

• GASTRIC CANCER •

# Effect of jianpiyiwei capsule on gastric precancerous lesions in rats

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**Received** 2002-04-09 **Accepted** 2002-06-15

## Abstract

**AIM:** To evaluate the therapeutic effect of compound Chinese drugs, Jianpiyiwei capsule (JPYW) on gastric precancerous lesions in rats and to explore its mechanism of action.

**METHODS:** Model of gastric precancerous lesions was constructed in male Wistar rats: a metal spring was inserted and fixed through pyloric sphincter. One week after recovery, each rat was given 50-60 °C hot paste containing 150 g/L NaCl 2 mL orally, twice a week for 15 weeks. Then 10 normal and 11 model rats were anaesthetized, after the measurement of gastric mucosa blood flow (GMBF), the rats were killed and the mucosal hexosamines and malonic dialdehyde (MDA) were measured. The morphological changes of gastric mucosa were observed macroscopically and microscopically, and by an automatic imaging analysis system. Other rats were treated with JPYW 1.5 g/kg·d<sup>-1</sup> or 4.5 g/kg·d<sup>-1</sup>, or distilled water as negative control respectively (*n*=10 in each group). After 12 weeks, all the rats were examined as above.

**RESULTS:** The gastric mucosa of model rats showed chronic atrophic gastritis with dysplasia and intestinal metaplasia (IM), GMBF and hexosamine content were reduced significantly and MDA was increased as compared to the normal group (*P*<0.01). After 12 weeks treatment, the pathological changes of the negative control group became worsened, while in JPYW treated groups the changes were modified with significant increase of GMBF and reduction of MDA, although the hexosamine concentration increased only mildly.

**CONCLUSION:** JPYW increases GMBF and reduces MDA content in gastric mucosa and has therapeutic effects on gastric precancerous lesions.

Shi XY, Zhao FZ, Dai X, Ma LS, Dong XY, Fang J. Effect of jianpiyiwei capsule on gastric precancerous lesions in rats. *World J Gastroenterol* 2002; 8(4):608-612

## INTRODUCTION

Jianpiyiwei capsule (JPYW), a compound Chinese drug, has the effect of replenishing *qi* (vital energy) and invigorate the spleen, promoting blood flow and regulating the stomach function. This study investigates the therapeutic effect of JPYW on gastric precancerous lesions in rats, and tries to elucidate its mechanism of action in part.

## MATERIALS AND METHODS

Male Wistar rats, weighed 250 g to 300 g, were purchased from the Laboratory Animal Center, Chinese Academy of Medical Science. The rats were housed in an air-conditioned animal room at 25±2 °C and 60 % humidity with food and water available *ad libitum*, and drinking water was changed every day.

The establishment of gastric precancerous lesions in rats was modified according to Zhao's method<sup>[1]</sup> with modification: a metal spring was inserted and fixed through pyloric sphincter. One week after recovery, each rat was given 50-60 °C hot paste containing 150g/L NaCl 2 mL orally, twice a week for 15 weeks. Then, all the survived rats were divided randomly into 4 groups: (1) model group (*n*=11), (2) negative control (*n*=10), (3) small dose JPYW group (*n*=10), (4) large dose JPYW group (*n*=10). Group 1 rats were killed 16 weeks after the operation, group 2-4 were given diluted water 2 mL/d, JPYW 1.5g/kg·d<sup>-1</sup> or JPYW 4.5g/kg·d<sup>-1</sup>, separately, 6 times per week for 12 weeks. Moreover, a group of normal rats (*n*=10) were raised for 16 weeks and killed together with group 1.

### Gastric mucosa blood flow (GMBF) assays

Before the rats were killed, they were forbidden from food except drinking freely for 24 hours, then they were anaesthetized. After incision of the abdominal wall, an ultrasonic detective probe was put to the junction between antrum pylori and corpus ventriculi in gastric curvature of stomach through an incision at the proventriculus, and the voltage number were read to represent the relative level of GMBF.

### Morphological study

After the detection of GMBF, the rat was killed and stomach was removed and cut off through the greater curvature, washed by physiological saline and residual liquid was absorbed. The whole stomach was first observed in gross, and then the gastric mucosa of posterior wall was scraped off and frozen to -20 °C immediately for the detection of hexosamines and malonic dialdehyde(MDA). The anterior wall was fixed in 10 % neutral buffered formalin over 24 hours, then were cut into 2 mm-wide pieces, all the pieces were embedded in paraffin. 5 μm sections, stained with hematoxylin and eosin and with alcian blue, periodic acid-schiff and high iron diamine (AB-PAS/HID), were examined under light microscopy.

Quantitative histologic evaluations were performed using

an automatic imaging analysis system (produced by Beijing University of Aeronautics and Astronautics). Ten cases of each group were selected for the morphometric measurements, including: (1) thickness of glandular layer *vs* thickness from surface to the end of muscular layer of mucosa ( $T_1/T_2$ , five low visual fields in corpus ventriculi and 5 in antrum pylori for each section), (2) nuclear area *vs* cytoplasm area of epithelioglandular cells(N/C), (3) intra-cavity periphery of a gland *vs* the diameter of the circle whose area was equal to the glandular cavity( $P_1/D_1$ ), (4) periphery of basal coil in a gland *vs* the diameter of the circle whose area was equal to the gland ( $P_2/D_2$ ). (2)-(4), each measured 10 middle visual fields in one section.

### Hexosamines and MDA assays

The achievement of gastric mucosa has been described as above. The hexosamine content in gastric mucosa was detected according to Neuhaus' s method<sup>[2]</sup>, and the measurement of MDA by thiobarbituric acid method.

### Statistical analysis

Numerical values were expressed as  $\bar{x} \pm s$ . Comparisons of data between groups were performed with *student's t* test or  $\chi^2$  test.  $P < 0.05$  was considered significant.

## RESULTS

### Effects of JPYW on the morphological changes of gastric precancerous lesions in rats

Gross examination of stomach in model and negative control groups showed thinning and paleness of gastric mucosa, with disarrayed plicae and small white nodules, while in the normal groups it was pink, moistened and smooth. In JPYW treated groups, the gross change of gastric mucosa was only modest as compared with negative control group, especially in 4.5 g/Kg·d<sup>-1</sup> group ( $P < 0.05$  or 0.01) (Table 1).

Histopathology of gastric mucosa in the model and the negative control groups revealed increased incidence of chronic superficial gastritis (CSG), chronic atrophic gastritis (CAG), intestinal metaplasia (IM) and gastric mucosa dysplasia, as compared with normal and JPYW treated groups ( $P < 0.05$  or 0.01)(Table 2,). AB-PAS/HID staining showed both complete and incomplete IM (Figure 1).

Data by automatic image pattern analysis were shown in table 3. The ratios of  $T_1/T_2$ , representing the degree of mucosal atrophy, were greatly reduced in model and negative control groups both at the gastric body and the antrum, and ratios of N/C,  $P_1/D_1$ ,  $P_2/D_2$  representing the degree of dysplasia, were increased more prominently in those two groups as compared with normal group ( $P < 0.01$ ). In JPYW treated groups, all the ratios approached normal, although there were still some difference between 1.5g/Kg·d<sup>-1</sup> group and normal ( $P < 0.05$  or 0.01). (Table 3)

**Table 1** Effects of JPYW on macroscopical changes of gastric precancerous lesions in rats

group	n	erosion / ulceration	Disarrayed plicae	thinning of mucosa	white nodules	xanthochromia of mucosa
normal	10	0	0	1	0	0
model	11	6 <sup>b</sup>	7 <sup>b</sup>	9 <sup>b</sup>	9 <sup>b</sup>	5 <sup>a</sup>
negative	10	6 <sup>a</sup>	6 <sup>a</sup>	9 <sup>b</sup>	10 <sup>b</sup>	5 <sup>a</sup>
1.5 g/Kg·d <sup>-1</sup>	10	2	1 <sup>ce</sup>	3 <sup>cf</sup>	3 <sup>cf</sup>	1 <sup>ce</sup>
4.5 g/Kg·d <sup>-1</sup>	10	0 <sup>de</sup>	0 <sup>de</sup>	1 <sup>df</sup>	2 <sup>df</sup>	0

n: number; normal: normal group; model: model group; negative: negative control group; 1.5 g/Kg·d<sup>-1</sup>: JPYW 1.5 g/

Kg·d<sup>-1</sup> group; 4.5 g/Kg·d<sup>-1</sup>:JPYW 4.5 g/Kg·d<sup>-1</sup> group <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , *vs* normal group; <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ , *vs* model group; <sup>e</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$ , *vs* negative control group.

**Table 2** Effects of JPYW on histological changes of gastric pre-cancerous lesions in rats

group	n	CSG	CAG	dysplasia	IM
normal	10	1	0	0	0
model	11	11 <sup>b</sup>	10 <sup>b</sup>	8 <sup>b</sup>	3
negative	10	10 <sup>b</sup>	10 <sup>b</sup>	8 <sup>b</sup>	5 <sup>b</sup>
1.5 g/Kg·d <sup>-1</sup>	10	3 <sup>df</sup>	5 <sup>ae</sup>	2 <sup>ce</sup>	1
4.5 g/Kg·d <sup>-1</sup>	10	2 <sup>df</sup>	1 <sup>df</sup>	1 <sup>df</sup>	0 <sup>e</sup>

n: number; normal: normal group; model: model group; negative: negative control group; 1.5 g/Kg·d<sup>-1</sup>: JPYW 1.5g/Kg·d<sup>-1</sup> group; 4.5 g/Kg·d<sup>-1</sup>:JPYW 4.5 g/Kg·d<sup>-1</sup> group <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , *vs* normal group; <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ , *vs* model group; <sup>e</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$ , *vs* negative control group.

**Table 3** Automatic imaging pattern analysis effects of JPYW ( $\bar{x} \pm s$ , n=10)

group	$T_1/T_2$ in gastric body	$T_1/T_2$ in antrum	N/C	$P_1/D_1$	$P_2/D_2$
normal	10.6±2.0	5.9±1.0	0.54±0.02	3.53±0.08	3.43±0.07
model	1.9±0.4 <sup>b</sup>	1.3±0.2 <sup>b</sup>	0.75±0.04 <sup>b</sup>	4.18±0.09 <sup>b</sup>	3.69±0.08 <sup>b</sup>
negative	1.8±0.5 <sup>b</sup>	1.1±0.1 <sup>bc</sup>	0.78±0.02 <sup>bc</sup>	4.39±0.10 <sup>bd</sup>	3.95±0.08 <sup>bd</sup>
1.5 g/Kg·d <sup>-1</sup>	4.3±0.6 <sup>bdf</sup>	3.1±0.7 <sup>bdf</sup>	0.62±0.02 <sup>bdf</sup>	3.62±0.09 <sup>bdf</sup>	3.55±0.06 <sup>bdf</sup>
4.5 g/Kg·d <sup>-1</sup>	8.9±1.8 <sup>df</sup>	5.3±0.9 <sup>df</sup>	0.55±0.03 <sup>df</sup>	3.57±0.07 <sup>df</sup>	3.46±0.07 <sup>df</sup>

normal: normal group; model: model group; negative: negative control group; 1.5 g/Kg·d<sup>-1</sup>: JPYW 1.5 g/Kg·d<sup>-1</sup> group; 4.5 g/Kg·d<sup>-1</sup>:JPYW 4.5 g/Kg·d<sup>-1</sup>group <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , *vs* normal group; <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ , *vs* model group; <sup>e</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$ , *vs* negative control group.

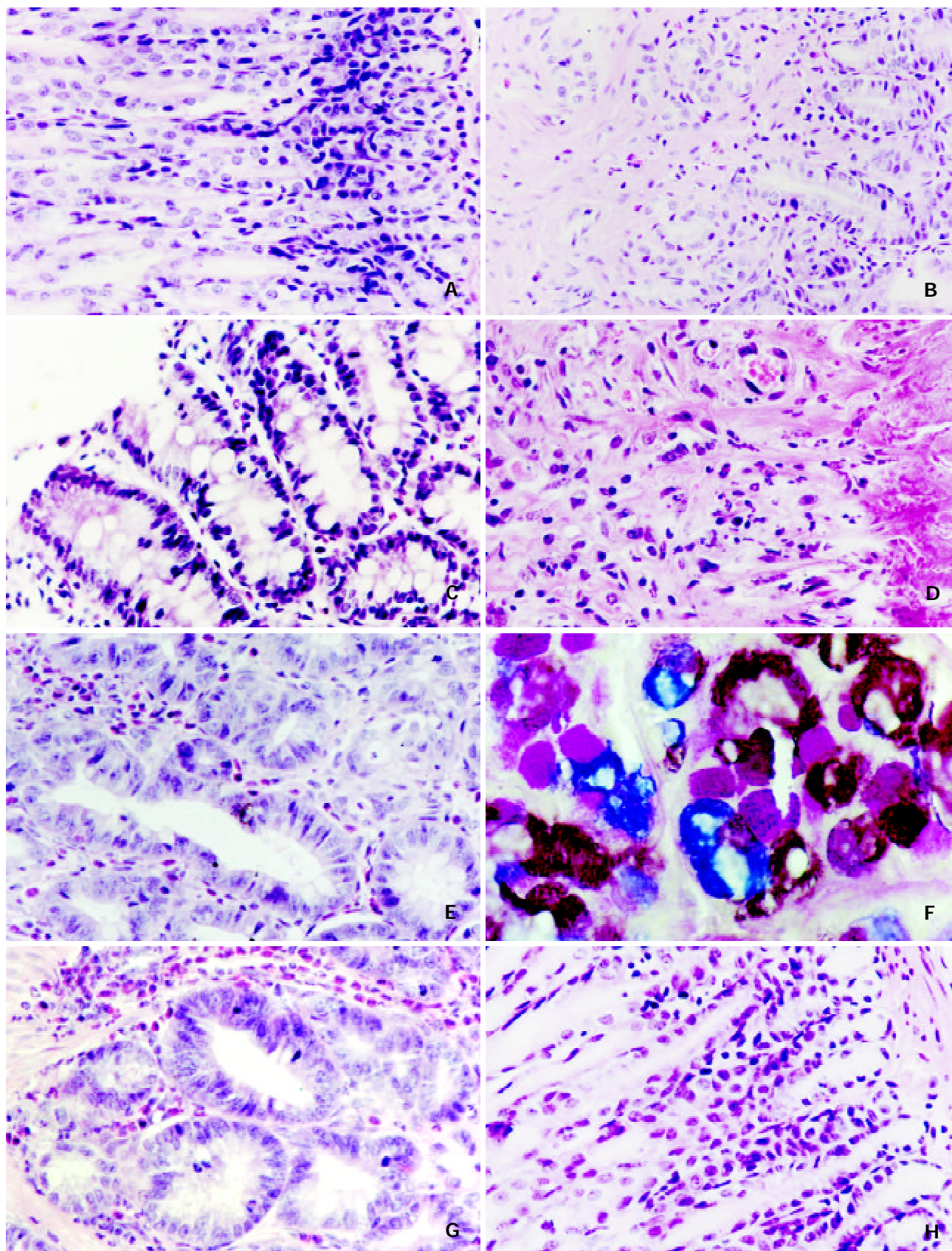
**Table 4** Effects of JPYW on GMBF and the gastric mucosal hexosamines and MDA contents ( $\bar{x} \pm s$ )

group	n	GMBF(mV)	Hexosamines(mg/g)	MDA(μmol/g)
normal	10	0.29±0.05	6.8±1.1	46±25
model	11	0.17±0.04 <sup>b</sup>	4.8±0.7 <sup>b</sup>	374±49 <sup>b</sup>
negative	10	0.18±0.04 <sup>b</sup>	4.9±0.8 <sup>b</sup>	419±43 <sup>bc</sup>
1.5g/Kg·d <sup>-1</sup>	10	0.27±0.07 <sup>de</sup>	5.2±1.2 <sup>b</sup>	312±56 <sup>bce</sup>
4.5g/Kg·d <sup>-1</sup>	10	0.29±0.05 <sup>de</sup>	5.6±0.9 <sup>ac</sup>	271±40 <sup>bce</sup>

n: number; normal: normal group; model: model group; negative: negative control group; 1.5g/Kg·d<sup>-1</sup>: JPYW 1.5g/Kg·d<sup>-1</sup> group; 4.5 g/Kg·d<sup>-1</sup>:JPYW 4.5 g/Kg·d<sup>-1</sup> group <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , *vs* normal group; <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$ , *vs* model group; <sup>e</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$ , *vs* negative control group.

### Effects of JPYW on GMBF

The GMBF in model group was reduced more obviously than that in the normal group ( $P < 0.01$ ). After 12wk treatment, there was no increase of GMBF in negative control group, but great improvements were seen in JPYW treated groups as compared with model or negative control group ( $P < 0.01$ ). (Table 4)



**Figure 1(A-D)** Effects of JPYW on histological changes of gastric precancerous lesions in rats A: Morphology of gastric mucosa in normal rat. HE 100× B-D Show the histologic changes of gastric mucosa in model group, B: CAG HE 100×, C: IM without dysplasia HE 100×, D: ulcer HE 100×.

**Figure 1(E-F)** Effects of JPYW on histological changes of gastric precancerous lesions in rats E-F Show the histologic changes of gastric mucosa in model group, E: Moderate dysplasia with IM HE 100×, F: Incomplete IM AB-PAS/HID 200× G: Moderate to severe dysplasia in gastric mucosa in negative control group HE 100× H: The histological figure of gastric mucosa in JPYW 4.5g/Kg·d<sup>-1</sup> group is close to normal HE 100×

### Effects of JPYW on gastric mucosal contents of hexosamines and MDA

In the model group, the gastric mucosa hexosamine content was lower and MDA was higher than that in the normal group. After 12 wk treatments, no differences were seen between them in the negative control group and model group, yet in 4.5 g/Kg·d<sup>-1</sup> group there was some increase in hexosamine content, significant difference was still present when compared with normal group ( $P<0.05$ ). Moreover, the MDA content in JPYW treated groups was greatly reduced ( $P<0.05$  or 0.01). (Table 4)

### DISCUSSION

Although the over all incidence of gastric cancer has steadily declined in the western world<sup>[3]</sup>, it is still the most common fatal malignancy in China<sup>[4]</sup>, therefore the treatment of gastric precancerous lesions should be one of the important measures for preventing gastric cancer.

Since 1988 Correa and his colleagues proposed a human model of gastric carcinogenesis, which is gastric cancer develops through a complex sequence of events from normal mucosa to superficial gastritis, chronic atrophic gastritis, IM, dysplasia and finally to intestinal type gastric cancer, numerous studies have found genetic alterations in gastric precancerous lesions and supported the hypothesis<sup>[5-10]</sup>. But the etiology of gastric precancerous lesions has not been fully elucidated. Many factors are thought to have close relations to gastric carcinogenesis, such as a diet high in salt<sup>[11,12]</sup> and starch<sup>[13]</sup>, consumption of high temperature food<sup>[14]</sup>, *Helicobacter pylori* (HP) infection<sup>[15-19]</sup>, drinking alcohol<sup>[19,20]</sup>, smoking<sup>[20,21]</sup> and bile reflux<sup>[21-23]</sup>.

Some experimental studies have shown that the high-salt diet may potentiate HP-associated carcinogenesis in mice by inducing proliferation, pit cell hyperplasia, and glandular atrophy<sup>[24]</sup>, and 50-week bile reflux may cause gastric cancer in rats<sup>[25]</sup>. In this article, we establish a model of gastric precancerous lesions in rats by inserted a metal spring through gastric pylorus sphincter and given hot paste containing high concentration of NaCl, which lead to the development of precancerous gastric lesions. The typical feature of this model are glandular atrophy, lamina propria infiltration of chronic inflammatory cells, thickening of muscularis mucosa, glandular dysplasia and IM, simulating human gastric precancerous lesions. JPYW was found to have obvious effects on gastric precancerous lesions in rats with thickening of gastric mucosa and decreased incidence of dysplasia and IM that firmly supported our hypothesis that JPYW has therapeutic effects on gastric precancerous lesions.

GMBF is an important part of mucosal defense system, bringing oxygen and nutrients to the mucosal cells, against penetration of carcinogenesis into gastric mucosa<sup>[26]</sup>, maintaining the structure and function of the stomach and is closely associated with the pathogenesis and the healing of gastrointestinal lesions<sup>[27]</sup>. In the JPYW treated groups, the GMBF recovered to normal, which indicates that the increase of GMBF may be one of the mechanisms of JPYW on gastric precancerous lesions.

Etiologic studies find that blood levels of natural antioxidants in patients with gastric precancerous lesions or cancer are much lower than healthy people<sup>[28]</sup> and low level of dietary vitamin C may contribute to the progression of precancerous lesions to gastric cancer<sup>[29]</sup>, whereas a dietary high intake of antioxidants may lower the risk for gastric cancer<sup>[30]</sup>. Farinati *et al.*<sup>[31]</sup> reported that there was an oxidative DNA damage accumulation with mutagenic and carcinogenic

potential in chronic gastritis. MDA content, an metabolite of lipid peroxidant, were found higher in gastric mucosa of patients with HP gastritis<sup>[32]</sup> and in N-methyl-N'-nitro-N-nitrosoguanidine induced gastric cancer in rats<sup>[33]</sup>. In our experiment, the MDA content of gastric mucosa in the model rats increased significantly, which implied that the free radicals participated in the occurrence of gastric precancerous lesions. And the reduction of mucosal MDA content in JPYW treated groups indicates that JPYW has a protective action on gastric mucosa.

We also noticed that the hexosamines content, which partially represented the function of gastric mucosal barrier<sup>[34]</sup> decreased obviously in gastric mucosa of model rats, and only increased slightly in JPYW treated groups with absence of statistical differences. This suggests that the therapeutic effects of JPYW in gastric precancerous lesions dose not involve hexosamine content.

In conclusion, JPYW has therapeutic effects on gastric precancerous lesions, it increases GMBF and reduces mucosal MDA content which may also play a role in its mechanism of action.

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Edited by Wu XN