

To Access the Anti-inflammatory Effects of Chamomile on 5-Fluorouracil Induced Oral Ulcers in Rabbits

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ABSTRACT

Objectives: The present study intended to find out the anti-inflammatory effects of chamomile on the oral mucosa of 5-Fluorouracil (5-FU) induced oral mucositis in rabbits by histopathological analysis.

Materials and Methods: The present Experimental interventional study was carried out at the Animal House, Department of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam. A sample of 18 rabbits was selected by convenient/purposive sampling according to inclusion and exclusion criteria. After induction of 5-FU –mucositis (60 mg/kg 5-FU), the animals were divided into three groups; Group A (n=6): Control– receive 0.9% normal saline, Group B (n=6): 5-FU 60 mg/kg body weight and Group C (n=6): 5-FU induced oral ulcers + Chamomile. At the end of the experiment, each rabbit was deeply anaesthetized by chloroform. A cotton swab was soaked in chamomile, and applied on the oral ulcer areas, topically 2 times daily. Oral Mucositis Scoring System (OMSS) and grading of Liver histological injury were noted. Tissue samples were fixed in neutral buffered 10% formalin and processed for H & E stains. Data were analyzed statistically for mean± SD and frequency and % by using Statistical Package for Social Science (SPSS) software, version 22.0.

Results: The mean ± SD of body weight in groups A, B, and C was noted as 530.83±15.30, 362.5±15.41 and 476.66±21.60 grams respectively ($p=0.0001$). The OMSS shows the control group A- animals were in grade 0, and B- grade 5 (which means virtual complete ulceration of oral mucosa) shows grade 3 and 4 injuries. The chamomile-treated experimental group C show mild and moderate injury compared to 5 FU-treated group B which shows severe injury in six rabbits ($p=0.0001$). Histological examination shows normal oral mucosa with intact surface epithelium in group A. While group B shows severe tissue injury, however, this was found low in group C (chamomile) treated animals.

Conclusion: Based on the evidence-based findings of the present study, it is concluded that chamomile decreases the 5-fluorouracil-induced oral mucositis. Oral mucositis score and histological examination showed that chamomile was effective in healing chemotherapy-induced oral mucositis ulcers.

Keywords: 5-Fluorouracil, Chamomile, Chemotherapy, Oral Mucositis

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INTRODUCTION

Inflammation of the mucous membrane of the gastrointestinal tract is a common side effect of anti-cancer chemotherapy. Mucositis defines an area of the inflamed mucous membrane of oral and oropharyngeal areas.¹ Oral Mucositis is the most common painful side effect of anti-cancer chemotherapy and radiotherapy.² It is characterized by ulceration of oral mucosa associated with erythema and inflammation. It causes a sore mouth and a feeling of severe discomfort. It interferes with chewing due to pain and may cause dehydration, parageusia and malnutrition as secondary complications.³ An effective strategy can substantially reduce the complications of oral mucositis such as secondary infections, pain and mucosa atrophy.⁴

5-Fluorouracil (5-FU) is an anti-metabolite anti-cancer drug which interferes with the biosynthesis of DNA and RNA. 5-FU inhibits the essential biosynthetic process of genetic material. 5-FU is a pyrimidine analog.⁵ 5-FU mimics nucleotides and becomes incorporated into the RNA and DNA and this is described as the mechanism of its cytotoxicity. 5-FU is termed a suicide inhibitor which causes irreversible inhibition of the enzyme “thymidylate synthase”. 5-FU is indicated for several solid cancer growths, particularly of the colon, rectum, breast, stomach, pancreas, uterus, ovaries, urinary bladder and liver.⁶

5-FU-chemotherapy is associated with oral mucositis. A prevalence of 40% oral mucositis with standard doses and 50% with high-dose chemotherapy is reported.⁷ 5-FU-associated oral mucositis is a painful condition. Painful chewing causes malnutrition due to no food intake by the patients. Narcotic analgesics and supplemental nutrition are often needed for these patients. The condition not only causes malnutrition but also longer hospital stays, weight loss and poor immune reactions.⁷

Currently, much interest has grown in herbal agents against drug-associated complications such as oral mucositis.^{7,8} Chamomile is a herb which belongs to the daisy-like plant family called the “Asteraceae”. Chamomile has been used since the ancient ages for its oral ulcer healing potential. Local application has been advocated in oral ulcerations. Chamomile “Asteraceae” family has been used for herbal infusions for various

ailments. Some common indications for which it is used include; oral ulcers, muscle spasms, hay fever, inflammation, insomnia, rheumatic pain, wounds and piles. It is used for a variety of gastrointestinal disorders.⁸

Anti-inflammatory, anti-oxidant, anti-bacterial and anti-fungal properties of Chamomile are reported in the medical literature.^{9,10} Anti-oxidant activity is attributed to its Phenolic compounds, which are highly applauded for their myriad biological effects.¹⁰

A search of the literature shows very few researches have been conducted on the efficacy of Chamomile against 5-FU-induced oral mucositis. Presently, topical steroids are prescribed for 5-FU-induced oral mucositis but they have adverse effects, hence there is a gap to explore new remedies available for the problem. The new remedy should be effective with minimal or no adverse effects for the 5-FU-induced oral mucositis.

The present study is the first of its design being conducted on the 5-FU-induced oral mucositis in a rabbit model where the wound-healing properties of Chamomile were examined scientifically. The study was conducted on the animal model with a scientific hypothesis to analyze the oral ulcer healing potential of Chamomile. As variables to evaluate the scientific grade of justification, we used histological examination to clarify the effects of Chamomile on oral mucosa cell proliferation and cell apoptosis. The objectives of the study were to induce Oral mucositis by 5-fluorouracil in rabbits. To find out the anti-inflammatory effect of chamomile on the oral mucosa of induced oral mucositis in rabbits by histopathological analysis. Nowadays, researchers are focusing on exploring the pharmacological profile of compounds from natural origin, where promising results are seen. The present research provides a naturally cheap and safe therapeutic opinion for the management of chemotherapy-induced oral mucositis which is agonizing and troublesome for patients and currently available drugs are either expensive or have toxic effects.

MATERIALS AND METHODS

This quasi-experimental interventional study was carried out at Animal House, Isra University Hyderabad and the Department of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam from January 2019 to June 2019. The rabbits

were housed and taken care of as indicated by the NIH Guide for the Care and Use of Laboratory Animals. Ethical approval was obtained from the university. 24 healthy male rabbits weighing above 400gm were included in the study. Female rabbits, diseased rabbits and rabbits below the weight of 400gm were excluded from this study. The sampling was done using non-probability purposive sampling and the sample size was 18. All rabbits were arbitrarily allocated into 3 groups control A and experimental B and C. Rabbits in group A were the control group which was not induced with 5-FU receiving 0.9% normal saline as a placebo + Normal diet two times a day. Rabbits in group B were experimental control which was 5-FU induced oral ulcers and were given a healthy diet and water two times a day. Group C was an experimental group, which was 5-FU-induced oral ulcers and was given chamomile two times a day.

For the enlistment of mucositis, 60 mg/kg of 5-FU was regulated intraperitoneal to every creature in the examination groups on day 0, and 40 mg/kg was managed on day 2, following the convention proposed by Sonis et al¹¹ and modified by Leitao et al.¹² The Body weight was measured on an electronic measuring balance at the end of the experiment period. Body weight was compared among different rabbit groups.

Each rabbit was deeply anaesthetized by an overdose of chloroform. A cotton swab was soaked in Chamomile and applied to the oral ulcer areas. It was applied topically 2 times daily. The experiment was done for twelve days. The administration of Chamomile oil was done for 7 days which was initiated on day 5 and ended on day 12. Oral mucositis was measured by the scoring system proposed by Elmansy and Elewa.^{13,15} The

histological criteria included vacuolar degeneration, inflammatory cell infiltration, congestion and necrosis measured by the grading system of Raoof.¹⁵ The rabbits were sacrificed by an overdose of anaesthesia on day twelve. In each experiment, oral mucosa was removed for histopathological analysis. Samples were fixed in neutral buffered 10% formalin and were processed for H & E stains. After examination, the data were tabulated and analyzed statistically for mean and standard deviation by using Statistical Package for Social Sciences software version 23. *p*-value less than or equal to 95% confident interval (*p*=0.05) was considered statistically significant.

RESULTS

The present experimental study studied the effects of chamomile on the 5-fluorouracil-induced oral ulcers in a rabbit model. The studied research variables included body weight, oral mucositis score and histological examination of oral ulcer tissue.

The mean ± SD of body weight in groups A, B, and C was noted as 530.83±15.30, 362.5±15.41 and 476.66±21.60 grams respectively (F = 48.43, *p*=0.0001). As shown in Table 1, significant weight loss was noted in group B; this proves that 5-FU induces weight loss. The chamomile group C rabbits showed 476.66± 21.60 grams which was proved as the least weight loss compared to group B. The evidence-based findings show that chamomile has positive effects on body weight in 5-FU-treated rabbits (*p*=0.0001). Table 1 shows the effects of 5-FU and chamomile on body weight in control and experimental rabbits. The chamomile-treated 5-FU-induced oral ulcer rabbits show less body weight loss compared to other groups.

Table 1: Effects of 5-FU and chamomile on body weight in control and experimental rabbits

	Mean	SD	F-value	p-value
Group A- Control (N/saline)	530.83	15.30	48.43	0.0001
Group B- 5-FU (60mg/Kg)	362.50	15.41		
Group C - Chamomile	476.66	21.60		

Table 2: Effects of 5-FU and chamomile on the oral mucositis score system in control and experimental rabbits

Grades	Group A- (N/saline)	Group B- 5-FU	Group C- Chamomile	X ²	p-value
0	6	0	0	54.73	0.0001
3.00	0	0	4		
4.00	0	0	2		
5.00	0	6	0		
Total	6	6	6	18	

Grading Score System: 0- Normal, 0.5- Slight Pink, 1-Slight Red, 2- Severe Reddening, 3-Focal desquamation, 4- Exudation covering <1/2 of the mucosa, 5-Virtual complete ulceration mucosa

Table 3: Effects of 5-FU and chamomile on the histological grading in control and experimental rabbits

Grades	Group A- (N/saline)	Group B- 5-FU	Group C- Chamomile	X^2	<i>p</i> -value
1.00	6	0	0	54.17	0.0001
2.00	0	0	5		
3.00	0	0	1		
4.00	0	6	0		
Total	6	6	6	18	

Grades: 1-No Abnormal finding, 2- mild injury, 3-moderate injury, 4-severe injury

Table 2 shows the effects of 5-FU and chamomile on the oral mucositis score system (OMSS) in control and experimental rabbits. The OMSS shows the control group A animals were in grade 0 which means normal looking oral mucosa, B shows all animals graded as 5 which means virtual complete ulceration of oral mucosa and chamomile group C shows grade 3 (4 animals) which means the focal desquamation and exudation covering <1/2 of the oral mucosa.

Table 3 shows the effects of 5-FU and chamomile on the histological grading in control and experimental rabbits. The chamomile-treated experimental group C shows mild and moderate injury compared to 5 FU-treated group B which shows severe injury in all six rabbits ($p=0.0001$).

DISCUSSION

The present experimental study reports on the effects of chamomile on 5-fluorouracil-induced oral ulcers in a rabbit model. The oral mucositis score and histological grading were detected scientifically. After induction of mucositis, a soaked cotton swab of chamomile was applied topically 2 times daily. The administration of Chamomile oil was initiated on day 5 and the experiment continued for 12 days. The findings of the present study suggest that chamomile exerts anti-inflammatory and ulcer-healing effects. 5-FU chemotherapy is associated with oral mucositis. A prevalence of 40% oral mucositis with standard doses and 50% with high-dose chemotherapy is reported.¹⁶ Currently, much interest has grown in herbal agents against drug-associated complications such as oral mucositis.¹³ Oral mucositis presents as recurrent aphthous ulceration or recurrent aphthous stomatitis and is the most common oral mucosal disease. Despite much clinical and experimental research, the cause remains poorly understood. The oral ulcers are not preventable and treatment is usually symptomatic.¹⁵

The body weight of animal groups (table 1) shows significant weight loss was noted in the 5-FU treated control rabbits. While the chamomile-treated rabbits showed less weight loss. The evidence-based findings show that chamomile has positive effects on body weight in 5 FU-treated rabbits ($p=0.0001$). The finding of body weight loss induced by 5-FU is in accordance with previous studies.^{3,17,18} Similarly, previous studies showed that Chamomile-treated animals displayed less body weight loss and these findings support our study.^{19,20} which have reported similar results. Study²¹ reported that topical chamomile lowers the severity of mucositis with low pain scores. The findings are in agreement with our study. Pourdeghatkar et al²² conducted a study to evaluate the effects of chamomile mouthwash on chemotherapy-induced oral mucositis in 31 children with acute lymphoblastic leukaemia from Iran. 15 drops of chamomile mouthwash in 10 ml water were prescribed to patients with oral mucositis. The result was that chamomile mouthwash was very effective in preventing the incidence and severity of pain and inflammation in children with oral mucositis. Hence it was concluded that chamomile may be used during chemotherapy. The findings of the above study support the present research. Another research reported by Schmidt et al²³ who investigated the effects of chamomile on chemotherapy-induced oral mucositis, their research showed better wound healing, this study is in agreement with our present research study. Another study by Thomsen²⁴ has reported conflicting results of no usage of mouthwash with a suitable treatment that could prevent chemotherapy-induced oral mucositis. The findings are inconsistent with our study.

Morales-Bozo et al⁹ reported on the comparative efficacy of chamomile and linseed in 74 elderly patients of xerostomia. They reported the chamomile-treated

group showed greater relief of dry mouth symptoms ($p < 0.05$). The findings of the above study are consistent with our study. Goes et al²⁵ investigated the effects of chamomile extract on the pathogenesis of 5-FU-induced intestinal mucositis in Albino rats. They injected 60 mg/kg of 5-FU intraperitoneal into each animal. Chamomile treatment started on day 5 and continued for 12 days. The results revealed a mucosal protective effect of 5-FU on the intestine. These findings are in agreement with our present study.

In the present study, we evaluated the anti-inflammatory effects of chamomile on 5-Fluorouracil-Induced Oral Ulcers in Rabbits; however, we cannot postulate that this effect is long-lasting or effective in humans also. Hence further studies are required to confirm these findings. Chamomile may be used for oral mucositis in clinical practice. However, future studies are recommended to further evaluate the efficacy of chamomile in combination with other agents in chemotherapy-induced oral mucositis.

CONCLUSION

Based on the evidence-based findings of the present study, it is concluded that chamomile decreases the 5-fluorouracil-induced oral mucositis. Oral mucositis score and histological examination show that chamomile was effective in healing chemotherapy-induced oral mucositis ulcers. Chamomile may be used for oral mucositis in clinical practice. However, future studies are recommended to further evaluate the efficacy of chamomile and other herbal agents alone and in combination with chemotherapy-induced oral mucositis.

DISCLAIMER

None to declare.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

ETHICAL STATEMENT

The ethical approval is provided by the Research Ethics Committee at Isra Dental College Hospital, Hyderabad (Ref: IU/DN (FD)/IDC/2018/728).

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