



Research Journal of
**Medicinal
Plant**

ISSN 1819-3455



Academic
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Dental Caries Inhibition in Albino Rats by *Breynia nivosus* Extract

¹E.S. Amadi, ²C.A. Oyeka, ²I. Okoli, ³J.I. Ihedioha and ¹I.R. Iroha

¹Department of Applied Microbiology,
Faculty of Applied and Natural Sciences, Ebonyi State University,
P.M.B. 053, Abakaliki, Ebonyi State, Nigeria

²Department of Applied Microbiology and Brewing,
Faculty of Natural Sciences, Nnamdi Azikiwe University,
Awka, Anambra State, Nigeria

³Department of Pathology and Microbiology,
Faculty of Veterinary Medicine, University of Nigeria,
Nsukka, Enugu State, Nigeria

Abstract: The study investigated the caries inductive capacities of different sucrose concentrations and the anti-caries activity of *Breynia nivosus* extract in experimental albino rats. Different concentrations (70, 50, 30 and 10%) of sucrose-in-diet, were respectively fed to caries-free albino rats harboring *Streptococcus rattus* in their oral cavity, to determine their caries induction effect. Subsequently, 200 mg mL⁻¹ of *Breynia nivosus* extract were intra-orally administered to the teeth surfaces of caries-free and non caries free rats to ascertain its possible caries curative and/or preventive effects. Direct observation, probing, microbial count and radiography were used to monitor the caries status of the rats. There were significant increase ($p < 0.05$) in the microbial count of dental plaque of rat groups fed with 70, 50 and 30% sucrose-in-diet. However, milky white spots were only observed among the rat groups fed with 70 and 50% sucrose-in-diet at the 6th week of observation. The results of this investigation suggests that *Breynia nivosus* extract possesses some degree of *in vivo* caries preventive and curative effect on the teeth surfaces of albino rats fed simultaneously with 70% sucrose-in-diet.

Key words: Sucrose-in-diet, teeth, plaque, probe, induction, microbial

INTRODUCTION

Dental caries is characterized by demineralization of the mineral portion of enamel and dentine, followed by disintegration of their organic material. It has been attributed to a dieto-bacterial interaction on the tooth-surface which produces acids such as lactic acid and pyruvic acid. These acids lowers the pH to below 5 which is enough to dissolve or decalcify the enamel (Talaro and Talaro, 2002; Ingraham *et al.*, 2001; Johnson and Beighton, 1992). Although glucose, fructose and other sugars are cariogenic in varying degrees, sucrose has been described as the main cause of dental caries. While decreased prevalence of dental caries is reported in some cities in Brazil (Tagliaferro *et al.*, 2008), it is however on the increase among adolescents in South Western Nigeria (Umesi-Koleoso *et al.*, 2007) as well as in Australian children, after marked improvements in the 1990s (Armfield and Spencer, 2008). Increased cases among adults and the elderly has also been reported in New Delhi (Patro *et al.*, 2008).

Corresponding Author: Dr. I.R. Iroha, Department of Applied Microbiology, Faculty of Applied and Natural Sciences, Ebonyi State University, P.M.B. 053, Abakaliki, Ebonyi State, Nigeria
Tel: +234(0)8037089448

Dental caries can pose numerous challenges to the sufferers including general discomfort, inconvenience, expense, masticatory difficulty and alteration of the facial skeleton and associated musculature (Talaro and Talaro, 2002; Johnson and Beighton, 1992).

Herbal remedies has been employed since ancient period in the prevention and treatment of diseases including dental diseases. Although works on the antimicrobial activities of medicinal plants on dental caries has been widely reported especially in the western countries (Yamamoto and Ogawa, 2002; Addai *et al.*, 2002; Sato *et al.*, 2003; Linke and LeGeros, 2003), not much reports are available on the effect of locally used plants in Southeastern Nigeria. In this report, we present findings on the caries inductive capacities of different concentrations of sucrose-in-diet and the effect of plant extract on the induction of dental caries in experimental rats.

MATERIALS AND METHODS

Selection of Experimental Rats

Plaque samples were collected from the anterior teeth surfaces of eighty 8-10 week old albino rats weighing between 60-120 g. The samples were cultured in crystal violet blood agar and incubated at 37°C for 24 h (Collins *et al.*, 1995). The colonies were sub-cultured by the streak plate technique to obtain pure culture. The isolates were identified using standard methods as described by Chessbrough (2002). Male rats harboring *Streptococcus rattus* were randomly selected from the eighty for use in the experiment.

Collection and Identification of Plant Materials

The fresh leaves and tender stems of *Breynia nivosus* (ice plant) were obtained from Enugu, Enugu State, Nigeria and authenticated by a taxonomist from the Applied Biology Department, Ebonyi State University, Abakaliki. This study was conducted in 2003.

Extraction of Plant Materials

The plant materials were dried at room temperature and pulverized using mechanical grinder (Fondicion, Corona). Exactly 40 g of the macerated plant material was introduced into 200 mL of 95% ethanol and mixed thoroughly. The mixtures were allowed to stand for 24 h with constant shaking. Whatman's No. 1 filter paper was used to sieve the extract and later evaporated to dryness in a water bath at 40°C (Ibrahim *et al.*, 1997).

Preparation of Diet

Granulated sugar (sucrose) obtained from Ogbete market, Enugu, Enugu State, were combined with commercially available rat feed purchased from Nsukka, Enugu State, Nigeria. The combinations were respectively prepared to produce 70, 50, 30 and 10% sucrose concentrations in the feed.

Feeding of Rats with Different Sucrose Concentrations in the Diet

Fifteen rats selected as previously described were divided into five groups (A-E). Prior to the commencement of sucrose-in-diet feeding, the upper and lower anterior teeth surfaces of each rats were visually observed and also examined using dental probe and torch. Plaque sample were also collected from the upper and lower anterior teeth surface of each of the rats using sterile swab sticks and dental probe. The radiography of the lower anterior teeth of the rats was also taken. Group A rats were fed with 70% sucrose-in-diet, group B 50%, group C 30% and group D 10%. Group E was fed with normal diet without sucrose. The anterior teeth surfaces of the rats were observed on weekly basis for signs of early dental carries. At the observation of milky white spots on the anterior teeth surface of any of the rat groups, dental plaque samples were collected from all the rats as previously stated, for post microbial count. The radiograph of the lower anterior teeth of the rats were also taken.

Curative Effects of *Breynia nivosus* on Experimental Dental Caries

Twelve 8-10 weeks old male albino rats weighing 62-120 g and showing no signs of dental caries but harboring *Streptococcus rattus* on their teeth surfaces were divided into four groups of three each (A-D). Base-line information was obtained by examining the upper and lower anterior teeth surface of each rat visually and with dental probe and torch. Plaque samples from the upper and lower anterior teeth surfaces of the rats were cultured to establish the pre-microbial count. Also the radiographs of their lower anterior teeth were obtained. The rat groups A and B were fed daily with 70% sucrose-in-diet while groups C and D were given normal diet. On confirmation of early caries signs among the rat groups A and B, the administration of herbal extract commenced. About 200 mg mL⁻¹ of *Breynia nivosus* extract was administered *via* intra-oral route, on the anterior teeth surfaces of group A rats. However, while group A were given normal drinking water (containing 0.1 mg L⁻¹ of fluoride), group B rats were given water containing 0.7 mg L⁻¹ of fluoride. Also commenced was the feeding of group D rats with 70% sucrose-in-diet to serve as control. Thus, whereas rat groups, A, B and D were given 70% sucrose-in-diet, those of group C continued with normal diet. On observation of early signs of caries among rat group D, dental plaque samples from all the rats in all the groups were collected for post-microbial count. The teeth surfaces were also examined visually and with dental probe and torch. The radiograph of the lower anterior teeth of the rats were also taken.

Preventive Effect of *Breynia nivosus* on Experimental Dental Caries

Fifteen 8-10 weeks old male albino rats weighing 60-120 g without signs of dental caries but harboring *Streptococcus rattus* on their anterior teeth surfaces were divided into 5 groups (A-E). The baseline information was determined by the examination of the anterior teeth surface of each rat visually and with dental probe and torch. Plaque samples were also collected for pre-microbial count as previously stated.

Rat groups A and B were fed with 70% sucrose diet. About 200 mg mL⁻¹ of *Breynia nivosus* extract were administered intra-orally to the anterior teeth surfaces of rat group A once daily and group B twice daily. While group C rats were fed with normal diet without medicinal plant preparation, group D were fed with 70% sucrose-in-diet and water containing 0.7 mg L⁻¹ of fluoride. Rat group E however, were given 70% sucrose-in-diet only, without plant extract treatment or fluoride. Upon observation of early signs of dental caries in rat group E, plaque samples from all the rats in all the groups were collected for post-microbial count. Their teeth were also observed visually and with dental probe and torch. The lower anterior teeth of the rats were also subjected to radiographic examination.

Detection of Dental Caries

The early signs of dental caries were determined using direct observation, probing, microbial count and radiograph (Talaro and Talaro, 2002; Ingraham *et al.*, 2001). In the direct observation, the colour and texture of the surface of the upper and lower anterior teeth were examined visually and by using dental probe and torch. The translucencies were also determined with the aid of dental torch and mirror. The pre and post microbial plaque counts were determined using dental plaque samples collected from the anterior teeth of the rats prior to the commencement of the experiment and after, respectively. The plate count technique as described by Ingraham *et al.* (2001) was used to quantify the microbial content of the plaque samples.

The radiographic examination of the lower anterior teeth of the rats was conducted at the Radiography Department, Ebonyi State University Teaching Hospital, Abakaliki.

Statistical Analysis

The t-distribution was used to analyze the microbial counts obtained in this study.

RESULTS

Effect of Different Sucrose Concentrations on the Anterior Teeth Surface of Experimental Rats

The appearance of the upper anterior teeth surface of the rats given 70, 50, 30 and 10% sucrose-in-diet were not different from that of the control group given normal diet without sucrose. However, the lower anterior teeth surface of the rats given 70 and 50% sucrose in diet showed presence of milky white spots and were sticky on probing by the sixth week of observation. The translucency and stickiness of the lower anterior teeth surface of the rats reduced with decrease in sucrose concentration. Similarly, the roughness of the teeth surface (texture) also reduced in the rats with decrease in the sucrose concentration in diet.

There was a significant increase ($p < 0.05$) in the microbial count in the dental plaque of rat groups fed 70, 50 and 30% sucrose-in-diet. The microbial count of the rat groups fed with 10% sucrose-in-diet and those of the control group did not increase significantly ($p < 0.05$) (Table 1).

Furthermore, the radiograph of the rats fed with 70% sucrose-in-diet suggested areas of radiolucency compared with those that were given 50, 30 and 10% sucrose-in-diet respectively. There was no evidence of radiolucency observed for the control rat group.

Curative Effects of *Breynia nivosus* on the Anterior Teeth of Experimental Rats

The appearance and texture of the upper anterior teeth surface of the rat groups showing signs of early dental caries and given *Breynia nivosus* extract and simultaneously fed with 70% sucrose in diet (group A) were not different from the control group given normal diet. However, while the appearances of lower anterior teeth surface of the same rat group were not different from the control group given normal diet, on probing, they were generally slightly sticky. Similarly, the translucency and texture of the lower anterior teeth surface of the same rat group were slightly dull and slightly rough respectively. However, the lower anterior teeth of the rat group given only 70% sucrose in diet were sticky on probing and had milky white spots.

There was no significant difference ($p < 0.05$) between the initial microbial count and the count at the end of the experiment for rat given *Breynia nivosus* and fluoride as well as the control group (Table 2). No sign of radiolucency was observed among the rat group showing early caries signal and given *Breynia nivosus* and 70% sucrose-in-diet as well as those given fluoride and the control group. However, the radiograph of those given 70% sucrose diet only, suggested possible area of radiolucency.

Table 1: Effect of different sucrose concentrations on the microbial count of albino rats

Sucrose concentrations (%)	Mean microbial counts ($\times 10^4$ cells mL ⁻¹)	
	Before sucrose admin	After sucrose admin
70	7.07 \pm 1.61 ^a	4666.67 \pm 1527.53 ^b
50	7.00 \pm 2.65 ^a	33.33 \pm 929.16 ^b
30	7.17 \pm 1.76 ^a	25.00 \pm 5.00 ^b
10	7.40 \pm 0.79 ^a	26.30 \pm 23.26 ^a
Control (no sucrose)	7.57 \pm 6.45 ^a	4.03 \pm 0.55 ^a

^{ab}Different alphabetical superscripts in a row indicates that the means are significantly different at the probability level $p < 0.05$ but similar superscripts in a row indicate a lack of significant difference between the means ($p < 0.05$)

Table 2: Effect of plant extracts and fluoride on the microbial counts of albino rats showing early signs of caries

Treatments	Mean microbial counts ($\times 10^4$ cells mL ⁻¹)	
	Before treatment	After treatment
<i>Breynia nivosus</i>	504.00 \pm 461.00 ^a	23.50 \pm 20.50 ^a
Fluoride	2930.00 \pm 4570.00 ^a	11.70 \pm 15.80 ^a
Control 1 (normal feed)	7.57 \pm 6.45 ^a	4.03 \pm 0.55 ^a
Control 11 (70% sucrose only)	4.83 \pm 3.01 ^a	1970.00 \pm 2630.00 ^a

^{ab}Different alphabetic superscripts in a row indicate that the means are significantly different at the probability level $p < 0.05$, but similar superscripts in a row indicate a lack of significant difference between the means ($p < 0.05$)

Table 3: Effects of plant extracts and fluoride on the microbial counts of caries-free albino rats

Treatments	Mean microbial counts ($\times 10^4$ cells mL ⁻¹)	
	Before treatment	After treatment
<i>Breynia nivosus</i> once daily +70% sucrose	4.67±1.94 ^a	5.77±8.04 ^a
<i>Breynia nivosus</i> twice daily+ 70% sucrose	4.00±1.00 ^a	6.44±5.59 ^a
Fluoride	7.57±6.45 ^a	4.03±0.55 ^a
Control 1 (70% sucrose only)	4.83±3.01 ^a	1966.67±2631.22 ^a
Control 11(no sucrose)	28.10±36.30 ^a	1.01±0.96 ^a

^{ab}Different alphabetical superscripts in a row indicate that the means are significantly different at the probability level $p < 0.05$, but similar superscripts in a row indicate a lack of significant difference between the means ($p < 0.05$)

Preventive Effect of *Breynia nivosus* on the Anterior Teeth of Experimental Rats

The appearance, translucency and texture on probing of the upper and lower anterior teeth surface of caries-free rat groups given simultaneously 70% sucrose-in-diet and *Breynia nivosus* once and twice daily were not different from those of the control group given normal diet and no medicinal plant preparation. The upper and lower anterior teeth surface of the rat group given fluoride were grayish-white in colour. However, those of the rat group given only 70% sucrose-in-diet were sticky on probing and had milky white spots.

There was no significant difference between the pre and post microbial count ($p < 0.05$) of the caries-free rats given 200 mg mL⁻¹ *Breynia nivosus* once and twice daily. Similarly, no significant difference was observed among the caries-free rats given simultaneously 70% sucrose-in-diet and fluoride as well as the control group given normal diet (Table 3).

Furthermore, there was no sign of radiolucency observed for the caries-free rat group given *Breynia nivosus* once and twice daily as well as those given fluoride and normal diet. However, the radiograph of the rats given 70% sucrose-in-diet only, suggested area of radiolucency.

DISCUSSION

The observation of milky-white spots on the lower anterior teeth surfaces of rat group fed 70% and 50% sucrose-in-diet for six weeks as well as their dull light reflection and rough texture in this work is noteworthy. Ooshima *et al.* (1983) reported the development of severe dental caries when specific pathogen-free rats infected with *Streptococcus mutans* was fed with a diet containing 56% sucrose. Further, Folayan *et al.* (2008) stated that children population who consumed sugar once a day were at risk of developing caries. Several studies have implicated sugared beverage and drinks (Levy *et al.*, 2003; Declerk *et al.*, 2008) in the presence of poor oral hygiene to contribute substantially to caries. Sugared free gum has been reported to significantly slow progression of approximal caries (Morgan *et al.*, 2008).

That the level of stickiness on probing decreased with decrease in the concentration of sucrose in the diet was not unexpected since the more the sucrose, the more the possibility of acid production which eventually demineralizes the tooth surface. Stickiness has been described to be an indicator of caries development. The discovery of a significant increase in *Streptococcus rattus* content of the plaque samples collected from the rats fed respectively with 70, 50 and 30% sucrose-in-diet in this study indicate possible development of dental caries. Previous studies has shown that *S. mutans* regularly increased at the sites that subsequently becomes carious especially in the presence of sucrose-in-diet (Loesche *et al.*, 1984; Staat *et al.*, 1975; Linossier *et al.*, 2003). It is not surprising however, that the rat group fed with 30% sucrose-in-diet in this study, though had significant increase in their *S. rattus* content, did not produce visible early caries signs after six weeks. This is probably

because, caries is a multifactorial disease and thus other factors such as sugar source, flow rate and composition of saliva and oral hygiene may come into play for caries initiation (Ingraham *et al.*, 2001; Johnson and Beighton, 1992).

The observation in this study of the absence of white spot lesion and presence of slightly sticky and rough lower anterior teeth of rat group given simultaneously extract of *Breynia nivosus* and 70% sucrose-in-diet is notable. Although may not be suggestive of absolute curative potential of the plant material, the fact that white spots lesions were absent at the end of the 6 weeks may indicate some level of early caries curative property. This was further corroborated by the fact that there was a drastic reduction in the microbial count compared with the rat group given only 70% sucrose-in-diet. A previous work reported that the feeding of *Streptococcus mutans* infected rats with polyphenol compound (designated Sunphenon) isolated from the leaf of *Camellia sinensis* reduced caries incidence (Saito, 1990). Also, fluoride containing dentifrices tested in another study reduced caries in rats (Guggenheim *et al.*, 1997). Further research in the area of isolation, identification and characterization of the active principles in the plant material used in this study is hereby advocated. The *in vitro* activity of *Breynia nivosus* on dental caries organism has been previously reported (Amadi *et al.*, 2007). Vanka *et al.* (2001) also reported the inhibitory effect of *Azadirachta indica* in *S. mutans* and its incipient caries reversing potential.

Furthermore, that the group of caries-free rats given *Breynia nivosus* extract generally did not show observable white spot lesions, reflected light brightly, had smooth texture and non-sticky anterior teeth surfaces is of significance given that the rats were all fed simultaneously with 70% sucrose-in-diet daily. This possibility of *in vivo* preventive effect of *Breynia nivosus* extract was further presented by the radiography of the rats given the plant extract which showed no signs of radiolucency compared with the control rat group given only 70% sucrose-in-diet. Addai *et al.* (2002) reported that brief chewing of *Garcinia manii* chewing stick confers a caries preventive/control benefit by reducing the salivary pH after glucose rinse. Also, Linke and LeGeros (2003) reported that frequent intake of black tea can significantly decrease caries formation, even in the presence of sugars in the diet. The result of the preventive study in the present work indicates that, this plant material, whether given once or twice daily, could exert some caries preventive effect on rats simultaneously fed with 70% sucrose-in-diet.

While the result of this study justifies the local use of this plant in the South-Eastern Nigeria in the treatment of dental diseases, it does appear that *Breynia nivosus* possesses more preventive effect than curative.

ACKNOWLEDGMENTS

The assistance of Dr. Okeke Clement of the Department of Botany, Nnamdi Azikiwe University Awka and the association for the identification, conservation and utilization of medicinal plants of Nigeria (ASICUMPON) in the recommendation and identification of the medicinal plant used in this study, is hereby appreciated.

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