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Gastroprotective activity and mechanism of novel dichlorido-zinc(II)-4-(2-(5-methoxybenzylideneamino)ethyl)piperazin-1-iumphenolate complex on ethanol-induced gastric ulceration

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ABSTRACT

Zinc complexes were reported to have anti-ulcer activity and used as drug for the treatment of gastrointestinal disorders. A novel compound dichlorido-zinc(II)-4-(2-(5-methoxybenzylidene amino)ethyl)piperazin-1-iumphenolate (ZnHMS) was synthesized, characterized and evaluated for its gastroprotective activity against ethanol-induced ulcer in rats. Gross and microscopic lesions, histochemical staining of glycogen storage, biochemical and immunological parameters were taken into consideration. Oral administration of ZnHMS (30 and 60 mg/kg; 14 days) dose-dependently inhibited gastric lesions. It significantly increased the mucus content and total acidity compared to the control group ($P < 0.01$). Serum levels of aspartate (AST), alanine (ALT) transaminases, pro-inflammatory interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and anti-inflammatory interleukin-10 (IL-10) in the rats exposed to ethanol induced ulceration have been altered. ZnHMS considerably enhances ($P < 0.05$) the protection of gastric epithelia by modulating the acute alterations of AST, ALT, IL-6, IL-10, TNF-α and stomach glycogen. Interestingly, ZnHMS did interfere with the natural release of nitric oxide. In addition, acute toxicity study revealed no abnormal sign to the rats treated with ZnHMS (2000 mg/kg). These findings suggest that the gastroprotective activity of ZnHMS might contribute in adjusting the inflammatory cytokine-mediated oxidative damage to the gastric mucosa.

1. Introduction

Gastric mucosal erosion was reported to be associated with the imbalance between the aggressive factors (physical, chemical or psychological) in the lumen and protective mechanisms [1] in the duodenal mucosa, causing chronic inflammation that leads to a defect in the regulation of gastrin production [2]. Gastrointestinal problems have now become a global problem, and many studies were conducted towards fixing it [3]. The ability of some ulcer models to suppress the production of prostaglandins and thromboxanes, and cause irreversible inactivation of the cyclooxygenases (which is essential for the production of prostaglandins) has provided a means for intense investigations [4–6]. It was suggested that, cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10) play important roles in the acute phase inflammation as well as in maintenance and regulation of the severity of gastric ulcers [7]. The ulceration bestowed by ethanol causes an intense infiltration in the submucosa, decrease mucus, depletes sulfhydryl groups and decrease blood flow, which resulted in serious damage to the gastric mucosa [8]. This ulcer model has provided a means of determining the mechanistic way by which many compounds protects gastric against ulcerations.

Zinc is an essential element which plays an imperative role in cell-mediated immune functions. It is employed as a cofactor for metalloenzymes, superoxide dismutase, collagenase, alcohol dehydrogenase, alkaline phosphate, children's growth and in spermatogenesis [9,10]. Zinc is required for the proper functioning of mucosal cells and can arrest the advancement of gastrointestinal disease by free radical scavenging and interruption of the inflammatory process as an antioxidant and anti-inflammatory agent. Zinc deficiency can cause poor wound healing, loss of taste and smell and elevation in ROS [11,12]. Zinc complex was reported to have anti-ulcer activity and used as drug for the treatment of gastrointestinal injuries in Japan [13]. This study considers the therapeutic potentials of piperazine derivatives, and the role played by zinc in wound healing to synthesize a novel compound with combine protective activity [14,15]. The study, therefore, reported for the first time the synthesis, characterization, acute toxicity and

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gastroprotective activity of Zinc(II) complex (ZnHMS) derived from the Schiff base (E)-4-methoxy-2-((2-(piperazin-1-yl)ethylimino)-methyl)phenol.

2. Materials and methods

2.1. Reagents

The primary amine 2-(piperazin-1-yl)ethanamine, 5-methoxysalicylaldehyde, Zn(II)chloride dihydrate and potassium hydroxide was purchased from Merck, Kuala Lumpur, Malaysia. ELISA kits for IL-6, IL-10 and TNF-α were purchased from Ray Biotech, USA. All other chemicals and reagents were of analytical grade.

2.2. Synthesis of dichlorido-zinc(II)-4-(2-(5-methoxybenzylideneamino)ethyl)piperazin-1-iumphenolate

A weighed amount of 2-(piperazin-1-yl)ethanamine (1.29 g, 10 mmol) dissolved in an absolute ethanol 25 mL was added drop wise to an ethanolic solution 25 mL of 5-methoxysalicylaldehyde (1.52 g, 10 mmol) at room temperature and refluxed for 3 h. An orange solution was formed which after evaporation gave a red gel. The product was isolated in solid form by adding few drops of diethyl ether. Recrystallization was carried out in methanol. To a stoichiometric amount of the synthesized Schiff base (0.26 g, 1 mmol) dissolved in 25 mL methanol, an equimolar quantity of Zinc(II) chloride dihydrate (0.14 g, 1 mmol) taken in 25 mL methanol was added at room temperature. In the presence of few drops of potassium hydroxide and stirring, a pale yellow precipitate was formed. The precipitate filtered, washed with ethanol–water mixture and dried in a vacuum desiccator. Recrystallization was performed in a mixture of methanol and dichloromethane.

2.3. Acute toxicity study

2.3.1. Animals

Adult male and female Sprague Dawley rats of 7–8 weeks old weighed (165 ± 15 g) were obtained from the Animal House, Faculty of Medicine, University of Malaya, Malaysia. In this study, 24 rats were assigned into two groups of twelve rats each (six male and six female rats per group) and labeled as control and treatment groups. All animals were given human care according to approved institutional guidelines and experiments articulated by the National Academy of Sciences and published by the National Institute of Health, Malaysia.

2.3.2. Protocol for acute toxicity

The overnight fasted animals received orally a single dose of ZnHMS 2000 mg/kg/body weight, and continued fasting for 3–4 h after dosing. Daily clinical examinations of the animals did not show any significant abnormal signs of toxicity like tremor, eyes mucus, body weight changes or autonomous saliva release. No mortality was recorded at any time of observation for the period of two weeks. The study was conducted according to the "International Guidelines for Testing of Chemicals Oral Toxicity" and was approved by the Animal Ethics Committee of University of Malaya.

2.4. Gastro-protective study

2.4.1. Ulcerogenic study animals

Sprague Dawley adult male rats (220 ± 30 g) 7–8 weeks old were for ulcerogenic study. Rats were distributed into eight groups of six rats each. The animals were caged individually. They were exposed to 12 h dark–light circles and fed with a standard pellet diet and tap water under controlled temperature conditions (23 ± 2 °C). All animals were given human care according to approved institutional guidelines and experiments articulated by the National Academy of Sciences and published by the National Institute of Health, Malaysia.

2.4.2. Dose selection

A preliminary study for dose fixation was conducted using 20 mg/kg of ZnHMS to 24 h fasted rats prior to ethanol induced ulceration. It was observed that 20 mg/kg of ZnHMS did not significantly prevent the damaging effects of ethanol. Increasing the dosage of ZnHMS to 30 mg/kg was observed to give a better protection. In addition, treatment with ZnHMS for 3, 7 and 10 days showed a continuous inhibition of the ulcer area. Thus, the dose of 30 mg/kg (ZnHMS) was then considered as the low dose and the treatment extend to 14 days to further ascertain the active dosage that can give the highest protection against the damages caused by ethanol.

2.4.3. Protocol for gastroprotectivity

Animals were fasted for 24 h before ethanol-induced ulceration (Scheme 1) and orally pre-treated as follows; Groups 1 and 2 received the vehicle, carboxymethylcellulose (CMC) after 14-days treatments with food and water alone. Group 3 rats were treated with omeprazole (20 mg/kg) dissolved in CMC prior to ethanol administration. Groups 4, 7 and 8 rats were given ZnHMS dissolved in CMC for two weeks. Group 5 and Group 6 were given ZnHMS single doses of 30 and 60 mg/kg, respectively, dissolved in CMC prior to ulcer induction. One hour after this treatment, animals in Groups 2, 3, 5, 6, 7 and 8 were orally gavaged with 95% ethanol at the dose of 5 mL/kg. Group 4 rats were not given ethanol and served as normal control group. All the animals were sacrificed after 30 min by cervical decapitation under anesthesia with xylazine and ketamine. Serum was collected for biochemical analyses.

2.4.4. Serum biochemical assays

Blood was collected through cardiac puncture. Serum samples obtained from the animals were analyzed using Hitachi Auto-analyzer at University of Malaya Medical Centre laboratory, Kuala Lumpur, Malaysia. Parameters analyzed include ALT, AST, C-reactive protein and HDL.

2.4.5. Measurement of gastric juice acidity

The stomachs removed from the experimental rats were opened along the greater curvature, and the content carefully transferred into a clean container. This was then centrifuged for 10 min at −4 °C and analyzed for hydrogen ion concentration by pH-meter titration using 0.1 N NaOH as titrant. The acid content was expressed as mEq/l [16].

2.4.6. Histological evaluation of gastric lesions

Specimens of the gastric walls removed from each rat were fixed in 10% buffered formalin and embedded in paraffin. Sections of the stomach (5 μm) were stained with hematoxylin and eosin for histological evaluation.

Animal groups	Pre-treatments	Duration	Description	Ethanol-induced ulcer
1	Distilled water	NA	Normal control	No
2	Vehicle (0.5% CMC)	14 Days	Ulcer control	Yes
3	Omeprazole (20 mg/Kg)	Single Dose	Positive control	Yes
4	ZnHMS (60 mg/Kg)	14 Days	Gastric tolerance group	No
5	ZnHMS (30 mg/Kg)	Single Dose	Gastroprotective group	Yes
6	ZnHMS (60 mg/Kg)	Single Dose	Gastroprotective group	Yes
7	ZnHMS (30 mg/Kg)	14 Days	Gastroprotective group	Yes
8	ZnHMS (60 mg/Kg)	14 Days	Gastroprotective group	Yes

Scheme 1. Experimental design.