

RESEARCH ARTICLE

Effect of pineapple core extract on growth and saliva stimulation in Stunted Rats

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ABSTRACT

Stunting refers to a condition of short stature in toddlers due to delayed growth from the prenatal period until the age of 24 months. Chronic malnutrition in stunted toddlers leads to protein deficiency; therefore, active substances are needed for maximum protein absorption. Pineapple core (*Ananas comosus*) contains bromelain enzymes and citric acid, which can enhance protein absorption in conditions of chronic malnutrition and increase saliva flow rate. This study aims to investigate the effect of pineapple core extract on growth parameters and saliva flow rate in stunted Wistar rats. This study involved 16 male Wistar rats, which were divided into four groups: a negative control group (no treatment), a treatment group (75% pineapple core extract), a positive control group 1 (FeSo4 supplements), and positive control group 2 (pilocarpine hydrochloride). Growth parameters and saliva flow rate were measured before and after pineapple core extract treatment. The research results were analyzed using one-way ANOVA followed by Tukeys test, which showed a significant difference between the treatment group and the other groups in body weight ($p = 0.037$), body length ($p = 0.028$), tail length ($p = 0.003$), and saliva flow rate ($p = 0.000$). Administration of 75% pineapple core extract resulted in increased growth parameters (body weight, body length, and tail length) and saliva flow rate in stunted rats.

Keywords: body length; body weight; bromelain enzyme; saliva flow rate; tail length

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INTRODUCTION

Growth failure in children under five caused by chronic malnutrition can result in shorter stature compared with children of the same age.¹ Stunting is a condition of growth delay (growth faltering) due to malnutrition from pregnancy to 24 months of age.² Conditions of growth failure in children under five as a result of chronic malnutrition can also lead to shorter stature compared to children of the same age.² Key preventive efforts include ensuring adequate food intake in terms of quantity and quality in children aged 2-5 years, exclusive breastfeeding for the first two years of life, and targeted nutritional interventions during the first 1,000 days of life.^{3,4}

Malnutrition in stunted children can lead to protein deficiency. Protein deficiency may reduce the activity of several enzymes, such as protease

enzymes, which can impair growth.^{5,6} Pineapple (*Ananas comosus*) contains bromelain enzymes, carbohydrates, fiber, and essential minerals, particularly Ca, P, Fe, Na and K. Pineapple also contains various vitamins, including vitamins A, B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic), B6 (pyridoxine), B9 (folate), and C (ascorbic acid).⁷ This study proves that administration of 75% pineapple core extract increases growth parameters, including body weight, body length, and tail length of stunted rats. The pineapple core contains the highest concentration of bromelain, which functions as a proteolytic enzyme to maximize protein absorption when consumed with other foods.^{8,9}

Stunting also affects the the oral cavity, particularly through atrophy of the salivary glands, resulting in reduced saliva production compared

with that of normal children.¹⁰ Previous study on epidemiology has shown that the average saliva flow rate (LAS) in stunted children (under five years) was 0.29 ml/minute, which falls into the very low category, while the average saliva flow rate for normal children was 0.90 ml/minute, classified as low. This difference in saliva flow rate between stunted and normal children is related to the condition of salivary gland atrophy in stunted children, leading to lower saliva secretion than that in normal children.⁵

A decreased saliva flow rate compromises the optimal function of saliva in maintaining oral health, including maintaining ecological balance, lubricating and protecting the oral cavity, buffering and cleansing the oral cavity, maintaining tooth integrity, and providing defense against bacterial activity.³ Previous research found that the majority of stunted children exhibited acidic salivary pH, primarily due to reduced saliva flowrate, which decreases the saliva's buffering capacity and leads to lower pH levels. A decrease in salivary pH can lead to demineralization of hard tooth tissue, which is closely related with dental caries. Reduced salivary secretion also impairs the function of saliva as a solvent, buffer, and anti-bacterial agent.¹¹ Pineapple core also contains citric acid, which increases salivary secretion.¹² This study proves that there is an increase in saliva flow rate in stunted rats following administration of 75% pineapple core extract. In line with these findings, research by Wanda et al. reported that consumption of pineapple fruit (*Ananas comosus*) influenced salivary secretion in elderly individuals with xerostomia.¹³

Similar studies remain limited, particularly in stunted populations, despite the relevance of pineapple core extract content in supporting nutritional improvement in stunted patients. In this study, local natural ingredients from Aceh were utilized, namely pineapple stem extract from Central Aceh, which has not been widely investigated. Therefore, this study aimed to explore the potential of pineapple core extract from Central Aceh in growth enhancement and saliva flowrate in stunted rats. The research question was whether pineapple core extract (*Ananas comosus*) has the potential to increase growth and saliva flow rate in

stunted rats? It was hypothesized that pineapple core extract (*Ananas comosus*) could increase growth and saliva flow rate in stunted male Wistar rats. Accordingly, the present study was conducted to investigate the effect of pineapple core extract on growth parameters and saliva flow rate in stunted Wistar rats and to compare its effects and those of other supplements used as positive controls across treatment groups.

MATERIALS AND METHODS

This study used a completely randomized design with 16 experimental units were obtained. The research was conducted from December 2023 to January 2024, at several locations, including the Natural Materials Pharmacy Laboratory for the preparation of pineapple core extract and the Animal Experiment Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University, for administering extracts and measuring growth and saliva flowrate. The research samples used in this study were pineapple core extract and 16 male Wistar rats. Ethical approval for this study was obtained from the Animal Care Committee of Syiah Kuala University (approval number: 183/KEPH/XI/2023).

The pineapple core was thoroughly washed and thinly sliced, then dried in an oven at 40–60°C for 24 hours. Following this, the pineapple core was extracted using the maceration method with 96% ethanol for seven days.¹⁴ Filtration was performed using filter paper, and the resulting extract was collected, concentrated, and evaporated.¹⁵ Evaporation was carried out at 45–50°C using a rotary evaporator until all solvent had evaporated, yielding a thick pineapple core extract.¹⁴ The extract was then diluted to a 75% concentration using the following dilution formula:

$$V1 \times C1 = V2 \times C2$$

Description:

V1: Initial solution volume

C1: Initial solution concentration

V2: Volume of solution after dilution

C2: Concentration of solution after dilution

Sixteen male Wistar rats (7 weeks old) were obtained from the Experimental animal center, Faculty of Veterinary Medicine, Syiah Kuala University, Aceh. The rats were retained under controlled environmental conditions, including a temperature of 23 ± 2 °C, relative humidity of $50 \pm 5\%$, and a 12-hour light–dark cycle. Rats were acclimatized for ten days prior to the experiment. During this period, they were kept in sanitized polypropylene cages containing sterile husk as bedding with free access to standard pellets as basal diet and water ad libitum. All procedures were approved by the Animal Care Committee of Syiah Kuala University (183/KEPH/XI/2023).

After the acclimatization period, the rats were randomly divided randomly into four groups. Group 1 (negative control, $n=4$) received a normal diet without treatment. Group 2 (intervention group, $n=4$) received 75% pineapple at a dose of 0.07 g/day. Group 3 (positive growth control, $n=4$) received FeSO₄ supplement. Group 4 (positive saliva control, $n=4$) received intraperitoneal injections of pilocarpine hydrochloride at a dose of 0.06 mg/kg body weight during saliva flowrate measurement after treatment. Body weight and food consumption for each group were recorded weekly throughout the experimental period of two weeks.

Growth measurements were conducted twice, before and after treatment with pineapple core extract. Prior to the measurement, rats were anesthetized

using a combination of Ketamine Hydrochloride and Xylazine administered intramuscularly into the hamstring muscles. Following anesthesia, growth measurements were performed. Rat growth was measured by measuring body weight, body length, and tail length.^{16,17} Body weight was measured using a Camry EK2150K digital scale.¹⁸ Body length was measured using a Kenko stainless steel ruler (cm), by measuring the length from the tip of the nose to the anus.¹⁹ Tail length was measured using a ruler in centimeters.¹⁶ Growth measurements were carried out in groups 1, 2 and 3.

Before saliva collection, rats were anesthetized using a combination of Ketamine hydrochloride and Xylazine.²⁰ Saliva flow rate was, then, measured using cotton pellets. Prior to placement in the oral cavity, cotton pellets were weighed using an analytical balance (Osuka). The pellets were placed in the rat's oral cavity.²¹ Rats were positioned at a 10° forward against the table. Saliva collection was performed for 15 minutes.²²

Total saliva volume was calculated based on the difference in cotton pellet weight before and after saliva collection.²¹ In the positive control group, pilocarpine hydrochloride (0.06 mg/kg body weight) was administered intraperitoneally during saliva flow rate measurement.²¹ Saliva flow rate measurements were conducted in groups 1, 2, and 4. Data were analyzed using one-way analysis of variance (ANOVA) with a significance level of 95%. When significant differences were observed, Tukey's Honest Significant Difference (HSD) post hoc test was applied.

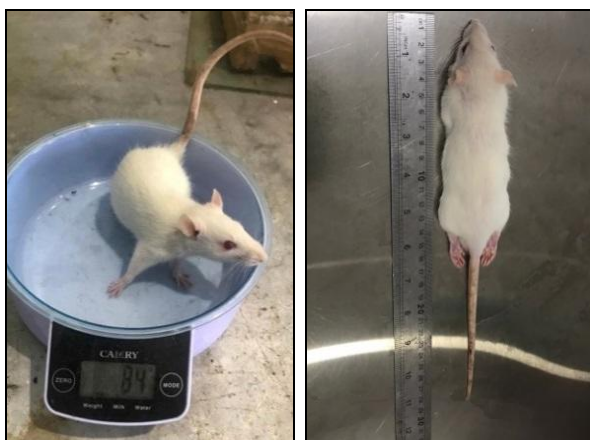


Figure 1. Growth measurement: body weight weighing, body length measurement, and tail length measurement

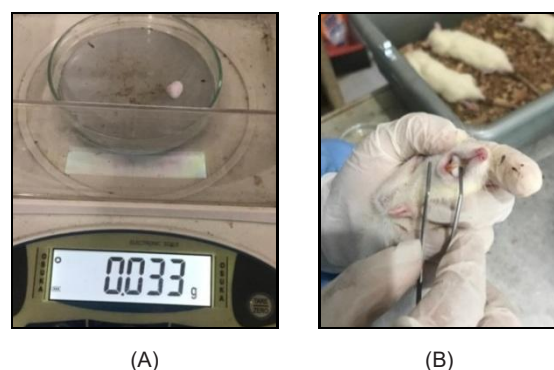


Figure 2. Saliva measurement (A) Cotton pellet weighing (B) Saliva flowrate measurement

RESULTS

One-way ANOVA results for body weight and body length showed p-values of 0.037 and 0.028, with effect sizes of 0.686 and 0.549, respectively, indicating significant differences in growth improvement among groups. Statistical analysis of tail length demonstrated a p-value of 0.003 with an effect size of 0.733, indicating a significant difference in tail length increase among groups. Tukey's post hoc test revealed significant differences between groups 1 and 3 ($p = 0.002$, $p < 0.05$) and between groups 2 and 3 ($p = 0.043$,

$p < 0.05$). However, no statistically significant difference was observed between groups 1 and 2.

One-way ANOVA analysis of saliva flow rate showed a significant difference among treatment groups ($p = 0.000$, $p < 0.05$) with an effect size of 0.8543. Tukey's test indicated significant differences between groups 1 and 3 ($p = 0.000$, $p < 0.05$) and between groups 2 and 3 ($p = 0.001$, $p < 0.05$). No statistically significant difference was found between groups 1 and 2. These results demonstrate that pineapple core extract administration influenced saliva flow rate in stunted male Wistar rats.

Table 1. Growth improvement in body weight (gr)

Sample	Body weight (gr)			p-value
	Group 1	Group 2	Group 3	
Rat 1	30	32	44	0.037
Rat 2	40	48	47	
Rat 3	28	45	45	
Rat 4	36	58	42	
Average	33.5	45.75	44.5	

Sig. < 0.05

Table 2. Growth improvement in body length (cm)

Sample	Body length (cm)			p-value
	Group 1	Group 2	Group 3	
Rat 1	0	0	0.3	0.028
Rat 2	0	0	0.7	
Rat 3	0	0.3	0.4	
Rat 4	1	1.2	1	
Average	0.25	0.375	0.6	

Sig. < 0.05

Table 3. Growth improvement in tail length (cm)

Sample	Tail length (cm)			p-value
	Group 1	Group 2	Group 3	
Rat 1	0.5	0.5	1.4	0.003
Rat 2	0.5	1	1.5	
Rat 3	0.7	0.7	1.4	
Rat 4	0	1	1	
Average	0.425	0.8	1.325	

Sig. < 0.05

Table 4. Increase in saliva flow rate (mg/20 mins)

Sample	Saliva flow rate (mg/ 20 mins)			p-value
	Group 1	Group 2	Group 3	
Rat 1	3	33	277	0.000
Rat 2	4	23	244	
Rat 3	1	59	263	
Rat 4	2	152	184	
Average	2.5	66.75	234.5	

Sig. < 0.05

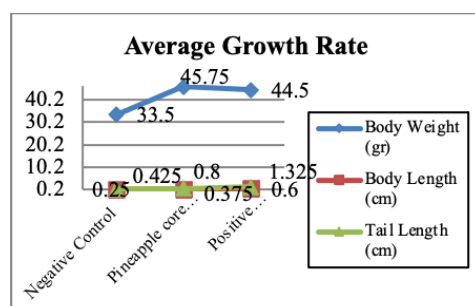


Figure 3. Diagram of average growth increase of stunted male Wistar rats

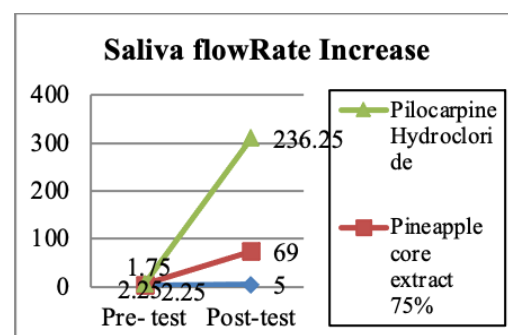


Figure 4. Diagram of average increase in saliva flowrate

Based on the average saliva flowrate diagram (Figure 4), the positive control group exhibited the highest saliva flow rate, followed by the treatment group, while the lowest value was observed in the negative control group. The highest saliva flow rate in the positive control group was due to the presence of salivary stimulating agent, pilocarpine.

DISCUSSION

In the present study, pineapple core, one of the major by-products of pineapple processing, was utilized as a source of bromelain. Pineapple core is often discarded as waste or used for animal feed, despite containing higher concentrations of bromelain compared to other pineapple residues and accounting for approximately 15% of total processing waste.²³ This study demonstrates that administration of 75% pineapple core extract significantly improves growth parameters (body weight, body length, and tail length) and saliva flow rate in stunted male Wistar rats. These findings highlight the dual nutritional and oral health potential of pineapple core extract, particularly in the context of chronic malnutrition-related growth impairment and salivary gland dysfunction.

Stunting is strongly associated with chronic protein and micronutrient deficiencies that impair linear growth, muscle mass accretion, and organ development. Protein deficiency leads to reduced synthesis of growth-related enzymes and hormones, ultimately limiting catch-up growth.²⁴ In this study, rats receiving pineapple core extract exhibited greater increases in body weight compared to negative controls and comparable improvements to the FeSO₄ positive control, suggesting that pineapple core extract supports growth through mechanisms beyond micronutrient supplementation alone. In line with findings by El-Shazly et al., pineapple administration has been shown to influence body weight in Wistar rats. Additionally, pineapple has been reported to suppress high-fat diet-induced obesity by reducing serum lipid levels and fat-binding capacity, indicating potential metabolic regulatory effects.²⁵ Moreover, pineapple core contains

carbohydrates, essential minerals (Ca, P, Fe, K), and vitamins that synergistically support energy metabolism and anabolic processes. While FeSO₄ supplementation directly targets iron deficiency and supports linear growth, pineapple core extract offers a broader nutritional profile combined with enzymatic activity, which may explain the relatively higher body-weight gain observed in the treatment group.

The observed growth enhancement can be partly explained by the **high bromelain content** of pineapple core. Bromelain is a proteolytic enzyme complex capable of hydrolyzing dietary proteins into smaller peptides and free amino acids, thereby improving intestinal protein digestibility and absorption.²⁶ Enhanced amino acid availability is critical for albumin synthesis, hemoglobin formation, and tissue growth, particularly in malnourished conditions. Previous studies have shown that bromelain supplementation increases serum albumin and hemoglobin levels in protein-energy malnutrition models, supporting its role in initiating catch-up growth.^{5,6} Similarly, research by Lufiasari et al. reported the effectiveness of MODISCO supplementation in increasing body weight of malnourished toddlers.²⁷ Adequate growth requires nutrients in sufficient and balanced amounts. The influence between nutrients and growth can be influenced by the low amount of substances consumed and the low content of microelements in food.¹⁶ Other factors that affect growth in rats include eating habits, energy intake, and energy expenditure.²⁸

At the biochemical level, bromelain functions by cleaving peptide bonds, reducing protein complexity, and facilitating enzymatic synergy with endogenous proteases such as pepsin and trypsin. In malnourished states, endogenous digestive enzyme production is often reduced; thus, exogenous proteolytic enzymes like bromelain can compensate for impaired digestive capacity.²⁹ Improved protein absorption enhances nitrogen retention, supports muscle protein synthesis, and contributes to skeletal growth, as reflected by increases in body and tail length observed in this study.

A key finding of this study is the significant increase in saliva flow rate following pineapple core extract administration. Salivary gland atrophy and reduced salivary secretion are well-documented consequences of chronic malnutrition and stunting. The salivary stimulation observed in the treatment group is primarily attributed to the citric acid content of pineapple core. Citric acid acts as a gustatory stimulant that activates chemoreceptors in the oral cavity, triggering parasympathetic reflexes that enhance salivary gland secretion.¹⁵ Previous studies have shown that there is an increase in the rate of saliva flow in the elderly suffering from xerostomia due to the content of citric acid in pineapple fruit even compared with other fruit.¹³ Furthermore, higher concentrations of citric acid are associated with greater increases in salivary volume compared with lower concentrations.³⁰

Beyond citric acid, bromelain may also contribute indirectly to improved salivary gland function. By enhancing protein absorption and reducing systemic inflammation, bromelain may support the structural and functional recovery of salivary glands affected by malnutrition. Improved nutritional status is known to restore glandular tissue integrity, which may explain the observed increase in salivary output in stunted rats. Although pilocarpine produced the highest saliva flow rate due to its direct muscarinic receptor agonism, pineapple core extract demonstrated a substantial physiological effect without pharmacological intervention.³¹

The findings of this study have important implications for stunting management, particularly in low-resource settings. Stunting interventions often focus primarily on macronutrient and micronutrient supplementation, while oral health consequences receive less attention. The demonstrated ability of pineapple core extract to simultaneously improve growth and saliva production suggests a novel, food-based adjunctive strategy that addresses both systemic and oral manifestations of chronic malnutrition.³²

Several limitations should be considered when interpreting these findings. First, the small sample size limits the generalizability of the results

and increases the risk of type II error. Second, the short intervention duration (two weeks) may not fully capture long-term growth trajectories or sustained salivary gland recovery. Additionally, the use of an animal model limits direct extrapolation to human stunted children, whose growth and oral health are influenced by complex socioeconomic and environmental factors. Comparative studies evaluating different doses of pineapple core extract and its combination with standard nutritional therapies are also warranted. Ultimately, clinical trials in stunted children are needed to assess safety, efficacy, and feasibility of pineapple core extract as a complementary nutritional and oral health intervention.

CONCLUSION

There was a significant increase in growth including body weight, body length, tail length and saliva flow rate in stunted male Wistar rats following administration of 75% pineapple core extract compared with the control group. These findings suggest that pineapple core extract may serve as a potential therapeutic candidate for stunting when administered at controlled doses. Further studies are required to evaluate its application and effectiveness in human therapy.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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