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Extract of *Juglandaceae regia* Inhibits Growth, In-vitro Adherence, Acid Production and Aggregation of *Streptococcus mutans*

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Abstract

Aqueous and alcoholic extracts from *Juglandaceae regia*, used as chewing sticks to maintain oral hygiene, were tested for their ability to inhibit the growth and some physiological functions of *Streptococcus mutans*.

Both the aqueous and the alcoholic extract strongly inhibited the growth, in-vitro adherence, acid production and glucan-induced aggregation of *S. mutans*. At a concentration of 8% w/v, the aqueous extract produced a 95% inhibition ($P < 0{\cdot}05$) of adherence of *S. mutans* to glass and a 40% inhibition ($P < 0{\cdot}05$) of adherence to tooth surface. The alcoholic extract at a concentration of 10% w/v produced a 95% inhibition ($P < 0{\cdot}05$) of adherence of *S. mutans* to glass and a 56% inhibition ($P < 0{\cdot}05$) of adherence to tooth surface. At concentrations of 2% w/v the aqueous and alcoholic extracts significantly inhibited ($P < 0{\cdot}05$) glucan-induced aggregation of *S. mutans* and the in-vitro salivary glycolytic reaction for up to 5 h. Bactericidal effects on *S. mutans* were also evident. At a concentration of 10% w/v, the zone of inhibition observed with the aqueous extract was $12 \pm 0{\cdot}01$ mm and that observed with the alcoholic extract was $12{\cdot}6 \pm 0{\cdot}02$ mm. As the in-vitro studies had shown that both the aqueous and the alcoholic extract of *J. regia*, at concentrations of 10% w/v, could inhibit the growth as well as the acid-producing ability of *S. mutans*, they were tested at the same concentration for their activity in-vivo. Three subjects were employed. Parameters monitored were salivary bacterial count and salivary glycolysis. Mouth-rinsing with the aqueous but not the alcoholic extract significantly reduced total streptococcal counts in the salivary samples obtained up to, and including, 3 h after rinsing, compared with the counts obtained pre-rinsing or after placebo rinsing. Mouth-rinsing with the aqueous extract produced a 65%, 27% and 78% reduction ($P < 0{\cdot}05$) in the streptococcal count in the salivary samples obtained 10 min, 1 h and 3 h after rinsing, respectively. Both the aqueous and the alcoholic extract also inhibited the glycolytic reaction by the salivary bacteria for up to 90 min post-rinsing.

This study provides evidence to justify the use of *J. regia* sticks as an aid to maintain oral hygiene.

Dental plaque has been implicated as the principal aetiological factor in dental caries and periodontal diseases (Wu-Yan et al 1988). The ability of *Streptococcus mutans* (a bacterium indigenous to the oral cavity and having its prime habitat in the plaque) to grow, synthesise adhesive glucan, aggregate in the presence of glucan and produce acid is responsible for its cariogenicity (Ciardi et al 1981). The search for an effective antiplaque agent has been extensive, though little attention has been given to plants used as chewing sticks for maintenance of oral hygiene, despite the fact that users attribute their dental health to their use. The precise method for use of these implements was recorded by the Babylonians in 5000 B.C., and the practice ultimately spread throughout the Greek and the Roman empires and elsewhere. The use of chewing sticks persists even today among many African and

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Southern Asian communities, as well as in isolated areas of tropical America and the southern United States (Walter et al 1977).

Some studies have suggested that caries rates are often very low among the users of chewing sticks despite the high carbohydrate diet they normally consume and the lack of exposure to modern dental procedures and prophylactic regimens (MacGregor 1963).

Among the many plants used as chewing sticks, the stem bark of *Juglandaceae regia* (walnut) is very popular and is used as a tooth-cleaning device by the people living in all of temperate Europe and in the Himalayas, China and Japan (Almas & Al-Lafi 1995). The bark is known to contain tannins, essential oils, a purgative and a naphthaquinone known as juglone (Chopra & Handa 1958). Homeopaths prescribe the tincture of its leaves for cutting wisdom teeth. It has also been claimed that a decoction of its leaves makes an effective mouthwash (Francesco 1977). The bark of *J. regia* is also known to contain 0·5 ppm fluorides and the role of fluorides in inhibiting dental caries has been well documented (Almas & Al-Lafi 1995). Studies performed on the powdered bark have demonstrated its effectiveness against gum disorders such as bleeding and swelling (Date & Kulkarni 1995).

The mechanical advantage offered by the fibrous nature of the chewing sticks cannot be used to explain the lower caries rates among the population using it, as one study has concluded that chewing sticks are 20% poorer than the toothbrush in reducing plaque scores (Talim, 1985). Therefore, the hypothesis that these chewing sticks contain some antiplaque constituents can not be overlooked.

No reports are presently available regarding the effects of the juice from the stem bark of *J. regia*, on the growth and physiological properties of *S. mutans*, the bacterium involved in dental caries. Thus we screened the crude alcoholic and the aqueous extracts of *J. regia* for their biological activity, in order to provide a scientific validity for its use as a chewing stick. The extracts were tested for their effect on the growth, in-vitro adherence, glucan-induced aggregation and the acid-producing ability of *S. mutans*.

Materials and Methods

Microorganism

The microorganism used for this study was *Streptococcus mutans*, originally isolated from the dental plaque of a healthy human volunteer. This strain was isolated onto the plates of Mitis Salivarius (MS) agar and incubated anaerobically at 37°C for 24 h. A cell suspension was prepared by removing the colonies of *S. mutans* into fluid thioglycolate medium and incubating at 37°C for 18 h.

Preparation of plant extracts

The dried stem bark of *Juglandaceae regia* was obtained from Yucca Enterprises Ltd, Mumbai and identified and authenticated by Dr K. S. Laddha, Department of Pharmacognosy, University Department of Chemical Technology, Mumbai.

Stem bark (400 g) was suspended in 10 times its quantity of sterile distilled water (50% v/v ethanol for the alcoholic extract) in a round-bottom flask and kept at 4°C for 72 h. The aqueous and the alcoholic extracts of the plant material were decanted, clarified by filtration through a muslin cloth and evaporated in a flat-bottom porcelain dish at 40°C. The dried extracts were stored at 4°C until use. Extracts were suspended in polyethylene glycol (PEG) 400 (20 %v/v) and sterile distilled water to give a final concentration of 30% w/v. The concentrated extracts were then diluted with sterile distilled water to give concentrations of 2, 4, 8, 10, and 15% w/v.

Antimicrobial activity of J. regia *extract*

To study the effect of the extract of *J. regia* on the growth of *S. mutans*, the 18-h-old culture of *S. mutans* was incorporated into Brain Heart Infusion (BHI) agar, poured into petriplates and allowed to set (0·1 mL of culture fluid for every 10 mL of agar). Sterile filter paper disks (6 mm diam.) were impregnated with known dilutions of the extract and placed on the agar. The zone of inhibition obtained with various concentrations of the extracts was observed after 24 h incubation at 37°C.

Effects on S. mutans *adherence*

The effect of the extract on the adherence of *S. mutans* to glass surfaces was studied by taking glass tubes containing 5 mL of Brain Heart Infusion (BHI) broth containing 2% sucrose and 0·1 mL of various concentrations of the extract (2–10%), inoculating them with the overnight cultures of *S. mutans* and incubating by standing for 24 h at 37°C. Controls consisted of cells grown in BHI broth containing sucrose and 0·1 mL of PEG 400 (20% v/v). Each culture was gently mixed in a cyclomixer for 5 s to remove the cells growing in close contact with the glass surface but not actually adhering, and the culture fluid poured off. The tubes were then scored on a scale of 0 to 4 for adherence as follows: 4, thick confluent coat of cells on the bottom and the sides of the tube; 3, thin

confluent coat of cells on the bottom and the sides of the tube; 2, thin confluent coat of cells on the bottom of the tube; 1, few visible cells adhering to bottom of the tube; and 0, no visible adherence.

To study the effects of the extracts on adherence by the spectrophotometric method, tubes containing 1·9 mL of BHI broth plus 2% sucrose and 0·1 mL of various concentrations of the extract (2–10%), were inoculated with the overnight culture of *S. mutans*. Controls consisted of cells grown in BHI broth containing sucrose and 0·1 mL of PEG 400 (20% v/v). All tubes were inclined at 30° and incubated at 37°C for 24 h. The adherent and non-adherent bacteria were quantitated spectrophotometrically as described by Segal et al (1985).

To study the effects of various concentrations of the extract on adherence of *S. mutans* to the surface of the tooth, saliva-coated sterile human teeth were kept in contact with 1 mL of the various concentrations of the extract (2–10%) for 1 min. The teeth were then added to 5 mL BHI broth containing 2% sucrose, inoculated with overnight cultures of *S. mutans* and incubated at 37°C for 24 h. Controls consisted of saliva-coated sterile teeth kept in contact with 1 mL of PEG 400 (20% v/v). After 24 h incubation the tubes were gently shaken between the palms for 5 s. The broth containing non-adherent bacteria was decanted and the tube gently washed with 0·5 mL saline. The decanted broth and the washings were pooled, centrifuged, the supernatant decanted and the sediment suspended in 3 mL saline. The cells adhering to the tooth and the glass were washed with 5 mL of 0·5 M NaOH separately. The washings were then pooled, centrifuged, the supernatant decanted and the sediment suspended in 3 mL saline. The adherent bacteria (cells adhering to the surface of the glass and tooth) and the non-adherent bacteria were quantitated spectrophotometrically at 540 nm.

Effects on glycolysis

The effect of the extracts on acid production by *S. mutans* was studied by 2 in-vitro methods.

To 2 mL of fresh clarified human saliva, was added 0·1 mL of 5% glucose and 0·1 mL of the various concentrations of the *J. regia* extracts (2–10%). Controls consisted of the saliva/glucose mixes containing 0·1 mL of PEG 400 (20% v/v). The pH of the saliva/glucose mixes was recorded immediately and at 60-min intervals for the next 5 h.

To study the effects of the extract on acid production after 24 h of incubation, 5 mL of BHI broth containing 2% sucrose and 0·1 mL of various concentrations of the extracts (2–10%) was inoculated with the overnight culture of *S. mutans* and incubated at 37°C for 24 h. Controls consisted of cells grown in BHI broth containing sucrose and 0·1 mL of PEG 400 (20% v/v). The pH of the bacterial broth was recorded at the onset and after 24 h incubation.

Effects on glucan-induced aggregation of S. mutans

Aggregation of *S. mutans* in the presence of dextran T4133 (Sigma Chemical Company, St Louis, MO) was studied as described by Murchison et al (1981). *S. mutans* cells were incubated with 2–10% of the extract for 1 h at 37°C before the addition of the dextran. Controls consisted of cells incubated with PEG 400 (20% v/v) for 1 h. The degree of aggregation was examined macroscopically and later confirmed microscopically after 1 h (37°C) and scored on a 0 to 4 basis as follows: 0, no visible aggregation; 1, slightly visible minute clumps of cells in turbid fluid; 2, easily visible small clumps of cells in turbid fluid; 3, well defined clumps of cells in clear supernatant fluid; and 4, very large flocculent clumps of cells in clear supernatant fluid.

For studying sucrose-induced aggregation, the cells were incubated in bacterial broth containing 2% sucrose in the presence of the extract for 24 h at 37°C. The degree of aggregation was observed after 24 h and scored on a zero to 4 basis as described above.

In-vivo study

Three adult subjects in good health and with twenty or more natural teeth free of dental caries were recruited for the study. The procedures, possible discomfort or risk were fully explained to the volunteers and their written consent obtained. Subjects continued their usual oral hygiene routines and no attempts were made to change or standardize diets or eating habits. Subjects gave a whole-mouth saliva sample that was not induced in any way. After collection the samples were placed immediately on ice. This saliva sample served as the control. Subjects rested for 15 min and then rinsed the mouth with 10 mL of the assigned extract for 1 min. The mouth-rinse was prepared by suspending the extract in PEG 400 (20% v/v) and water to give a concentration of 10%. None of the subjects rinsed with water afterwards. Further samples were collected after 10 min, 1 h and 3 h post-rinsing. Subjects were not allowed to eat between sample collections. Placebo rinses consisted of vehicle only, with no active ingredient.

The vehicle was a solution containing water and polyethylene glycol.

Each saliva sample was immediately diluted 10^3 times with sterile saline and streaked on BHI agar plates and MS agar plates to determine the total bacterial and total streptococcal counts, respectively. The plates were incubated at 37°C for 24 h and the number of bacterial colonies was counted visually.

To determine inhibition of glycolysis, saliva samples were collected both pre-rinsing and at 1, 15, 30, 45 and 90 min post-rinsing. Two millilitres of each saliva sample were pipetted into test tubes containing 0·5 mL of 5% glucose. All test tubes were placed in a 37°C water bath for 1–2 min and the pH value of the saliva/glucose mixes was determined immediately and at 60-min intervals thereafter for the next 5 h.

Analysis of data

Data were analysed for inhibition of adherence, aggregation and acid production. The adherence index and the aggregation index were analysed by Wilcoxon's matched-pairs signed ranks test. For in-vitro adherence, data from the test and the control groups were compared using Student's *t*-test. For in-vitro glycolysis, data from the test at the fifth hour in the glycolytic reaction were compared with data from the control, at the same time in the glycolytic reaction, using Wilcoxon's matched-pairs signed ranks test. All values were considered significant when $P < 0{\cdot}05$.

Data were analysed for the mouth-rinse, for the inhibition of bacterial growth, by comparing the pre-rinsing values with the post-rinsing values using paired *t*-test. All values were considered significant when $P < 0{\cdot}05$. Data were analysed for the mouth-rinses for the inhibition of salivary glycolysis by comparing the pH values of the post-rinsing saliva samples collected at various time intervals (10, 60 and 180 min at their fifth hour in the glycolytic reaction with the pH values of the pre-rinsing saliva sample, also at the fifth hour in the glycolytic reaction, using Wilcoxon's matched-pairs signed ranks test. All values were considered significant when $P < 0{\cdot}05$.

Results

When *S. mutans* was grown in agar containing 2–15% of extract, both the aqueous and the alcoholic extract of *J. regia* were found to be bactericidal. With the alcoholic extract the minimum inhibitory concentration for *S. mutans* was 8% while for the aqueous extract it was 10% (Table 1).

As seen in Figure 1, inhibition of in-vitro adherence of *S. mutans* to glass was evident when the cells were grown in BHI broth containing sucrose and various concentrations of the aqueous extract of *J. regia*. Similar results were observed even with the alcoholic extract of *J. regia*. At a concentration of 2% the aqueous extract showed more than 65% inhibition of adherence while at the same concentration the alcoholic extract produced an inhibition greater than 85% (Table 2). The aqueous extract inhibited adherence of *S. mutans* to the tooth surface at a concentration of 4%, 8% and

Table 2. Inhibitory effects of aqueous and alcoholic extracts of *J. regia* on sucrose-induced adherence of *S. mutans* to glass surface.

Concn of the extract	% Inhibition of adherence	
	Aqueous extract	Alcoholic extract
2%	65·30 ± 8·93*	84·73 ± 1·20*
4%	83·19 ± 5·81*	86·59 ± 2·19*
8%	95·04 ± 0·71*	95·50 ± 0·36*
10%	95·70 ± 1·10*	95·50 ± 0·40*

Values are expressed as the mean ± s.e.m. (n = 10). *$P < 0{\cdot}05$, compared with control. Tubes containing BHI broth (1·9 mL), sucrose (2%) and *J. regia* extract (0·1 mL) were inoculated with *S. mutans* overnight culture (control was *S. mutans* in BHI broth containing sucrose and 0·1 mL of PEG 400 (20% v/v)). Tubes were incubated at 37°C for 24 h and the adherent and non-adherent bacteria were quantified spectrophotometrically by the method of Segal et al (1985).

Table 1. Diameter of the zone of inhibition obtained with various concentrations of aqueous and alcoholic extracts of *J. regia* when *S. mutans* was grown on BHI agar.

Extract	Concn of the extract					
	2%	4%	6%	8%	10%	15%
Aqueous	–	–	–	–	12·0 ± 0·01	12·2 ± 0·1
Alcoholic	–	–	–	10·5 ± 0·1	12·6 ± 0·02	13·1 ± 0·01

Values are given in mm and expressed as means ± s.e.m. (n = 10). *J. regia* extracts were applied on filter-paper disks to plates of *S. mutans* growing on BHI agar and incubated for 24 h at 37°C.

Table 3. Effect of various concentrations of aqueous and alcoholic extract of *J. regia* on sucrose-induced adherence of *S. mutans* to tooth surfaces.

Extract	Optical density at 540 nm				
	Control	2%	4%	8%	10%
Aqueous	0·46 ± 0·10	0·31 ± 0·09	0·24 ± 0·05*	0·28 ± 0·04*	0·26 ± 0·02*
Alcoholic	0·43 ± 0·06	0·60 ± 0·20	0·35 ± 0·02	0·37 ± 0·10	0·18 ± 0·09*

Values are expressed as the mean ± s.e.m. (n = 6). *$P < 0·05$ compared with control. Sterile human teeth pretreated for 1 min with *J. regia* extracts were added to BHI broth containing 2% sucrose, inoculated with overnight cultures of *S. mutans* and incubated at 37°C for 24 h. Controls were performed with teeth pretreated with 1 mL of PEG 400 (20% v/v).

Table 4. Effect of aqueous and alcoholic extracts of *J. regia* on glycolysis in-vitro by *S. mutans* after various periods of incubation.

Incubation time (h)	Extract	pH value of bacterial broth				
		Concn of extract				
		Control	2%	4%	8%	10%
0	Aqueous	6·84	6·67	6·35	6·19	5·86
	Alcoholic	7·69	6·77	6·66	6·56	6·46
5	Aqueous	5·29	5·62*	5·86*	5·94*	5·86*
	Alcoholic	4·92	5·33*	5·64*	5·95*	6·15*
24	Aqueous	4·34	4·52*	4·49*	4·41*	4·51*
	Alcoholic	4·33	4·32	4·35	4·37	4·37

Values are expressed as the mean (n = 3 for 0- and 5-h data; n = 6 for 24-h data). *$P < 0·05$, compared with control. For the 0- and 5-h data: Clarified human saliva (2 mL) was mixed with 0·1 mL of 5% glucose and 0·1 mL of *J. regia* extract; control was saliva/glucose mix with 0·1 mL PEG 400 (20% v/v). For the 24-h data: BHI broth (5 mL) containing 2% sucrose and 0·1 mL of *J. regia* extract was inoculated with overnight culture of *S. mutans* and incubated at 37°C for 24 h; control was cells grown in BHI broth containing sucrose and 0·1 mL of PEG 400 (20% v/v).

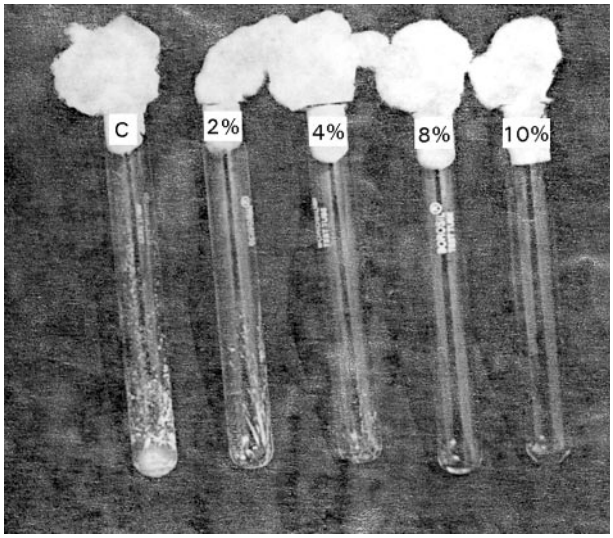

Figure 1. Appearance of *S. mutans* adhering to glass surface after treatment with various concentrations of the aqueous extract of *J. regia*. Arranged from left to right are control (score 4), 2% (score 0·5), 4% (score 0·5), 8% (score 0) and 10% (score 0). BHI broth (5 mL) containing 2% sucrose and 0·1 mL of extract was inoculated with overnight culture of *S. mutans* and incubated for 24 h at 37°C. Controls were BHI broth containing sucrose and 0·1 mL of PEG 400 (20% v/v) inoculated with *S. mutans*.

10% (Table 3). The alcoholic extract produced the same effect but only at a concentration of 10% (Table 3).

Both the aqueous and the alcoholic extract inhibited acid production by the salivary bacteria for up to 5 h at all the concentrations tested (Table 4); with the aqueous extract the antiglycolytic effect persisted for up to 24 h.

When cells of *S. mutans* were incubated with the aqueous and the alcoholic extract of *J. regia* for 1 h, the subsequent addition of dextran resulted in a rapid flocculation followed by sedimentation of the cells and the dextran particles (Table 4). When the flocculated suspension was examined microscopically it was seen that the particles of dextran were joined by individual bacterial cells or single chains of cells and that massive complexes of cells, as seen when the cells of *S. mutans* were grown in the presence of dextran only, were absent. Similar results were observed even with sucrose-induced aggregation of cells.

Mouth-rinsing with 10 mL of 10% aqueous extract of *J. regia* brought about a significant reduction in the total salivary bacterial count at 10 min post-rinsing ($P < 0·05$) but not at 1 h or 3 h post-rinsing. However, a significant reduction in the streptococcal count was obtained at 10 min, 1 h and 3 h post-rinsing ($P < 0·05$). Mouth-rinsing with 10 mL of a 10% alcoholic extract of *J. regia*, however, brought about no significant difference in either the total salivary bacterial count or the total streptococcal count at any time interval as compared with the pre-rinsing count (Table 5). Rinsing with the vehicle produced no difference in either the total bacterial or the total streptococcal count at any time interval (data not shown).

The pH of the saliva samples collected at 1 min and at 15, 30, 45 and 90 min post-rinsing with the

Table 5. Temporal effects of mouth-rinses with aqueous and alcoholic extracts of *J. regia* (10%) on the total salivary bacterial counts and total streptococcal counts.

Extract	% Reduction of total salivary bacterial count after mouthrinsing (min)			% Reduction of total salivary streptococcal count after mouthrinsing (min)		
	Time			Time		
	10	60	180	10	60	180
Aqueous	63·2 ± 16·0*	28·3 ± 11·2	53·3 ± 49·7	64·9 ± 24·5*	27·3 ± 13·3*	77·5 ± 19·3*
Alcoholic	46·1 ± 24·5	43·9 ± 13·3	33·6 ± 19·3	47·9 ± 32·5	48·8 ± 32·0	58·4 ± 23·1

Values are expressed as the mean ± s.e.m. of the readings obtained from three volunteers. *$P < 0.05$ compared with pre-rinsing values. Subjects rinsed their mouth for 1 min with 10 mL of a solution containing 10% extract and PEG 400 (20% v/v).

Table 6. Temporal effects of mouth-rinses with aqueous and alcoholic extracts of *J. regia* on in-vivo salivary glycolysis.

Incubation time (h)	Extract	Time post-rinsing (min)					
		Pre-rinse	1	15	30	45	90
0	Aqueous	6·63	6·30	6·64	6·95	6·95	6·63
	Alcoholic	7·07	6·72	6·72	6·84	7·19	6·96
5	Aqueous	5·17	5·33*	5·34	5·66*	5·82*	5·82*
	Alcoholic	5·68	6·38*	6·26*	6·03*	6·49*	6·26*

Values are expressed as the mean of readings obtained from three volunteers. *$P < 0.05$ compared with control. Saliva samples were taken at the times shown after rinsing and were incubated with glucose (final concentration 2%) at 37°C for 5 h.

aqueous and the alcoholic extract of *J. regia* were significantly higher ($P < 0.05$) than the pH of the saliva samples collected pre-rinsing (analyses performed on the data at the fifth hour into the glycolytic reaction; Table 6). Rinsing with the vehicle produced no effect on salivary glycolysis (data not shown).

Discussion

In examining data from the various in-vitro and in-vivo studies it is evident that the therapeutic rationale behind selection of the stem bark of *Juglandacaea regia* as an aid to oral hygiene holds. Results of the in-vitro studies indicate that the aqueous as well as the alcoholic extract of *J. regia* can inhibit the growth and various physiological functions of *Streptococcus mutans*.

Mouth-rinsing with the aqueous extract produced an immediate and prolonged antibacterial effect in the oral cavity. This effect must have been exerted through a high initial oral retention of the extract, with a strong bactericidal activity, during and immediately after rinsing combined with a prolonged bacteriostatic activity. The failure of the alcoholic extract to reduce the bacterial count in the oral cavity on mouth-rinsing, despite demonstrating antibacterial activity in the in-vitro bacteriological tests, reflects on its poor substantivity in the oral cavity. The alcoholic extract must have either adsorbed weakly to the hydroxyapatite of the tooth enamel or to the salivary mucins or desorbed quickly producing a low concentration in the oral cavity over a period of time.

Apart from having bactericidal effects the *J. regia* extracts also interfered with the non-vital processes of *S. mutans* during its growth, such as plaque formation on the surface of the tooth. The aqueous extract, however, inhibited plaque formation at a lower concentration than the aqueous extract.

The reduced adherence of *S. mutans* to the surface of the tooth in the presence of the *J. regia* extracts could result from the effect of the tannins in the extract. Tannins, by virtue of hydrogen bonding through their phenolic groups, could significantly alter the initial attachment of *S. mutans* in-vitro by binding to proline-rich proteins found in salivary pellicle or with the lipoteichoic acid on the bacterial surfaces.

This inhibition of the initial adsorption of *S. mutans* to the surface of the tooth has been considered important for the prevention of plaque formation. However, establishment of *S. mutans* on the surface of the tooth is rendered irreversible only after the synthesis of sticky water-insoluble glucan from sucrose by the enzymatic action of glucosyltransferase (GTF) (Wolinsky & Sote 1984).

The ability of the *J. regia* extracts to prevent the adherence of *S. mutans* to the glass surface in the presence of sucrose can be related to their inhibitory effects on the activity of GTF, whereby they prevent the synthesis of insoluble glucan. Tannins have been reported to have a non-competitive inhibitory effect on the activity of GTF.

It may be suggested that the tannins physically interact with the insoluble enzyme, binding to an

active site or changing the quaternary structure. This would affect the enzyme ability to aggregate and cause the shift in molecular weight which is required for its activity. Alternately it may that the tannins prevent the adsorption of GTF to the surface of *S. mutans* cells. GTF can synthesize the insoluble glucan polymer only after adsorption to the cell surface. The glycoprotein-like character of GTF suggests that the protein on the surface of the cell is involved in the binding of the enzyme. The inhibition of adherence observed could therefore be the result of some tannin–protein interaction owing to which the enzyme could not adsorb to the surface of the cell and synthesize the insoluble glucan.

It was observed that in the absence of insoluble enzyme activity, enhanced synthesis of the soluble glucan from the cell-free enzyme occurred. This could be inferred from the flocculation of the cells of *S. mutans* on incubation with sucrose in the presence of the extract. The cells of *S. mutans* flocculate in the presence of soluble glucan as it contains glucose primarily in the α-1, 6-linkage with no branching, whereas the insoluble glucan, which possesses a high degree of branching involving α-1, 3-linkage, would lead to aggregation of the cells.

Our findings have been corroborated by the results of previous studies, which have demonstrated that loss of in-vitro and in-vivo plaque-forming ability of *S. mutans* can be correlated with the synthesis of increased amounts of extracellular water-soluble dextran and decreased amounts of insoluble cell-associated dextran (Mukasa & Slade 1974).

As stated previously, the alcoholic extract of *J. regia* was less efficient than the aqueous extract in inhibiting plaque formation on the surface of the tooth. As alcohol solubilizes significantly higher amounts of tannins as compared with the aqueous extraction, we anticipated a greater suppression of plaque formation on the surface of the tooth with the alcoholic extract. However, it was more efficient than the aqueous extract in inhibiting the adherence of *S. mutans* to the surface of glass. The difference in sensitivity could be explained by the addition of the extract directly to the glass adherence system whereas the saliva-coated teeth were pretreated with the extract for one minute. For an agent to inhibit plaque formation on the surface of the tooth, it must adsorb in sufficient amounts to the hydroxyapatite of the tooth, or to the salivary mucins, during the one-minute exposure time. We believe that the low efficiency of the alcoholic extract is due to competitive antagonism of the tannins with some other constituent in the extract for the same binding site on the surface of the tooth. Presently, competitive antagonism appears the only plausible explanation for the failure of the alcoholic extract to inhibit plaque formation on the tooth surface and its failure to reduce the bacterial count in the oral cavity after mouth-rinsing with the extract for one minute.

Besides inhibiting sucrose-induced adherence, the extracts also prevented glucan-induced aggregation, an important mechanism in the accumulation of *S. mutans* on the surface of the tooth. The mutans streptococci aggregate by virtue of their cell-surface glucan-binding protein that is capable of binding high molecular weight glucan, thereby facilitating aggregation. However, in the presence of sucrose the GTF enzymes adsorb to the surface of the cell and increase the dextran-binding capacity of the cell by serving as a second type of glucan receptor. These cell-bound GTF enzymes are present in the form of aggregates formed by virtue of protein–protein interaction (Germaine & Schachtele 1976). We observed that pretreatment of *S. mutans* with *J. regia* extract resulted in flocculation of the cells on subsequent addition of dextran. If, as suggested earlier, the tannins interfered with the aggregation of the enzyme, it would lead to a reduction in the dextran-binding capacity of the cells. This helps us explain the observed decrease in the tendency of the cells to aggregate in the presence of dextran.

Although the role of aggregation in cariogenicity has been less emphasized than the sucrose-induced adherence, it nevertheless contributes to the accumulation of the oral streptococci on the tooth surface. Thus, by inhibiting the glucan-induced aggregation of the cells, the cariostatic potential of the extracts is further substantiated.

Apart from inhibiting the synthesis of insoluble glucan from sucrose, the *J. regia* extract even inhibited the decomposition of glucose to lactic acid, which could be attributed to the presence of fluorides in the stem bark. Some studies have shown that a low level of fluorides can inhibit carbohydrate metabolism by oral microorganisms through inhibiting several steps in carbohydrate metabolism (Schee 1989).

Thus, when considering the overall effect of the extracts on *S. mutans*, it can be said that the use of the stem bark of *J. regia* as a chewing stick will have an effect on the growth, adherence, aggregation and acid-producing ability of *S. mutans*.

We now have a scientific reason to justify the use of these *J. regia* sticks as an aid to maintain oral hygiene.

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References

Almas, K., Al-Lafi, T. R. (1995) The natural toothbrush. World. Health. Forum. 16: 206–210

Chopra, R. N., Handa, K. L. (1958) Chopras Indigenous Drugs in India. 2nd Edn, U. N. Dhurs & Sons Pvt Ltd, Calcutta, pp 658–659

Ciardi, J. E., Rosenthal, A. B., Bowen, W. H. (1981) Rapid quantitative determination of the effect of anti-plaque agents and antisera on the growth, acid production and adherence of *S. mutans*. J. Dent. Res. 60: 756–762

Date, B. B., Kulkarni, P. H. (1995) Assessment of Rasadant in various oral disorders. Ayurveda Res. Papers 2: 167–175

Francesco, B. (1977) Health plants of the world. In: Atlas of Medicinal Plants. Newsweek Books, New York, pp 156–220

Germaine, G. R., Schachtele, C. F. (1976) S. mutans dextransucrase: mode of interaction of high molecular weight dextran and role in cellular aggregation. Infect. Immun. 13: 365–372

MacGregor, A. B. (1963) Increasing caries incidence and changing diets in Ghana. Int. Dent. J. 13: 516–522

Mukasa, H., Slade, H. D. (1974) Mechanism of adherence of S. mutans to smooth surfaces, nature of binding and adsorption of dextran levan synthetase enzymes on the cell wall surface of streptococcus. Infect. Immun. 9: 419–429

Murchison, M., Larrimore, S., Curtis, R. (1981) Isolation and characterization of *S. mutans* mutants defective in adherence and aggregation. Infect. Immun. 34: 1044–1055

Schee, A. (1989) Modes of action of currently known chemical antiplaque agents other than chlorhexidine. J. Periodontal Res. 5: 102–109

Segal, R., Pisanty, S., Wormser, R., Azaz, E., Sela, M. N. (1985) Anticariogenic activity of licorice and glycyrrhizine I: inhibition of in vitro plaque formation by *S. mutans*. J. Pharm. Sci. 74: 79–81

Talim, S. T. (1985)MD Thesis, University of Mumbai, Nair, Dental College, Mumbai

Walter, H. L., Memory, P. F., Elvin-Lewis (1977) Medical Botany Plants Affecting Man's Health. John Wiley & Sons, New York, p. 230

Wolinsky, L. E., Sote, E. O. (1984) Isolation of natural plaque inhibiting substances from Nigerian chewing sticks. Caries Res. 18: 216–225

Wu-Yan, C. D., Chen, C. Y., Wu, R. T. (1988) Gallotannins inhibit growth, water insoluble glucan synthesis and aggregation of mutans streptococci. J. Dent. Res. 67: 51–55