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*Full Length Research Paper*

# Evaluation of gastric ulcer model based on gray-scale image analysis

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**Absolute alcohol, stress, pyloric ligation and others are well-rounded experimental gastric ulcer models widely used to evaluate the anti-ulcer activity of drugs (Wang et al., 2007; Zhang et al., 2007; Huang et al., 2004; Oyagi et al., 2010). In the model evaluation process, ulcer index is a more important indicator. So in this experiment, the absolute alcohol gastric ulcer model is taken as an example to discuss a model of gastric ulcer index, which can be used as a new method to calculate the reduction of the systematic errors that exist in the experiment. The oral ethanol approach was used to establish an absolute alcohol gastric ulcer model, and impose certain medication, while traditional methods and the establishment of grayscale image analysis methods were used for calculating its ulcer index respectively, before comparing the differences between the two methods. Grayscale image analysis methods and traditional methods have significant correlation (r = 0.819, P＜0.01) and similarity (r = 0.951). A grayscale image analysis method not only proves to be a more scientific approach to measure rat gastric ulcer area, but also provide a new method to evaluate drug efficacy to some extent.**

**Key words:** Gastric ulcer, ulcer index, gray-scale image analysis, image J.

## INTRODUCTION

Ulcer index is a visualized indicator used to reflect the gastric ulcer model injury or evaluate the extent of ulcer, which is commonly used in the anti-ulcer study of pharmacodynamics. The traditional evaluation method is used to measure and calculate the ulcer index in an artificial way by ruler or Vernier caliper, although it seems fast and easy. However, due to some subjective factors of humans, the errors are demonstrated to be relatively large. Currently, the digital camera which is combined with computer software analysis techniques has been widely used in medical image processing fields (Irving et al., 2007; Proceedings of Information Optics and Photonics Technologies II, 2007). Image J processing software possesses powerful functions, including the target count and classification, object measurement, motion capture and tracking of the standard curve fitting and regression equation optimization, etc (Wang, 2005). The researchers write programs by themselves and intend to develop the application level of the Image J analysis software to a higher level. In addition, they established the evaluation systems for the ulcer index of gray-scale images by trying some new methods. In contrast with the traditional methods, the main purpose focuses on a scientific, accurate and convenient evaluation method.

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## MATERIALS AND REAGENTS

### Experimental animals

Female SD rats in good health, with a weight of 200 ± 20 g, were provided by Dalian Laboratory Animal Center with certificate number SCXK (Liaoning) 2008-0002. Animals were raised in the air conditioning room, at a room temperature of 20 ± 2°C, and a relative humidity of 50 to 60%, by feeding pellet and free drinking.

### Drug and reagent

The drugs and reagents used in this study are omeprazole

**Table 1.** Ulcer index calculation results ($\bar{x}$±S).

| | Sample size (n) | Ulcer index (mm) | Suppression ratio (%) |
|---|---|---|---|
| Model group | 8 | 70.5±17.51 | - |
| Omeprazole group | 8 | 13.03±5.76 | 81.52* |
| Ranitidine group | 8 | 93.94±22.83 | - |
| Colloidal bismuth pectin group | 8 | 42.75±14.06 | 39.36 |
| Gas-delayed stomachache group | 8 | 13.41±9.70 | 80.98* |

Suppression ratio = (1-ulcer index in experimental group/ ulcer index in model group) × 100%. Note: A comparison is made with the model group, * signifies that P < 0.01.

enteric-coated capsule (lot number: 100430327, Harbin pharmaceutical group, Sanjing Nuojie co., Ltd.), ranitidine hydrochloride capsules (lot number: 20100101, Shanghai Wong-like tie- blue sky pharmaceutical co., Ltd.), colloid pectin and Bismuth SubCarbonate capsules (lot number: 09101210123, Qingdao Chia Tai Haier pharmaceutical co., Ltd.), gas-delayed stomachache particles (lot number: Tianshifu three medicine co., Ltd), formaldehyde solution (lot number: 20081108, Dandong city, sea reagent factory), ethanol (lot number: 20100116, North specialty Chemical Reagent Development Center), sodium dihydrogen phosphate (lot number: 20100318, Tianjin City Comet-European Chemical Co., Ltd.) and disodium phosphate (lot number: 20081127, Kaixin chemical industry co., Ltd. in Tianjin).

#### Main instrument

The main instruments used in this study are a digital camera (Kodak N137, Eastman Kodak Company) and Image J medical image analysis software (United States National Institutes of Health).

## METHODS AND RESULTS

### Preparation of neutral formalin solution

In preparing neutral formalin solution, anhydrous (6.5 g) of dihydrogen phosphate, 4 g of disodium phosphate and 40% of formaldehyde solution are weighed and the volume is set at 1000 ml by dissolving it in water.

### Ethanol gastric ulcer model

Fifty healthy SD female rats, weighing 200 ± 20 g, were obtained and randomly split into five groups. Each group has 10 rats and the groups comprise the model group, Omeprazole group (0.012 g/kg), Ranitidine (0.09 g/kg), Colloidal Bismuth Pectin group (0.18 g/kg) and Qi stagnation stomachache particle group (5 g/kg). Various groups were administered for the first time different doses of daily gavages, while the model groups were taken in distilled water and irrigated for 15 days (from intragastric 14 days of fasting, water could not help the group for 24 h, but 15 days after delivery, the group was assisted by 1 h intragastric 1 ml ethanol/only). After the death of 1 h cervical rats, the gastric cardiac caesarean, ligation and helicobacter, in neutral formalin 5 ml solution, were removed from the stomach by the gastric parietal stomach cavity injection and placed in a neutral solution of formalin for 15 min, after which a big cut was made along the stomach. Stomach ulcer index were calculated for each member groups and photographs were taken (Jiang et al., 2009).

### Traditional ulcer index calculation method (method A)

First, the gastric mucosa injury length (mm) is measured by a ruler. If the length of the strip's injury is greater than 1 mm, it is calculated by 1 point per mm gauge; but if the width exceeds 1 mm, multiply the score and then sum up the score as the animal's ulcer index. SPSS16.0 software is used to process the data, while the results are identified by means and standard deviation. However, analysis approach of single factor is adopted to distinguish differences among groups. The results can be seen in Table 1 (Jiang et al., 2009).

### Calculation methods of gray-scale image analysis ulcer index (B)

#### *Image acquisition*

Place the sample in the black box (length × width × height = 20 × 20 × 20 cm), and fix light source on all sides of the box in order to create a state without shadow as much as possible. Fix the focal length of the digital camera in one direction and take photos in the same distance from the sample, before acquiring color (RGB) images.

#### *Image conversion*

The pre-processing pictures (Figure 1) are opened in Image J analysis software, and the self-programming No. 1 are operated, before the images are transmitted into grayscale images standardized in a 8-bit form (Figure 2). The functional pictures in the polygonal area of the software are selected, after which the gastric mucosa

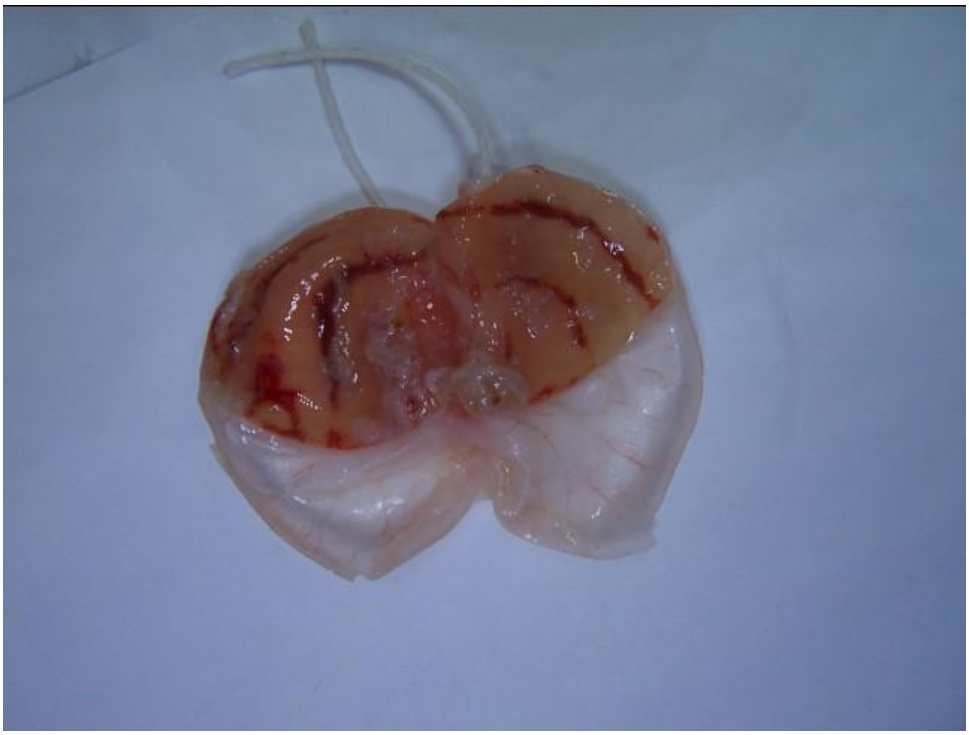

**Figure 1.** Original captured images by digital camera.

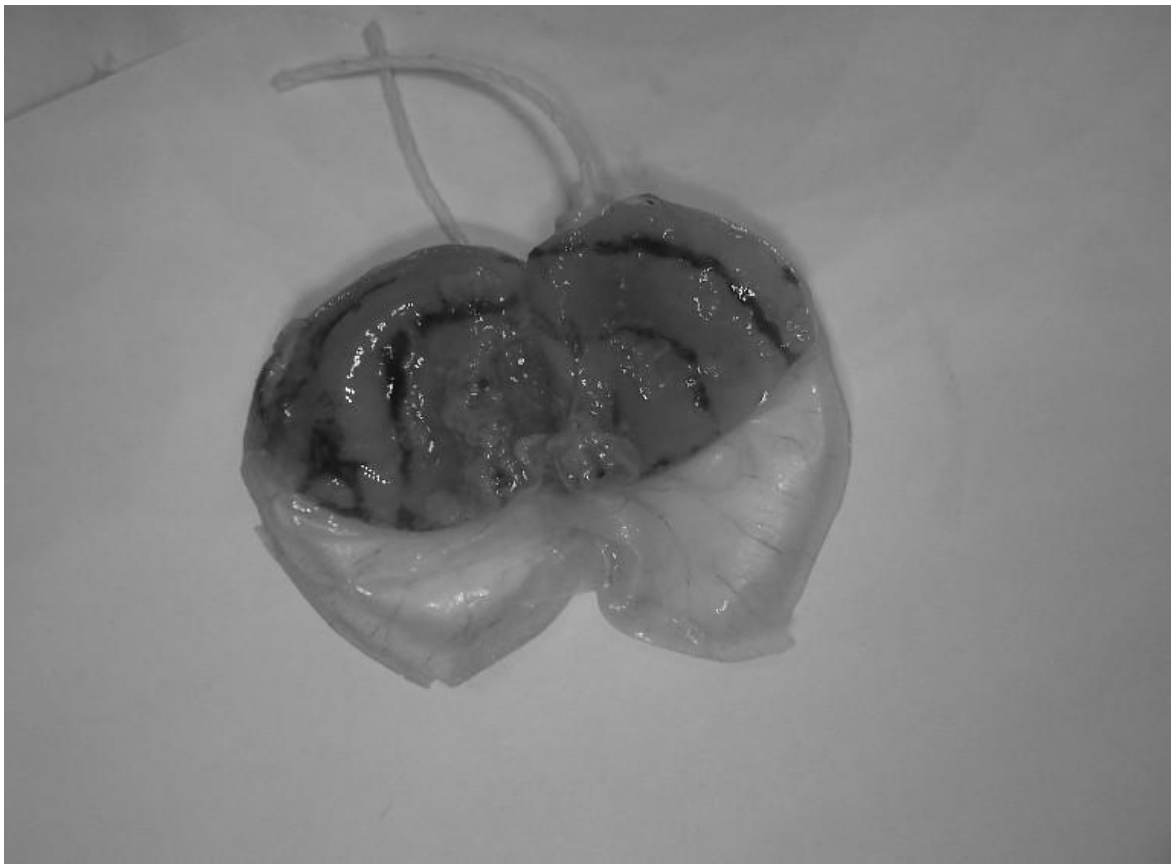

**Figure 2.** Converted gray-scale images.

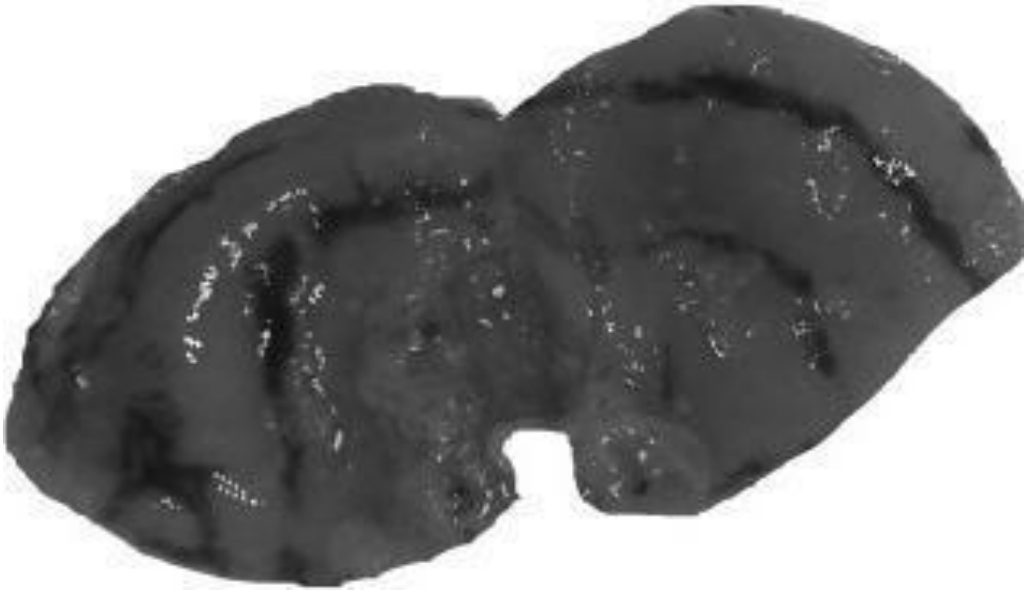

**Figure 3.** Gastric mucosal region image.

**Figure 4.** Ulcer area picture.

areas in the picture are manually chosen and replicated. In implementing the self-programming No. 2, the total number of pixels is shown and regarded as gastric mucosal areas, while the data are stored in Excel sheet 1. Consequently, a new mucosal region is built in gray-scale images automatically and the data are saved there (Figure 3). In implementing self-programming No.3, a new image is opened and at the same time, the threshold is shown, while the threshold is adjusted to the ulcer area that is to be selected. In implementing self-programming No.4, the ulcer area is saved in grayscale images automatically (Figure 4), and the total number of pixels is displayed in the ulcer area as the surface area of ulceration, before storing the data in Excel sheet 2 (Table 2).

***Data processing***

Calculate the ratio of data in Excel sheet 2 to the data in

**Table 2.** Ulcer index calculation results ($\bar{x}\pm S$).

| | Sample size (n) | Ulcer index (mm) | Suppression ratio (%) |
|---|---|---|---|
| Model group | 8 | 8.89±3.16 | - |
| Omeprazole group | 8 | 1.36±0.39 | 84.70* |
| Ranitidine group | 8 | 7.04±1.85 | 20.81 |
| Colloidal bismuth pectin group | 8 | 6.82±2.32 | 23.28 |
| Gas-delayed stomachache group | 8 | 2.14±0.98 | 75.93* |

Note: A comparison is made with the model group, * signifies that $P < 0.01$.

Excel Table 1, which is equal to the ratio of areas of ulceration to the areas of gastric mucosa. SPSS16.0 software is used for dataprocessing, and the result can be reflected by means and standard deviation, then the analysis approach of the single factor is adopted to compare the differences among groups. The results can be seen in Table 2.

## Correlative analysis

### *Analysis between the correlations of two methods*

SPSS16.0 statistical software is used to analyze the correlation data that is converted by the logarithmic approach between methods A and B. The data are calculated and concluded by the simple Pearson's correlation coefficient r = simple 0.819, and test P = 0.000 by double-side inspection. Consequently, if the level is above 0.01, there is a significant positive correlation between the two approaches (Wang et al., 2008).

### *Similarity analysis between two methods*

One of the series of symbols having unique meaning in a fixed order can be derived by counting. The SPSS16.0 statistical software is used to analyze the correlation data which is converted by the logarithmic approach between methods A and B. Thus, the similarity coefficient r = 0.951 can be derived by the cosine law which demonstrates an apparent similarity between the two methods.

## DISCUSSION AND CONCLUSION

Peptic ulcer including gastric ulcer and duodenal ulcer is a common illness in internal medicine which affects a considerable number of people worldwide (Oyagi et al., 2010). Studies on such illness and drugs are rarely warm at home and abroad. In this study, image analysis techniques are used to improve the evaluating method, so that it makes the evaluation methods more scientific and objective.

The method to calculate the traditional ulcer index is based on the ruler measurements for the length of cable damage of gastric mucosa and the conclusion of the total number of statistics; however, this method fails to take into account different sizes among rats' gastric mucosa areas. The grayscale image analysis method takes advantage of its specialty to measure the proportion of areas of all ulcer points to the total areas of gastric mucosa areas which are proved to be more scientific.

According to imaging principles, the actual object of imaging is proportional to the size of the pixels, in that the image in the pixels can indirectly reflect the size of the area of actual objects. Thus, the ratio of the total number of pixels in ulcer areas' image to those in the gastric mucosa areas can truly represent the ratio of ulcer areas to gastric mucosa areas.

The rat's gastric mucosa structure consists of two zones: the mucous membrane area and the serous membrane area. Ulcers or injuries only occur in mucous membrane area, so it is natural to select the mucous regions to operate statistics, which turns out to be of more practical significance.

The digital camera, in general, can serve the function of the image acquisition process; moreover, it is easy to operate. During the image acquisition process used to analyse the ulcer index in gray-scale images, the sample can be shot in the same light source, adopting the same focal length of the camera, while the photographer is put in the same distance. The materials are collected and calculated in the same threshold condition during the image conversion process, so that human errors associated with it can be reduced to a large extent.

This experiment is implemented by selecting traditional medicine and Western medicine in clinic for the treatment of gastric ulcer, and the statistical software is used to evaluate the relevance and comparability of the results between the two approaches for the evaluation of ulcer index. The result shows that these two approaches are of significant relevance and similarity; therefore, it plays a positive role for the firm's scientific basis of the wider application of a new gray-scale image analysis method. Image J analysis software is provided by the United States National Institutes of Health (NIH) (Jiang and Ran, 2008). It is kind of free, accessible, and an irregular image analysis software that deals with simple Image J (download: HTTP:／／RSB. INFO. NIH. GOV／IJ (Li and Yang,

2009). The digital camera combined with the function of the image analysis software has been reported in the literature for the evaluation of ulcer index. In addition, researchers have improved software application by designing new programs on the higher level, so that it makes the evaluation methods more scientific and convenient.

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