



EFFECT OF GENISTEIN PHENYLPIPERAZINE DERIVATIVE ON TRAUMATIC ORAL ULCER

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ABSTRACT

The present study was performed to probe into the effect of genistein phenylpiperazine derivative (GPD) on traumatic oral ulcer so as to provide theoretical guidance and practical data for such areas as food and drug. A traumatic oral ulcer model in guinea pig was built by phenol (90%) burn method and the indicators of guinea pig were determined in this study to investigate the pharmacological mechanism of GPD's effect on traumatic oral ulcer. The findings showed that high-dose GPD brings about remarkable therapeutic effect on traumatic oral ulcer ($P < 0.01$). Furthermore, concentrations of SOD (47%), AMPK (84%), IL-10 (132%) and PEG-2 (12%) increase significantly ($P < 0.01$) while the concentrations of MDA (40%), mTOR (62%), ROS (36%), NO (20%), NF- κ B (8%) and TNF- α (33%) in guinea pig plasma ($P < 0.01$) obviously decreased as compared with the vehicle. GPD has a significant effect on treatment of traumatic oral ulcer by inhibiting inflammatory reaction, improving oxidative stress, and suppressing the growth and reproduction of inflammatory cells.

KEYWORDS: Genistein, Derivative, Piperazine, Traumatic oral ulcer, Guinea pig.

INTRODUCTION

As one of the most common oral diseases, oral ulcer, with a population incidence of 10%-17%, is characterized by periodicity and high recurrence rate.^[1] Existing studies are not yet adequate to interpret the pathogenesis of oral ulcer and the factors inducing oral ulcer may

include inheritance, viral or bacterial infections and food allergies and deficiency of vitamins or trace elements etc.^[2] At present, the treatment of oral ulcer includes systemic treatment and local treatment; most studies tend to recommend local treatment of oral ulcer since systemic treatment, for example, with prescription of traditional Chinese medicine features complicated medication, diversified side effects and low efficiency.^[3]

Genistein phenylpiperazine derivative (GPD) is the combination product of genistein and 1-(2,4)-dimethyl phenyl piperazine. Some studies have shown that GPD has antitumour effect and could thus inhibit the activity of gastric cancer MGC-803 and hepatocellular carcinoma cell HepG2; when it comes to anti-microbial effect, GPD has remarkable inhibitory effect on staphylococcus aureus and bacillus subtilis etc.; additionally, GPD has a protective effect on gastric mucosa and could thus enhance the anti-inflammatory effect and defensive protection thereof.^[4,5]

To exploit GPD function and widen its application spectrum, this paper provides technical support and theoretical basis for its development in the field of food or pharmaceutical by probing into the effect of genistein phenylpiperazine derivative on local treatment of traumatic oral ulcer and the associated mechanism of action.

MATERIALS AND METHODS

Chemicals

Guinea pigs TNF- α , mTOR, PGE-2, ROS, NF- κ B, AMPK, NO, IL-10, MDA and SOD kits were purchased from Shanghai Lanpai Biological Technology Co., Ltd. GPD (99%) was lab-synthesized. Ethanol etc. (analytically pure) was purchased from Yantai Shuangshuang Chemical Co., Ltd. 1-(2,4-dimethylphenyl) piperazine (analytically pure) was obtained from Xiya Reagent. Mirabilitum praeparatum (MP) was purchased from Guilin Sanjin Pharmaceutical Co., Ltd.

Phenol induced oral ulcer

84 healthy guinea pigs (250-270g, 42 males and females, respectively) were purchased from Jinan Jinfeng Experimental Animals Co., Ltd. (production license: SCXK (Shandong) 2014006) and randomly divided into normal vehicle, vehicle, DMSO group, MP group (45 mg/kg), low-dose GPD group (LGPD, 0.45 mg/kg), medium-dose GPD group (MGPD, 4.5

mg/kg) and high-dose GPD group (HGPD, 45 mg/kg). Except for the normal group, the other ones were anesthetized with ether. The cotton ball was placed at one end of the glass tube of 5mm in diameter and dipped it with 90% phenol solution to burn the left cheek of guinea pig for 60s. All guinea pigs were found with ulcers of about 5mm in diameter in the oral mucosa when observed in 24h, indicating the vehicle was successfully built.^[6] Cover the oral ulcer area of each guinea pig from the treated group with a piece of sterilized cotton with 0.5mL of corresponding medical solution (0.5mL of 0.9% sodium chloride solution for the vehicle) for 3 min and clean administration area with warm normal saline. Apply the solution once a day for 5 consecutive days. The blank group was left untreated. Take pictures of oral ulcer in guinea pig with a digital camera on d1, d3 and d5 after application.

Macroscopic oral mucosa damage

The evaluation of the degree of ulcer is divided into 5 grades. “0” means the oral mucosa is in good condition without ulcer; “I” means ulcer develops but without obvious pseudomembrane on its surface; “II” means a yellowish white pseudomembrane is on the surface of ulcer but without edema around; “III” indicates a thick pseudomembrane is on the surface of ulcer with inflammatory edema around; “IV” indicates a thick pseudomembrane on ulcer surface with obviously inflammatory edema around.^[7] Each figure in the table indicates the number of guinea pigs that correspond to each degree.

Index Measurement

Blood samples were taken from guinea pig through cardiac puncture and were collected into heparinized eppendorf tubes and centrifuged at 2500 rpm for 15 min. The supernatant were packing separated and stored at 80°C until analysis. The levels of TNF- α , mTOR, PGE-2, ROS, NF- κ B, AMPK, NO, IL-10, MDA and SOD were determined using the guinea pig enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions, respectively.^[8]

Data analysis

All data were expressed as mean \pm standard error of mean(S.E.M). Statistical analysis was performed using SPSS 17.0 statistical software. Differences among groups were analyzed by an oneway analysis of variance (ANOVA) followed by Dunnett's test. P values less than or

equal to 0.05 were considered statistically significant. Rank data is checked by Ridit.

RESULTS AND DISCUSSION

Macroscopic examination

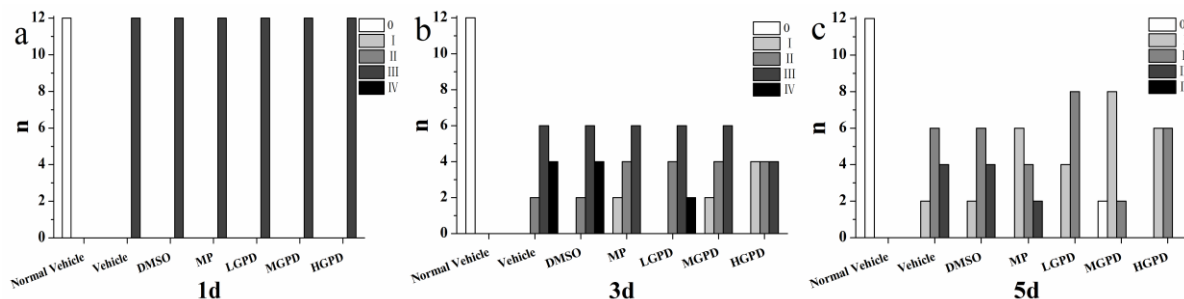


Fig. 1. Curative effect of GPD on oral ulcer of guinea pig at different times. “n” means the number of guinea pigs at each of the different levels.

Fig. 1 showed the effect of GPD on degree of oral ulcer in guinea pig, and the data was subjected to Ridit test.^[6] No ulcer developed in the normal vehicle, when compared with the normal vehicle, vehicle exhibited obvious local hyperemia and edema ($P < 0.01$) within 1-5d, which indicated the vehicle was successfully built. As compared with the vehicle, the degree of ulceration in all administration groups showed no significant difference on d1. On d3, MP extremely significantly alleviated the degree of oral ulcer ($P < 0.01$), while GPD could obviously alleviate the degree of oral ulcer ($P < 0.05$), HGPD offered the most remarkable alleviation of oral ulcer. On d5, guinea pigs treated with MP and GPD exhibited significant alleviation of the degree of oral ulcer ($P < 0.01$, $P < 0.05$). This indicated that, GPD helps to alleviate the degree of oral ulcer, and promote the skin healing and recovery at the site of oral ulcer, thereby significantly curing oral ulcer. The curative effect of GPD complies with a dose-effect relationship, which is to say, the curative effect of GPD on oral ulcer gets remarkably enhanced with the increase of GPD dose. The curative effect of GPD is time-efficient, remarkable therapeutic action takes place within 3d, the cure rate within 5d is up to 16.7% and the degree of oral ulcer falls below level 3 in all cases studied.

Effect of GPD on inflammatory reaction

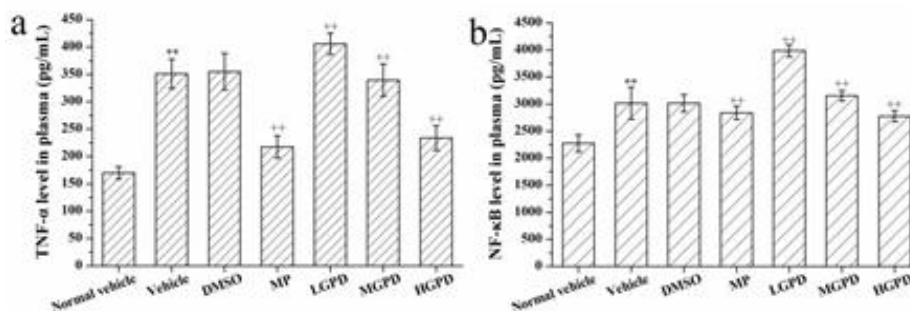


Fig. 2: Effect of GPD on the levels of TNF- α (a) and NF- κ B (b). +P<0.05 and ++P<0.01 compared with vehicle, *P<0.05 and **P<0.01 compared with normal vehicle.

Inflammatory factor: Tumor necrosis factor- α (TNF- α) is a proinflammatory factor that can facilitate T cells produce a variety of inflammatory reactions.^[8,9] As a transcription factor with several transcription regulation effects, NF- κ B could participate in the regulation of many inflammation-related genes.^[10]

Since statistical analysis of data showed that DMSO had no effect on the inflammatory factor, thus DMSO had no effect on treatment of oral ulcer and had no effect on the objective evaluation of GPD in this experiment. As shown in Fig. 2, compared with the normal vehicle, phenol administration increased inflammatory factors TNF- α (106%, Fig. 2a) and NF- κ B (32%, Fig. 2b) significantly in plasma. As compared with the vehicle, HGPD could significantly reduce the levels of TNF- α and NF- κ B and the inhibition rate were 33% and 8%, respectively. MGPD-based treatment reduced the TNF- α content by 3%, and brought about no significant effect on NF- κ B. LGPD exhibited no significant effect on the expression of inflammatory factors. The content of NF- κ B in plasma of guinea pigs treated with HGPD was lower than in the MP group.

In this study, GPD inhibited proinflammatory factor TNF- α , thereby inhibiting the inflammatory reaction that may lead to oral ulcer. On the other hand, GPD was in a position to inhibit NF- κ B activation, thereby regulating complicated proinflammatory cytokine network and alleviating the severity of oral ulcer and mucous membrane injury.

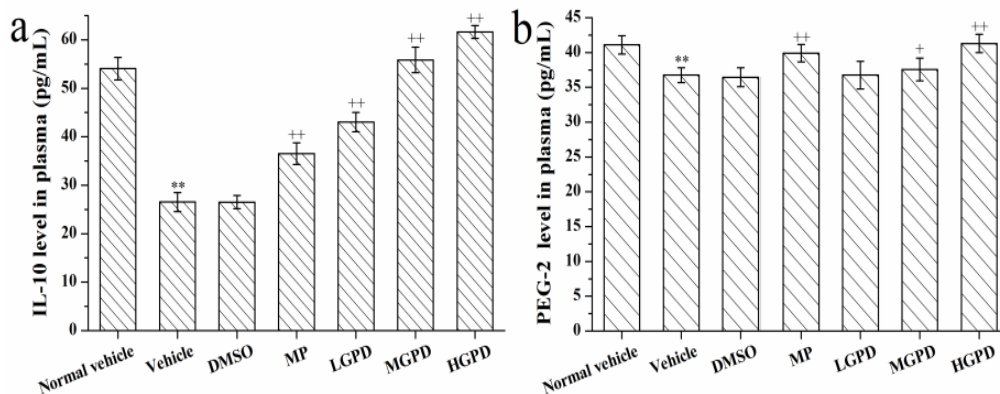


Fig 3: Effect of GPD on the levels of IL-10 (a) and PEG-2 (b). +P<0.05 and ++P<0.01 compared with vehicle, *P<0.05 and **P<0.01 compared with normal vehicle.

Anti-inflammatory cytokine: As an anti-inflammatory factor secreted by Treg cells to regulate the growth and differentiation of immune cells, interleukin 10 (IL-10) negatively regulates immune response in immune responses.^[11,12] Prostaglandin E₂ (PGE-2), as an important regulatory factor and growth factor in cells, is immunosuppressive and anti-inflammatory.^[13]

Data analysis showed that DMSO had no effect on the levels of IL-10 and PGE-2, so the evaluation of the effect of GPD on oral ulceration would not be affected by DMSO. As compared with the normal vehicle, the levels of anti-inflammatory cytokines IL-10 (51%, Fig.3a) and PEG-2 (11%, Fig. 3b) significantly decreased in vehicle. The treatment with GPD in the three dose groups significantly enhanced the contents of IL-10 and PEG-2. The biggest amplitude of increase was observed in the high dose group, where the contents of IL-10 and PEG-2 increased by 132% and 12%, respectively. Such contents increased by 110% and 2% in medium dose group and the content of IL-10 increased by 62% in the low dose group, where PEG-2 content was not remarkably affected. The effect of GPD on IL-10 and PEG-2 was dose dependent. When compared with MP group, the contents of IL-10 and PEG-2 in HGD group was higher than MP group and the content of IL-10 was extremely remarkably higher, which substantially indicated that the inhibitory effect of HGD on oral ulcer of guinea pig was superior to that of MP.

After the treatment with MP and GPD in this study, the IL-10 expression was significantly higher than the vehicle, indicating that MP and GPD promoted the secretion of IL-10 by Treg

cells and the negative feedback effect of IL-10 helped to inhibit inflammation. In addition, MP and GPD could promote the production of PEG-2, facilitate the blood flow in the oral mucosa, inhibit the incidence of inflammation and facilitate the recovery from oral mucosa.

Effect of GPD on signaling pathway factor

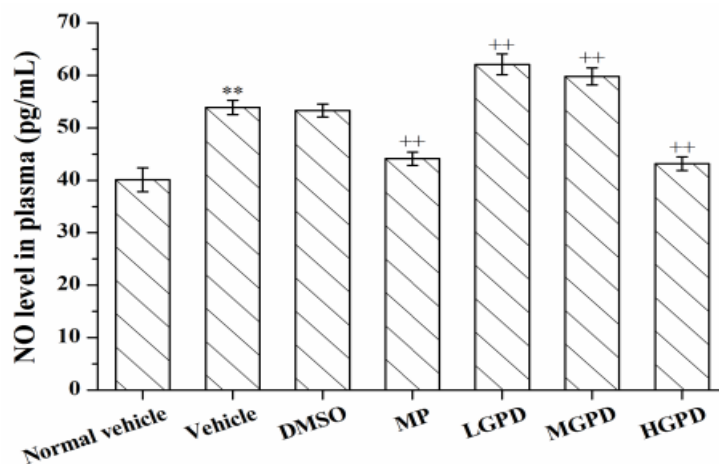


Fig. 4 Effect of GPD on level of NO. + $P < 0.05$ and ++ $P < 0.01$ compared with vehicle, * $P < 0.05$ and ** $P < 0.01$ compared with normal vehicle.

NO signaling pathway: As a gas signaling molecule involved in signal transduction in vivo, NO plays an important role in the nervous system, immune system and cardiovascular system. NO exhibits cytotoxicity, high-concentration NO may lead to adverse effect on the body due to its involvement in infection, inflammatory response, and autoimmune response.^[14,15]

According to data analysis, there was not significantly difference between DMSO group and vehicle in the level of NO, so the influence of DMSO on experimental results should be eliminated. As shown in Fig. 4, the NO content in vehicle was significantly higher than in normal vehicle. The treatment with GPD for 5 consecutive days could significantly inhibited the overexpression of NO and the inhibiting effect was more remarkable in high dose group, where NO content dropped by 20%. GPD exhibited no inhibitory effect on NO in the low and medium dose groups. The comparison between GPD group and MP group showed that the NO content in HGPD group was significantly lower than in MP group.

In this study, HGPD could remarkably reduce the NO content with a result that it could inhibit the regulating effect of NO as gaseous signaling molecule on inflammatory reaction,

deaden cytotoxicity and contribute towards the recovery of oral cells.

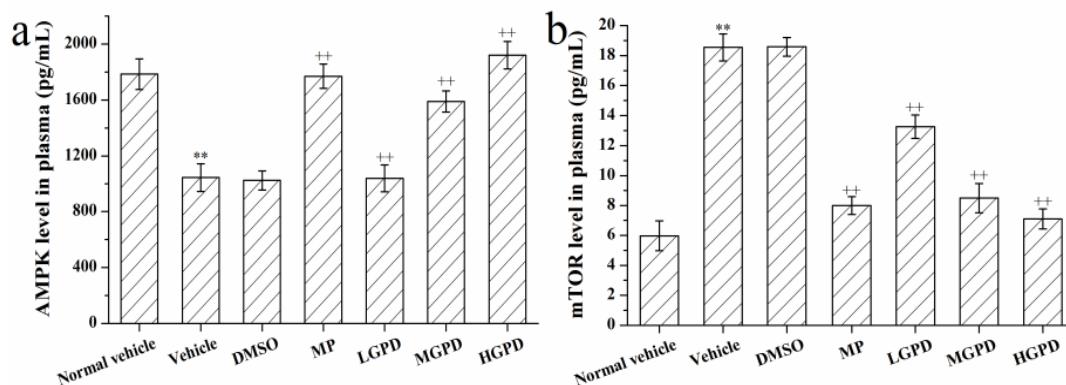


Fig: Effect of GPD on the levels of AMPK (a) and mTOR (b). +P<0.05 and ++P<0.01 compared with vehicle, *P<0.05 and **P<0.01 compared with normal vehicle.

AMPK-mTOR signaling pathway: AMPK is an “energy receptor”, the change of energy (ATP/AMP) in body could activate the AMPK in cells.^[16] mTOR signaling pathway can regulate cell growth and reproduction, and is closely related to the onset of inflammation, and can transfer inflammatory information, while AMPK could inhibit mTOR signaling pathway.^[17]

The data analysis showed that the difference of AMPK (Fig. 5a) and mTOR (Fig. 5b) contents between the vehicle and the DMSO group is not obvious, so the influence of DMSO on the experimental results could be eliminated. As shown in Fig. 5a, the mTOR level in vehicle was significantly higher than in normal vehicle. Treatment with GPD for 5 consecutive days could significantly inhibit the overexpression of mTOR, and the inhibiting effect was more remarkable in high dose group, where mTOR level dropped by 62%. In addition, mTOR level decreased by 29% and 54% respectively in the low and medium dose groups. So the effect of GPD on mTOR was dose dependent. As shown in Fig. 5b, AMPK level in vehicle was significantly lower than in normal vehicle, but the AMPK level significantly increased in GPD group. Moreover, the optimal result was observed in high dose group, where AMPK level dramatically increased by 84%, and the AMPK level increased by 52% in medium dose group, but no promoting effect on AMPK was found in the low dose group. The comparison of HGD group with MP group proved

that the effect of HGPD was superior to that of MP.

This research have shown that GPD significantly reduced the mTOR level through promoting the production of AMPK and inhibited the growth and reproduction of inflammatory cells and the transfer of inflammatory information, thereby suppressing oral ulcer.

Effect of GPD on oxidative stress marker

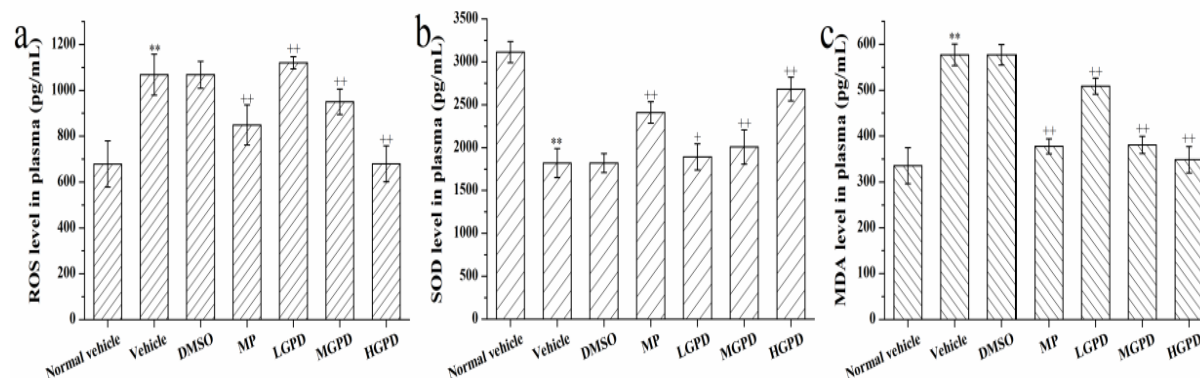


Fig. 6 Effect of genistein derivative on concentrations of ROS (a), SOD (b) and MDA (c). + $P < 0.05$ and ++ $P < 0.01$ compared with vehicle, * $P < 0.05$ and ** $P < 0.01$ compared with normal vehicle.

ROS are a series of reactive oxygen species produced by aerobic cells in metabolic process. Due to its strong oxidizing property, excessive ROS may cause damage to the structures of cells and genes.^[8,9] As one of the important products of membrane lipid peroxidation, malondialdehyde (MDA) may cause damage to membrane, and its content could be used to determine the degree of membrane lipid peroxidation and indirectly determine the extent of damage to membrane system.^[20] Superoxide dismutase (SOD) is an antioxidative substance that could eliminate the harmful substances produced during metabolism of organisms, constant replenishment of SOD helps to enhance the resistance of body to aging.^[21] Fig. 6 showed that, there was not significantly difference between vehicle and DMSO group in terms of the levels of ROS, SOD and MDA, so the effect of DMSO on experimental result could be excluded. Under normal circumstances, the levels of ROS (Fig. 6a) and MDA (Fig. 6c) in plasma of guinea pigs were lower and the level of SOD (Fig. 6b) was higher. In the plasma of guinea pigs with oral ulcers, the levels of ROS and MDA increased significantly and SOD level decreased significantly. When compared with vehicle, HGPD

could notably improved in terms of the excess of ROS and MDA and the suppression of SOD and its effect was significantly superior to LGPD and MGPD. The ROS and MDA levels in plasma of guinea pigs treated with HGPD decreased by 36% and 40% respectively, while the SOD content increased by 47%. In the MGPD group, the levels of ROS and MDA decreased by 11% and 34% respectively, and the SOD content increased by 10%. In the LGPD group, the MDA level decreased by 12%, and SOD content increased by 4%, but no inhibitory effect was observed on ROS. The effect of GPD on ROS, SOD and MDA was dose dependent. ROS and MDA levels in HGPD group was significantly lower than in MP group, while the level of SOD in HGPD group was higher than in drug group, which indicated the inhibitory effect of HGPD on oral ulcer of guinea pig was superior to that of MP.

In this study, the exposure to phenol resulted in excessive ROS in body, and the ROS converted lipid into MDA through oxidation, which led to ulceration of oral mucosa. GPD could enhance the ability of body to remove ROS and other harmful substances by increasing SOD content, thereby inhibiting lipid oxidation, as a result, lipid was prevented from generating MDA, which helped to reduce the extent of damage of ulcer.

CONCLUSION

In this study, a model for oral ulcer in guinea pig was built through phenol burning, and the inhibitory effect of GPD on ulcer was investigated through inflammatory factor, oxidative stress and signaling pathway. Studies showed that treatment with GPD brought about significant increase in the levels of SOD, IL-10, PGE-2 and AMPK in guinea pig plasma, as well as significant decrease in the levels of TNF- α , NF- κ B, NO, MDA, mTOR and ROS as compared with vehicle, which indicated that GPD was in a position to remarkably inhibit oral ulcer. Moreover, GPD of various concentrations could suppress oral ulcer, and HGPD (45 mg/kg) exhibited the best inhibitory effect on oral ulcer, and its effect was better than that of commercially available drug (MP) for treatment of oral ulcer.

To sum up, this study showed that GPD could significantly suppress phenol-induced traumatic oral ulcer by inhibiting inflammatory reaction, improving oxidative stress, and

inhibiting the growth and reproduction of inflammatory cells. The protective effect of HGPD on oral mucosa was significantly stronger than that of LGPD and MGPD. Thanks to its strong resistance to inflammation and oxidation, HGPD exhibited excellent property of alleviating oral mucosa injury.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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