

Cinnamon Extract Effect on Osteoblast Activity in Diabetic Wistar Rats

Fahrin Ramadan Andiwijaya¹, Fundhy Sinar Ikrar Prihatanto², Hermawan Susanto³

¹ Faculty of Medicine, Airlangga University

² Anatomy and Histology Department, Faculty of Medicine, Airlangga University

³ Endocrine and Metabolism Division, Internal Department RSUD dr. Soetomo, Airlangga University

*Corresponding: E-mail: fahrin.andiwijaya@gmail.com

Background: Diabetes is clinically known to cause prolongation to bone fracture healing. This research aims to find the effect of cinnamon in the process of bone remodeling, and changes in the activity of osteoblast cells in diabetic bone after giving cinnamon as a supplement in diabetic rats. **Method:** The design employed was experimental with *randomized post-test group design research*. A total of 24 Wistar Rats was randomly divided into four groups, which consist of normal without treatment, normal with treatment, diabetic without treatment and diabetic with treatment. Treatment of 300 mg/kg cinnamon extract was given per-orally. Upon euthanizing the samples, femur samples were taken and processed to histopathological slides. All slides were analyzed under light microscopes to find the osteoblast cells. The Kruskal-Wallis method was used to test the results; due to the population of sample was not normally distributed. **Results:** The osteoblast cells found were scored in mean ranks. The normal group mean rank 12.25, normal with treatment group 12.17, diabetic without treatment group 8.58, and diabetic with treatment group 17.00. Asymptotic significance was 0.195. **Conclusion:** This research concludes that there is no significant increase in osteoblast activity in diabetic Wistar rats after the administration of 300 mg/kg cinnamon extract.

Keywords: Diabetes, Wistar Rats, Cinnamon, Histopathological, Osteoblast Cells.

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INTRODUCTION

Globally, 347 million people have diabetes, whether it is type 1 or type 2 diabetes.¹ The global prevalence of diabetes was 8.3% and from this data, approximately there will be one in twelve persons who has diabetes.² A research conducted in Indonesia, shows the prevalence of diabetes among the Indonesian population is 2.1% compared to other non-communicable diseases (RISKESDAS 2013).³

Untreated diabetes increases the possibility of developing further health complications.² Furthermore, diabetic patients show prolongation in bone fracture healing time.⁴ In further notice, based on RISKESDAS 2013, bone fractures were ranked fourth highest on the prevalence rate of injury types with 5.8%.³

The Indonesian National Diabetes Management suggests the attainment and maintenance of fasting blood glucose (FBG) <100 mg/dL.

Correspondence author:

Fahrin Ramadan Andiwijaya, Jalan Jendral
Sudirman Gang Solor Nomor 3 Rembiga,
Mataram, NTB. +6282336069091.
Fahrin.andiwijaya@gmail.com

However, according to a research in Indonesia in 2008, 47-69% of type 2 diabetes mellitus patients did not achieve the suggested levels of FBG.⁵

Natural remedies such as cinnamon has been vastly used as supplementation for diabetes, countries such as China, Korea and Russia commonly use this type of herb for diabetes.⁶ A research conducted by Kim et al, (2005)⁷ showed that by giving cinnamon extract can increase the serum insulin level and provides a significant difference to the control group that does not receive any extra supplementation. Polyphenol as one of the active substances in cinnamon plays a role in activating insulin receptors and decreasing phosphatase activity that inactivates insulin receptor.⁸ Moreover, insulin is one of the key molecular link between bone remodeling and energy metabolism, this is achieved by insulin signaling to the osteoblast by favoring osteocalcin decarboxylation.⁹

MATERIALS AND METHODS

Animal model

This study was ethically approved by the Ethical Board Airlangga University. A total of 26 male Wistar Rats were used as the sample. Wistar

rats represents a close homology to human type-1 diabetes, and has been used for studies on the effect of diabetes on bone density. The rats were randomly divided into four groups, with equal amount in each groups, and the groups are normal without treatment, normal with treatment, diabetic without treatment, and diabetic with treatment. For diabetic groups, every rats were given streptozotocin injection. Streptozotocin (STZ) injection is a toxin that can damage the pancreatic β cells, thus inducing a diabetic condition to the rat. The amount of STZ induced was 50mg/kg the weight of the rats, dissolved with 10% sucrose and dextrose each.¹⁰ Blood glucose levels of the diabetic group were checked two day post injection of STZ.

Cinnamon Extract

The cinnamon extract used in this research was processed at the pharmacology laboratory, Airlangga University. There were two groups that received the treatment, each from the non-diabetic

and the diabetic group. Extracts for a minimum of 300 mg/kg were given by using an oral gavage.

Histopathological Examination

The analyzed rats were euthanized on the 10th day, and the bone tissues of the femur was taken from the right leg of the euthanized rats, subsequently, processed by Sodium Citrate decalcifying agents making it possible to be sliced into slides and then stained in hematoxylin and eosin staining. Upon completion of histopathological staining, each specimen was analyzed under a light microscope and a clinical pathologist recorded the total amount osteoblast cells in total of every field of view.

RESULT

Histopathological results of the femur bone taken from each groups of Wistar rats. The green arrows show an active osteoblast in the bone are shown in Table 1.

Table 1. Histopathological results of the femur bone taken from each groups of Wistar rats. The green arrows show an active osteoblast in the bone.

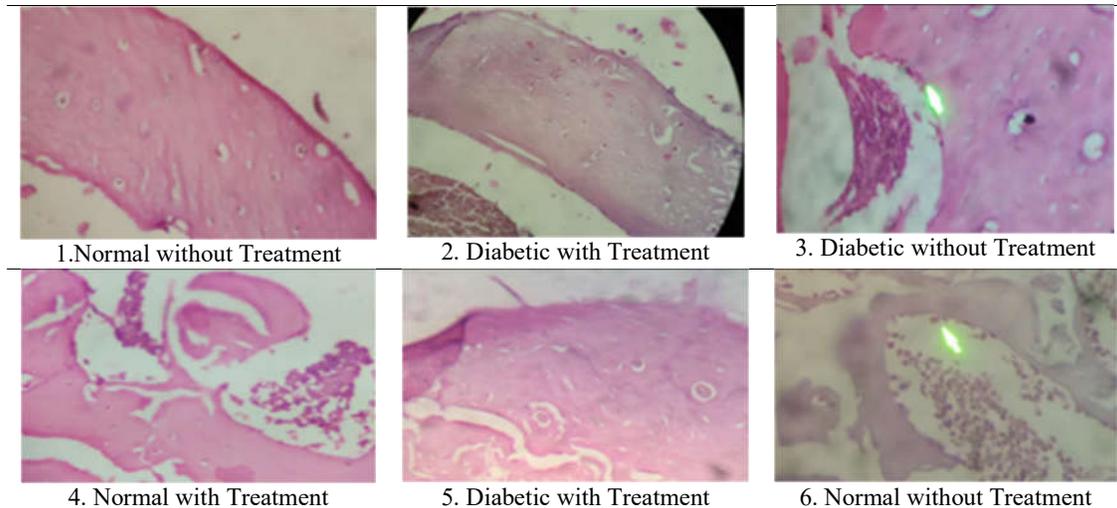


Table 2. Results showing the amount of osteoblast in each group, also comparison between each mean amounts of each group. Diabetic without treatment shows the highest mean, however with a high amount of standard deviation, Diabetic with Treatment shows more activity compared to Normal with Treatment.

Rat Number	Number of Osteoblast			
	Control	ND+T	D	D+T
1	0	2	0	3
2	0	2	0	4
3	4	3	0	1
4	2	0	0	2
5	8	0	46	2
6	0	2	0	12
Mean \pm SD	2.33 \pm 3.20	1.5 \pm 1.22	7.66 \pm 18.77	4.00 \pm 4.04

In comparison towards each group, the control group reached a mean of 2.33 ± 3.20 , only

higher than the normal with treatment group 1.5 ± 1.22 , this group also scored the lowest mean. The

highest mean number was achieved by the diabetic group; however, the standard deviation number is also high. In the diabetic group, osteoblast activity is only seen in the fifth sample, where it is noted 46 active osteoblasts. On the other hand, the diabetic with treatment group scored second highest, and in all sample osteoblast activities are seen. This group scored the second highest mean numbers, with 4.00 ± 4.04 .

Analysis of Research Results

Previous research showed evidence that diabetes clinically affects the progression of bone healing, where insulin impairment results in the decrease of osteoblast activity.⁴ This research was intended to prove giving cinnamon extracts as supplementation would improve this condition. Results showed the diabetic treated group has a higher mean score compare to both non-diabetic group, where the mean score of the diabetic treated group is 4.00 ± 4.04 . This shows the supplement given could have a direct effect on the dependent variable. However, this result was not supported by the statistical significance calculation. The result of the statistical analysis using the Kruskal-Wallis method was 0.195 or (>0.01).

DISCUSSIONS

It is well known that diabetes is a gateway to other complications and if left untreated, one will have higher possibilities in developing further complication and one of which is prolonged bone healing time due to disrupted signaling pathways of insulin to the osteoblast cells. Result shows, the lowest mean rank goes to the diabetic group without treatment by scoring 8.58. This is supportive to previous research, where it was suggested that insulin impairment in diabetic in-vitro model reduces osteoblast activity.¹¹

The treated diabetic group showed positive suggestion and is in-line with the hypothesis. It should be considered that the highest mean rank amount of osteoblast is the diabetic treated group with (17.0), which supports the postulate where cinnamon works in various way of boosting insulin signaling to increase the activity of osteoblast¹² and may also give direct effect to the cell itself.

This research showed indications of irregularities, which is possibly caused from either procedural or unexpected errors. One perspicuous example is the number osteoblast found in the 5th sample, which is significantly higher than other sample of the same group. One possible explanation was that the fifth sample could have developed other complication due to streptozotocin, as it is known diabetes could lead to other metabolic syndrome and nephrotic syndromes.¹³ Furthermore, it was suggested that nephrotic syndrome could increase osteoblastic activity.¹⁴ Another plausible explanation is that the 5th sample underwent

unexpected inflammation, where previous research have shown a resolving inflammation can cause an increase in osteoblast activity and function.¹⁵

Methods such as giving the supplementation by an oral gavage tube can increase stress levels of the rats thus could interfere with desired results in the blood glucose levels. As studies shows, blood glucose levels in the majority of type 1 diabetics, were raised due to the increase of stress.¹⁶

Assessments on the issues towards the results may suggest that homogeneities among subject should be stricter, to prevent detrimental confounding factors that could interfere the results. However, prevention of other complication that could impede desired outcomes are more often hard to attain, since it is hard to deliver routine checkups on the subjects.

Limitations of the Research Model

The main subject in this research was Wistar Rats, and it is known that this animal shows homogeneity to human and its intended pathological condition, which in this case is diabetes thus making this subject a reliable source of data.¹⁷ The raw Cinnamon in this research has certification by the National Health Department for its content and nutritional facts, by this the authenticity of the raw Cinnamon materials are proven to be trustworthy.

Several real-time limitations in this research are methodological, and the main limitation is the total population observed. By rooting from previous researches, each group required a minimal of 6 samples. The implication was felt with the normality of distribution, and insignificance in each results when compared. If more samples were included, there is a high probability that result could show higher significance, by hindrance of faulty results. Further concerns may rise from the bone processing where the length of decalcifying and the substance used in the procedure could be a major contributor to errors if not treated correctly. The Sodium Citrate is faster in decalcifying, in other words stronger than other chelating agent such as EDTA¹⁸, meaning, if processed too long more minerals would disappear thus making it more prone to annihilate the bone cells. As suggested before another option would be to use EDTA as the decalcifying agent, however with EDTA it would require more time to reach the level where the preparations can actually be processed.

CONCLUSIONS

This research concludes that there is no significant increase in osteoblast activity in diabetic Wistar rats after the administration of 300 mg/kg cinnamon extract. However, there are indications that the hypothesis could be proven if it is judged solely on the mean ranks and neglecting statistical significance tests (>0.01).

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