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ORIGINAL ARTICLE – CANCER RESEARCH

Investigation of the protective effect of gel incorporating *Eugenia jambolana* leaf extract on 5-fluorouracil-induced oral mucositis: an animal study

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Abstract

Purpose The study aimed to evaluate the possible preventive effect of two concentrations (3 and 5% w/w) of *Eugenia jambolana* (EJ) extract against 5-FU-induced mucositis.

Method Sixteen adult rats were separated into four groups: two control and two preventive groups. Animals in Groups 1, 2, and 3 were injected intraperitoneally with 60 mg/kg/day of 5-FU on Day 1 followed by 150 mg/kg/day on Day 5. The rats in Group 4 (negative control) were given physiological saline at the same times and doses. Furthermore, on the fifth day of the study, the cheek and sublingual mucosa were irritated by external superficial scratches using the tip of an 18-G needle, followed by the application 15 µL of 20% acetic acid, after which 3 and 5% EJ w/w gels were applied topically for animals in Groups 2 and 3, respectively.

Results The weight and the mucositis scores were recorded. Antioxidant and anti-inflammatory markers and biochemical tests were analyzed. Significant differences were found between the study groups in weight loss, clinical mucositis scores, mortality rates, and antioxidant and anti-inflammatory parameters.

Conclusion The preventive effect of 3% gel was significant, with no mortality rate, making it an option for preventive strategies.

Keywords Mucositis · Chemotherapy · *Eugenia jambolana* · Antioxidant · Anti-inflammatory · Gel

Introduction

Cancer has been identified as one of the most serious public health threats in the world. According to Turkish Statistical Institute (2019) data, cancer is the leading cause of death in Turkey, followed by cardiovascular diseases (Turkstat 2019). Cancer cases are expected to rise significantly in the future as the population ages and human lifestyles change (Sung et al. 2021). Given the high mortality rate and the threat to public health, it is crucial to conduct research aimed at improving both cancer therapy and the quality of life of cancer patients.

Over the years, significant progress has been made in cancer treatment in terms of identifying cancer cells and reducing their harmful influence on normal healthy cells. Furthermore, targeted cancer therapy is now widely used to develop treatment models that are compatible with the biology of existing cancers (Collins and Varmus 2015; P. Sharma et al. 2022). Despite this progress, the side effects of chemotherapy drugs continue to have a substantial impact on the quality of life of patients. One of the undesirable effects of chemotherapy is mucositis. Patients with mucositis of the oral cavity and gastrointestinal tract may develop a wide range of clinical symptoms include pain, local infection, malnutrition, and weight loss. Moreover, with the increased severity of these symptoms, physicians may be obliged to delay or reduce chemotherapy courses, resulting in a poor therapeutic outcome (Pulito et al. 2020).

Mucositis affects about 30–40% of chemotherapy patients; however, this percentage can reach 60–85% with some

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treatments, such as the hematopoietic stem cell regimen (Villa and Sonis 2016). The rate of mucositis can be influenced not only by the anti-cancer agent's capabilities, but also by the patient's characteristics. One of the chemotherapeutic agents that has been linked to oral mucositis is 5-fluorouracil (5-FU) (Vanlancker et al. 2016). The active metabolite of 5-FU destroys cancer cells by inhibiting thymidylate synthase and disrupting RNA synthesis. In addition to its vast range of chemotherapeutic effects, 5-FU can produce a wide range of gastrointestinal symptoms, including mucositis, inflammation, ulceration, bleeding, diarrhea, nausea and vomiting (O'Reilly et al. 2020). Many natural or herbal remedies like aloe vera, chamomile, honey, kefir, and black mulberry syrup have been utilized for the prevention of mucositis in cancer patients (Sahebjamee et al. 2015; Dos Reis et.al. 2016; Münstedt et al. 2019). The Java plum (*Eugenia jambolana or Syzygium cumini)*, which is native to India, grows well in the tropical regions of South America and the Asian subcontinent. The presence of anthocyanins, including glycosides of delphinidin, petunidin, and malvidin, gives the fruit its black-purple color (B. Sharma et al. 2008; R.J. Sharma et al. 2016).

Numerous different extracts of *Eugenia jambolana* (black plum or jamun) have shown various pharmacological characteristics such as antibacterial, antifungal, antiviral, anti-inflammatory, cardioprotective, anti-ulcerogenic, anti-cancer, chemo preventive, anti-allergic, antioxidant, anti-cancer, anti-hypoglycemic, and anti-diabetic effects (Baliga et al. 2011; Rani et al. 2020). Its anti-inflammatory and antioxidant properties as well as its traditional use in the treatment of mucositis made it an ideal candidate for this research. The possible preventive effect of two concentrations (3% w/w and 5% w/w) of *Eugenia jambolana* extract against 5-FU-induced mucositis was evaluated in this study.

Materials and methods

Gel preparation

The Eugenia jambolana (EJ) gel was prepared from Eugenia jambolana water extract in the laboratory of the International Islamic University, Bagan Kedah, Malaysia, then incorporated into Carbopol® gel before being transferred to Istanbul for the in vivo trial. The leaves were collected from Wonosobo, Central Java, Indonesia and extracted using the sequential cold percolation extraction method. The following solvents were used in order: petroleum ether, toluene, ethyl acetate, acetone, and finally water (Sukmasari et al. 2018). The collected solvents were then dried using a rotary evaporator (model IKA RV8) until dry. The crude extract collected from the round bottom flask was left under the fume hood for another 2 days until completely dry from any remaining solvents. To protect the extracts from light during this process, they were wrapped in aluminum foil. The crude extract was then collected and stored at – 20 ºC. The gel contains 3 or 5% EJ water extract, 4% carbopol powder, 0.1% methyl and 0.03% prophyl paraben as preservative and water.

Experimental animals and induction of oral mucositis

Sixteen adult *Sprague Dawley* rats were separated into four groups after being isolated for 3 days. The gel groups contained six rats and the control groups two rats. All rats used in this experiment were 6 month-old males. The groups were divided into Group 1 (positive control), Group 2 (3% w/w EJ), Group 3 (5% w/w EJ), and Group 4 (negative control). The baseline weights were recorded and then all the animals in Groups 1, 2, and 3 were injected intraperitoneally with 60 mg/kg/day of 5-FU on the first day, followed by 150 mg/kg/day on the fifth day. The rats in Group 4 (negative control) were given physiological saline at the same times and doses.

Furthermore, on the fifth day of the study, the cheek and sublingual mucosa were irritated by external superficial scratches using the tip of an 18-G needle, followed by the application of 15 µL of 20% acetic acid. Oral mucositis was predicted to occur on the fourth day after injection (Day 9 of the experiment). The 3% EJ and 5% EJ w/w gels were applied topically to animals in Groups 2 and 3, respectively, on the 1st, 5th, 9th, 10th, 11th, and 13th days. On Days 1, 5, 9, 11, and 13, the weight of the animals was recorded, and the Parkins et al. mucositis score was utilized for clinical evaluation of mucositis (Parkins et al. 1983). The scoring was carried out by two independent evaluators as follows: score 0: Normal; Score 0.5: light pink; Score 1: slightly red; Score 2: dark red; Score 3: concentrated; Score 4: increased exudate and crusting partially overflowing to the lip; Score 5: excessive exudate increase and crusting over most of the lip.

At the end of the 13th day, blood was drawn from the heart under anesthesia, and tissue samples were taken from the cheek, kidney, and liver after killing. Oxidative stress and inflammation biomarkers were investigated.

Biochemical analysis of the samples

The blood samples were collected in a yellow blood collection tube containing gel to separate the serum. Serum samples were kept at room temperature for 30 min and were then centrifuged at 4000 rpm for 15 min. The obtained serum samples were stored at − 20 °C until the study was conducted.

Determination of serum glucose and urea levels

The levels of serum glucose and urea were measured quantitatively using assay kits (Biolabo, Maizy, France), and spectrophotometry (BioTek, Winooski, VT, USA) at 550 and 600 nm, respectively.

Determination of serum ALT and AST levels

Serum alanine transaminase (ALT) and aspartate transaminase (AST) levels were determined using ELISA kits (YL Biotech, Shanghai, China). The obtained results were calculated using standard graphs and were given as U/L.

Biochemical analysis of cheek, kidney, and liver tissue

Tissues were collected as soon as possible after decapitation, washed with PBS buffer, weighed, and kept at − 80 °C until measurements were taken. The tissues removed were homogenized with cold 150 mM KCl solution and 10% tissue homogenates were freshly prepared.

Determination of serum MDA levels

Serum malondialdehyde (MDA) analysis, an indicator of lipid peroxidation, was performed spectrophotometrically at 532 nm (Richard et al. 1992). Using nmol/g of tissue prepared with 1.1′,3,3′ tetraethoxypropane, the MDA levels were measured using standard graphs.

Determination of tissue GSH levels

In this study, the method developed by Beutler was used for the determination of glutathione (GSH) in the tissues (Beutler 1963). The GSH in the analysis tube reacted with 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) to produce a yellowish color, which was then measured spectrophotometrically at 401 nm.

Analysis of tissue myeloperoxidase (MPO), superoxide dismutase (SOD), catalase (CAT) and nitric oxide (NO) levels

For the determination of MPO, SOD, CAT, and NO, assay kits for rat MPO (YL Biotech, Shanghai, China).), rat SOD (ElabScience, Houston, Texas, USA), rat CAT (YL Biotech, Shanghai, China).), and total NO (Thermofischer, Waltham, MA, USA) were used, respectively. At the end of the procedures, standard graphs were created, and results were given as ng/mL for MPO, ng/mL for SOD, ng/mL for CAT, and μM for NO.

Analysis of tissue cytokine levels

Rat IL-6, rat IL-10, and rat IL-1β assay kits (YL Biotech, Shanghai, China) were used for the measurement of IL-6, IL-10 and IL-1β, respectively, in the cheek, kidney, and liver tissues. To determine the cytokine concentration, standard graphs were created, and the results were given in ng/mL.

Histological examination

After fixing in 10% buffered formalin, the cheeks of the rats were dissected, and 3-micron-thick sections were taken from the paraffin blocks prepared by routine tissue follow-up. The samples, stained with hematoxylin–eosin (H&E), were examined under a light microscope. The surface epithelium was inspected for ulcers, atrophy, acanthosis, and inflammation.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 11. The results were evaluated at a 95% confidence interval and a significance level of $p < 0.05$. The significant differences between the groups were determined using analysis of variance (ANOVA).

Results

Animals in the positive control group (chemotherapy + acetic acid) lost an average of 81 ± 12.33 g from their weight during the experiment lifetime. The EJ preventive group (3 and 5% w/w EJ) lost an average of 44.4 ± 7.8 g and 42.1 ± 21.70 g, respectively. Weight follow-ups were performed on Days 1–13 in the experimental animal model and the mucositis scores for the cheek and sublingual region of the rats were taken on Days 5 and 13. All results according to the lesions are given in (Table 1). Means and standard deviations of ALT, AST, urea, and glucose values for the four groups are given in (Table 2). The changes in body weight during the experiment are given in (Fig. 1).

The MDA, GSH, SOD, and CAT levels in the cheek, liver, and kidney were significantly different among the groups ($p < 0.05$), whereas the level of MPO was significantly different only for the kidney. The tissue MDA and other antioxidant levels in the cheek, kidney, and liver are shown in (Table 3).

For the inflammatory parameters, among the groups, significant differences were found in the cheek tissue for IL-6 and IL-1β. (Table 4) presents the concentration results in the

Table 1 Mucositis score for rat groups according to Parkins et al. (1983) criteria

		Group 1 Positive control		Group 2 3% EJ gel		Group 3 5% EJ gel		Group 4 Negative control	
		Cheek	Sublingual	Cheek	Sublingual	Cheek	Sublingual	Cheek	Sublingual
Day 5	1	0.5	0.5	0.5	0	1	0.5	0	0
	2	1	0	0	0.5	0.5	0	0	0
	3			0	0.5	0.5	0		
	4			0	0	0.5	0		
	5			0	0	0	0.5		
	6			0.5	0	1	0.5		
Day 9	1	3–4	2	4	2–3	3	3–4	0	0
	2	4–5 W + H	3	4 H + NL	3	3–4 NL + E	2	0	0
	3			4	3	4 E	2		
	4			1–2	2	Ex			
	5			3	1	5 E + NL	3 LL + N		
	6			4–5 NL	3	3	3–4		
Day 11	1	3–4 H + NL	2	4	2	3 NL	3	0.5	0 -O
	2	4–5 NL	2	4NL + H	3	4 NL + E + NL	3	0	0
	3			4NL + H	3	4 NL + E + NL	2		
	4			4NL + H	3				
	5			2	1	4 E + NL	3 LL + NL		
	6			4NL	2				
Day 13	1	EX		0	0	0	0	0	0
	2	4 NL + E + H	3	2	0			0	0
	3			0	0				
	4			0 T	0				
	5			0	0	0 E	0		
	6			1 E + H	0				

LL lip lesion, *W* white lesion, *NL* nasal lesion, *H* hair loss under lip, *B* bleeding, *O* oedema, *E* eye lesion

Table 2 Blood biochemical parameters for the study groups

Biochemical Parameter	Group 1 Positive control	Group 2 3% EJ gel	Group 3 5% EJ gel	Group 4 Negative control	*p*-values
ALT	13.1 ± 1.03	14.07 ± 0.79	13.40 ± 0.48	14.87 ± 1.41	0.37
AST	27.57 ± 3.39	11.84 ± 1.51	18.04 ± 2.94	21.74 ± 0.86	< 0.05*
Urea	73.43 ± 6.11	46.27 ± 5.80	60.57 ± 4.96	40.17 ± 4.51	< 0.05*
Glucose	127.33 ± 6.25	210.78 ± 31.19	232.97 ± 1.89	150.76 ± 7.15	< 0.05*

*$P < 0.05$ is considered significant

tissues. The mortality rate was obtained with an interval of two days, and as of the 3rd day, no mortality was seen in the rats ($N = 6$) in the 3% EJ group, whereas the mortality rate in the 5% EJ group was 66.6%.

Histological results are seen in (Figs. 2, 3, 4, 5). Significant hyperkeratosis, hypergranulosis, and irregularity in the basal layer were noted in the surface epithelium in Group 1 (positive control). Mild inflammatory infiltration was seen in the underlying connective tissue. Mast cells, significant parts of which were degranulated, were observed in the superficial and deep regions. In Group 2 (3% EJ), thin, light, hyperkeratosis and active basal cells and dilated capillaries were seen in the surface epithelium, as well as mast cells in the connective tissue. In the Group 3 (5% EJ) animals, the surface epithelium was thin and relatively smooth, but showed marked hyperkeratosis. Basal cells appeared active, although the basal layer exhibited natural thickness. Connective tissue characteristics showed similarities with the 3% EJ group. In Group 4 (negative control), the surface epithelium exhibited irregular thickening, papillamatosis, and hyperkeratosis. The basal layer was prominent and the basal cells were active. The connective tissue contained mild lymphocyte infiltration and mast cells.

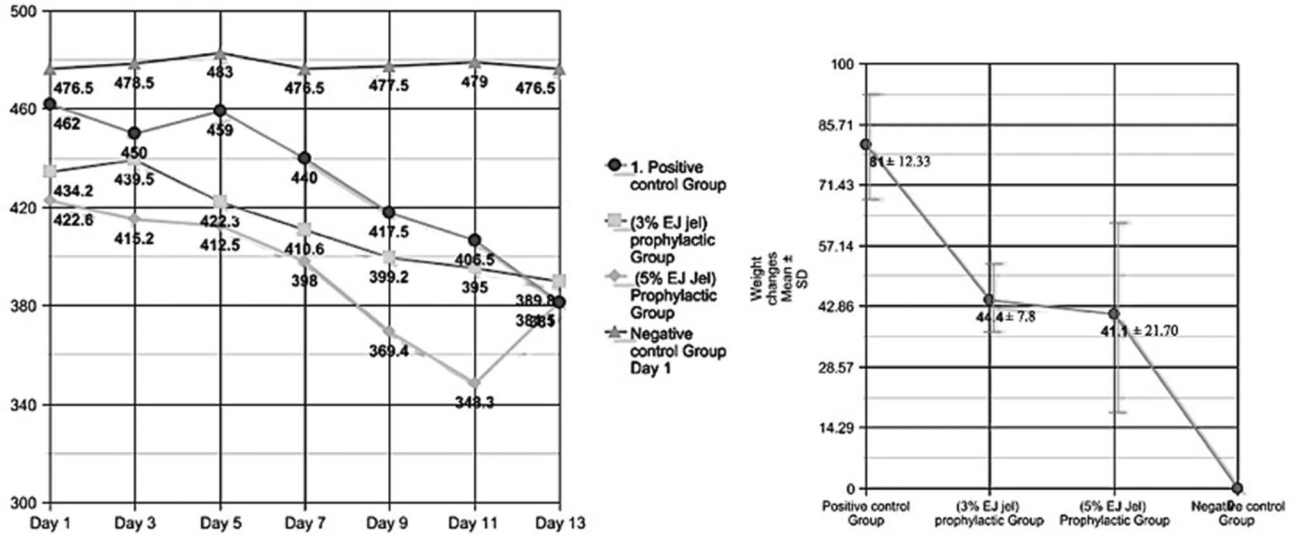

Fig. 1 Follow-up of rat weights for Days 1–13 and main weight changes for Days 5–13

Table 3 Tissue antioxidant enzyme levels (MDA, GSH, SOD, CAT, and MPO) for the study groups

	Group 1 Positive control	Group 2 3% EJ gel	Group 3 5% EJ gel	Group 4 Negative control	*p*-values
MDA					
Cheek	66.67	32.12 ± 5.39	46.54 ± 4.90	32.6 ± 0.05	< 0.05*
Liver	46.79	40.34 ± 5.96	40.77 ± 2.90	32.81 ± 2.92	< 0.05*
Kidney	54.36	42.71 ± 4.34	49.49 ± 0.36	40.77	< 0.05*
GSH					
Cheek	15.36	25.47 ± 1.63	27.41 ± 3.31	32.4	< 0.05*
Liver	39.82	40.47 ± 3.59	41.09 ± 1.19	53.5	< 0.05*
Kidney	34.56	43.33 ± 2.30	50.54 ± 1.67	50.76	< 0.05*
SOD					
Cheek	1.04	1.59 ± 0.12	2.73	2.24	< 0.05*
Kidney	5.73	11.16 ± 0.45	10.42 ± 0.83	9.87	< 0.05*
Liver	5.15	7.06 ± 0.70	5.04 ± 1.13	7.89 ± 0.27	0.18
CAT					
Cheek	21.26	38.42 ± 1.95	94,.5	91.87	< 0.05*
Kidney	3.58	109.81 ± 2.01	112.18 ± 1.19	112.05	< 0.05*
Liver	5.49	95.36 ± 2.80	98.05 ± 1.79	100.29 ± 3.95	< 0.05*
MPO					
Cheek	11.63	8.09 ± 0.58	8.14	8.34	0.15
Kidney	7.65	7.47 ± 0.34	10.24 ± 0.04	7.46 ± 0.48	< 0.05*
Liver	11.44	8.26 ± 1.25	7.68 ± 0.27	7.33 ± 0.03	0.13
NO					
Cheek	113.07	44.70 ± 0.80	54.34	62.59	0.189
Kidney	102.41	102.83 ± 13.71	82.24	87.23	0.25
Liver	77.11	93.54 ± 14.91	173.93 ± 35.79	92.09 ± 0.28	0.09

*$P < 0.05$ is considered significant

Table 4 Tissue concentration results for inflammatory parameters IL-6, IL-10, and IL-1β

	Group 1 Positive control	Group 2 3% EJ gel	Group 3 5% EJ gel	Group 4 Negative control	*p*-values
IL-6					
Cheek	2.63	1.76 ± 0.05	1.77	1.66	< 0.05*
Kidney	3.88	2.29 ± 0.16	2.93 ± 0.03	2.34	< 0.05
Liver	2.54	2.54 ± 0.12	2.15 ± 0.18	2.33 ± 0.12	0.55
IL-10					
Cheek	49.78	40.02 ± 6.75	39.81	35.73	0.29
Kidney	46.83	45.77 ± 3,00	43.97 ± 0.07	20.18	< 0.05*
Liver	47.78	40.55 ± 5.95	43.75 ± 4.36	45.33 ± 5.09	0.31
IL-1β					
Cheek	1019.97	706.81 ± 88.29	522.32	357.81	< 0.05*
Liver	2079.89	1756.58 ± 185.15	1973.84 ± 67.72	999.99	< 0.05*
Kidney	2050.52	2171.72 ± 35.07	2143.35 ± 3.32	2093.99	0.27

*$P < 0.05$ is considered significant

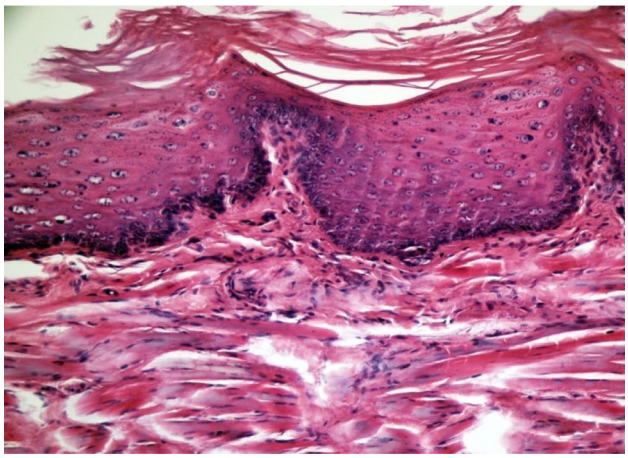

Fig. 2 Group 1: surface epithelium showing acanthosis, mild papillomatosis, hypergranulosis, and hyperkeratosis (H&E × 200)

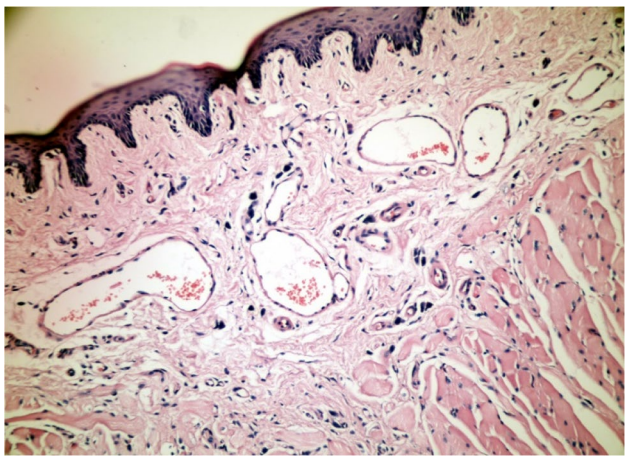

Fig. 3 Group 2 (3% EJ): thin, light, hyperkeratosis and surface epithelium with active basal cells; dilated capillaries and mast cells in connective tissue (H&E × 200)

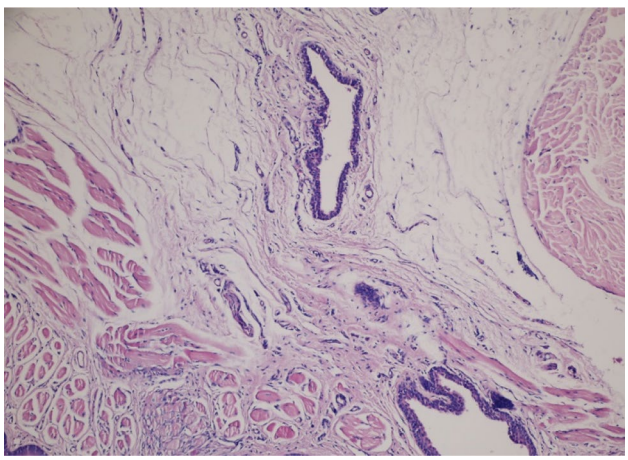

Fig. 4 Group 3(5% EJ): thin hyperkeratotic surface epithelium and underlying loose connective tissue (H&E × 100)

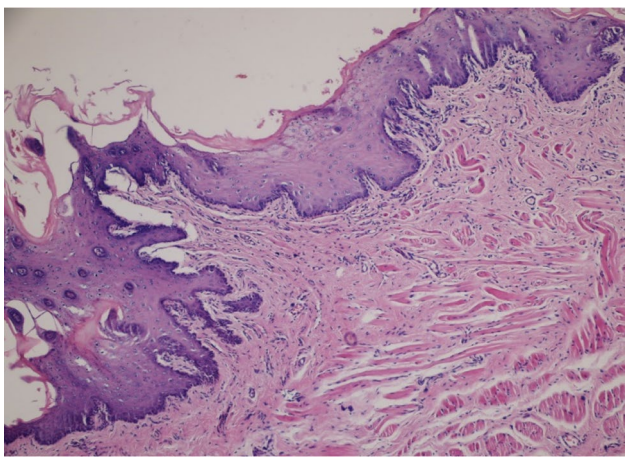

Fig. 5 Group 4 (control): surface epithelium showing irregular acanthosis, papillomatosis, and hyperkeratosis, and underlying fiber-rich, striated muscle fibers (H&E × 200)

Discussion

The pathogenesis of oral mucositis appears to involve five biological phases including initiation, primary damage response, signal amplification, ulceration, and healing. Chemotherapy such as 5-FU leads to the generation of reactive oxygen species (ROS) which in turn activate several signaling pathways in the submucosa and epithelium (Sonis 2004a). The ROS superoxide anion (O_2^-) causes oxidative stress via different mechanisms and is involved in the pathogenesis, onset, and progression of oral mucositis (Siomek et al. 2006). Thus, reducing oxidative stress and ROS can prevent the initiation and progression of oral mucositis, which suggests that the use of the 3% EJ gel in our study acts as a preventive against oral mucositis in rats.

Starting from the clinical observation, when compared to the positive control (chemotherapy group + acetic acid), rats in the 3 and 5% *Eugenia jambolana* preventative groups lost less weight and had lower mucositis scores. Furthermore, after nine days, the rats in the EJ preventative groups began to gain weight again, whereas the rats in the positive control group continued to lose weight. These findings imply that the *Eugenia jambolana* groups may have benefitted from the protective effect, resulting in a milder form of mucositis. The researchers also noticed that rats given EJ had a greater willingness to eat than rats who were not given EJ, which raises the question of whether this impact was related to the mild mucositis or rather to the specific effect of EJ as an appetite enhancer. This effect was also corroborated by the much lower urea level in the preventive groups, indicating that these groups, particularly the 3% EJ rats, suffered from less dehydration (Cheng 2007; Silverman 2007). Weight loss has been considered as one of the markers of mucositis in many studies. Da Cruz Campos et al. (2021) investigated the preventive effect of several drugs on rat oral mucositis and concluded that there was significant variation in weight loss among the different groups. They associated that with the different preventive effects of these drugs. In the EJ preventative groups, there was an unexpected increase in glucose levels compared to the positive control group. Given that the EJ was administered as a gel and that systemic absorption of EJ is unusual, this increase above the normal glucose level may have been due to increased appetite; however, further investigation is required. Moreover, the numerous studies conducted using EJ extracts have yielded contradictory effects. Many have found that EJ extracts had an anti-diabetic effect and reduced glucose levels (Rahman and Baishnab 2016). The researchers also noticed that animals in the 3% gel group were active throughout the lifetime of the study in comparison with those in the positive control and 5% EJ groups. The mortality results also supported this observation, with a zero mortality rate in the 3% EJ gel group, although the 5% group was associated with 66.6% mortality and the positive control group with a rate of 50%.

These clinical data were supported by the tissue antioxidant and anti-inflammatory results. When the positive control group was compared to the 3 and 5% EJ preventative groups, the MDA levels in the positive control group were seen as extremely high, whereas the lowest value was seen in the 3% EJ group. A similar finding was obtained when MDA levels in the kidney and liver were measured, with the kidney tissue showing the sole significant difference. Based on these findings, we may conclude that the preventive gels containing 3 and 5% EJ reduced oxidative stress, particularly in the cheek and kidney. Compared to the preventative groups, there was a substantial difference in GSH level reduction in the cheek, kidney, and liver tissue of the positive control group. These findings point to a reduction in oxidative stress in the 3 and 5% EJ gel groups. The same result was reported for SOD levels, with the cheek tissue showing a more significant result. All the oxidative stress parameters indicated a decrease in oxidative stress in the 3 and 5% EJ gel groups, with the 5% gel group having the advantage. When we compared the cheek CAT levels in the 3 and 5% EJ groups, we found that the CAT level was reduced. Although a better result was obtained with the 5% EJ, the positive control group had a higher decrease. The same findings occurred for the kidney and liver tissue.

When comparing the positive control group to the 3 and 5% EJ preventive groups, a higher concentration of MPO was found in the cheek, kidney, and liver, indicating less oxidative stress in the 3 and 5% EJ groups. When the cheek tissue NO levels were examined, an increased level was seen in the chemotherapy group, whereas the level was significantly decreased in Group 3 (3% EJ) and to a lesser extent in Group 5 (5% EJ). Although the effects of *E. jambolana* on oxidative stress and oral mucositis have not been studied in the literature, its effects have been evaluated and demonstrated in studies examining oxidative stress in diabetes, cardiovascular disease, and others (Jay et al. 2006; Stephens et al. 2009; Ito et al. 2019).

Eugenia jambolana aqueous seed extract has been shown to ameliorate diabetes-induced oxidative stress when compared to glimepiride (Nandi et al. 2019). Other studies have demonstrated the protective role of *Eugenia jambolana* seed extract (EJSE) on the cardiac and hepatic oxidative stress of high cholesterol diet (HCD)-induced hyperlipidemia / hypercholesterolemia (Raval et al. 2019; Anatoliotakis et al. 2013; Sankhari et al. 2012). An in vitro study also revealed the cytoprotective effects of *Eugenia jambolana* fruit pulp on H_2O_2-induced oxidative stress and apoptosis in rat Leydig cells (Anand et al. 2012).

The tissue expression of nuclear factors IL-6, IL-10, and IL-β, which is indicative of the T helper inflammatory response, was significantly lower in the 3 and 5% EJ preventive groups than in the positive control group, especially for IL-6 in the cheek tissue. These differences were significant for IL-6 and IL-β. Differences in inflammation were significant in the cheek tissue, but not in the kidney or liver tissue. We can conclude from this finding that the inflammatory response was lower in the preventive groups, with no differences between 3 and 5% EJ except for IL-β, which was lower in the 3 EJ preventive group.

Previous studies have directly implicated the presence of TNF, IL-6, and IL-1β pro-inflammatory cytokines in the pathogenesis of a number of inflammatory diseases, such as inflammatory bowel disease (IBD), rheumatoid arthritis, sepsis, and most importantly, in mucositis (Sonis 2004b, 2007; Keefe et al. 2000; Logan et al. 2007, 2008). In particular, cytotoxic drug administration results in the upregulation of NFκB and subsequently, pro-inflammatory cytokine (TNF, IL-6, and IL-1β) levels. In further support of this, Logan et al. (2008) reported a significant rise in serum NFκB, TNF, IL-6, and IL-1β levels following administration of three different chemotherapeutic drugs known to cause mucositis (Asadullah et al. 2003; Moore et al. 2001). Additionally, IL-10 receptor activation induces a wide range of inflammatory controlling genes during tissue injury. The IL-10 controls inflammatory processes by suppressing the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules, as well as antigen-presenting and costimulatory molecules in monocytes/macrophages, neutrophils, and T cells. In addition to these data from animal studies, the total phenolic and flavonoid contents and antioxidant capacity of EJ collected in Indonesia have been previously investigated in vitro (Sukmasari et al. 2018).

Evaluation of the clinical data and the results of all the oxidative (MDA, GSH, SOD, CAT, MPO, NO) and inflammatory (IL-6, IL-10. IL-β) parameters found that increased concentration of EJ extract was accompanied by less weight loss and a greater reduction of oxidative stress and inflammatory response. However, a higher mortality rate (66.6%) resulted with the 5% EJ gel, whereas mortality for the 3% EJ gel was 0%. The preventive effect of the 3% gel, with no mortality, was significant, thus making it an option for preventive strategies. Moreover, EJ concentrations of between 3 and 5% need to be studied, and further investigations should be carried out on the increased mortality rate at the 5% w/w concentration.

The magnified images of the 3 and 5% EJ groups were similar, but with more prominent acanthosis and mild hyperkeratosis in the epithelium of the 5% EJ gel group animals. Although basal cells in both groups were active and budding into the connective tissue, thin, light, hyperkeratosis was observed in the 3% gel group. These minor differences, however, are insufficient to demonstrate the superiority of one concentration over the other.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of ınterest All authors declare that they have no known competing ınterests or personal relationships that could appear to have ınfluenced the work reported ın this paper.

Ethical approval Approval was obtained from the ethics committee of the Turkish ministry of health Bağcılar hospital ın Istanbul (No. 2019–34/37/38).

Consent to participate Written ınformed consent was obtained before performing the experiments on the animals.

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