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The Effect of Carrageenan on Burning Mouth Syndrome in Diabetes Patient Type 2, IL-1 and *Candida* Species Studies

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ABSTRACT

Introduction: *A sulfated polyglycan, carrageenan, is found in 3 different types Iota, Lambda, and Kappa, all of which differ in their sulfation degree. Diabetes disease affects all the vital body organs in the body by changing the metabolic activity of the body. Some diabetic patients may suffer from burning mouth syndrome which has multiple causative factors and can be diagnosed clinically by a hot, burning feeling in the oral cavity but there is no noticeable lesion.* ***Objective:*** *The present study was designed to determine the effect of topical carrageenan on the symptom of burning mouth syndrome in diabetes patients' type 2 and to determine the effect of topical carrageenan on the levels of salivary interleukin-1beta and Candida species.* ***Results:*** *The results showed no effect of carrageenan on the immunological marker (Interleukin-1) after carrageenan usage. For candida, the results showed a decline in the number of colonies forming units after carrageenan utilization. Regarding Candida, the dramatic decrease in the colony forming unit which was observed in the present study was supported by many previous studies that concerned about antifungal activities of red seaweeds and these activities are suggested to be due to biological activities of red seaweeds (carrageenan). Carrageenan was applied topically for the relief of symptoms of burning mouth syndrome as it has been proved by the previous study that carrageenan has the ability to relieve the pain associated with mucositis. Carrageenan does not exert any effect on IL1 since it was never absorbed from oral mucosa.* ***Conclusion:*** *The effect of carrageenan gel on the burning mouth syndrome may allow utilizing of kappa carrageenan as a good compound to relief pain and hot sensation in burning mouth syndrome.*

Keywords: Carrageenan, *Candida* species, Burning mouth syndrome

INTRODUCTION

The sulfated polyglycan, carrageenan, is drawn from various genera of red seaweed such as *Chondrus, Iridaea, Eucheuma* and *Gigartina* [1]. It is a widely used as a food additive because of its thickening and emulsifying properties. Carrageenan is also used as an inactive substance that bears the active substance such as in medications, cosmetics, and oral medication products [1,2]. Carrageenan usage has been approved by the Food and Drug Administration in the United State [3]. The extraction of carrageenan can be done by heating the seaweed in water with a dilute alkali, and then recovered by alcohol or gel pressing [4,5]. Diabetes mellitus (DM) is a chronic multisystemic disorder characterized by deregulation in the metabolic action of insulin as a result of a relatively or absolutely inadequate insulin discharge and/or concomitant resistance of particular body tissues to insulin [6,7]. It is a worldwide developing problem associated with healthiness, social and financial disability. International Diabetes Federation in 2015 showed a dramatic increase in diabetes and proposed it as a main global problem which needs to focus on. Burning mouth syndrome (BMS) can be defined as a dysaesthesia of oral cavity with the exclusion of organic causes that may cause a similar symptom with an obvious cause [8,9]. BMS can be considered as a complex disease that is described by burning and/or warm sensation in the mucosa with no obvious changes physically. The most affected people are middle-aged and elderly women and the most often affected part is the tip of the tongue, lateral borders, lips, hard and soft palate. BMS is probably a condition of multi-factorial origin with unknown etiopathogenesis [10]. Peripheral neuropathy is a complication of long-standing DM which results in the secondary BMS due to improper signaling along the sensory nerve fibers and/or due to de-epithelialization resulting from dry mouth induced by DM [11].

Interleukin-1 beta (IL-1β) is a proinflammatory cytokine which plays a fundamental role in immunity as well as in inflammation.

PATIENTS AND METHODS

The ethics committee of the Ministry of Health, Iraq had approved this study. The study samples consisted of 50 diabetes patient with burning mouth syndrome and 25 healthy subjects. Carrageenan is extracted from the seaweeds by heating them in water containing a dilute alkali which increases the gel strength of the product. It is then recovered either by alcohol precipitation usually isopropanol or gel pressing (only for kappa-carrageenan) [4,5]. All of the processes follow the same basic steps [4,5,12].

Preparation of a K-Carrageenan Gel

The pharmaceutical formula in percent was carrageenan powder (0.9%), sucrose (20%), potassium citrate (0.35%), citric acid (0.45%). The use of carrageenan was for a 1-week period and the patient was instructed to use carrageenan after finishing breakfast and before bedtime. All diabetes patients were diagnosed by a specialist in endocrine glands and confirmed by 2 laboratories test, the fasting blood glucose and the HbA1c. Burning mouth syndrome is diagnosed according to scale criteria and laboratory examination to exclude other causative factors for burning mouth syndrome. Carrageenan gel was prepared and then utilized by patients to relieve the burning sensation associated with burning mouth syndrome. The collection of saliva was done to determine the levels of salivary immunological markers which are measured by Enzyme-Linked Immunosorbent Assay (ELISA), saliva was taken on two intervals for the study groups and at one interval for the controlled groups.

Regarding the *Candida*, the quantity of the colonies was calculated by the number of CFU/ml, which was derived by the formula: CFU/ml = 1000 × number of colonies/4.

Samples were inoculated on sterile Sabouraud's Dextrose Agar (SDA) plates and surface streaking was done, for the estimation of the CFU count. The plates were incubated overnight at 37°C and were observed the next day. The number of colonies was counted and the average was calculated. The counts were expressed in CFU/ml. The presence of *Candida* was confirmed by the presence of creamy white colonies. It was reconfirmed by gram staining and observing the presence of ovoid yeasts.

RESULTS

Distribution of Elementary Parameters

The subjects in this study are divided into 3 groups like the following:

- Study group: It consists of 25 diabetes patients with burning mouth syndrome that were receiving carrageenan
- Control diabetics group: It consists of 25 diabetes patients with burning mouth syndrome that were not receiving carrageenan
- Control healthy group: It consists of 25 healthy subjects

Demographical Characteristics Variables

Table 1 shows observed frequencies, and their percentages distribution of studied demographical characteristics variables (DCv.), age groups, gender, and marital status with comparisons significant. Table 2 shows observed frequencies and their percentages distribution of studied general information variables (GIv.)

Table 1 Distribution of demographical characteristics variables of the studied groups with their relationships

DCv.	Groups	Study		Control-Diabetic		Control-Healthy		C.S. p-value
		No.	%	No.	%	No.	%	
Age Groups	30-39	4	16%	7	28%	12	48%	C.C.=0.322 p=0.193 NS
(Years)	40-49	13	52%	13	52%	11	44%	
	50-59	5	20%	3	12%	2	8%	
	60-69	3	12%	2	8%	0	0%	
	Total	25	100%	25	100%	25	100%	
Mean ± SD		47.12 ± 9.18		44.36 ± 7.92		39.32 ± 7.86		

Gender	Male	12	48%	12	48%	11	44%	C.C.=0.038 p=0.948 NS
	Female	13	52%	13	52%	14	56%	
	Total	25	100%	25	100%	25	100%	
Marital Status	Married	21	84%	17	68%	21	84%	C.C.=0.181 p=0.280 NS
	Single	4	16%	8	32%	4	16%	
	Total	25	100%	25	100%	25	100%	
NS: Not Significant at p>0.05; C.C.; Contingency Coefficient								

Table 2 Distribution of general information related to studied groups with comparisons significant

GIv.	Groups	Study		Control - Diabetic		C.S. p-value
		No.	%	No.	%	
Duration time of BMS	1-2	6	24%	2	8%	C.C.=0.268 p=0.276 NS
	3-4	9	36%	11	44%	
	5-6	6	24%	10	40%	
	7-8	4	16%	2	8%	
	Total	25	100%	25	100%	
HbA1c	6.5	12	48%	7	28%	C.C.=0.374 p=0.017 S
	7	12	48%	9	36%	
	7.5-8.0	1	4%	9	36%	
	Total	25	100%	25	100%	
Duration of BMS Pain	15	13	52%	18	72%	C.C.=0.284 P=0.112 NS
	50	6	24%	6	24%	
	85-120	6	24%	1	4%	
	Total	25	100%	25	100%	
Onset of pain	Morning	17	68%	21	84%	C.C.=0.184 p=0.185 NS
	Afternoon	8	32%	4	16%	
	Total	25	100%	25	100%	
Family History	-ve	19	76%	19	76%	C.C.=0.000 p=1.000 NS
	+ve	6	24%	6	24%	
	Total	25	100%	25	100%	
Age of onset of BMS	20-29	1	4%	2	8%	C.C.=0.206 P=0.697 NS
	30-39	8	32%	9	36%	
	40-49	9	36%	11	44%	
	50-59	5	20%	2	8%	
	60-69	2	8%	1	4%	
	Total	25	100%	25	100%	
S: Significant at p<0.05; NS: Not Significant at p>0.05; C.C.; Contingency Coefficient						

Results reported weak relationships amongst distribution of studied groups with no significant differences at p>0.05, except with HbA1c, with a significant difference at p<0.05, with increase outcomes in the controlled-diabetic group.

Candida CFU Parameter

Results show strong relationship amongst distribution of studied groups since highly significant differences are accounted at p<0.01, while the weak relationship is found between diseased groups, with no significant different at p>0.05, rather stating that the level of pos. ++(100) accounted for more cases in the study group in contrast to the controlled-diabetic group. This can be shown in Table 3. Results show a strong relationship amongst distribution of study group along pre-post periods since significant differences are accounted at p<0.05, as well as most individuals who have pos. ++(100) diagnosed at pre-period are reduced to pos. +(10) at the post period, and all individuals who have pos. +++(1000) diagnosed at pre-period are reduced to pos. ++(100) in post period (Tables 3 and 4).

Table 3 Distribution of *Candida* CFU parameter outcomes in studied groups with comparisons significant

Candida CFU-pre	No. and %	Groups			Total	C.S. p-value
		Study	Control Diabetic	Control Healthy		
Neg.	No.	2	0	9	11	Amongst Groups C.C.=0.519 P=0.000 HS Between Diseased Groups C.C.=0.316 P=0.137 NS
	%	0.080%	0.000%	0.360%	0.147%	
Pos. +:(10)	No.	5	11	13	29	
	%	0.200%	0.440%	0.520%	0.387%	
Pos. ++:(100)	No.	13	8	3	24	
	%	0.52%	0.32%	0.12%	0.32%	
Pos. +++:(1000)	No.	5	6	0	11	
	%	0.200%	0.240%	0.000%	0.147%	
Total	No.	25	25	25	75	
	%	1.000%	1.000%	1.000%	1.000%	
HS: Highly Significant at p<0.01; NS: Not Significant at p>0.05; C.C.; Contingency Coefficient						

Table 4 Distribution of *Candida* CFU parameter outcomes in the study group along (pre-post) periods with comparisons significant

Period	Group	No. and %	Candida CFU-post			Total	C.S. p-value
			Neg.	Pos. + : (10)	Pos. ++ : (100)		
Candida CFU-pre	Neg.	No.	1	1	0	2	C.C.= 0.631 p=0.011 S
		%	0.167%	0.056%	0.000%	0.080%	
	Pos. + : (10)	No.	4	1	0	5	
		%	0.667%	0.056%	0.000%	0.2.000%	
	Pos. ++ : (100)	No.	1	12	0	13	
		%	0.167%	0.667%	0.000%	0.520%	
	Pos. +++ : (1000)	No.	0	4	1	5	
		%	0.000%	0.222%	1.000%	0.200%	
	Total	No.	6	18	1	25	
		%	1.000%	1.000%	1.000%	1.000%	
S: Significant at p<0.05; C.C.; Contingency Coefficient							

Normal Distribution Function (Goodness of Fit test)

The one-sample Kolmogorov-Smirnov test was used to observe the cumulative distribution function for studied IL-1 to specified theoretical distribution, which proposed normal shape (i.e. bell shape) (Table 5).

Table 5 Normal distribution function fitness for studied (IL-1)

Groups	Test Statistic and Comparison's Significant	IL-1	
		Pre	Post
Study	No.	25	25
	Kolmogorov-Smirnov Z	0.795	0.625
	Asymp. Sig. (2-tailed)	0.553	0.83
	C.S.*	NS	NS
Control-Diabetic	No.	25	-
	Kolmogorov-Smirnov Z	0.672	-
	Asymp. Sig. (2-tailed)	0.757	-
	C.S.*	NS	-
Control-Healthy	No.	25	-
	Kolmogorov-Smirnov Z	0.62	-
	Asymp. Sig. (2-tailed)	0.837	-
	C.S.*	NS	-
*Non-Significant at p>0.05			

And with respect to test statistical hypothesis, with a highly significant difference at p<0.01, there were 10% readings are decreased as a result of effectiveness studied treatment (Table 6).

Table 6 Statistics of IL-1 in the study group

Parameters	Period	No.	Mean	SD	SE	MP- test	p-value*
IL1	Pre	25	161.3	38.6	7.7	3.405	0.002
	post	25	141.2	38.3	7.7		
*HS: Highly Significant at p<0.01; C.C.; NS: Not Significant at p>0.05; Contingency Coefficient; MP-test: Matched Paired Student t-test							

The use of cut off point that separate response levels between each control groups (diabetic and healthy) and study group have been subjected to treatment, and that for studied parameters (IL-1) through applying Stem-Leaf plot and ROC curve shown in Tables 7-9 (Figure 1).

Table 7 Estimation of cutoff points for the suggested methods by applying the suggested technique

Studied Parameters	Cutoff points
	Healthy Control
IL-1	100

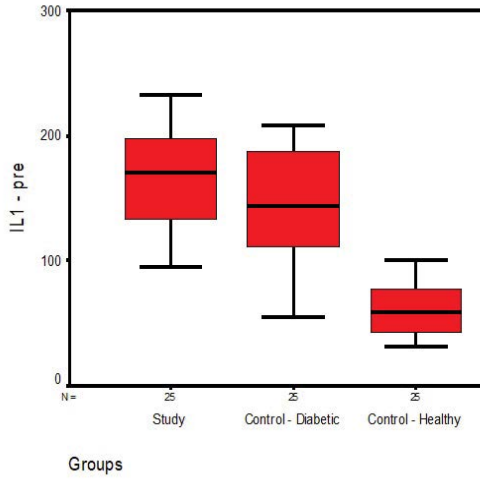

Figure 1 Stem-Leaf plots for estimation a cutoff point for studied parameters

Table 8 Redistribution (under/upper) a cutoff point for (IL-1) in pre-period parameter amongst studied groups

Groups	No. and %	IL – 1 (Pre Period)		Total	P-value*
		Under	Upper		
Study	No.	2	23	25	CC=0.170 p=0.221 NS
	%	0.08%	0.92%	1.00%	
Control – Diabetic	No.	5	20	25	
	%	0.20%	0.80%	1.00%	
Study	No.	2	23	25	CC=0.661 p=0.000 HS
	%	0.08%	0.92%	1.00%	
Control – Healthy	No.	24	1	25	
	%	0.96%	0.46%	1.00%	
Control – Diabetic	No.	20	5	25	CC=0.610 p=0.000 HS
	%	0.80%	0.20%	1.00%	
Control – Healthy	No.	1	24	25	
	%	0.04%	0.96%	1.00%	
*HS: Highly Significant at p<0.01; C.C.; NS: No Significant at p>0.05; Contingency Coefficient "IL - 1"					

Table 9 ROC curve statistics for the "IL - 1" readings amongst studied groups

Studied Parameters	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Study X Diabetic	0.597	0.081	0.240	0.438	0.755
Study X Healthy	0.997	0.004	0.000	0.988	1.000
Diabetic X Healthy	0.954	0.030	0.000	0.896	1.000
*S: Significant at $p<0.05$; Non-Significant at $p>0.05$; The positive actual state is Pos					

DISCUSSION

Carrageenan and Burning Mouth Syndrome

The use of carrageenan as a topical gel for pain relief in BMS is effective as almost all the patients in the present study showed dramatic relief in the burning sensation immediately after application of carrageenan. This result is not unusual, the usage of carrageenan on oral lesions in patients with Hodgkin lymphoma and non-Hodgkin lymphoma receiving chemotherapy showed a profound relief of pain and healing [13].

Many studies showed that orally administrated carrageenan will not be absorbed and this is confirmed with the present study as the immunological salivary marker (IL-1β) in this study is not affected by carrageenan application [14-16]. The use of carrageenan is proved to be safe on the mucosal epithelium, all these studies are confirmed with the present study since all patient showed no complaint from the treatment [17-19]. The patients in this study reported a prolonged relief and a good routine lifestyle performance when carrageenan gel was utilized as a topical gel. This prolongation in relieving time is confirmed by many studies, carrageenan was used in many gel formulations to prolong the buccal drug delivery since carrageenan has gelling and thickening properties. Carrageenan can be used in extended release medication and can prolong the medication release about 24 hours which give the more profound and prolonged effect of the medication [20].

Candida

Many previous studies concern the antifungal activity of red seaweeds [21-29]. In the present study, the antifungal activity of carrageenan is obvious since the results showed that there was a reduction in the CFU of *Candida* after carrageenan treatment. Regarding the antifungal activity of carrageenan, many studies confirmed the present study [30-34] all these studies matched with the study which has proved a significant decrease in CFU of *Candida* after application of carrageenan. The procedure used in this study proposed or followed as the bases of action of the antifungal properties of carrageenan has been suggested to be due to halogenated compounds, particularly bromophenols, brominated terpenoids and acetylenic compounds, produced by many members of this group of algae [35-37]. The cell wall, as well as the cell membrane of fungi, has an important role in communication with the environment and in metabolic processes, constituting an important target for antifungal drugs [38-40]. A widely accepted scientific theory is that the marine algae have the ability to produce bioactive metabolites [32,34,41,42) with high biomedical potential which enhances their antifungal activity [34]. In the last decade, many polysaccharides with interesting bioactive and functional properties have been extracted from seaweeds [43-45]. The logic based knowledge representable for the Carrageenan is the naturally sulfated polysaccharides which occur as a cell wall matrix in various species of red seaweed and contribute to a wide range of biological activities [46,47]. The carrageenan molecular chains are composed of galactose and possess a high content of sulfate esters, which are responsible for the negative charge of the compound [48]. There are many pieces of evidence supported that the sulfate groups number and the molecular weight of carrageenan can influence their biological action [49-51].

Carrageenan cause morphological change in the hyphal *Candida,* which resembles the effect of antifungal medication on *Candida* [52]. The morphological changes in *Candida* hyphae can be demonstrated by structural hyphal swollen, which are indicative of fungal cell wall weakness that combined with changing in the structural organization or composition [53]. Antifungal and antibacterial activities can be contributed to an initial oxidative burst with activation of salicylic acid, jasmonic acid and/or ethylene signaling pathways in fungal species [54].

Salivary Interleukin-1 Beta

The study of the fundamental nature of knowledge reality and existence of the Interleukin-1 beta (IL-1β),

proinflammatory cytokine exert various biological functions. One of these functions is regulating the inflammatory response [55]. In the present study, the interleukin-1 level was higher in a diabetic patient in comparison with non-diabetic and this matched with the study by Al-Askar et al., in 2018 [56]. Interleukin-1 is raised in diabetics' patient compared with non-diabetic persons [57]. Injection of carrageenan in hind paw of rat leads to release of IL-1 and IL-6 and cause hyperplasia. But this is due to injection of carrageenan while in the present study carrageenan is used as a topical applicable gel that will not act systematically. This study was very close to one that has been obtained in 2017 by Al-Mamory and Al-Aswad, by using the same gel formulation in this study on patients with Hodgkin and Non-Hodgkin lymphoma to determine the topical effect of carrageenan on the levels of salivary immunoglobulin A, interleukin-6 and tumor necrosis factor-α [13]. The result showed a slight decrease in the saliva level of the immunological markers after taking carrageenan which leads to a conclusion that carrageenan is not absorbed through the oral mucosa.

CONCLUSION

The effect of carrageenan gel on the burning mouth syndrome may allow utilizing kappa carrageenan as a good compound to relief pain and hot sensation in burning mouth syndrome. The biological activity of carrageenan which is reported in this study belonged to the physiochemistry of polysaccharides. The effect of carrageenan on the oral cavity may allow the use of carrageenan as a primary material in dental practice. Carrageenan has a powerful antifungal activity. Carrageenan will not affect the immunological markers if it is utilized topically.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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