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Efficacy and safety of the compound Chinese herb medicine mouthwash on oral ulcer model in rats

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Abstract: The present study aimed to evaluate the effect of a mouthwash containing a novel compound Chinese herbal medicine (artemisia capillaris, chrysanthemum, honeysuckle, angelica dahurica and asarum sieboldii) on oral ulcers and analyze sub chronic oral toxicity in rats. For efficacy study, mouthwash was administered on the ulcer area twice daily. Compared with the control group, healing time in the test group was shorter and the ulcer area was smaller. Histological analysis showed less inflammatory cell infiltration in the test group. For sub chronic oral toxicity, mouthwash was administered by oral gavage for 93 consecutive days. There were no significant differences in body weight, food consumption or organ coefficients between the test and control groups. Some parameters of haematology and serum chemistry were statistically different but within normal physiological ranges. No obvious abnormalities were found in the necropsies and histopathological observations. In conclusion, the compound Chinese herbal medicine mouthwash promoted oral ulcer healing in rats with no obvious sub chronic toxicity, providing a potential alternative therapeutic strategy for oral ulcers.

Keywords: Chinese herbal medicine mouthwash, oral ulcer, wound healing, safety, sprague-dawley rat.

INTRODUCTION

Oral ulcers, a common mucosal disease that affects 5% to 25% of the population (Chavan *et al*., 2012), are characterised as a localised defect on the surface of the oral mucosa with destructive epithelial continuity and exposed connective tissue. Clinically, the lesion is covered with a yellowish pseudo membrane on its surface, surrounded by an erythematous halo (Woo and Sonis, 1996). Oral ulcers can cause mild to severe pain, affecting patients' nutritional intake, oral hygiene and even quality of life (Al-Omiri *et al*., 2015).

The pathogenesis of oral ulcers is multifactorial and significant risk factors include trauma, infection, immune disorders, heredity and psychological factors (Saikaly *et al*., 2018). Oral ulcers can be caused by single or multiple factors and sometimes it is difficult to identify the definite causes (Jurge *et al*., 2006). Due to these multiple factors, the gold standard treatment protocol has not yet been established (Eisen and Lynch, 2001). At present, the main treatment is topical symptomatic treatment, with the purpose of providing anti-inflammatory effects, preventing secondary infections and promoting healing (Barrons, 2001; Tarakji *et al*., 2015). Mouthwash is a preparation method that is well accepted by the public, with advances in convenience and safety. Chlorhexidine (CHX) mouthwash is commonly used in dental clinical practice because of its anti-inflammatory effect (James *et al*., 2017; Teixeira *et al*., 2019). However, the long-term use of chlorhexidine is limited because of taste disturbance, temporary staining of teeth and tongue and local adverse effects on the oral mucosa (Jones, 1997; Lorenz *et al*., 2006). In addition, other antiseptic mouthwash products containing cetylpyridinium chloride (CPC) or chlorhexidine digluconate (CXD) may be related to genetic and cellular toxicity (Rodrigues *et al*., 2007). Some studies have indicated that CPC is cytotoxic (Burgalassi *et al*., 2001; Chetoni *et al*., 2003) and that CXD can induce DNA damage (Eren *et al*., 2002; Ribeiro *et al*., 2004). Therefore, it is necessary to develop a novel mouthwash not only against oral ulcers, but also with fewer side effects.

Traditional Chinese medicine (TCM), with a long history of application, plays an increasingly important role in the prevention of diseases and in health recovery, as well as in its synergy with Western medicine in the diagnosis and treatment of major diseases (Xue and Roy, 2003). Chinese herbal medicine (CHM) is under the guidance of TCM. The compound CHM is an overlay of multi-herbsto, which improves the holistic efficacy. Various studies have shown that Chinese patent medicines are beneficial for patients with oral ulcers in relieving ulcer pain and reducing the duration and frequency of attacks (Jin *et al*., 2017; Guo *et al*., 2018; Zhu *et al*., 2018). Therefore, CHM has wide application prospects in the treatment of oral ulcers.

Artemisia capillaris, chrysanthemum, honeysuckle, Angelica dahurica and Asarum sieboldii are common Chinese herbal medicines with varying efficacies. A. capillaris, chrysanthemum and honeysuckle have antibacterial and anti-inflammation properties (Quang *et al*., 2012; Park *et al*., 2012; Cha *et al*., 2005). Some

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studies have suggested that A. dahurica extract could affect antioxidant activity (Lin *et al*., 2014; Xu *et al*., 2011) and A. sieboldii showed an analgesic effect (Kim *et al*., 2003). To clarify the effects of these herbs, our research team applied the method of steam distillation to extract these five herbs and found that the extracts of each herb can inhibit *Staphylococcus aureus*, *Escherichia coli,* and *Porphyromonas gingivalis*. Furthermore, we adopted a uniform design to confirm the optimal ratio and then compounded the extracts of these five herbs to develop a new Chinese herbal medicine mouthwash according to the optimal ratio (Xu *et al*., 2018).

Nevertheless, some studies have reported that the adverse effects of Chinese medicine include stomach ache, abdominal distention, diarrhoea, mild nausea and gastrointestinal discomfort (Zhou *et al*., 2017). This safety problem has caused widespread concern; thus, safety issues are essential for the clinical application of Chinese medicine.

In this study, we aimed to explore the effect of a compound Chinese herbal medicine mouthwash, on oral ulcers and analyse its sub chronic oral toxicity in rats.

MATERIALS AND METHODS

Preparation of the compound Chinese herb medicine mouthwash

Honeysuckle (production batch: 150205391), chrysanthemum (production batch: 141210661), *Artemisia capillaris* (production batch: 150301461), *Angelica dahurica* (production batch: 150109911), and *Asarum sieboldii* (production batch: 141211061) were purchased from Kangmei Pharmaceutical Co., Ltd. and Nanfang Hospital, Southern Medical University. The compound Chinese herbal medicine mouthwash was prepared according to the method described below. Briefly, we selected the optimal ratio by uniform design, and binomial regression was applied to obtain the optimal ratio (honeysuckle 40mg/mL, chrysanthemum 45mg/mL, Artemisia capillaris 55mg/mL and the mixture of Angelica dahurica and Asarum sieboldii (33:17) 40mg/mL). Artemisia capillaris, chrysanthemum, honeysuckle and a mixture of Angelica dahurica and Asarum sieboldii (in a ratio of 33: 17) were extracted and sterilised by the boiling method (boiling for 30 min). A compound Chinese herbal medicine mouthwash was prepared according to the optimal ratio.

Animals

Healthy Sprague-Dawley (SD) rats (Laboratory Animal Center of Southern Medical University, Guangzhou, China), aged 6-7 weeks, weighing 180-220g, were maintained in polypropylene cages with a pine-sawdust-covered floor in a temperature- and humidity-controlled room under a light-dark cycle (L:D, 12:12-h). All animals had free access to food and drink. 45 female rats were used for the efficacy study of oral ulcers and 60 rats (half male and half female) were used for the sub chronic oral toxicity test. The present study was approved by the Institutional Animal Care and Use Committee of Southern Medical University (44002100014087). All methods were carried out in accordance with the guidelines laid down by the revised Animals (Scientific Procedures) Act 1986 in the UK and Directive 2010/63/EU in Europe.

Establishment of the oral ulcer model

The oral ulcer models were established through a chemical method adopted in a previous study with slight modifications (Guo *et al*., 2012). All animals were acclimated for seven days before induction. Under anaesthesia with 2% pentobarbital sodium (0.3ml/100g, intraperitoneally), seven-week-old rats were treated vertically with a plastic tube (diameter: 5mm, length: 4cm) containing three pieces of cotton sheets and soaked with 70μl of 20% sodium hydroxide solution on the right cheek mucosa for 30 seconds. To standardise the area of oral ulcers, all animal models were induced by the same author using the same plastic tube.

Grouping and treatment

The method for the efficacy study of oral ulcers was described previously and modified (Shang *et al.*, 2020). In brief, oral ulcer models were established in 45 female rats. After one day, ulcers were visible in the right oral mucosa. Animals were randomly divided into three groups: compound Chinese herbal medicine mouthwash (test group), compound gargle solution chlorhexidine gluconate (positive control group) and sterile water (negative control group). After inhalation anaesthesia, the rats were treated with 20μL of the corresponding liquids applied to cover the surface of the ulcers twice daily throughout the experimental period until the ulcers healed.

Clinical evaluation

The diameter (d) of the ulcers was measured daily using a digital Vernier caliper (0.01 mm precision) to calculate the area of the ulcers ($A=\pi\cdot(d/2)^2$). Intraoral photographs were taken to record the change in oral ulcers on days 0 (before treatment), 3, 6 and 9.

Histopathological evaluation

After anaesthesia, the animals were sacrificed on days 3, 6 and 9 and right cheek mucosal specimens were collected and fixed in 4% paraformaldehyde for 24h. The specimens were washed with water, dehydrated using gradient ethanol, diaphanized with dimethylbenzene and embedded in paraffin. Tissue slices were 3-μm thick, stained with haematoxylin and eosin (H&E) and observed using an optical microscope.

Subchronic oral toxicity study

The method of the sub chronic oral toxicity study was described in a previous study (Kunanusorn *et al*., 2011; Liu *et al*., 2021). In the current study, after acclimatisation

for one week, 60 rats were randomly divided into two groups (15 rats per sex per group): test and control groups. Animals in the test group were administered the compound Chinese herbal medicine mouthwash via gavage once a day for 93 days, whereas those in the control group received sterile water. The intragastric dose was 1000mg/kg body weight. After 93-day drug administration, the animals were observed without treatment for 28 days as the recovery period. The timeline of the subchronic oral toxicity study is shown in fig.1.

During the experimental period, the animals were observed once a day for mortality and any abnormal clinical signs, including changes in the fur, skin, mucous membranes, respiratory systems, circulatory systems, somatomotor activity and behavioural patterns. If any abnormal signs were observed, the emergence time, type, frequency and duration of the symptoms were recorded. Body weight and food consumption were measured weekly for each animal during the drug administration and recovery periods.

Haematological analysis, serum chemistry analysis and necropsy evaluation were conducted on days 45, 93 and 121 during the experimental period. All animals were fasted overnight prior to anaesthesia with 2% pentobarbital sodium. After reaching the anaesthetic stage, blood samples were obtained from the abdominal aorta. The following parameters of haematology were measured using a haematological autoanalyzer: White blood count (WBC), neutrophil count (Neut), lymphocyte count (Lymph), eosinophil count (Eos), basophil count (Baso), monocyte count (Mono), percentage of neutrophils (Neut%), percentage of lymphocytes (Lymph%), percentage of eosinophils (Eos%), percentage of basophils (Baso%), percentage of monocytes (Mono%), red blood count (RBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW).

Blood samples were collected into coagulation-promoting tubes and centrifuged to obtain serum at a speed of 3000 rpm/min for 10 min, and serum chemistry was detected using an automated analyser: Potassium (K), sodium (Na), chloride (Cl), calcium (Ca), phosphate (P), urea, creatinine (Cr), total protein (TP), albumin (Alb), globulin (Glo), albumin/globulin (A/G), total bilirubin (Tbil), alanine aminotransferase (ALT), aspartate aminotransferase (AST), aspartate aminotransferase/ alanine aminotransferase (AST/ALT) and gamma-glutamyl transpeptidase (GGT).

Necropsies were performed following blood collection. The organs examined included the brain, thymus, heart, liver, kidney, adrenal gland, spleen, testis, epididymis, uterus and ovary. The absolute organ weights were measured immediately after isolation. Organ coefficients were calculated using the following formula: Organ coefficient= (absolute organ weight) / (final body weight). All organs were fixed in 4% paraformaldehyde for 24h. The specimens were washed with water, dehydrated using gradient ethanol, diaphanized with dimethylbenzene and embedded in paraffin. Tissue slices were made into 3-μm thick, stained with H&E and observed using an optical microscope.

STATISTICAL ANALYSIS

All values were presented as means ± standard deviations (SDs). Data analysis was performed using SPSS 20.0. The ulcerated areas were compared among the three groups using one-way analysis of variance and followed by the SNK-q post-test. Body weight, food consumption, organ coefficient, parameters of haematology and serum chemistry were analysed using the t-test. Statistical significance was set at $p<0.05$.

RESULTS

Healing time and ulcerated area evaluation

The oral ulcer model was established using 20% sodium hydroxide solution. A rounded oral ulcer was clinically visible in the right cheek mucosa of the rat, with a delineating erythematous border and covered with a faint yellowish pseudo membrane after one day of induction. During the period of drug administration, it could be observed that the ulcers in the test group and positive control group healed on day 9 of treatment, while ulcers in the negative control group healed on day 11. (fig. 2).

According to the evaluation of the ulcerated area, there was no significant difference among the three groups regarding the area of oral ulcers measured before treatment and after three days of treatment ($P>0.05$). However, on days 4 to 7, the mean ulcer areas in both the test and positive control groups were significantly smaller than those in the negative control group ($P<0.05$). (fig. 3)

Histopathological evaluation

When the oral ulcer model was established, microscopic analysis of the ulcer lesion sections showed local sunken destruction with broken continuity of the epithelial layer. The surface of the lesion was covered with a mixture of fibrin exudate and inflammatory cells. In the connective tissue, obvious hyaline degeneration and inflammatory cell infiltration were observed. At the margin of the lesion, pathological observations showed a decrease in epithelial cell layers and coagulation necrosis of cell debris.

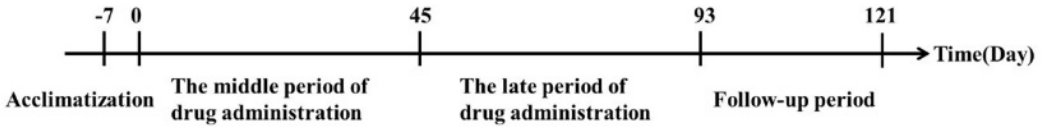

Fig.1: The timeline of sub chronic oral toxicity study.

Table 1: The parameters of hematologic analysis with statistical difference in the sub chronic oral toxicity test.

Day	Parameters	Female		Male	
		Test group	Control group	Test group	Control group
45	MPV (fL)	6.50 ±0.08*	6.77±0.15	6.78 ±0.22	6.90±0.36
93	Baso (10^9/L)	0.02±0.01*	0.01±0.01	0.03±0.01	0.02±0.01
	Baso %(%)	0.28±0.08*	0.10±0.08	0.38±0.08	0.34±0.18
	Hb (g/L)	134.60±1.52*	140.25±3.30	143.40±3.29	138.60±4.22
121	Neut %(%)	21.84±5.33	21.43±4.46	28.98±4.27*	20.33±4.08
	Lymph %(%)	74.92±6.12	76.75 ±4.53	67.84±5.14*	76.55±4.42
	MCH (pg)	19.60±0.49	19.33 ±0.66	18.26±0.34*	18.75±0.13

Data expressed as mean ± SD.
Significant differences are indicated by $^*P<0.05$ compared to the control group.

Table 2: The parameters of serum chemistry with statistical difference in the sub chronic oral toxicity test

Day	Parameters	Female		Male	
		Test group	Control group	Test group	Control group
45	Alb (g/L)	37.86±2.81	36.50±5.34	33.78±0.82*	32.40±0.54
93	Tbil (μmol/L)	4.96±0.67*	6.40±0.45	4.58±1.73	5.08±2.23
121	Na (mmol/L)	141.72±0.60	142.55±0.93	145.44±0.55*	143.73±1.34
	Cl (mmol/L)	103.08±0.93	103.13±1.28	105.70±1.45*	103.33±1.22
	Urea (mmol/L)	10.93±1.15	11.54±1.85	7.24±0.49*	8.84±1.28

Data expressed as mean ± SD.
Significant differences are indicated by $^*P<0.05$ compared to the control group.

Table 3: Organ coefficient of rats in the sub chronic oral toxicity test

		Day 45		Day 93		Day 121	
		Test group	Control group	Test group	Control group	Test group	Control group
Female	Brain	0.71±0.08	0.70±0.12	0.69±0.04	0.65±0.03	0.71±0.05	0.72±0.06
	Heart	0.37±0.04	0.34±0.02	0.38±0.06	0.39±0.05	0.41±0.06	0.38±0.05
	Thymus	0.15±0.04	0.15±0.03	0.14±0.04	0.14±0.06	0.11±0.03	0.14±0.06
	Liver	2.88±0.23	3.00±0.64	2.92±0.43	3.57±1.01	3.17±0.90	2.81±0.39
	Spleen	0.23±0.03	0.28±0.09	0.25±0.04	0.24±0.02	0.27±0.07	0.26±0.05
	Kidney	0.67±0.06	0.76±0.10	0.75±0.10	0.78±0.11	0.77±0.13	0.69±0.03
	Adrenal	0.02±0.01	0.02±0.00	0.04±0.01	0.04±0.00	0.04±0.01	0.04±0.00
	Uterus	0.25±0.05	0.20±0.02	0.26±0.11	0.25±0.06	0.24±0.03	0.23±0.02
	Ovary	0.05±0.01	0.05±0.01	0.08±0.02	0.09±0.03	0.07±0.01	0.10±0.08
Male	Brain	0.48±0.05	0.50±0.06	0.50±0.07	0.44±0.07	0.48±0.04	0.45±0.04
	Heart	0.36±0.06	0.34±0.03	0.35± 0.04	0.34±0.03	0.38±0.08	0.33±0.02
	Thymus	0.10±0.03	0.08±0.01	0.09±0.02	0.08±0.03	0.05±0.02	0.07±0.02
	Spleen	0.21±0.04	0.20±0.01	0.18±0.02	0.18±0.03	0.21±0.07	0.22±0.09
	Liver	2.62±0.30	2.97±0.47	3.04±0.85	3.09±0.80	2.59±0.15	2.41±0.06
	Kidney	0.69±0.06	0.68±0.04	0.70±0.11	0.72±0.11	0.74±0.03	0.69±0.07
	Adrenal	0.02±0.01	0.02±0.01	0.01±0.01	0.02±0.01	0.02±0.01	0.02±0.00
	Testis	0.86±0.10	0.84±0.06	0.80±0.09	0.72±0.04	0.75±0.06	0.76±0.06
	Epididymis	0.30±0.05	0.34±0.03	0.34±0.04	0.33±0.06	0.33±0.04	0.32±0.05

Table S1: The parameters of hematologic analysis in the sub chronic oral toxicity test.

Day	Parameters	Female		Male	
		Test group	Control group	Test group	Control group
45	WBC (109/L)	5.59±1.11	4.93±3.02	9.50±3.08	8.18±1.64
	Neut (109/L)	1.57±0.68	1.55±1.18	3.55 ±1.92	1.85±0.64
	Lymph (109/L)	3.87 ±1.29	3.27±2.20	5.70± 1.25	6.15±1.48
	Eos (109/L)	0.06 ±0.01	0.06±0.03	0.10 ±0.05	0.09±0.01
	Baso (109/L)	0.03 ±0.01	0.01±0.02	0.04 ±0.02	0.03 ±0.02
	Mono (109/L)	0.08±0.04	0.03±0.03	0.11 ±0.08	0.08 ±0.05
	Neut% (%)	28.55±13.12	32.17±11.20	29.36 ±12.67	22.78±6.92
	Lymph% (%)	68.50±13.00	65.43±11.77	62.06 ±10.62	74.90±6.73
	Eos% (%)	0.93 ±0.19	1.77± 1.17	1.00± 0.28	1.10±0.34
	Baso% (%)	0.50 ±0.08	0.20±0.20	0.40 ±0.14	0.28±0.17
	Mono% (%)	1.53 ±1.08	0.43±0.25	1.18 ±0.86	0.95±0.55
	RBC (1012/L)	6.52 ±0.47	6.45±0.28	7.20 ±0.49	7.40 ±0.22
	Hb (g/L)	136.50±7.90	132.00±6.25	140.20±2.59	142.00±4.90
	Hct (%)	0.40 ±0.02	0.39± 0.02	0.41 ±0.02	0.43±0.02
	MCV (fL)	61.60 ±3.06	59.87 ±1.93	57.70 ±1.90	57.30±1.36
	MCH (pg)	21.03 ±1.32	20.43±0.68	19.46 ±1.06	19.10±0.42
	MCHC (g/L)	341.75±7.50	341.67±3.21	339.20± 10.43	332.50±7.94
	RDW (%)	14.43±0.78	14.30±0.36	14.54 ±0.56	15.25±0.19
	PLT (109/L)	1130.50±100.26	1115.67±51.39	1082.00± 64.14	1066.25±92.50
	MPV (fL)	6.50 ±0.08*	6.77±0.15	6.78 ±0.22	6.90±0.36
	PCT (%)	0.73 ±0.06	0.75±0.05	0.73 ±0.05	0.73±0.02
	PDW (%)	14.85 ±0.06	14.90±0.10	14.90±0.07	14.83±0.15
93	WBC (10^9/L)	6.35±2.34	5.51±0.96	7.80±2.93	6.74±1.63
	Neut (10^9/L)	1.30±0.28	1.14±0.09	2.09±0.83	2.03±0.80
	Lymph (10^9/L)	4.89 ±2.09	4.23±0.95	5.51±2.13	4.57±0.91
	Eos (10^9/L)	0.06±0.01	0.07±0.03	0.06±0.01	0.05±0.02
	Baso (10^9/L)	0.02±0.01*	0.01±0.01	0.03±0.01	0.02±0.01
	Mono (10^9/L)	0.09±0.08	0.07±0.05	0.11±0.07	0.08±0.02
	Neut% (%)	21.88±6.59	21.38±5.08	27.02±3.69	29.24±5.49
	Lymph% (%)	75.48±6.83	76.10 ±4.95	70.24±4.87	68.42±4.77
	Eos% (%)	0.98±0.33	1.20±0.45	0.86±0.34	0.72±0.31
	Baso% (%)	0.28 ±0.08*	0.10±0.08	0.38±0.08	0.34±0.18
	Mono% (%)	1.38 ±1.14	1.24±0.81	1.50±1.09	1.28±0.52
	RBC (10^{12}/L)	6.52±0.36	6.83±0.38	7.81±0.20	7.47±0.27
	Hb (g/L)	134.60±1.52*	140.25±3.30	143.40±3.29	138.60±4.22
	Hct (%)	0.40±0.00	0.41±0.02	0.43±0.01	0.42±0.01
	MCV (fL)	61.26±3.08	60.48±2.30	55.48±0.98	55.98±1.28
	MCH (pg)	20.66±1.14	20.58±0.82	18.36±0.40	18.56±0.25
	MCHC (g/L)	338.40±3.21	340.25±9.43	332.20±2.68	331.60±3.85
	RDW (%)	14.36±0.44	14.73±0.33	15.28±0.53	15.18±0.27
	PLT (10^9/L)	1065.60±63.56	1063.00 ±48.44	1079.40±17.10	1052.60±126.53
	MPV (fL)	6.35±2.34	5.51±0.96	6.86±0.27	6.64±0.24
	PCT (%)	1.30±0.28	1.14±0.09	0.74±0.03	0.69±0.08
	PDW (%)	4.89 ±2.09	4.23±0.95	14.82±0.08	14.78±0.16
121	WBC (10^9/L)	5.15±0.60	6.00±1.60	4.65±0.65	6.54±1.76
	Neut (10^9/L)	1.12±0.29	1.24± 0.17	1.33±0.18	1.03 ±0.38
	Lymph (10^9/L)	3.87 ±0.63	4.66±1.49	3.17±0.60	5.06±1.59
	Eos (10^9/L)	0.05 ±0.02	0.06±0.03	0.04±0.02	0.03±0.01
	Baso (10^9/L)	0.02 ±0.01	0.02±0.01	0.02±0.01	0.03±0.01
	Mono (10^9/L)	0.09 ±0.05	0.03±0.02	0.08±0.06	0.14±0.08
	Neut% (%)	21.84 ±5.33	21.43±4.46	28.98±4.27*	20.33±4.08
	Lymph% (%)	74.92±6.12	76.75 ±4.53	67.84±5.14*	76.55±4.42
	Eos% (%)	1.12±0.46	0.93±0.50	0.94±0.23	0.55± 0.24
	Baso% (%)	0.40± 0.28	0.33± 0.13	0.38±0.13	0.43±0.10
	Mono% (%)	1.72 ±1.05	0.58±0.17	1.86±1.43	2.15±0.82
	RBC (10^{12}/L)	6.95 ±0.42	6.86 ±0.29	7.73±0.41	7.89±0.11
	Hb (g/L)	135.80±5.40	132.25±5.62	140.80±6.18	147.75±1.71
	Hct (%)	0.39± 0.02	0.40 ±0.01	0.43±0.02	0.44±0.01
	MCV (fL)	56.90±2.18	57.20 ±2.21	55.04±1.81	55.08±1.30
	MCH (pg)	19.60±0.49	19.33 ±0.66	18.26±0.34*	18.75±0.13
	MCHC (g/L)	344.60±4.39	347.50±27.23	330.60±7.83	339.75±5.32
	RDW (%)	14.90±1.22	14.35± 0.34	14.98±0.51	15.33±0.71
	PLT (10^9/L)	1092.00±135.02	1138.75±52.70	1009.20±128.25	1074.00±31.89
	MPV (fL)	7.12±0.29	7.03±0.21	7.06±0.27	6.85±0.06
	PCT (%)	0.77±0.07	0.80±0.05	0.72±0.07	0.73±0.02
	PDW (%)	14.90±0.07	14.83± 0.17	14.82±0.15	14.80±0.08

Data expressed as mean ± SD. Significant differences are indicated by *P<0.05 compared to the control group.

Table S2: The parameters of serum chemistry in the sub chronic oral toxicity test.

Day	Parameters	Female		Male	
		Test group	Control group	Test group	Control group
45	K (mmol/L)	5.09 ±1.18	4.88±0.36	4.81±0.15	4.81±0.13
	Na (mmol/L)	142.96±2.08	144.70±2.46	144.82±2.04	144.85±1.36
	Cl (mmol/L)	104.08±0.93	104.33±1.88	103.80±0.46	103.38±0.92
	Ca (mmol/L)	2.80±0.10	2.73±0.05	2.65±0.10	2.69±0.06
	P (mmol/L)	2.50±0.47	2.59 ±0.81	2.78±0.20	2.69±0.25
	Urea (mmol/L)	8.44±2.22	7.21 ±0.78	9.07±2.53	7.91±2.24
	Cr (μmol/L)	60.10±21.61	50.33±5.59	59.44±16.36	48.63±18.49
	TP (g/L)	63.34±4.21	62.83±4.04	61.04±2.57	56.75±4.07
	Alb (g/L)	37.86± 2.81	36.50±5.34	33.78±0.82*	32.40±0.54
	Glo (g/L)	25.48±2.46	26.33±1.31	27.26±2.42	24.35±3.95
	A/G	1.48±0.15	1.37±0.25	1.24±0.11	1.35±0.21
	TBil (μmol/L)	4.72±1.21	4.97± 0.74	3.72±0.33	4.88±1.25
	ALT (U/L)	23.60±6.43	24.33±11.85	32.60±8.56	31.00±8.76
	AST (U/L)	231.60±74.73	252.00±59.51	224.40±56.20	197.25±38.06
	AST/ ALT	10.00±2.84	11.20± 2.95	7.00±0.88	6.85±2.40
	GGT (U/L)	0.80±0.45	1.00±0.00	1.00±.000	1.00±.000
93	K (mmol/L)	4.43±0.18	4.42±0.19	5.10±0.29	5.17±0.32
	Na (mmol/L)	142.14±0.86	141.95±0.47	144.62±0.97	144.08±1.65
	Cl (mmol/L)	104.90±0.63	103.43±2.47	104.88±1.38	104.22±1.37
	Ca (mmol/L)	2.63±0.02	2.67±0.06	2.55±0.03	2.56±0.03
	P (mmol/L)	1.98±0.26	1.98±0.36	2.68±0.24	2.65±0.10
	Urea (mmol/L)	9.66±1.11	8.95±2.67	7.53 ±1.01	7.31±0.70
	Cr (μmol/L)	59.54±12.13	61.45±16.71	45.34±6.49	42.42±10.81
	TP (g/L)	68.26 ±3.51	67.58±2.87	59.96±1.35	58.28±2.20
	Alb (g/L)	35.64±1.65	36.08±1.35	30.88±0.64	29.74±1.56
	Glo (g/L)	32.62±1.91	31.50±1.69	29.08±1.51	28.54±1.72
	A/G	1.10±0.00	1.15±0.06	1.06±0.09	1.06±0.11
	TBil (μmol/L)	4.96±0.67*	6.40±0.45	4.58±1.73	5.08±2.23
	ALT (U/L)	38.60±17.66	29.75± 8.66	34.20±7.36	36.60±4.88
	AST (U/L)	218.60±49.82	193.75±68.05	205.60±12.36	203.40±46.72
	AST/ ALT	6.12±1.84	6.48±1.23	6.26±1.47	5.52±0.58
	GGT (U/L)	0.80±0.45	0.75±0.50	0.80 ±0.45	1.00±0.00
121	K (mmol/L)	4.55±0.28	5.02±0.35	4.81±0.11	4.85±0.07
	Na (mmol/L)	141.72±0.60	142.55±0.93	145.44±0.55*	143.73±1.34
	Cl (mmol/L)	103.08±0.93	103.13±1.28	105.70±1.45*	103.33±1.22
	Ca (mmol/L)	2.80± 0.06	2.86±0.12	2.66±0.07	2.71±0.10
	P (mmol/L)	2.21±0.39	2.68±0.32	2.39±0.26	2.35±0.21
	Urea (mmol/L)	10.93±1.15	11.54±1.85	7.24±0.49*	8.84±1.28
	Cr (μmol/L)	52.08±5.89	57.03±10.24	37.78±7.86	45.08±8.26
	TP (g/L)	68.42±2.07	65.88±5.44	58.86±2.41	58.55±2.85
	Alb (g/L)	36.82± 1.39	35.75±2.48	31.58±1.19	31.35±0.58
	Glo (g/L)	31.60±1.02	30.13±3.21	27.28±1.68	27.20±2.35
	A/G	1.14±0.05	1.18±0.10	1.18±0.04	1.15±0.10
	TBil (μmol/L)	5.42±1.60	5.38±0.76	4.12±1.26	5.65±1.44
	ALT (U/L)	38.00±8.37	41.50±18.14	37.60±11.10	29.00±2.94
	AST (U/L)	167.60±19.81	185.50±32.76	203.40±63.24	180.25±32.90
	AST/ ALT	4.58±1.18	5.03±1.85	5.60 ±1.70	6.25±1.13
	GGT (U/L)	0.60 ±0.55	0.5±0.577	0.20±0.45	0.25±0.50

Data expressed as mean ± SD. Significant differences are indicated by *P<0.05 compared to the control group.

Histopathological changes in oral ulcers during drug administration are shown in fig. 4. On day 3, the lesions in all three groups had similar histological features, exhibited clearer borders, and were covered with thicker pseudo membranes. H&E staining of the sections showed that the thick pseudo membrane had a large amount of fibrin exudation. An infiltrative zone filled with inflammatory cells was clearly observed below the pseudo membrane. In addition, epithelial cells at the margin of the ulcer proliferated to the centre of the lesion. On day 6, in the test and positive control groups, the lesions became smaller and were surrounded by a whitish halo. The pseudo membranes became thinner and disappeared. Moderate inflammatory cell infiltration and large amounts of fibroblast proliferation were evident and neovascularization which was perpendicular to the surface of the ulcer lesion, was visible. Moreover, epithelial cells at the margin of the ulcer proliferated to the centre of the

ulcer. In contrast, in the negative control group, the pseudo membrane was still apparent and there was a small amount of neovascularization and more inflammatory cell infiltration, which indicated that it was still in the chronic inflammation stage. On day 9, the test and positive control groups demonstrated complete epithelial regeneration, whereas epithelial continuity still appeared broken in the negative control group.

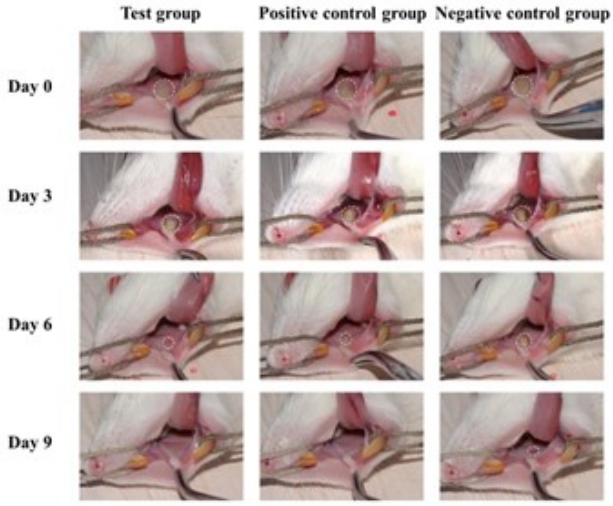

Fig. 2: Macroscopic changes of the oral ulcers on the right buccal mucosa during the period of drug administration.

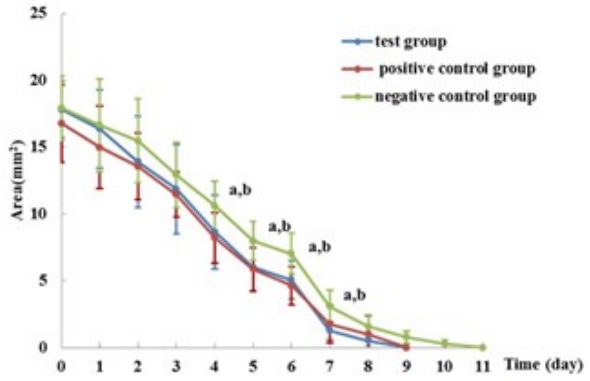

Fig. 3: Area of the oral ulcers during the period of drug administration. (a: There was a statistically significant difference between test group and negative control group, $P<0.05$; b: There was a statistically significant difference between positive control group and negative control group, $P<0.05$).

Subchronic oral toxicity study

Clinical observation

No mortality or abnormal clinical signs, including changes in the fur, skin, mucous membranes, respiratory systems, circulatory systems, somatomotor activity, or behavioural pattern, were observed in any group during the 121-day sub chronic oral toxicity study. The changes in body weight and weekly food consumption of male and female rats are shown in fig. 5 and fig. 6. There were no statistical differences in body weight or food consumption between the test and control groups ($P>0.05$).

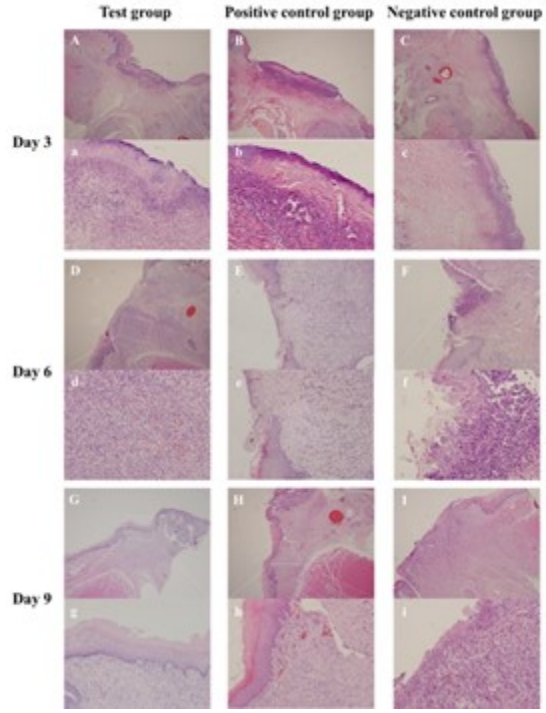

Fig. 4: Histopathological evaluation of oral ulcers in rats with H&E staining during the period of drug administration. (A-I: ×100 magnification; a-i: ×400 magnification).

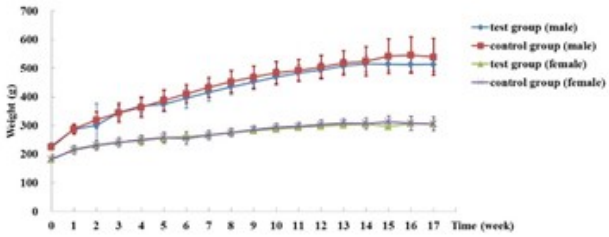

Fig. 5: Changes of weight of rats during the sub chronic oral toxicity test. (test group: compound Chinese herb medicine mouthwash; control group: sterile water)

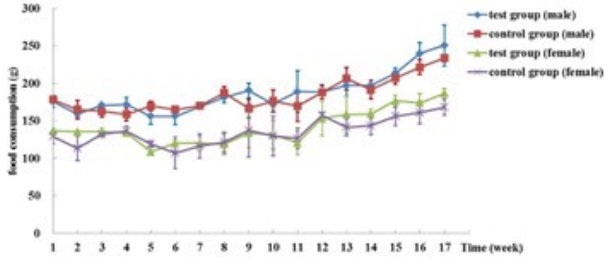

Fig. 6: Changes of food consumption of rats during the sub chronic oral toxicity test. (test group: compound Chinese herb medicine mouthwash; control group: sterile water).

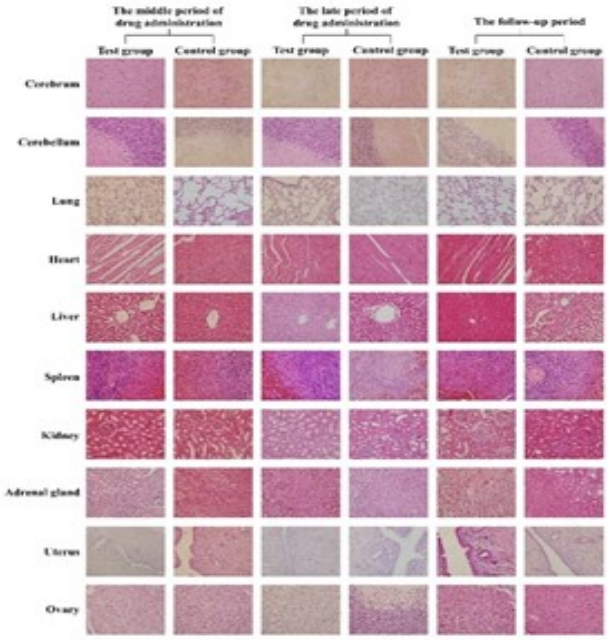

Fig. 7: Histopathological evaluation of organs in female rats with H&E staining in sub chronic oral toxicity test. (×400 magnification).

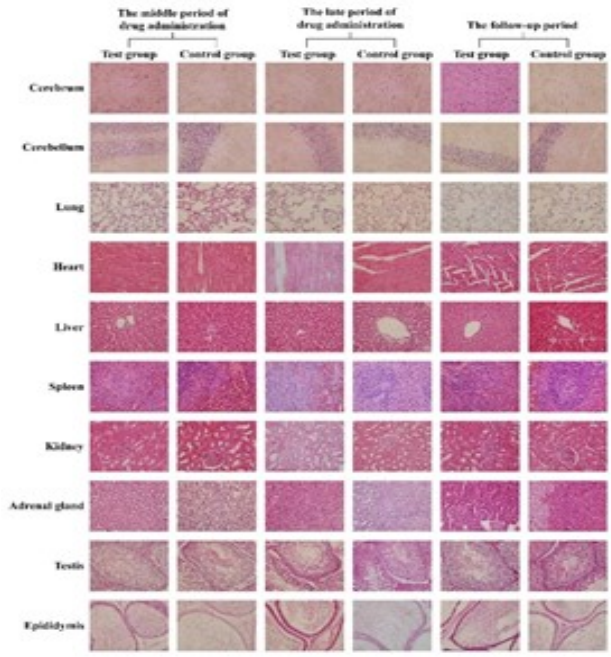

Fig. 8: Histopathological evaluation of organs in male rats with H&E staining in subchronic oral toxicity test. (×400 magnification).

Hematologic analysis

Table 1 summarises the parameters of haematology with statistically significant differences between the test and control groups on days 45, 93 and 121 of the sub chronic oral toxicity test (P<0.05). We found that female rats in the test group had a slight but statistically significant decrease in MPV on day 45 and a slight increase in Baso and Baso % on day 93. The Hb level of female rats also slightly decreased compared to that of the control group on day 93. However, these changes were not observed on Day 121. After the follow-up period, male rats showed a statistically significant increase in Neut % and a decrease in Lymph% and MCH on day 121 in the test group. All haematologic data are shown in Supporting Information. (table S1).

Serum chemistry analysis

The parameters of serum chemistry with statistically significant differences between the test and control groups on days 45, 93 and 121 of the sub chronic oral toxicity test are presented in table 2 (P<0.05). Compared with the control group, male rats in the test group showed a slight but statistically significant increase in Alb on day 45, whereas female rats showed a decrease in Tbil on day 93, which was no longer apparent after the follow-up period. In addition, a slight decrease in urea and an increase in Na and Cl were observed on day 121 in the male rats of the test group. All serum chemistry data are shown in Supporting Information (table S2).

Necropsy evaluation

The necropsies showed that the brain, thymus, heart, liver, kidney, adrenal, spleen, testis, epididymis, uterus and ovary had normal texture in rats of all groups and no abnormal signs, including hyperaemia, oedema, haemorrhage and lump, were found in gross appearance. As shown in table 3, there were no significant differences in the organ coefficients of the above organs between the test and control groups (p>0.05). H&E staining of all organs mentioned above after the middle period of drug administration, late period of drug administration, and follow-up period are shown in fig. 7 and fig. 8. Histopathological evaluation did not show any obvious abnormal pathological changes and no histopathological findings were considered to be related to the treatment.

DISCUSSION

Oral ulcers are a common pathological condition with a high incidence in the oral mucosa. In this study, we established an animal model using 20% sodium hydroxide solution to create uniform oral ulcers and found that the histological changes of ulcers in the model resembled those of oral ulcers in humans. In previous studies, oral ulcer models were usually established using phenol or acetic acid (Hitomi *et al*., 2016; Miao *et al*., 2019). The results from our study suggest that sodium hydroxide solution could be an alternative method which can successfully construct an oral ulcer model in rats.

Due to the indeterminate aetiology of oral ulcers, no satisfactory curative treatment exists, and the aim of current treatments is to ameliorate symptoms, reduce ulcer size and number, promote healing and relieve

episode duration and frequency (Barrons, 2001; Tarakji *et al*., 2015). A recent systematic review concluded that Chinese patent medicines were beneficial for patients with recurrent aphthous stomatitis in relieving ulcer pain and reducing the duration and frequency of attacks (Zhou *et al*., 2017). Since traditional Chinese herbs are becoming increasingly popular and accepted worldwide, compound Chinese herbal medicine mouthwash may be likely to become a potential option for the treatment of oral ulcers. In this study, a novel compound, Chinese herbal medicine mouthwash containing Artemisia capillaris, chrysanthemum, honeysuckle, Angelica dahurica and Asarum sieboldii, was prepared according to the optimal ratio confirmed previously (Xu *et al*., 2018).

Based on the findings of healing time and ulcerated area evaluation, we suggest that local application of this compound in Chinese herbal medicine mouthwash can accelerate the mucosal healing process of oral ulcers in rats, and its effect was similar to that of the compound gargle solution chlorhexidine gluconate. Histological analysis showed that on the sixth day of treatment, many inflammatory cells infiltrated the surface of the lesion in the negative control group, while this phenomenon was not obvious in the test and positive control groups. We speculated that the components of this compound, the Chinese herbal medicine mouthwash, might have anti-inflammatory effects. Zdarilova *et al*. (2010) found that honeysuckle extract could inhibit LPS-induced up regulation of interleukin-1beta (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α). Excessive expression of TNF-α and IL-1β is associated with cytolysis, degeneration, and interstitial fluid pressure lowering, which facilitates oedema in the oral mucosa and prolongs the inflammatory reaction (Nedrebø *et al*., 1999; Bletsa *et al*., 2006). A prolonged inflammatory response may also inhibit the wound healing process (Dubay and Franz, 2013), which could explain the difference in healing time between the test and negative control groups. In addition, Pyee *et al*. (2014) showed that wild-type lactone could down regulate the NF-κB signalling pathway and inhibit the production of inflammatory factors. In addition, Jing *et al*. (2017) isolated 37 compounds from Asarum and found that 21 of them have different levels of anti-inflammatory effects and can inhibit the release of β-glucuronidase from neutrophils.

In addition, the oral cavity is an environment that contains complex microorganisms. Bacterial infections can aggravate the inflammatory response; thus, preventing secondary infections is an important part of oral ulcer treatment. In previous studies, we found that this Chinese herbal medicine mouthwash had antibacterial effects on the growth of *Staphylococcus aureus*, *Escherichia coli,* and *Porphyromonas gingivalis* (Xu *et al*., 2018). Moreover, as an important component of this mouthwash, honeysuckle extract has been shown by Chen *et al*. (2012) to promote epithelial cell proliferation, which may serve as one of the mechanisms in promoting the healing of oral ulcers. However, the composition of traditional Chinese medicine compound preparations is complex and its mechanism of action may be multi channel and multifaceted. Therefore, further investigations are needed to provide new insights into the mechanism of action of this Chinese herbal medicine mouthwash in oral ulcers.

Toxicological evaluation is necessary prior to launching the mouthwash in the market as it provides a reference for determining the safety level of human exposure. Sub chronic oral toxicity was analysed at a dose of 1000 mg/kg/day by gavage for 93 days. During the sub chronic oral toxicity test, all animals survived and no abnormal signs of toxicity were observed. The weights of male and female rats in both the test and control groups showed an increasing trend and the difference was not statistically significant, revealing that the Chinese herbal medicine mouthwash did not affect the normal growth and development of rats. In addition, there was no statistical difference between the feed consumption of the test and control groups, suggesting that this Chinese herbal medicine mouthwash did not affect the appetite of rats.

Haematologic and serum chemistry analyses showed that only a few parameters had statistically significant differences between the test and control groups in the middle and late phases of drug administration in the sub chronic oral toxicity test. However, these differences were all within the normal physiological range for SD rats, and these changes did not depend on time. Furthermore, there were no statistical differences in organ coefficients between the test and control groups and no obvious abnormalities were found in histopathological observations. The evidence reported here supports the conclusion that this Chinese herbal medicine mouthwash has no obvious sub chronic oral toxicity at a dose of 1000 mg/kg/day for 93-day treatment. To further understand whether the compound Chinese herbal medicine mouthwash had delayed toxicity, all surviving rats were observed for 28 days after gavage. During the follow-up period, none of the animals showed signs of toxicity. The haematology and serum chemistry parameters with statistical differences were also within the normal physiological ranges for SD rats. At the same time, no abnormal changes were observed in gross autopsy and histopathological examination, suggesting that the compound Chinese herbal medicine mouthwash had no delayed toxicity.

CONCLUSION

The present study established an oral ulcer model using 20% sodium hydroxide solution which provided easily reproducible operations in rats. Based on the findings of the efficacy study, the topical application of this compound in Chinese herbal medicine mouthwash could effectively reduce the ulcerated area and healing time of

oral ulcers, and the potential mechanisms may be related to reducing inflammation and promoting healing. Furthermore, according to the sub chronic oral toxicity study, this mouthwash did not affect the normal growth and appetite of rats, and no abnormal changes were observed in haematologic and serum chemistry analyses or necropsy evaluation. In summary, the compound Chinese herbal medicine mouthwash promoted healing of oral ulcers and had no obvious sub chronic toxicity, and can be considered as an efficient and safe product for a potential alternative therapeutic strategy, while the exact mechanism and functions in the human body require further investigation.

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REFERENCES

Al-Omiri MK, Karasneh J, Alhijawi MM, Zwiri AM, Scully C, Lynch E (2015). Recurrent aphthous stomatitis (RAS): A preliminary within-subject study of quality of life, oral health impacts and personality profiles. *J. Oral Pathol. Med.,* **44**(4): 278-283.

Barrons RW (2001). Treatment strategies for recurrent oral aphthous ulcers. *Am. J. Health Syst Pharm.,* **58**(1): 41-50.

Bletsa A, Nedrebø T, Heyeraas KJ and Berggreen E (2006). Edema in oral mucosa after LPS or cytokine exposure. *J. Dent Res.,* **85**(5): 442-446.

Burgalassi S, Chetoni P, Monti D and Saettone MF (2001). Cytotoxicity of potential ocular permeation enhancers evaluated on rabbit and human corneal epithelial cell lines. *Toxicol Lett.,* **122**(1): 1-8.

Cha JD, Jeong MR, Jeong SI, Moon SE, Kim JY, Kil BS and Song YH (2005). Chemical composition and antimicrobial activity of the essential oils of Artemisia scoparia and A. capillaris. Planta Med., **71**(2): 186-190.

Chavan M, Jain H, Diwan N, Khedkar S, Shete A, Durkar S (2012). Recurrent aphthous stomatitis: A review. *J. Oral Pathol. Med.,* **41**(8): 577-583.

Chen WC, Liou SS, Tzeng TF, Lee SL and Liu IM (2012). Wound repair and anti-inflammatory potential of Lonicera japonica in excision wound-induced rats. *BMC Complement Altern Med.,* **12**: 226.

Chetoni P, Burgalassi S, Monti D and Saettone MF (2003). Ocular toxicity of some corneal penetration enhancers evaluated by electrophysiology measurements on isolated rabbit corneas. *Toxicol In Vitro.,* **17**(4): 497-504.

Dubay DA and Franz MG (2003). Acute wound healing: the biology of acute wound failure. *Surg Clin North Am.,* **83**(3): 463-481.

Eisen D and Lynch DP (2001). Selecting topical and systemic agents for recurrent aphthous stomatitis. *Cutis.,* **68**(3): 201-206.

Eren K, Ozmeric N and Sardas S (2002). Monitoring of buccal epithelial cells by alkaline comet assay (single cell gel electrophoresis technique) in cytogenetic evaluation of chlorhexidine. *Clin. Oral. Investig,* **6**(3):150-154.

Guo L, Zhao ZY, Bai J and Han SY (2012). Preparation and treatment for oral ulcer of quercetin and the drug-loaded chitosan composite material. *Adv. Mater. Res.,* **583**(2012): 44-48.

Guo Q, Yang J and Yu M (2018). Observing the effect of traditional Chinese medicine Kouchuang Xiaotong powder on patients with recurrent aphthus ulcer. *Pak J Pharm. Sci.,* **31**(4): 1687-1690.

Hitomi S, Ono K, Yamaguchi K, Terawaki K, Imai R, Kubota K, Omiya Y, Hattori T, Kase Y and Inenaga K (2016). The traditional Japanese medicine hangeshashinto alleviates oral ulcer-induced pain in a rat model. *Arch Oral Biol.,* **66**(Issue): 30-37.

James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, Whelton H and Riley P (2017). Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. *Cochrane Database Syst Rev.,* **3**(3): CD008676.

Jin Y, Lin X, Song L, Liu M, Zhang Y, Qi X and Zhao D (2017). The effect of pudilan anti-inflammatory oral liquid on the treatment of mild recurrent aphthous ulcers. *Evid Based Complement Alternat Med.,* 6250892.

Jing Y, Zhang YF, Shang MY, Liu GX, Li YL, Wang X and Cai SQ (2017). Chemical constituents from the roots and rhizomes of *Asarum heterotropoides* var. mandshuricum and the *in vitro* anti-inflammatory activity. *Molecules,* **22**(1): 125.

Jones CG (1997). Chlorhexidine: Is it still the gold standard? *Periodontol.,* **15**: 55-62.

Jurge S, Kuffer R, Scully C and Porter SR (2006). Mucosal disease series. Number VI. Recurrent aphthous stomatitis. *Oral Dis.,* **12**(1): 1-21.

Kim SJ, Gao Zhang C and Taek Lim J (2003). Mechanism of anti-nociceptive effects of *Asarum sieboldii* Miq. radix: potential role of bradykinin, histamine and opioid receptor-mediated pathways. *J. Ethnopharmacol.,* **88**(1): 5-9.

Kunanusorn P, Panthong A, Pittayanurak P, Wanauppathamkul S, Nathasaen N, Reutrakul V (2011). Acute and sub chronic oral toxicity studies of *Nelumbo nucifera* stamens extract in rats. *J Ethnopharmacol.,* **134**(3): 789-795.

Lin HH, Charles AL, Hsieh CW, Lee YC and Ciou JY (2014). Antioxidant effects of 14 Chinese traditional

medicinal herbs against human low-density lipoprotein oxidation. *J. Tradit Complement Med.,* **5**(1): 51-55.

Liu C, Liu J, Zheng Y, Qu J, Yang W, Tang X, Bai H and Fan B (2021). Subchronic oral toxicity study of rhubarb extract in sprague-dawley rats. Regul Toxicol Pharmacol., **123**(8): 104921.

Lorenz K, Bruhn G, Heumann C, Netuschil L, Brecx M, Hoffmann T (2006). Effect of two new chlorhexidine mouthrinses on the development of dental plaque, gingivitis, and discolouration. A randomized, investigator-blind, placebo-controlled, 3-week experimental gingivitis study. *J. Clin. Periodontol.,* **33**(8): 561-567.

Miao M, Peng M, Xing Z and Liu D (2019). Effect of Shuangjinlian mixture on oral ulcer model in rat. *Saudi J. Biol. Sci.,* **26**(4): 790-794.

Nedrebø T, Berg A and Reed RK (1999). Effect of tumor necrosis factor-alpha, IL-1beta, and IL-6 on interstitial fluid pressure in rat skin. *Am. J. Physiol.,* **277**(5 Pt 2): H1857-862.

Park Y, Noh HJ, Han L, Kim HS, Kim YJ, Choi JS, Kim CK, Kim YS and Cho S (2012). Artemisia capillaris extracts as a green factory for the synthesis of silver nanoparticles with antibacterial activities. *J. Nanosci. Nanotechnol.,* **12**(9): 7087-7095.

Pyee Y, Chung HJ, Choi TJ, Park HJ, Hong JY, Kim JS, Kang SS and Lee SK (2014). Suppression of inflammatory responses by handelin, a guaianolide dimer from Chrysanthemum boreale, via down regulation of NF-κB signaling and pro-inflammatory cytokine production. *J. Nat. Prod.,* **77**(4): 917-924.

Quang TH, Ngan NT, Minh CV, Kiem PV, Tai BH, Thao NP, Song SB and Kim YH (2012). Anti-inflammatory and PPAR transactivational effects of secondary metabolites from the roots of Asarum sieboldii. *Bioorg Med Chem Lett.,* **22**(7): 2527-2533.

Ribeiro DA, Bazo AP, da Silva Franchi CA, Marques ME, Salvadori DM (2004). Chlorhexidine induces DNA damage in rat peripheral leukocytes and oral mucosal cells. *J Periodontal Res.,* **39**(5): 358-361.

Rodrigues F, Lehmann M, do Amaral VS, Reguly ML, de Andrade HH (2007). Genotoxicity of three mouthwash products, cepacol, periogard and plax, in the drosophila wing-spot test. *Environ Mol. Mutagen.,* **48**(8): 644-649.

Saikaly SK, Saikaly TS and Saikaly LE (2018). Recurrent aphthous ulceration: A review of potential causes and novel treatments. *J. Dermatolog Treat.,* **29**(6): 542-552.

Shang Y, Yao S, Qiao X, Wang Z, Huang Z, Gu Q, Wang N and Peng C (2020). Evaluations of marine collagen peptides from tilapia skin on experimental oral ulcer model of mice. *Mater Today Commun.,* **26**(Suppl. 121): 101893.

Tarakji B, Gazal G, Al-Maweri SA, Azzeghaiby SN and Alaizari N (2015). Guideline for the diagnosis and treatment of recurrent aphthous stomatitis for dental practitioners. *J. Int. Oral Health,* **7**(5): 74-80.

Teixeira DDS, de Figueiredo MAZ, Cherubini K, de Oliveira SD and Salum FG (2019). The topical effect of chlorhexidine and povidone-iodine in the repair of oral wounds. A review. *Stomatologija*., **21**(2): 35-41.

Woo SB, Sonis ST (1996). Recurrent aphthous ulcers: A review of diagnosis and treatment. J Am Dent Assoc. **127**(8): 1202-1213.

Xu SF, Ye YP, Li XY and Chen FY (2011). Chemical composition and antioxidant activities of different polysaccharides from the roots of *Angelica dahurica*. *Chem Biodivers.,* **8**(6): 1121-1131.

Xue T, Roy R (2003). Studying traditional Chinese medicine. *Science,* **300**(5620): 740-741.

Zdarilova A, Rajnochova Svobodova A, Chytilova K, Simanek V and Ulrichova J (2010). Polyphenolic fraction of Lonicera caerulea L. fruits reduces oxidative stress and inflammatory markers induced by lipopolysaccharide in gingival fibroblasts. *Food Chem Toxicol.,* **48**(6): 1555-1561.

Zhou P, Mao Q, Hua H, Liu X and Yan Z (2017). Efficacy and safety of Chinese patent medicines in the treatment of recurrent aphthous stomatitis: A systematic review. *J. Am. Dent Assoc.,* **148**(1): 17-25.

Zhu S, Shi Q and Lu J (2018). Curative effect of oral ulcer powder on the treatment of recurrent aphthous ulcer. *Pak. J. Pharm. Sci.,* **31**(3): 1175-1178.