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Efficacy of *Juglans regia* extract as a mouthwash in the treatment of periodontal disease

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***Abstract*---**The study was aimed to evaluate the efficacy of *Juglans Regia* extracts in treatment of periodontal diseases. An extract of *Juglans regia* bark was preparation by mixing *Juglans regia* bark powder with distilled water. Phytochemicals tests were carried out for all the extract, detection of glycosides, tannins, terpenes, Steroid, Anthraquinones and, coumarines, alkaloids and saponins. 51 patients suffering from gingivitis were subjected to an experiment for 28 days, the parameters used were Gingival Index (GI), percentage of bleeding on probing (BOP) and presence of inflammatory signs and symptoms. The results show that the group that received the extract was superior compared to the chlorohexidine group, and the patients recovered at a greater rate, with reduction in signs as redness, swelling and pain, also any clinical signs or side effects.

***Keywords*---**gingivitis, Juglans Regia, chlorhexidine, Gingival Index, bleeding on probing.

Introduction

Gingivitis is caused by microbial plaque accumulating at or near the gingival sulcus; suspected local and systemic etiologic factors either enhance accumulation or retention plaque, or enhance the susceptibility of the gingival tissue to microbial plaque effect. The classic experiments of Loe *et al.* (1965) demonstrated that the accutnulation of microbial plaque results in the develop of ginvititis and that its removal and control results in resolution of the lesions

in humans.(Moore *et al.*, 1984) Under the classification system of the American Academy of Periodontology, gingivitis was defined as the inflamma-tory lesion confined to the tissues of the marginal gingiva, and periodontitis was the term accepted to describe inflamma-tory lesions extending into the deeper tissues. (Lyons *et al.*, 1959) The severe form of the disease often affects only people with compromised immune systems (It also occurs with HIV infection or immunosuppressive drugs (Abooj, 2021). Infection is caused by an overgrowth of bacteria that are normally present in the mouth without causing any problems. Poor oral care is often a contributing factor to acute necrotizing ulcerative gingivitis, as is physical stress, psychological stress, poor nutrition and lack of sleep. Infection occurs most often in people with gingivitis who are facing psychological stress (such as preparing for a final exam or undergoing intensive military courses). Acute necrotizing ulcerative gingivitis is more common in smokers than in non-smokers (Srivastava, *et al.*, 2019). Gingival enlargement an increase in the size of the gums is a common feature of periodontal disease. The currently accepted term for this condition is enlargment gingival, or gingival overgrowth (Uppal *et al.*, 2020). Chronic inflammatory hypertrophy begins as a mild swelling of the papilla between the dental and marginal gingiva. In the early stages results in swelling of the tent around the tooth. This swelling can increase in size until it covers part of the crowns. This enlargement can be localized or generalized and develops slowly and without pain unless it is mixed with a chronic infection. It may be attached to the gums. They are lumps that grow slowly and are usually painless. It may spontaneously decline in size after its continuous exacerbation and inflation and surgical intervention is often used in treatment (Deschamps-Lenhardt *et al.,* 2019).

The problem of antibiotic resistance and the development of antibiotic-resistant strains is one of the most problems and serious threat to the global health, as well as the side effects of antibiotics and high cost, which made attention turn to find safe natural alternatives to antibiotics (Yu *et al.,* 2020). Among the most important natural alternatives are plant extracts for their wide diversity, bio-availability, diversity of chemical composition and effectiveness in inhibiting the pathogen. Research studies have proven that *Juglans regia* bark extracts have the effect of inhibiting the growth of many pathogenic microorganisms such as Gram-positive bacteria (S*taphylococcus aureus* and *Streptococcus mutans*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and pathogenic yeast (*Candida albicans*). It has also been proven that these extracts have a synergistic effect or an adjuvant effect on a variety of anti-bacterials also increase saliva pH , studies reported that *J. regia* extracts are contain monoterpenes and sesquiterpenes, and the bark contains ketones like juglone, regiolone, sterol, and flavonoid (Shah *et al,* 2013).

Materials and Methods

Prepare *Juglans regia* bark extract

Juglans regia bark was collected from the market in Samawah city. The preparation of the extract by mixing separately 10, 5 and 2.5 gm of *Juglans regia* bark powder with 100 mL of distilled water for four hours on the magnetic stirrer. The extract were filtered through filter paper (Whatmann No. 1) and the filtrates

were dried and concentrated using the oven at 30°C (Muhammad and Muhammad, 2005).

Preliminary phytochemical screening for *Juglans regia* bark extract

The preliminary phytochemicals tests were carried out for all the extract as per standard methods, Detection of glycosides, tannins, terpenes and Steroid, Anthraquinones and flavonoids (Shihata, 1951) Detection of coumarines (Harborne, 1984), Detection of alkaloids and saponins (Stahl, 1969).

Study design

Fifty one patients (both males and females, age range (20 − 55 years) were selected from the outpatients who visited the specialized dental clinic As Samawa City –Iraq complaining from gingival inflammation were diagnosed by specialized dentists. The design and nature of the clinical trial was explained to the patients and written consent was obtained for their participation. At baseline patients diagnosed to have generalized gingivitis, who had not undergone any form of non surgical or surgical periodontal therapy in the last 6 months and not received any topical or systemic antimicrobial treatment for the past 6 months were included in the study. Exclusion criteria included patients having systemic diseases, pregnant and lactating mothers, smokers, and patients with less than 20 scorable teeth. Patients were divided randomly under case group (*Juglans regia* bark extract) and control group (0.12% chlorhexidine). Following a complete dental prophylaxis, subjects started rinsing twice daily with their respective mouthrinse as an adjunct to their usual mechanical oral hygiene procedures. Each of the groups were assessed for gingival index (GI) (Loe and Silness, 1963), bleeding on probing (B0P) and reexamined at baseline, 14 and 28 days. The treatment groups were compared with respect to baseline clinical variables. The primary efficacy variables were BOP, GI and signs of gingival inflammation.

Results and Discussion

The results (Table-1) of the phytochemical screening found that *J. regia* bark extract containing Alkaloids, Glycosides, Flavonoids, Tannins, Saponins, Phenols, Steroids and Anthraquinones, while negative results for Terpens, Resins, and Coumarines. AL-Sa'ady *et al* (2017) found that phytochemical, presence in *J. regia* bark extract. Shah *et.al* (2013) found that the *J. regia* bark extracts containing carbohydrates, cardiac glycosides, flavonoids, steroids, and tannins, also Djaalab *et.al* (2014), found that *J. regia* bark extract containing tannins, flavonoids, sterols, terpenoids and saponins.

Table-1: Phytochemical analysis *J. regia* bark extracts

Phytochemicals	*J. regia* bark extract
Alkaloids	+
Glycosides	+
Flavonoids	+
Tannins	+
Saponins	+
Terpens	-
Phenols	+
Steroids	+
Resins	-
Coumarines	-
Anthraquinones	+

Experiment results

All the patients subjected to scaling and polishing at baseline, with oral hygiene motivations and instructions (OHI). Then divided randomizlly into two groups, control (25 subjects) and case group (26 subjects), evaluated at interval of baseline,14 and 28 days. The parameters used Gingival Index (GI) and percentage of bleeding on probing (BOP) and presence of inflammatory signs and symptoms. The GI uses the following scoring system:

0 = normal gingiva.
1 = mild inflammation: slight change in color, slight edema, no bleeding on probing.
2 = moderate inflammation: redness, edema, and glazing, or bleeding on probing.
3 = severe inflammation: marked redness and edema, tendency toward spontaneous bleeding, ulceration.

Table (2) parameters of control group

Parameters	Scores	Group A (CHX) n=25		
		Baseline	14 Days	28 Days
Gingival Index GI	0	-	-	2
	1	4	5	21
	2	10	15	2
	3	11	5	-
B.O.P	≤10-29%	21	22	25
	≥30%	4	3	-

Table (3) parameters of case group

Parameters	Scores	Group B (*Juglans regia* bark extract) n=26		
		Baseline	14 Days	28 Days
Gingival Index GI	0	-	3	24
	1	4	7	2
	2	9	12	-
	3	13	4	-
B.O.P	≤10-30%	22	26	26
	>30%	4	-	-

Bleeding on probing (BOP) or gingival bleeding is bleeding caused by gentle manipulation of periodontal probe deep into the gingival sulcus, or the interface between the gum and tooth. It is an indicator of gingivitis and refers to a type of destruction and erosion of the sulcus lining, or ulceration of the sulcus epithelium, as a result of erosion of the lining (Pietropaoli *et al,* 2020). The results in the table 2 and 3 show that the GI index is at the base line of the group A, meaning the number of patients were 11,10,4and 0 at the grades 3,2,1 and 0 respectively, while the group B had 13,9,4and 0 at the same grades, after 14 days the the number of patients was 5,15,5 and 0 at the grades 3,2,1 and 0 respectively, while the group B had 4, 12, 7 and 3 at the same grades, at the last of experience after 28 days the the number of patients was 0,2 ,21 and 2 at the grades 3,2,1 and 0 respectively, while the group B had 0, 0, .21 and 2 at the same grades. Also, Bleeding on probing (BoP) results appered that 4 patients in both growpes were >30% and 21 patients were ≤10-30% in group A, while 22 in group B at base line, after 14 days 3 patients in group A were >30% and 22 patients were ≤10-30%, while all patients in group B were ≤10-30% after the same period, after 28 days all patients in both groups were ≤10-30%. Inflammatory signs and symptoms results in table 4. At 14th day of evaluation for patients of both groups the results shifting the condition from generalized gingivitis (BOP >30%) to localized gingivitis (BOP>10% -- <30%) with reduction in signs as redness, swelling and pain. At 14 th and 28th day results for Juglans group were difference reduction in pain and swelling in comparison to CHX group.

Table (4) Signs of inflammation for both groups

Signs	Group A (CHX) n=			Group B (Juglans regia bark extract) n=		
	Baseline	14 Days	28 Days	Baseline	14 Days	28 Days
Redness	25	20	3	26	22	2
Swelling	15	4	-	16	4	-
Pain	3	1	-	4	-	-
Bleeding	15	7		14	-	-

The study reveal uses of Juglans extract with less side effect (no staining or calculus depsition) as in the chlorhexidine mouthrinse group which had more calculus depsition and extrinsic tooth stain than case group. The result of this study reveal that reduction of percentage of BOP. After use the *Juglans regia* as

mouthwash due to its biological antimicrobial activity which effect the microflora of dental plaque and cause inhibition of bacteria growth leading to prevent of inflammation, destruction of the gingival tissue as showen in tab 2&3 and shifting the inflammation of gingival tissue from generalized to localized more likely than CHX mouthwash do. The other effect of *Juglans regia* mouthwash is the improve the healing process of periodontal tissue as it strengthing the gingiva and prevent the laceration and erosion the lining of gingival sulcus and furthermore prevent destruction of epithelial walls which reduce the signs of swelling and pain in comparism with CHX mouthwash. *Juglans regia* mouthwash with less side effects as staining of oral cavity tissues (teeth and mucosa) as in CHX mouthwash which include non enzymatic browning and production of melanoidin, also denaturation of protiens, in comparison with *Juglans regia* mouthwash, and no Ca and P ions deposition after using *Juglans regia* mouthwash so less calculus formation than using CHX more than 14 days.

Conclusion

The patients that received the extract was superior compared to the chlorohexidine group, and the patients recovered at a greater rate,with reduction in signs as redness,swelling and pain, *Juglans regia* mouthwash has no side effects as staining of oral cavity tissues(teeth and mucosa) as in CHX mouthwash which include nonenzymatic browning and production of melanoidin. We wer that *Juglans regia* mouthwash important natural alternatives to treatment periodontal diseases.

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