

Evaluation of Anti-inflammatory and Antimicrobial Effects of Iraqi Propolis Mouth Wash in Mucositis Patients Induced by Chemotherapy

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ABSTRACT

The study was done on 48 subjects which divided into three groups; two groups each one consisted of 16 cancerous patients with mucositis induced by chemotherapy. First group was treated with propolis mouth wash and second group was treated with chlorhexidine mouth wash. The third group consisted of 16 healthy volunteer subjects without mucositis. Blood samples were taken pre and post treatment from each patient in first and second groups while blood samples were taken from each patient in third group for once time. The level of IL-6 and TNF- α was assessed by enzyme-linked immuno-sorbant assay (ELISA) and the data collected were analyzed using paired t-test, ANOVA test and Duncan's multiple analysis range test. Saliva samples were taken pre and post treatment from each patient in first group and by using serial dilution method, the numbers of colony forming units (CFU/ml) of microorganisms were counted. Treatment by propolis mouth wash led to highly significant decrease in the means of serum TNF- α level in the propolis group; whereas the means of serum and TNF- α level were increased by chlorhexidine mouth wash in chlorhexidine group after two weeks of treatment. Moreover there were significant decrease in the means of serum IL-6 level in mouth wash propolis group; but the means of serum IL-6 was increased by chlorhexidine mouth wash in chlorhexidine group after two weeks of treatment. The patient's microbiological study showed significant decrease in means of colony forming units for cancerous patients after used propolis mouth wash 10% as treatment for mucositis.

INTRODUCTION

Oral mucositis is defined as an injury of the oral mucosa in cancer patients, either induced by chemotherapy, or due to irradiation of patients who have head and neck cancer. This has painful and debilitating side-effects and adversely affects the nutritional status of the patient ⁽¹⁾. In mucositis the level of proinflammatory cytokines tumour necrosis factor α , interleukin-6 and interleukin-1B are increased and there is an influx of inflammatory immune cells into submucosa ⁽²⁾. Haddad et al. 2009 found a raise in serum levels of cytokines IL-6, TNF- α and IL-1 β which associated with mucositis severity. They confirmed the positive correlation between cytokine levels and mucositis ⁽³⁾. There is relationship between development of mucositis and micro-organisms ⁽⁴⁾. Staph.aureus was isolated from the oral microbiota of chemotherapeutic patients. Smith et al. in 2001 further state that the role of Staphylococcus in the etiology of oral mucositis also there is diversity of the oral microbiota in the oral cavity of mucositis patients. Involvement of fungi in the development of oral mucositis has been subject. The most common finding among patients receiving myelo-ablative chemotherapy is candidiasis. Thus, it is not unexpected to isolate Candida from patients with mucositis as a parallel condition, rather than causal ⁽⁵⁾.

For thousands of years, natural products have been used for folk medicine purposes throughout the world. Many of them have demonstrable medical, pharmacological and dental properties, such as antimicrobial, anti-inflammatory, anesthetic, antiviral and antioxidant ⁽⁶⁾. Propolis, a natural nontoxic resinous substance collected by Apis mellifera bees from various plant sources, has been recognized to have several properties that may confer health benefits to humans, including prevention of oral diseases ⁽⁷⁾. Propolis is a low-cost potential anti-inflammatory agent for both acute and chronic phases ⁽⁸⁾. Propolis, as an anti-inflammatory agent stimulates the immune system by promoting phagocytic activity and cellular immunity and improves the healing effects on epithelial tissues ⁽⁹⁾. As well as, propolis contains elements, such as iron and zinc that are important for the synthesis of collagen ⁽¹⁰⁾. The antimicrobial effects of propolis consist of over 100 species of numerous bacteria, fungi, and viruses, including the causative agents of influenza, syphilis, tuberculosis and diphtheria ⁽¹¹⁾. The purpose of this study was to develop a new palliative drug to cancerous patients with mucositis according to anti-inflammatory activity and antimicrobial effects of propolis mouth wash.

MATERIALS AND METHODS

A. PREPARATION OF PROPOLIS MOUTH WASH

10% by weight of ethanolic propolis extract was used to prepare propolis mouth wash⁽¹²⁾.

B. PATIENTS

The investigation was carried out on a total number of sixty two subjects (fourteen patients were excluded due to dying or the failure of the follow up or not using the recommended drugs). Mean age was (47.04 ± 10.396) years with a range of (28-65) years and their sex (21 males, 27 females). Patients were divided into 3 groups :first group consisted of (16) mucositis patients (study group), received 150 ml of propolis mouth wash this group taking propolis mouth wash three times a day for two weeks. The second group consisted of (16) mucositis patients, received 150 ml of chlorhexidine mouth wash., this group taking chlorhexidine mouth wash three times a day for two weeks. In group1 and group2 all patients attended Oncology and Molecular Medicine hospital at Nineveh Health Office who revealed a presence of mucositis according to the recommended criteria: No history of all allergies to propolis, non hypertensive, non diabetic, not take antibiotics, non- smoker. The third group (control) consisted of (16) healthy volunteer individuals, this group had no signs of any systemic disease or any type of mucositis in their oral cavity. Clinical examination was performed for assessment of mucositis according to World Health Organization Oral Mucositis Scale⁽¹³⁾ and pain assessment according Verbal Pain Scale (VPS)⁽¹⁴⁾.

C. IMMUNOLOGICAL EXAMINATION (ELIZA)

Four ml venous blood sample was taken from treated patient for measurement of TNF- α and IL-6 before and after two weeks of receiving medication. IL-6 and TNF- α were measured by Boster's human IL-6 (ELISA) kit, while TNF- α was measured by using Boster's human TNF- α (ELISA) kit (BOSTER Immunoleader USA).

D. ANTIMICROBIAL EFFECTS OF PROPOLIS MOUTH WASH (INVIVO STUDY):

The investigation was carried out on (first group) a total number of twenty three cancerous mucositis (seven patients were excluded due to dying or the failure of the follow up or not using the recommended drugs) with a range of (28-65) years and their sex (7 males, 9 females). A 0.5 ml of saliva samples were taken from each patient in the first group before and after taking propolis mouth wash and placed in screw -capped vials containing 4.5ml of brain heart infusion broth ,to determine the number of microorganisms in the last dilutions replicate specimens 10^{-7} and 10^{-8} ,100 μ l of each dilution were transferred to 2 plates of blood agar. The plates were incubated at 37°C for 48 hours. The colony forming units per milliliter (CFU/ml) were then calculated⁽¹⁵⁾.

STATISTICAL ANALYSIS

SPSS program version 19 was used to analyze the obtained data. ANOVA test and Duncan's multiple range tests were used for the comparison between the study groups. Data expressed as a mean and standard deviation values. The level of significance at $p < 0.05$

RESULTS

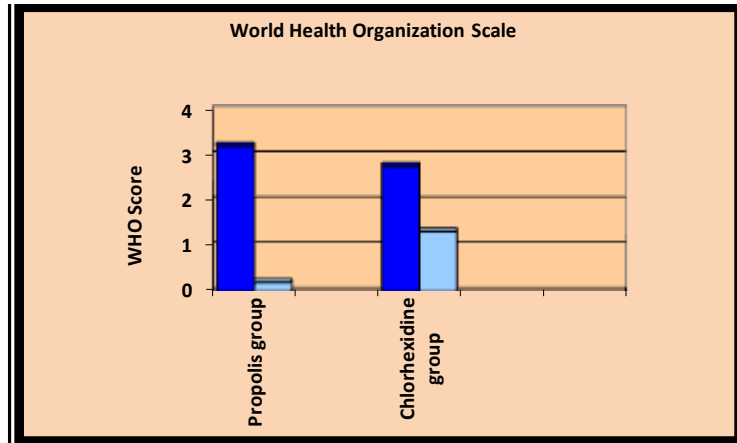
WORLD HEALTH ORGANIZATION ORAL MUCOSITIS SCALE (WHO SCORE):

The means of WHO Score according to World Health Organization Oral Mucositis Scale were highly decreased significantly in both propolis and chlorhexidine groups from (3.19 ± 0.403) and (2.75 ± 0.775) before treatment respectively to (0.19 ± 0.403) and (1.31 ± 0.873) after two weeks of treatment, at $p \leq 0.01$ as shown in Table (1) and Figure (1).

Table (1): Wilcoxon Signed Ranks Test of comparison between the means of WHO score before and after two weeks of treatment in both propolis and chlorhexidine groups.

	WHO Score before treatment	WHO score after treatment	Z	P-value
Propolis group	3.19 ± 0.403	0.19 ± 0.403	-4.000	0.000**
Chlorhexidine group	2.75 ± 0.775	1.31 ± 0.873	-3.624	0.000**

** indicated highly significant difference at $P < 0.01$.



■ WHO score before treatment. ■ WHO score after treatment.

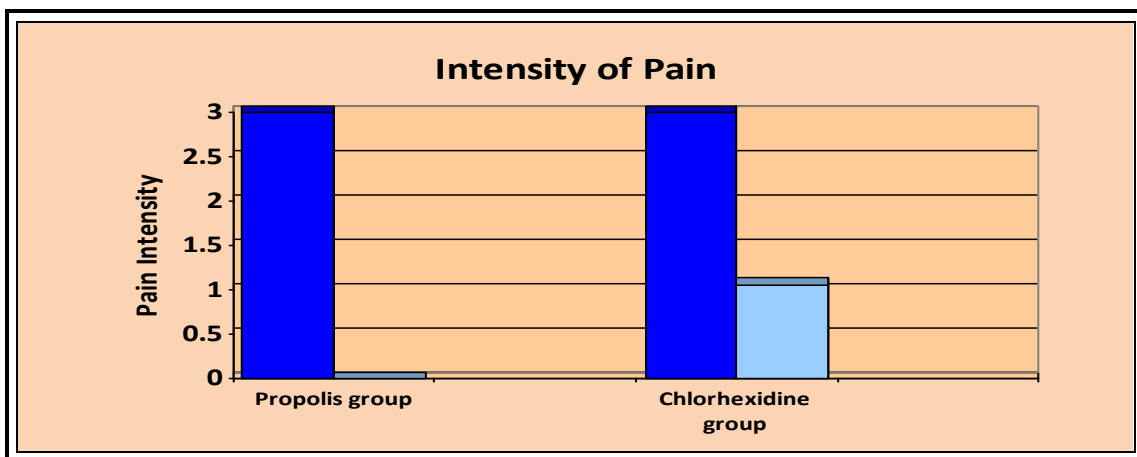
Figure (1): WHO score in both propolis and chlorhexidine groups before and after two weeks of treatment.
 Pain Intensity

The means of pain intensity according to Verbal Pain Scale were highly decreased significantly from score (3) in both propolis and chlorhexidine groups before treatment to (0) and (1.06±0.574) in propolis and chlorhexidine group respectively after two weeks of treatment, at p≤0.01 as shown in Table(2) and Figure (2).

Table (2): Wilcoxon Signed Ranks Test of comparison between the means of intensity of pain before and after two weeks of treatment in both propolis and chlorhexidine groups according to (VPS).

	Pain intensity score before treatment	Pain intensity score after treatment	Z	P-value
Propolis group	3	0	-4	0.000**
Chlorhexidine group	3	1.06±0.574	-3.656	0.000**

** indicated highly significant difference at P < 0.01.



■ Intensity of pain before treatment. ■ Intensity of pain after treatment.

Figure (2): Intensity of pain in both propolis and chlorhexidine groups before and after two weeks of treatment.

SERUM TNF-A

The results showed that the mean ±SD of serum TNF-α concentrations were significant difference in both propolis and chlorhexidine groups (143.06±70.347) pg/ml, (146.5±62.24) pg/ml respectively before treatment comparing with mean ±SE of serum TNF-α concentrations of control group(92.13±44.248)pg/ml at P≤0.05, while there were no significant difference between propolis and chlorhexidine group at P≤0.05 before treatment as shown in Table (3) an Figure (3).

Table (3): One way ANOVA of the means of serum TNF- α concentrations before treatment of all groups.

S.O.V.	Sum of Squares	df	Mean Square	F	p-value
Between Groups	29669.792	2	14834.896	4.128	0.023*
Within Groups	161706.688	45	3593.482		
Total	191376.479	47			

* indicated significant difference at $P < 0.05$.

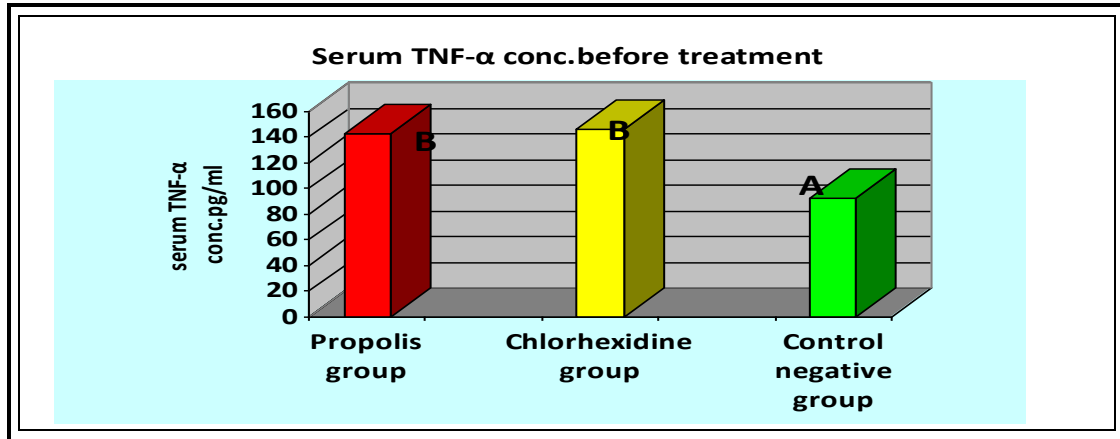


Figure (3): Duncan's multiple range test of serum TNF- α concentrations before treatment of all groups.

The mean \pm SD of serum TNF- α concentrations were decreased but not significant difference at $P \leq 0.05$ in the propolis group from (143.06 ± 70.347) pg/ml to (109.13 ± 48.163) pg/ml after two weeks of treatment by propolis mouth wash, whereas the mean \pm SD of serum TNF- α concentrations increased but not significant at $P \leq 0.05$ in chlorhexidine group from (146.50 ± 62.240) pg/ml to (174.00 ± 166.049) pg/ml after two weeks of treatment by chlorhexidine mouth wash as shown in Table (2)

Table (4): Paired Sample T-Test of comparison between the mean \pm SD of serum TNF- α concentrations before and after treatment.

	Serum TNF- α conc. before treatment pg/ml	Serum TNF- α conc. after treatment pg/ml	T	df	P-value
Propolis group	143.06 ± 70.347	109.13 ± 48.163	1.391	15	0.184
Chlorhexidine group	146.50 ± 62.240	174.00 ± 166.049	-0.769	15	0.454

The results showed that the mean \pm SD of serum TNF- α concentrations were not significant difference in propolis group (109.13 ± 48.163) pg/ml compared with chlorhexidine group (174 ± 166.049) pg/ml and control negative group (92.13 ± 44.248) pg/ml at $P \leq 0.05$ respectively while there were significant difference between mean \pm SD of chlorhexidine and control negative group at $P \leq 0.05$ after treatment as shown in Table (5) and Figure (4).

Table (5): One way ANOVA of the means of serum TNF- α concentrations after treatment of all groups.

S.O.V.	Sum of Squares	df	Mean Square	F	p-value
Between Groups	59740.167	2	29870.083	2.814	0.071
Within Groups	477747.500	45	10616.611		
Total	537487.667	47			

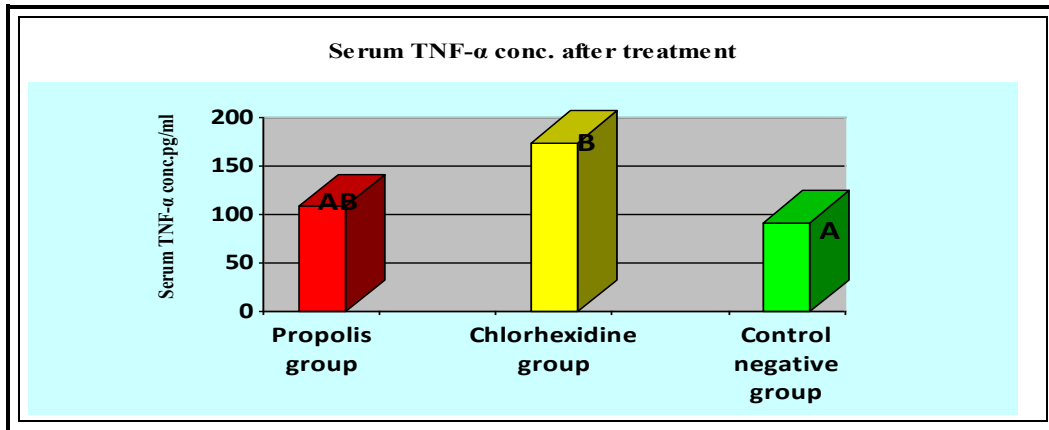


Figure (4): Duncan's multiple range test of serum TNF- α concentrations after treatment of all groups.

SERUM IL-6

The results showed that the mean \pm SD of serum IL-6 concentrations of propolis group (398.50 \pm 184.057) pg/ml were not significant difference compared with chlorhexidine group (331.50 \pm 244.355) pg/ml but it significant difference compared with control negative group (248.88 \pm 92.413) pg/ml and the latter were not significant difference when compared with chlorhexidine group at $P \leq 0.05$ before treatment shown in Table (6) and Figure (5).

Table (6): One way ANOVA of the means of serum IL-6 concentrations before treatment of all groups.

S.O.V.	Sum of Squares	df	Mean Square	F	p-value
Between Groups	179752.167	2	89876.083	2.640	0.082
Within Groups	1531903.750	45	34042.306		
Total	1711655.917	47			

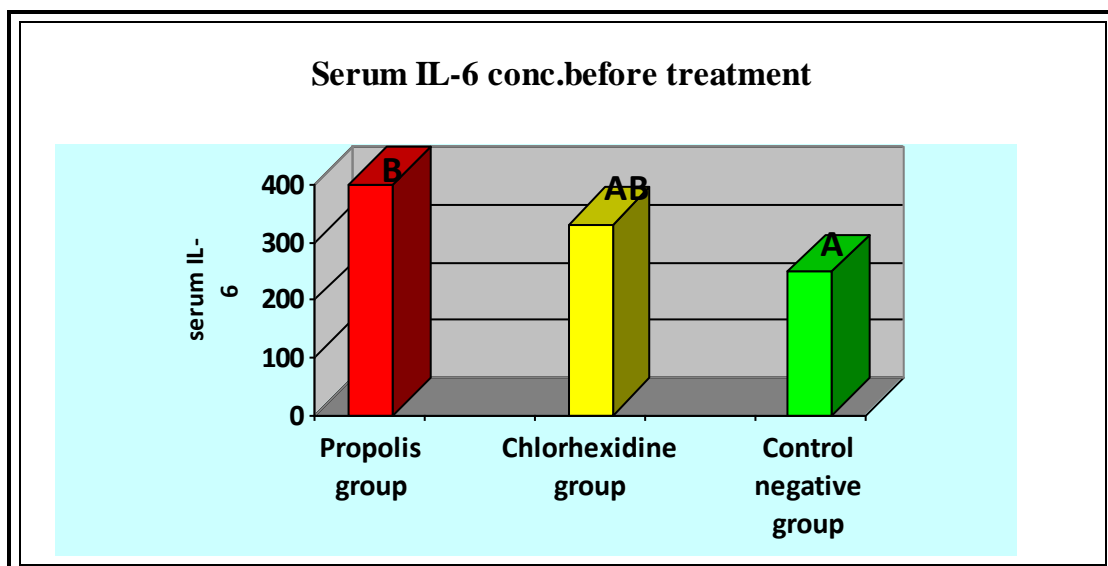


Figure (5): Duncan's multiple range test of serum IL-6 concentrations before treatment of all groups.

The mean \pm SD of serum IL-6 concentrations were highly decrease significantly in the propolis group from (398.50 \pm 184.057) pg/ml to (289.25 \pm 147.637) pg/ml after two weeks of treatment by propolis mouth wash at $P \leq 0.01$, whereas the mean \pm SD of serum IL-6 concentrations increased but not significantly in chlorhexidine group from (331.50 \pm 244.355) pg/ml to (391.25 \pm 359.241) pg/ml after two weeks of treatment by chlorhexidine mouth wash as shown in Table (7).

Table (7): Paired Sample T-Test of comparison between the mean \pm SD of serum IL-6 concentrations before and after treatment.

	Serum IL-6 conc. before treatment pg/ml	Serum IL-6 conc. after treatment pg/ml	T	Df	P-value
Propolis group	398.50 \pm 184.057	289.25 \pm 147.637	4.324	15	0.001**
Chlorhexidine group	331.50 \pm 244.355	391.25 \pm 359.241	-1.82	15	0.089

**indicated highly significant difference at $P < 0.01$.

The results showed that the mean \pm SD of serum IL-6 concentrations were not significant difference in both propolis and chlorhexidine groups (289.25 \pm 147.637) pg/ml, (391.25 \pm 359.241) pg/ml respectively comparing with mean \pm SD of serum IL-6 concentrations of control negative group (248.88 \pm 92.413) pg/ml at $P \leq 0.05$ after treatment as shown in Table (8) and Figure (6).

Table (8): One way ANOVA of the means of serum IL-6 concentrations after treatment of all groups.

S.O.V.	Sum of Squares	df	Mean Square	F	p-value
Between Groups	172292.167	2	86146.083	1.621	0.209
Within Groups	2390861.750	45	53130.261		
Total	2563153.917	47			

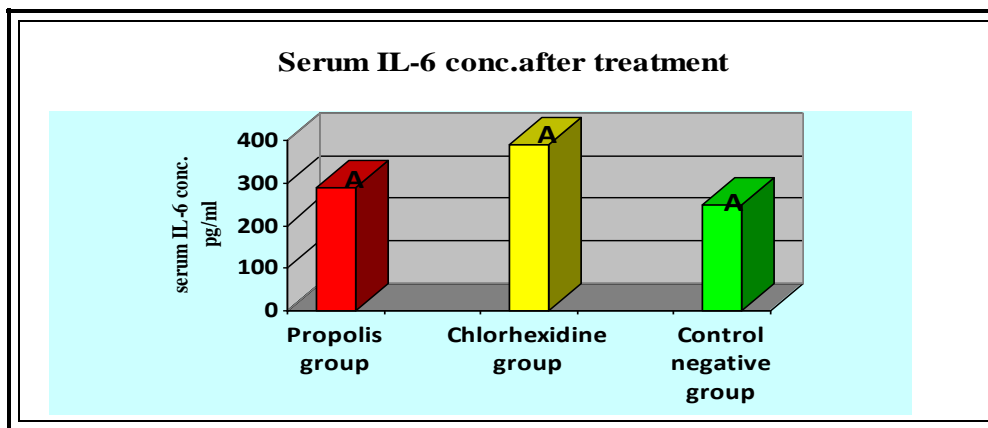


Figure (6): Duncan's multiple range test of serum IL-6 concentrations after treatment of all groups.

Antimicrobial effects of propolis mouth wash (Invivo study)

Table (9): Paired Sample T-Test of comparison between the mean of colony forming units per milliliter (CFU/ml) before and after treatment with propolis mouth washes.

	Mean \pm SD before treatment	Mean \pm SD after treatment	t-value	df	P-value
Propolis group	21.625*10 ⁸ \pm 1.99	8.3188*10 ⁸ \pm 1.24	7.704	15	0.000**

**indicated highly significant difference at $P < 0.01$. (Data described by mean \pm SD)

•Number of patients included in this study sixteen patients.

The results of table (9) showed the means of colony forming units of propolis mouth wash group decrease after treatment with propolis mouth washes from (21.625*10⁸ \pm 1.99188) CFU/ml to (8.3188*10⁸ \pm 1.24101) CFU/ml i.e. there is a highly significant difference at p value < 0.01 .

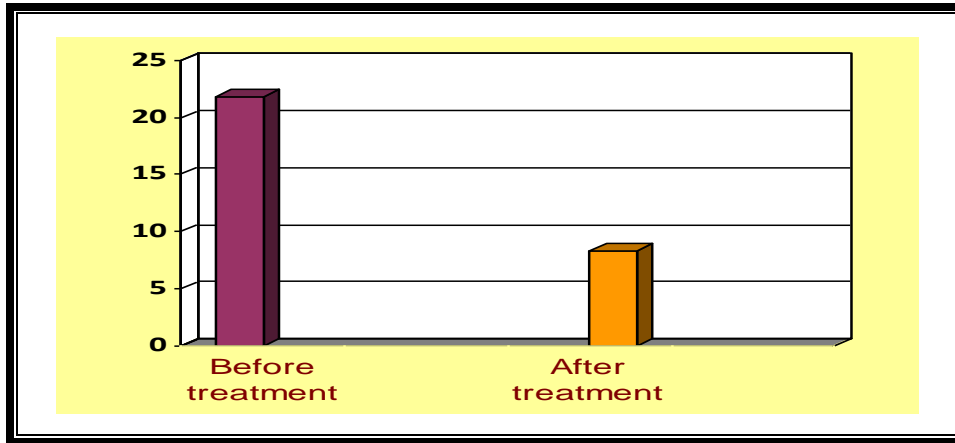


Figure (7): Means of colony forming units per milliliter (CFU/ml) for microorganisms in propolis mouth wash group before and after treatment.

DISCUSSION

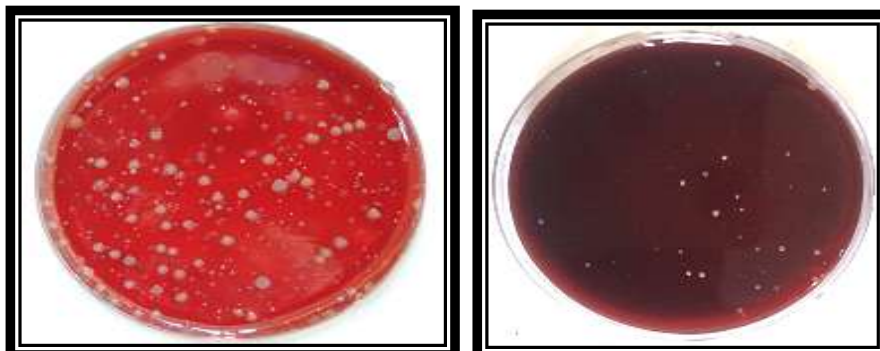
ROLE OF PROPOLIS MOUTH WASH AS ANTI-INFLAMMATORY DRUG

After the administration of chemotherapy, cytokines (IL-1, IL-6, TNF- α and transforming growth factor -3) are released from the mucosal tissue, causing local tissue damage and leading to ulcer formation and pain ⁽¹⁶⁾. In our study the level of serum IL-6, TNF- α were drawn after treatment this accordance with many researches, in the last 30 years, which have pointed out the anti-inflammatory properties of honey and propolis properties ⁽¹⁷⁾, due principally to the presence of flavonoids that inhibit the development of inflammation triggered by a variety of agents ^(18,19). Propolis, as an anti-inflammatory agent stimulates the immune system by promoting phagocytic activity and cellular immunity and improves the healing effects on epithelial tissues ⁽⁹⁾. As well as, propolis contains elements, such as iron and zinc that are important for the synthesis of collagen ⁽¹⁰⁾. Some anti-inflammatory substances (flavonoids) found in propolis, these compounds contribute to the suppression of prostaglandins and leukotrienes synthesis by macrophages ⁽²⁰⁾ and these findings are typical to our results.

ANTIMICROBIAL EFFECTS OF PROPOLIS EXTRACTS

Propolis is one of the most powerful natural antibiotics characterized by a very wide spectrum of effect. Propolis is a non-toxic antimicrobial preparation influencing Gram-positive and Gram-negative bacteria ⁽²¹⁾. Propolis has a fungicidal effect on a number of species of fungi, including *C.albicans*, *Aspergillus niger*, *Botrytis cinerea*, *Ascosphaera apis*, and *Plasmopara viticola* ⁽²²⁾. The result of present the study showed that the propolis mouth wash have antimicrobial effect on the oral micro-organisms in cancerous patients with mucositis (Fig11 and Fig10), the inhibitory effects of propolis mouth wash on the oral micro-organisms may be due to presence of multiple aromatic compounds (mainly phenolics and flavonoids). In 2006, Katircioglu and Mercan were informed that flavonoids were the greatest important group of compound with propolis biological activity ⁽²³⁾. A possible description for propolis mechanism of action may be attributed to the fact that one or some of its constituents caused a significant inhibition of bacterial motility, besides ion permeability also alteration on the inner bacterial membrane ⁽²⁴⁾. Propolis breaks down bacterial cell wall and cytoplasm; therefore, it prevents bacterial cell division ⁽²⁵⁾.

Patient No.1



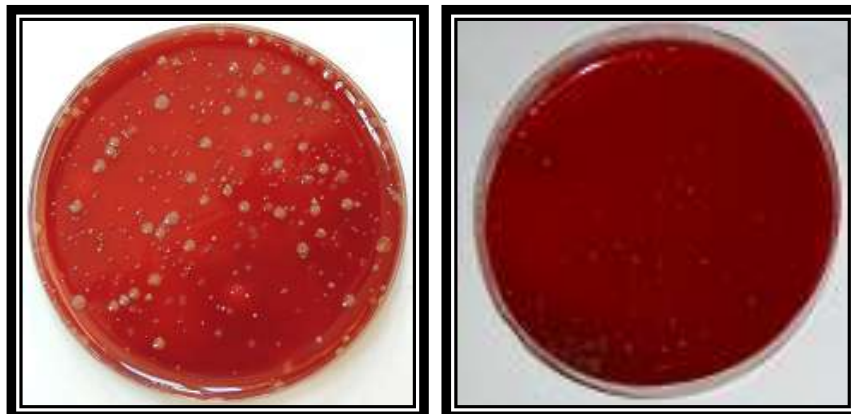
Before treatment with propolis mouth wash. After treatment with propolis mouth wash.

Patient No.2



Before treatment with propolis mouth wash. After treatment with propolis mouth wash.

Patient No.3



Before treatment with propolis mouth wash. After treatment with propolis mouth wash.

Figure (8): Blood agar plates exhibit microorganism's growth on saliva for three mucositis patients before and after treatment with propolis mouth wash

CONCLUSION

Propolis mouth wash has good anti-inflammatory and antimicrobial effects without adverse effects after two weeks of treatment of mucositis patients.

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