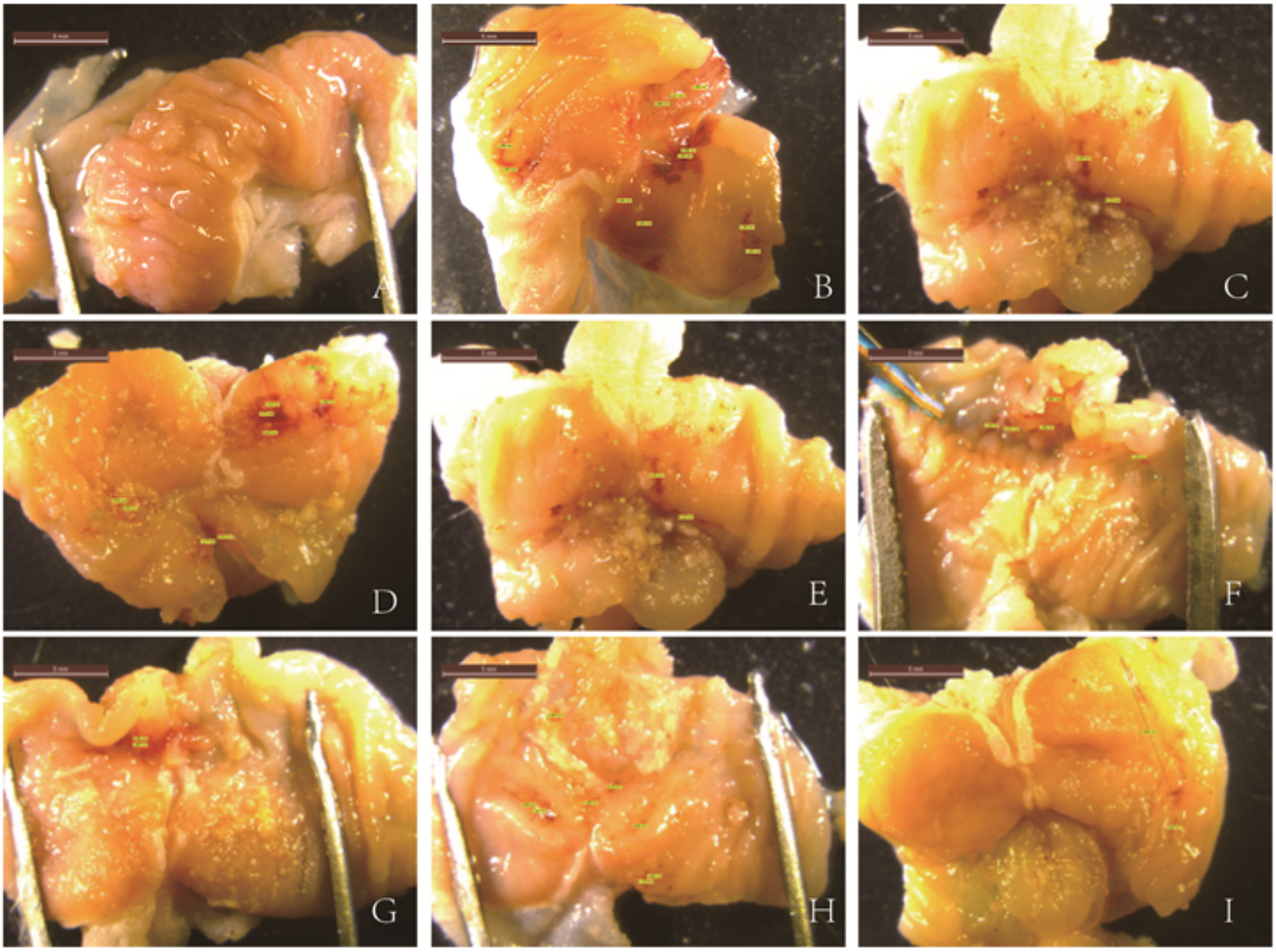

Figure 1

The representative macroscopic appearance of gastric mucosa in different groups. Note: A: normal group; B: model group; C: BBR (50 mg/kg); D: 8-BBR-C_4 (50 mg/kg); E: 8-BBR-C_8 (50 mg/kg); F: 8-BBR-C_{12} (50 mg/kg); G: 8-BBR-C_{16} (25 mg/kg); H: 8-BBR-C_{16} (50 mg/kg); I: 8-BBR-C_{16} (100 mg/kg).

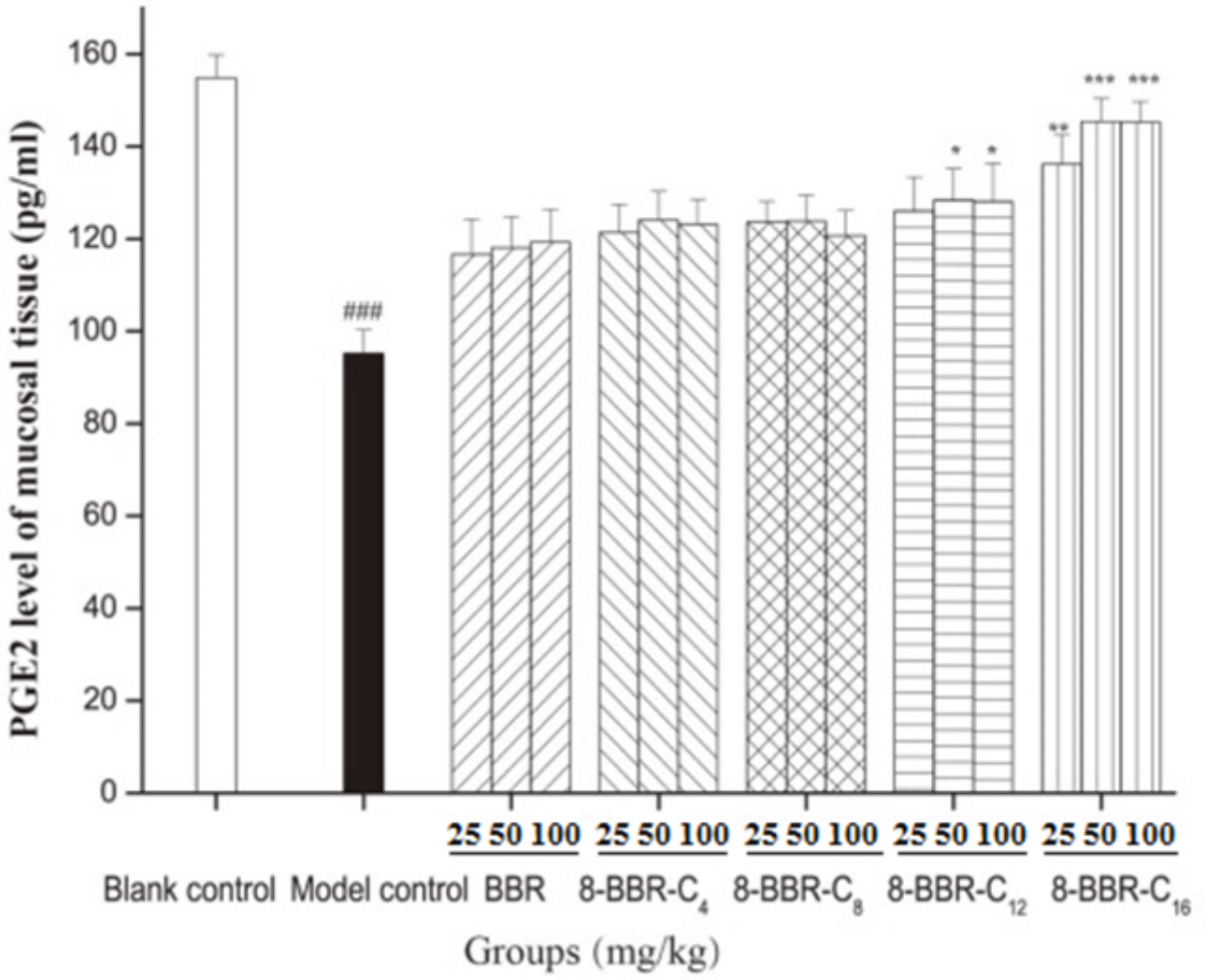

Figure 2

Effect of 8-BBR-Cn derivatives on PGE2 level of mucosal tissue in rats. Values presented are mean ± SEM of 10 rats in each group (n=10). Multiple comparisons between treatment groups were performed by Tukey's test. a: Group 1 compared with Groups 2, $^{\#}P<0.05$ $^{\#\#}P<0.01$ $^{\#\#\#}P<0.001$; b: Group 2 compared with Groups 3–17, $^{*}P<0.05$ $^{**}P<0.01$ $^{***}P<0.001$.

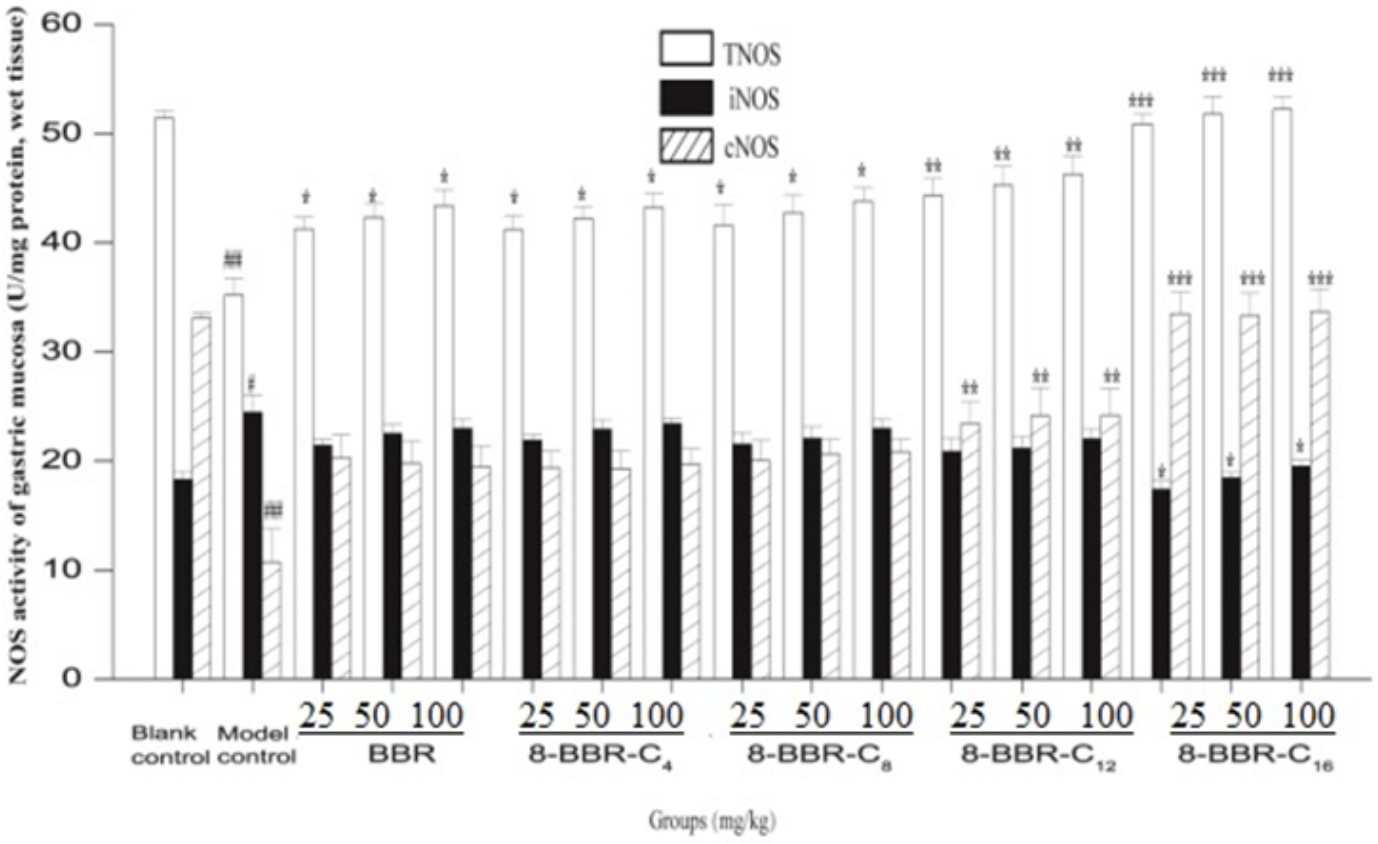

Figure 3

Effect of 8-BBR-Cn derivatives on NOS activity in mucosal tissue of rats. Multiple comparisons between groups were performed by Tukey's test, mean ± SEM, n=10, a: Group 1 compared with Groups 2, $^{\#}P<0.05$ $^{\#\#}P<0.01$ $^{\#\#\#}P<0.001$; b: Group 2 compared with Groups 3–17, $^{*}P<0.05$ $^{**}P<0.01$ $^{***}P<0.001$.