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RESEARCH ARTICLE

Study on the effects of varying concentrations of xylitol on the weight, and blood glucose, and cholesterol levels of *Mus musculus L.* (ICR mice)

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ABSTRACT

Background: High sugar intake has increased the risk of having diabetes and cardiovascular diseases. Many sugar substitutes have surfaced to combat these problems; however, there is risk associated with increased frequency of intake. **Aims and Objectives:** With the known effects of frequent sugar intake, varying concentrations of the sugar substitute, and xylitol were used to determine its effects on the weight, blood glucose, and blood cholesterol levels on ICR mice. **Materials and Methods:** In this study, 25 mice were used as subjects that were split into five groups randomly: Group 1 (negative control) was not given any treatment, Group 2 (positive control) was given 10% sucrose solution, and Groups 3–5 (treatment groups) were given 10%, 15%, and 20% xylitol solutions, respectively. Before the treatments were given, baseline levels were determined for glucose and cholesterol using glucose cholesterol uric acid Easy Touch® multifunction monitoring system, and an analytical balance for the weight. The tests were determined again after giving the treatments to the groups for 3 weeks. The results of these tests after treatment were then compared with the baseline. **Results:** Results showed that the change in weight and change in blood cholesterol level in xylitol-treated groups were not significantly different with the positive control, while the change in blood glucose level showed that xylitol-treated groups were significantly different with the positive control. It was also shown that the tests done in varying concentrations of xylitol did not differ from each other. **Conclusion:** This shows that xylitol does not greatly affect the blood glucose level, but is shown to have the same effects with sucrose on the weight and blood cholesterol level.

KEY WORDS: Cholesterol; Glucose; *Mus musculus*; Sucrose; Weight; Xylitol

INTRODUCTION

High sugar consumption has increased the risk of having health problems such as diabetes, heart diseases, and being overweight, which could also affect the urinary, nervous, and immune system functions.[1] Sugar that is usually added to consumables is the disaccharide sucrose, which consists of two monosaccharide units, glucose, and fructose.[2] When too much sucrose is consumed regularly, the cells may become resistant to insulin.[3] This would then cause the increase in glucose levels in the bloodstream, which could lead to damaging blood vessels and organs.[4] Recent studies suggest that taking in too much sugar not only raises blood glucose level but may also increase the blood cholesterol level.[5] Taking in too much sugar would result to the storing of excess sugar as triglycerides and synthesis of cholesterol.[6] High concentrations of triglycerides raise the low-density lipoproteins (LDL) high-density lipoprotein (HDL), which causes the deposit of cholesterol to the arterial walls. This also lowers the HDL concentration, which counteracts the LDL. These cholesterol deposits may lead to atherosclerosis and other cardiovascular diseases such as

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high blood pressure.[7] Other effects of excess intake of sugar include obesity, diabetes, and heart diseases.[6] With these diseases becoming apparent, many sugar substitutes, such as sucralose, stevia, aspartame, sugar alcohols, and many more, have emerged. These substitutes claim to be better as these have lesser calories compared to regular sugar and that they neither increase nor decrease blood sugar levels significantly.[8,9] Although these substitutes may prevent the adverse effects of sugar, many studies also prove that some may not be a better substitute to regular sugar, specifically there are some adverse effects that xylitol may bring.

Xylitol, with a molecular formula of $(CHOH)_3$ $(CH_2OH)_2$, is a sugar alcohol that occurs naturally usually in fruits and vegetables and can be acquired from birch wood by the chemical reduction of xylose. Another means of producing xylitol is the assimilation and fermentation of xylose to xylitol using microorganisms. A few bacteria, such as *Corynebacterium* spp., *Enterobacter liquefaciens*, and *Mycobacterium smegmatis*, and a few fungi, yeasts to be exact, are able to produce xylitol by reduction of xylose by either NADH or NADPH-dependent xylose reductase.[10] It is usually found in sugar-free chewing gums, mints, and candies, and even claim that it does not promote tooth decay.[11,12] This is because xylitol, unlike glucose, is not converted into its acidic form, which is the cause of tooth decay, because bacteria in the mouth do not feed on xylitol, which may lead to decreased plaque formation.[11] Although it looks and tastes like table sugar, it has 40% lesser calories because the body cannot fully break down and only absorb 50% of xylitol.[8,9] This means that it intake will not increase the blood sugar and production of insulin.[12]

When xylitol is ingested, it is barely absorbed by the small intestine by passive diffusion, while most of it is transferred to the large intestine. It can bind with calcium, which facilitates calcium absorption through the gut wall that contributes to the remineralization of enamel and prevention of osteoporosis.[11] Xylitol is a non-digestible sugar since it has not much readily available enzymes to break it down found in the digestive tract. Furthermore, bacteria found in the digestive tract also have no means of digesting xylitol.[13] With this, osmotic retention of fluid due to indigestion of xylitol in the small and large intestines cause diarrhea, which may only be resolved by excreting the material.[14] However, the body can adapt to tolerate ingestion of xylitol by increasing the activity levels of polyol dehydrogenase that greatly increases the rate of xylitol absorption after a few days.[13] The absorbed xylitol, on the other hand, is phosphorylated and metabolized in the liver to xylulose 5-phosphate, an intermediate branch of the pentose phosphate pathway, which connects the pathway of xylitol to glucose and cholesterol.[15] Although preliminary research suggests that xylitol induces diarrhea, it was proven in the study of Mattila *et al.*[16] that adaptation of the intestines can occur. In the study, it was seen that the Sprague-Dawley rats were able to adapt to the feeding of xylitol for 1 week. Only sorbitol was shown to continuously cause diarrhea. The adaptation to xylitol was also in the study of Ellwood *et al.*[17] where they had an adjustment period, by feeding mice xylitol by increments of 5% concentration per week up until the desired concentration was achieved. It was seen that feeding the mice the desired amount at once caused serious problems to the mouse's health. In another study which looked into the effects of xylitol on gastric emptying and food intake, it showed that the participants who consumed 25 g xylitol pre-loads consumed lesser calories compared to the control by 25%.[18]

A study on Sprague-Dawley rats were done to determine the effects of xylitol on blood glucose and glucose tolerance.[19] It showed that after 5 weeks of feeding non-diabetic rats with sucrose, diabetic rats with sucrose, and diabetic rats with xylitol, the body weight and blood glucose significantly decreased, and the glucose tolerance ability of their bodies significantly increased in the group treated with xylitol compared to diabetic rats fed with sucrose. It showed that xylitol could be used as an anti-diabetic sugar substitute that can lower blood glucose levels. It was proven as well that xylitol may decrease levels of plasma triglycerides and cholesterol.[17] It was as well seen that accumulation of visceral fat was significantly smaller in xylitol-fed groups compared to the control group that was fed high-fat diet.[15] However, it was seen that xylitol activates the carbohydrate response element binding protein (ChREBP), which upregulates gene transcription of lipogenic enzymes. Accumulation of visceral fat that was significantly smaller in the xylitol-fed group could be associated with the lower expression levels of the sterol regulatory element binding protein 1c (SREBP-1c) and higher fatty acid oxidation-related genes in the liver.[15,17]

With the known effects of frequent intake of sugary food and drinks, varying concentrations of the sugar substitute, xylitol, were used to determine its effects on the blood glucose and blood cholesterol levels of the body and weight, which was then compared with sucrose. Specifically, the study aimed to determine whether varying concentrations of xylitol would have a significant effect on the weight, blood glucose level, and blood cholesterol level of mice and to determine the correlation between weight, blood glucose level, and blood cholesterol level of mice.

MATERIALS AND METHODS

Test Subjects

Twenty-five 9-week-old male ICR mice were obtained from food and drug administration, Muntinlupa City, Philippines. The mice were housed individually at room temperature (27 ± 2°C) having 58% humidity with controlled 12:12 h of light/dark cycle. The mice were acclimatized in laboratory conditions for at least 7 days. The study was conducted in the animal house in De La Salle University where the temperature is controlled by the air conditioner; proper ventilation with the use of an exhaust fan, and a dim and quiet environment was observed. All experimental procedures were approved by the Institutional Animal Care and Use Committee of De La Salle University following the standard guidelines for animal care as recommended by the Philippine Association of Laboratory Animal Science and the Department of Agriculture Bureau of Animal Industry. The experiment from extraction to administration of samples and extrapolation of results was performed from September 2013 to April 2015.

Experiment Procedures

At the start of the experiment, weight and the non-fasting blood glucose and cholesterol levels were determined as baseline using the Easy Touch® glucose cholesterol uric acid (GCU) Blood GCU multi-function monitoring system. The mice obtained were then split into five groups of 5 mice each, namely, negative control group, positive control group, and xylitol treated groups. The negative control group was given distilled water, while the positive control group was given 10% sucrose solution. The three treatment groups were given 10%, 15%, and 20% xylitol solutions, respectively. All solutions were administered *ad libitum*. For the xylitol treatments, increments of 5% per week starting at 10% were administered until the desired concentration has been reached, which lasted for 3 weeks. This was to let the mice adapt to the treatment since introducing high concentrations of xylitol would cause death.[17] The weight, blood glucose, and cholesterol levels were again measured after 21, 28, and 35 days of treatment.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine significant differences between each treatment groups. Significant differences were then compared using Tukey's test to at $P < 0.05$. All statistical analysis was performed using STATA version.12.

RESULTS

The obtained values for weight, blood glucose level, and blood cholesterol level at baseline 21, 28, and 35 days were plotted in line graphs as seen in Figures 1-3. The values for the latter 3 weeks were then compared to the baseline to determine how much change the varying treatment groups caused. Mean and standard deviation of the change in values were determined and bar graphs were then made to show the effects of varying xylitol groups to the positive and negative control groups, as seen in Figures 4-6.

As seen in Figure 1, the means of the different treatments are shown to be increasing at the same time as each week passes. Although the positive control group has a higher weight in all weeks compared to the other groups, the mean increase in weight is not different from each other. It is also seen in the graph that varying concentrations of xylitol did not differ in the increase in weight. It could also be seen that toward the

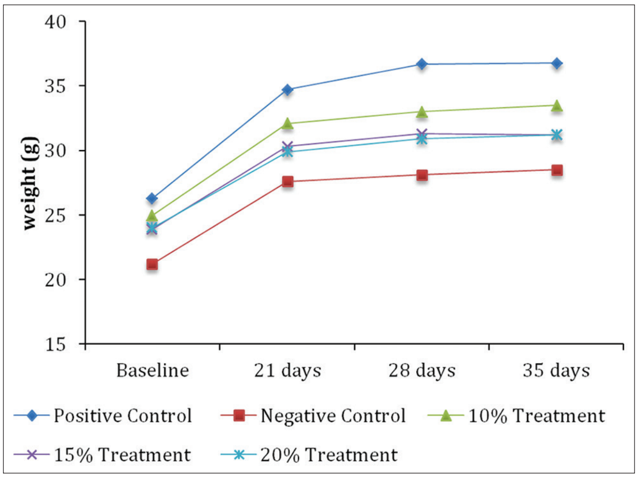

Figure 1: Line graph showing the mean weight of each group at baseline, 21 days, 28 days, and 35 days after treatment

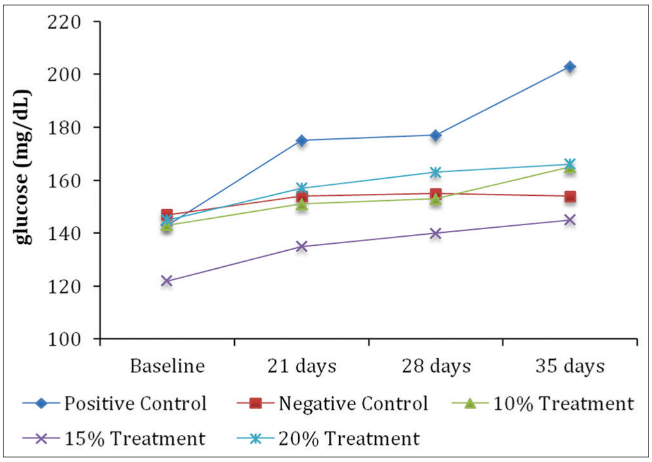

Figure 2: Line graph showing the mean glucose of each group at baseline 21 days, 28 days, and 35 days after treatment

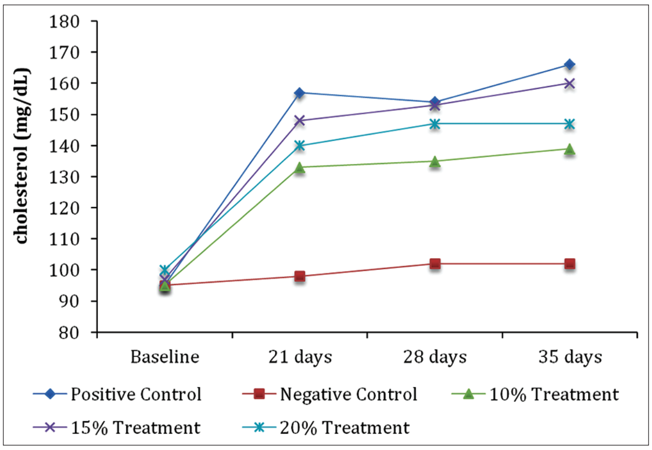

Figure 3: Line graph showing the mean cholesterol of each group at baseline 21 days, 28 days, and 35 days after treatment

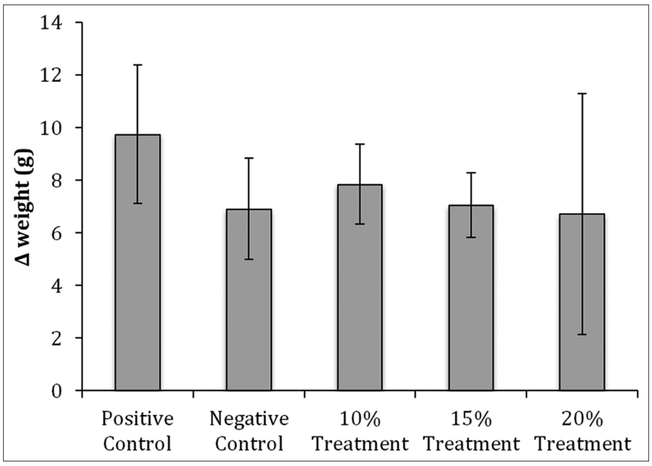

Figure 4: Bar graph showing the average of the mean change in weight between the different groups

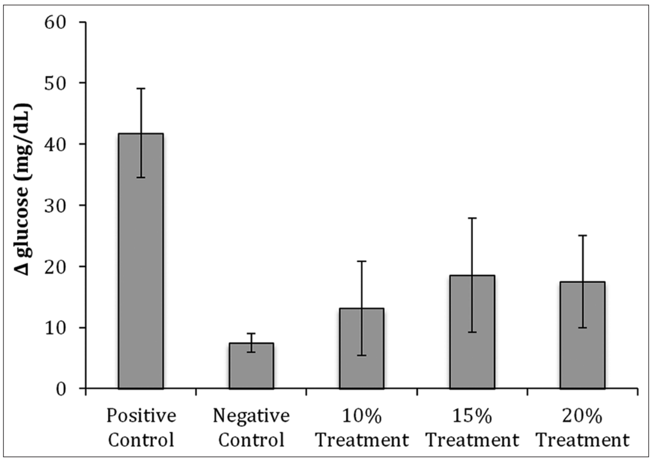

Figure 5: Bar graph showing the average of the mean change in glucose between the different groups

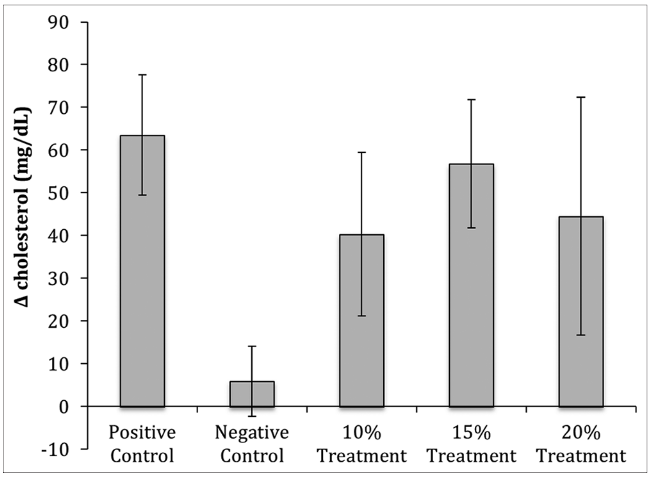

Figure 6: Bar graph showing the average of the mean change in cholesterol between the different groups

end of treatment period that the increase in weight reaches a plateau period. The changes in weight were then calculated 21, 28, and 35 days compared to the baseline. As seen in Figure 2, the positive control group had the highest mean change in weight, while the 20% xylitol-treated group had the lowest; however, it could be seen that the bar graphs for each treatment are not that far apart. Also seen in this graph is the error bars, which show that all treatment and control groups are not significantly different from each other. This means that, in this experiment, there was not much difference in the effects of xylitol on the weight of mice compared to the effects of sucrose and effects without treatment. Statistical analysis using one-way ANOVA showed that there is an interaction between the different treatments and control groups, which means that at least one of the means is different from one another. *Post hoc* analysis using Tukey-Kramer showed that all means of the groups did not differ from each other except the positive and negative control. Varying concentrations of xylitol were, again, shown to be not significantly different from each other.

The normal blood glucose level in the adult mice is 106–278 mg/dl according to Danneman *et al.*[20] As seen in the line graph plotted in Figure 3, all the blood glucose levels measured are within the normal range. It also showed that all treatment groups did not increase as much except for the positive control. Varying concentrations of xylitol, again, did not seem to have a significantly different increase in blood glucose level. As seen in Figure 4, it could be seen that there is a disparity in the mean change of the positive control group compared to the other treatment groups, which shows that the positive control group is significantly different to all other treatment groups. This means that sucrose greatly affects the blood glucose level compared to all the xylitol-treated groups. Moreover, the negative control is seen to be slightly significantly different to the 15% and 20% xylitol-treated groups, while the negative control group is not significantly different with the 10% xylitol-treated group. This means that xylitol does not greatly increase the blood glucose level and could be seen to not be significantly different with the negative control group. Statistical analysis showed that there are differences among the different treatment groups. Tukey-Kramer test showed that all the means are not different with the other except the positive control group and 15% xylitol-treated group. This is because the blood glucose level of the 15% xylitol-treated group is already lower compared to the other groups.

Finally, the normal blood cholesterol level in mice is 63–174 mg/dl.[20] As seen in Figure 5, the line graph shows that the increase in blood cholesterol level in all treatment groups are not significantly different except for the negative control group. It can also be seen that the varying concentrations of xylitol are not significantly different from each other. As seen in Figure 6, the mean change in blood cholesterol in the positive control group is not significantly different with all other groups except negative control group. The negative control group, on the other hand, is significantly different to all groups. This shows that the effect of xylitol on the blood cholesterol level of mice is not very different with the effects of sucrose on the blood cholesterol level. Statistical analysis shows that there are differences between the different groups. Tukey-Kramer test showed that the mean of the negative control group is significantly different to other treatments, while the mean of the positive control group and the xylitol-treated groups are not significantly different. Again, the difference in concentration of xylitol-treated groups did not differ significantly in its effects on the blood glucose cholesterol.

DISCUSSION

A study by Schulze *et al.*[1] showed that high sugar intake was associated greatly with weight gain. Since it was shown that effect of xylitol on weight was not significantly different with the effect of sucrose, it may be said that xylitol intake due to increase in weight like sugar. It was shown that accumulation of fat was significantly lower in xylitol-fed groups because the presence of xylitol lowers the SREBP-1c and increases the fatty acid oxidation-related genes in the liver.[15] However, it was also shown to increase the ChREBP, which upregulates the lipogenesis enzyme. Since accumulation of fat is directly related to weight gain, it was shown in Figure 1 that the weight of xylitol-treated groups did not decrease significantly due to the contradictory effects of xylitol. It was shown in this experiment that xylitol does not increase the blood glucose level compared to sucrose. This means that although xylitol has a pathway in converting xylitol to glucose, it does not occur.[15] This may be due to the fact that the mice had enough food for them to resort to other means of glucose production. Glucose can be converted to cholesterol through the HMG-CoA pathway.[21] Xylitol can also be converted to cholesterol when xylitol is metabolized in the liver to become xylulose-5-phosphate, which is an immediate branch of the pentose phosphate pathway.[15] Since cholesterol in xylitol-treated groups did not differ with the positive control group, it could be possible that the synthesis of cholesterol is promoted or xylitol is metabolized to become cholesterol.

In a study on the effects of xylitol in Type 2 diabetic rat models, it was shown that it decreased food and fluid intake, as well as the body weight, blood glucose, and serum lipids.[19] This effect is due to the insulin-independent metabolism of xylitol that can be used to replace sugar on weight/weight basis.[22] This further shows that xylitol does, in fact, can be used as a supplement both in preventing unwanted effects in normal as well as diabetic populations. In another study on obese mice, xylitol was seen to be able to reduce body weight and lowers the blood glucose levels and increasing the HDL levels,[23] particularly, when used regularly in the diet. This is different with the results of the experiment, which shows that xylitol increases the weight of the animal. This difference may be attributed to the use of different models where the latter used obese mice, whereas this experiment used a normoglycemic mouse.

The effects of varying concentrations of xylitol on the weight, blood glucose level, and blood cholesterol level were determined by treating male ICR mice with xylitol solutions as their water source. After the acclimatization of the weight, blood glucose level, and blood cholesterol level were determined in all mice to establish a baseline. Data were then again collected at 21, 28, and 35 days after treatment. It was seen that xylitol-treated mice are not significantly different with the group given 10% sucrose. As such, it may be seen that xylitol may still be able to increase weight and blood cholesterol, but not blood glucose. As a sugar replacer,[22] xylitol did not, in fact, raise the value of blood glucose, but prudence should be observed when using xylitol since it may still increase the weight and blood glucose.

CONCLUSION

The effects of xylitol treatment on weight, blood glucose, and blood cholesterol were determined in normoglycemic mice. In this experiment, it was shown that the xylitol-treated groups and the positive control group were not significantly different from each other in reference to weight and in blood cholesterol level. In contrast, the blood glucose level of xylitol-treated mice and positive control group was shown to be significantly different. It was also seen in all tests that varying xylitol concentrations did not significantly differ from each other. Since xylitol is marketed as a sugar replacer, which does not increase blood glucose, this experiment further supports the claim. However, it should be noted that the weight and blood cholesterol still is similar in the effects with sugar, which may post problems to those using it solely.

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