

# Effect of Different Doses of Sorbitol on Hematological Parameters and Blood Glucose of Rats

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## Abstract

The current study was conducted in the animal house of the college of veterinary medicine, Al-Qasim green university during December (2021) to February (2022) to investigate the effect of sorbitol as one of the most worldwide used chemical sweeteners on hematological parameters and on blood glucose. For this purpose, a total 40 male Wistar rats of 60 days age old and 300±10 grams weight were used, acclimated divided randomly to 4 groups [control (GC) given distilled water and 3 experimental tested (T1, T2 and T3) which given respectively 50, 100 and 200 mg/kg B.W of sorbitol for 60 days], and finally subjected for direct collection of blood samples. Using the automatic blood analyzer, the findings of hematology were revealed a significant increase in values of red blood corpuscles (RBCs), packed cell volume (PCV), hemoglobin (Hb), white blood cells (WBCs), lymphocytes, neutrophils, monocytes, eosinophils and basophils of all treated groups when compared to value of CG. Among values of treated groups, significant higher value was reported in T3 when compared to those of T1 and T2. The results of blood glucose were noted a significant elevation in values of treated groups when compared to value of CG. Moreover, there was an ascended elevation in values of blood glucose throughout the treated groups since significant higher level was reported in T3 and then in T2 and T1 values. In conclusion, the findings of this research sound a cautionary note about the impact of sorbitol on RBCs and blood glucose. However, we recommended to extending the scope of investigations to include all relevant aspects.

**Keywords:** Experimental study, Saccharin, RBCs, WBCs, Iraq.

## INTRODUCTION

Sorbitol, less commonly known as glucitol, is a sugar alcohol with a sweet taste which discovered firstly by a French chemist in the berries of the mountain ash in 1872. Also, it can be naturally found in apples, pears, peaches, apricots and nectarines as well as in dried fruits such as prunes, dates and raisins and in some vegetables [Awuchi, 2017; Zhang et al., 2019]. Also, several industrial processes were known for production of sorbitol in addition to few microorganisms including *Zymomonas mobilis* and *Candida boidini* [Nicoud et al., 2015; Mooradian et al., 2017]. Sorbitol supplies fewer calories than sugars, and characterizes with a 20-fold higher solubility in water than mannitol [Aprea et al., 2017]. Similarly to xylitol and erythritol, it has a negative heat of solution and thus gives a cooling sensation in the mouth and pleasant taste [Awuchi, 2017]. Sorbitol can combine well with other food components and reveal synergistic effects with other sweeteners that result in greater sweetness and better taste [Bakshi et al., 2020]. However, it is resistant to digestion by oral bacteria which break down sugars and starches to release acids which may leading to cavities or erodes of tooth enamel [Adkison et al., 2018].

The metabolism of sorbitol has been studied since 1933, and the available data bearing on the formation of glycogen both in the liver and muscles, appearance of hyperglycemia after feeding, elimination through kidney, determination of extracellular fluid volume, effect on respiratory quotient, antiketogenic agents, and relieving of insulin shock [Silveira et al., 1999]. In small intestine, only 25% of sorbitol is absorbed while the rest is fermented by bacteria. The absorbed sorbitol can impact blood sugar levels because it is fully metabolized [Kloub et al., 2022]. In normal cases, sorbitol did not have a significant effect on blood sugar levels [Yang et al., 2019]; but abdominal pain and diarrhea can occur in large doses of sorbitol [Kaplan et al., 2013]. Even though enormous amounts of sorbitol are consumed, only a trace quantity penetrates the small intestine; the bulk of sorbitol is ejected into the colon, resulting in gastrointestinal discomfort due to its high molecular weight [Shin et al., 2019]. In all diabetic patients, significant increases in blood-sugar concentrations occurred after sorbitol administration [Kondo et al., 2021]. Additional direct negative consequences of pathway hyperactivity under diabetic or hyperglycemic conditions include

intracellular sorbitol accumulation that resulting in osmotic stress [Darko et al., 2019], and generation of fructose that being 10 times more potent glycation agent than glucose [Al-Kadhi et al., 2020]. Due to inability of sorbitol to be carried via kidneys, it can result in 10% renal losses [HU et al., 2021]. This study was aimed to investigate the effect of sorbitol as one of the most worldwide used chemical sweeteners on hematological parameters and blood glucose.

## Materials and methods

### Ethical approval

The current study, carried out during December (2021) to February (2022), was performed under the license of the Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, Al-Qasim Green University (Al-Qasim, Babil, Iraq).

### Study animals

A total 40 mature male Wistar rats of 60 days and old and  $300 \pm 10$  grams weight were purchased, kept in plastic cages in the animal house of the College of Veterinary Medicine (Al-Qasim green University), and subjected to preparation period; during which, they received a standard feeding and tap water supply.

### Study design and sample collection

The study rats were divided randomly and equally into four groups as following:

1. CG (GC): Animals of this group were received distilled water for 90 days.
2. Experimental tested group 1 (T1): Animals of this group were received 50 mg/kg B.W of sorbitol for 60 days by gavage.
3. Experimental tested group 2 (T2): Animals of this group were received 100 mg/kg B.W of sorbitol for 60 days by gavage.
4. Experimental tested group 3 (T3): Animals of this group were received 200 mg/kg B.W of sorbitol for 60 days by gavage.

After 60 days, blood samples were collected directly from each rat into EDTA-labeled tubes.

### Hematology

The analysis of blood samples for measurement of RBCs, WBCs, PCV and Hb was carried out as soon as possible using an automatic blood analyzer (Mindary, China). Totally, 25  $\mu$ l of whole blood sample was aspirated via the sampling needle, mixed with 4 ml of diluent and stored in the chamber (primary dilution). Then, 25  $\mu$ l of the primary dilution was aspirated into and stored in the needle during WBC measurement and the hemoglobin analysis. Lysing reagent was added to the primary dilution held in the chamber for WBC differential analysis. This amount of lysing reagent is species dependent and varied with operator within "Limits" menu. After WBC counting and washing process, 5 ml of diluent was added to the second dilution using the 25  $\mu$ l of primary dilution stored in the needle. Throughout this portion, RBC count and their parameters were analyzed.

### Blood glucose

Following the manufacturers' instructions of the Roche Calibrator for Automated Systems, blood glucose of study animals was measured.

### Statistical analysis

All collected data were analyzed by ANOVA at  $P \leq 0.05$  in SAS (2012), [Al-gharban and Dhahir, 2015; Gharban et al., 2019].

## Results

The results of this study demonstrated that there was a significant increase ( $P < 0.05$ ) in values of total RBCs in all experimental treated groups; T1 ( $7.22 \pm 0.03$ ), T2 ( $7.16 \pm 0.12$ ) and T3 ( $7.78 \pm 0.02$ ) when compared to value of CG ( $6.47 \pm 0.06$ ). In comparison between values of treated groups; significant higher value ( $P < 0.05$ ) was reported in T3, and then in T1 and T2 (Figure 1).

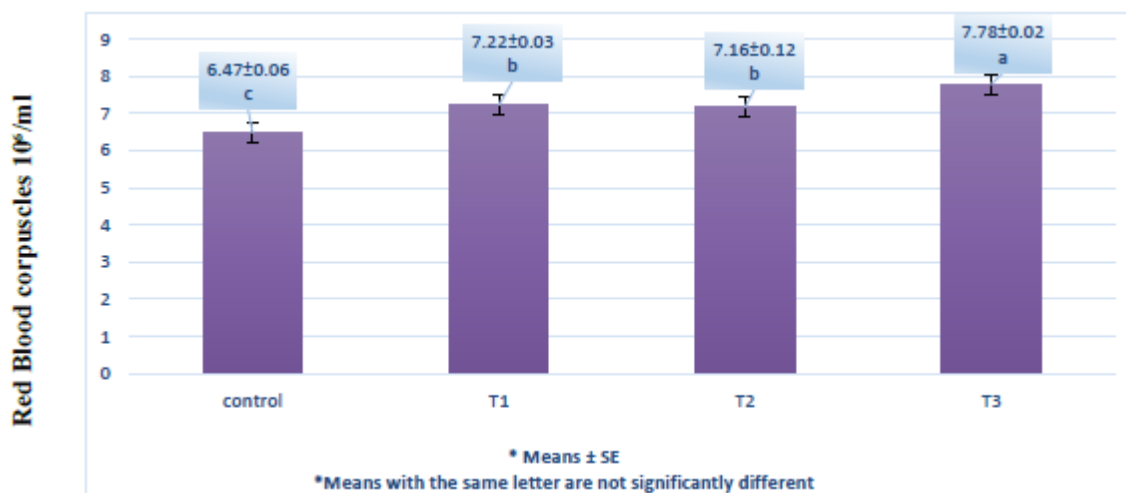


Figure (1): Effect of different dose of sorbitol on Red Blood corpuscles

The current investigation found a substantial ( $P < 0.05$ ) increase in Hb in all treatment groups when compared to the CG, as well as a significant increase ( $P < 0.05$ ) in T1 ( $13.5 \pm 0.02$ ) when compared to the CG ( $13.32 \pm 0.05$ ) and between the same group and T2 ( $13.82 \pm 0.01$ ). While the findings of this study revealed a substantial drop in T2 ( $13.82 \pm 0.01$ ) compared to T3 ( $14.62 \pm 0.03$ ), as well as a significant decrease ( $P < 0.05$ ) in T1 ( $13.5 \pm 0.02$ ) compared to T2 ( $13.82 \pm 0.01$ ), (Figure 2).

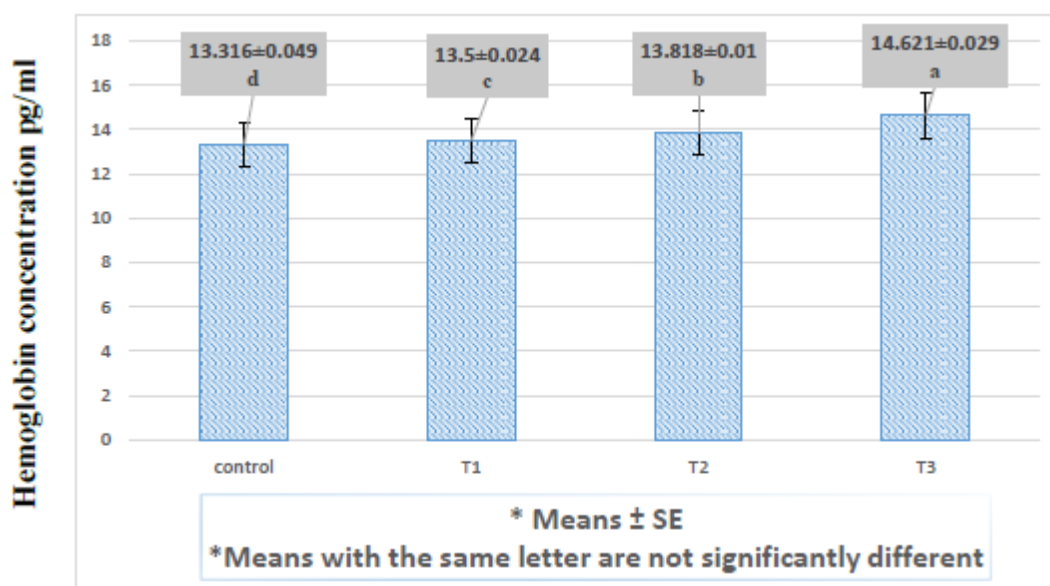


Figure (2): Effect of different dose of sorbitol on Hb

The current study findings revealed a significant ( $P < 0.05$ ) decrease in PCV in all treatment groups when compared to the CG, whereas the results of this research revealed a significant decrease ( $P < 0.05$ ) in PCV percent in the T1 group when compared to the CG and between the same group and T2 group. In addition, a significant decrease ( $P < 0.05$ ) between T3 and T2 groups as

well as the T2 and T1 groups (Figure 3).

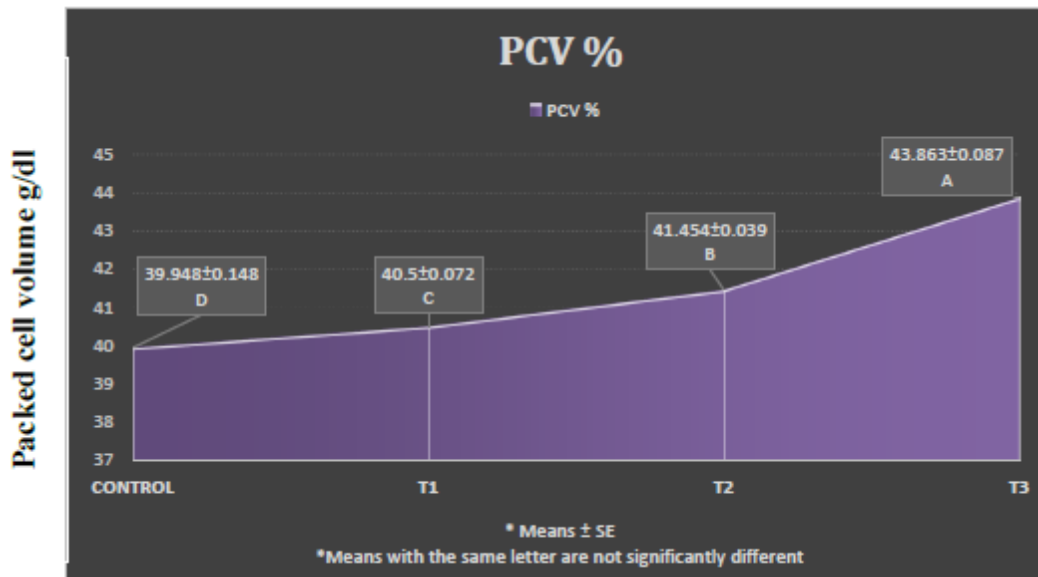


Figure (3): Effect of different dose of sorbitol on PCV

The results of the present study showed that there was a significant increase ( $P < 0.05$ ) in white blood cells in all treatment groups compared with CG, this study showed that there is a significant increase ( $P < 0.05$ ) in T1 ( $9.47 \pm 0.02$ ) compared with the CG ( $8.35 \pm 0.04$ ) and the same group with T2 ( $9.86 \pm 0.03$ ). Also, there is a significant increase ( $P < 0.05$ ) in T3 ( $10.68 \pm 0.11$ ) compared with T2 and T1 ( $9.86 \pm 0.03$  and  $9.47 \pm 0.02$ ), as well as a significant increase ( $P < 0.05$ ) in T2 ( $9.86 \pm 0.03$ ) compared with T1 ( $9.47 \pm 0.02$ ), (Figure 4).

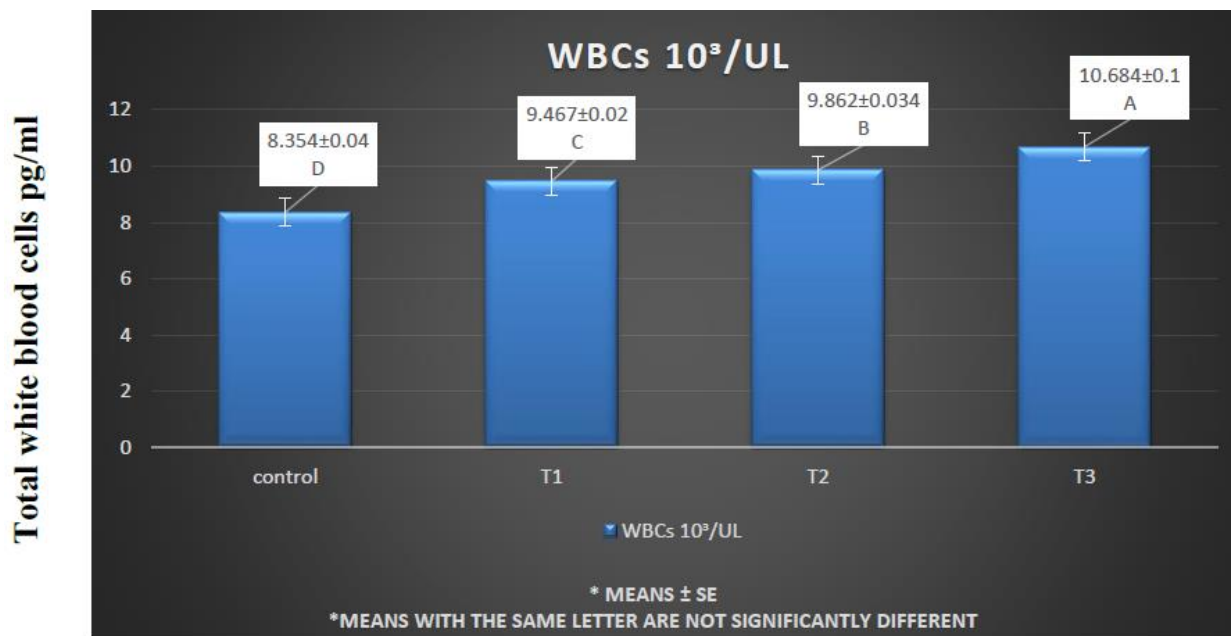


Figure (5): Effect of different dose of sorbitol total leucocytes count

The results demonstrated a significant increase ( $p < 0.05$ ) in lymphocytes of T1 ( $66.34 \pm 0.25$ ), T2 ( $69.81 \pm 0.27$ ) and T3 ( $71.43 \pm 0.32$ ) when compared with CG ( $65.56 \pm 0.15$ ). Furthermore, the results showed a significant increase ( $p < 0.05$ ) in lymphocytes concentration in T3 ( $71.43 \pm 0.32$ ) compared with T1 ( $66.37 \pm 0.25$ ) and T2 ( $69.81 \pm 0.27$ ) and a significant increase ( $P < 0.05$ )

in T2 ( $69.81 \pm 0.27$ ) when compared with T1 ( $66.37 \pm 0.25$ ). Also, the results showed that there was a significant decrease ( $p < 0.05$ ) in neutrophils of treated groups compared with CG. By comparing among treatment groups, the results showed that there is a significant decrease ( $p < 0.05$ ) in T3 ( $20.50 \pm 0.21$ ) compared with T1 ( $21.19 \pm 0.15$ ) and T2 ( $21.81 \pm 0.21$ ). In addition, the results were recorded a significant increase ( $p < 0.05$ ) in monocytes of three treated groups compared with CG. Moreover, by comparing between treatment groups the results showed a significant increase ( $P < 0.05$ ) in monocytes concentration in T3 ( $6.97 \pm 0.02$ ) compared with T1 ( $6.47 \pm 0.03$ ) and T2 ( $6.90 \pm 0.01$ ) and a significant increase ( $P < 0.05$ ) in T2 ( $6.90 \pm 0.01$ ) compared with T1 ( $6.47 \pm 0.03$ ). Likewise, the results illustrated a significant increase ( $p < 0.05$ ) in eosinophils of T1, T2 and T3 compared with CG. Moreover, by comparing between treatment groups the results showed that there was a significant increase ( $p < 0.05$ ) in eosinophils concentration in T3 compared with T1 and T2. In a similar vein, our data indicated an increase ( $p < 0.05$ ) in number of basophils found in T1, T2, and T3 samples when compared with the CG. Furthermore, the findings indicated that there was an increase ( $p < 0.05$ ) in concentration of basophils in treatment group T3 when compared with treatment groups T1 and T2 (Figure 5).

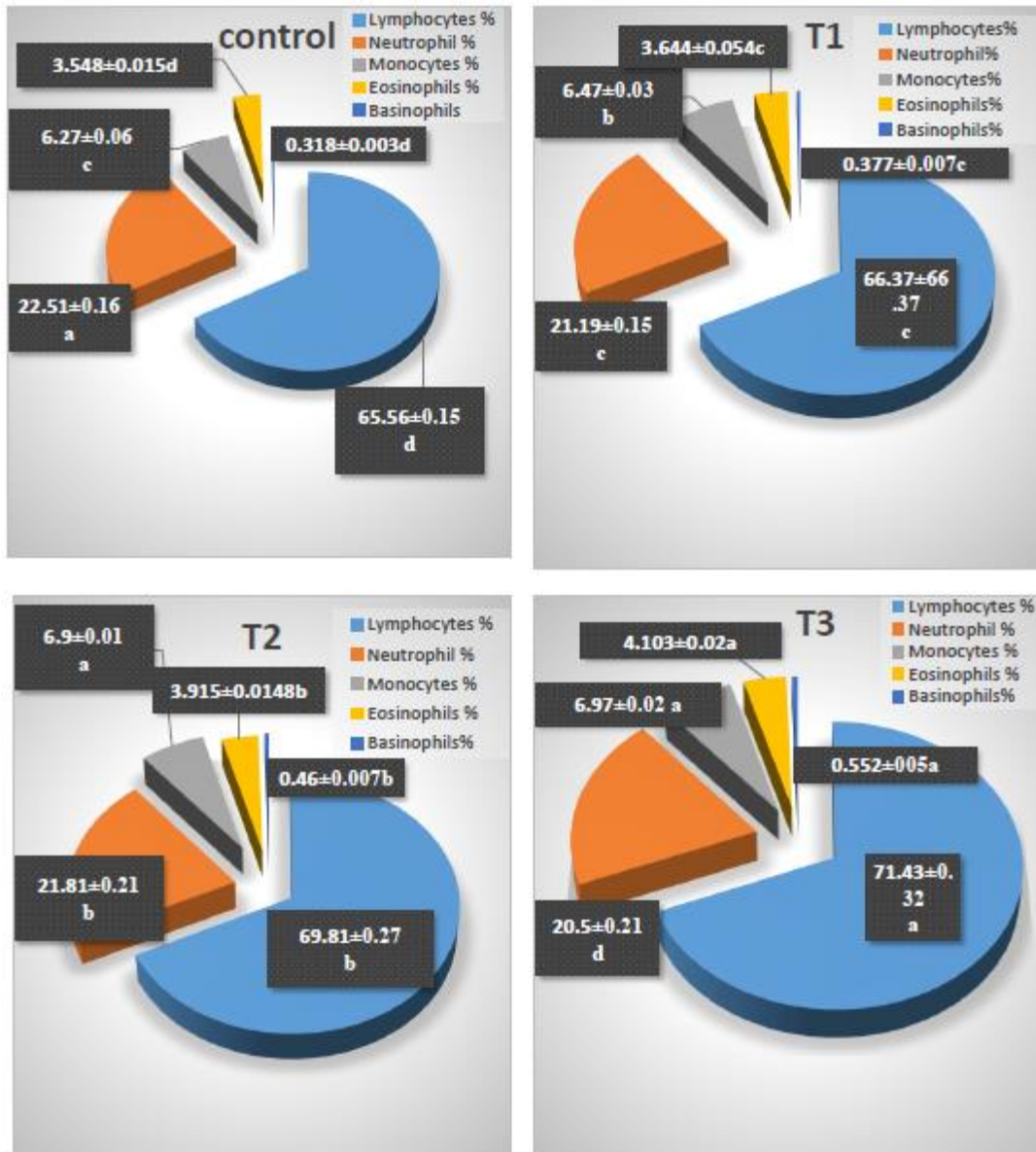


Figure (5): Effect of different dose of sorbitol on lymphocytes, neutrophils, monocytes, eosinophils and basophils

The results of blood glucose were noted a significant increase ( $p < 0.05$ ) in values of treated groups; T1 ( $99.9 \pm 0.57$ ), T2 ( $116 \pm 1.49$ ) and T3 ( $121 \pm 0.53$ ) when compared to value of CG. Moreover, by comparing between treatment groups the results showed that there is a significant increase ( $p < 0.05$ ) in glucose concentration in T3 ( $89.3 \pm 0.52$ ) compared with T1 ( $99.9 \pm 0.57$ )

and T2 ( $116 \pm 1.49$ ). While, significant decrease ( $P < 0.05$ ) was showed in T1 ( $99 \pm 0.57$ ) when compared with T2 ( $116 \pm 1.49$ ), (Figure 6).

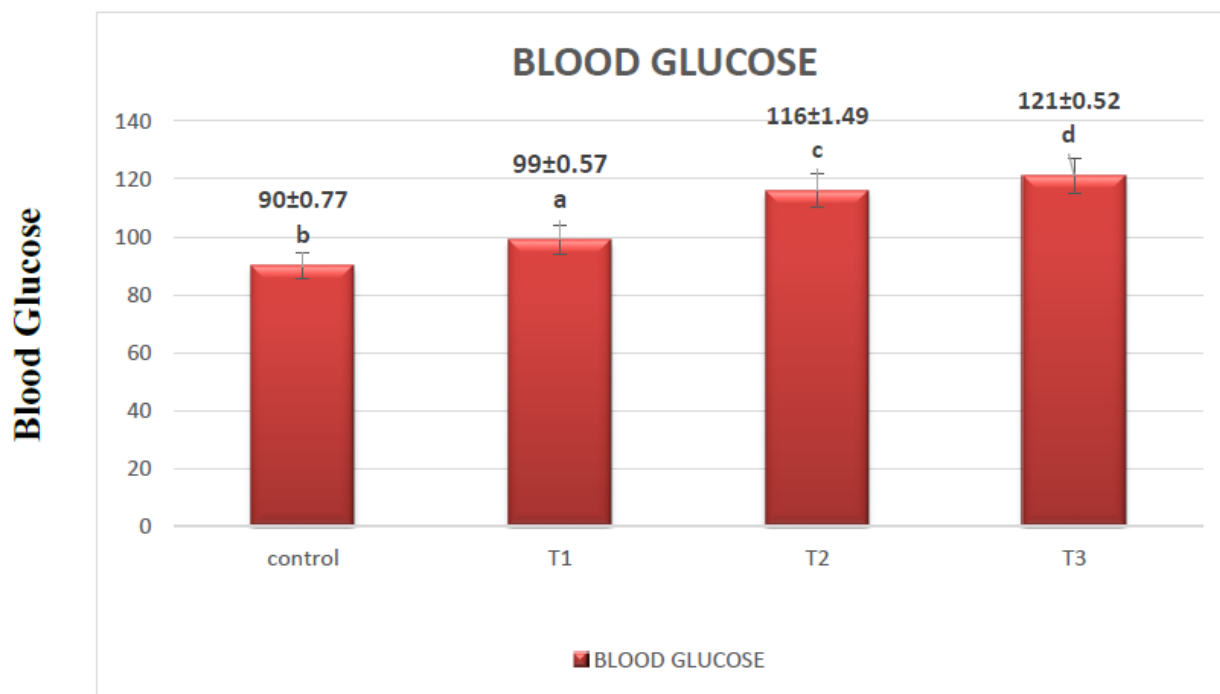


Figure (6): Effect of different dose of sorbitol on blood glucose

## Discussion

The study showed an increase in RBC compared to the control. However, the increasing of blood parameters of experimental rats could be related to the probably produced oxidative damage by sorbitol. Hematological indicators provide an excellent basis for judging disease, the extent of tissue damage, the response of the antioxidant defense mechanism, and for describing the health status. The PCV is the percentage of blood volume occupied by red blood cells. PCV is one of the factors that influence RBC sedimentation. Increased PCV essentially means more red blood cells per unit volume of suspension, so increased PCV would result in increased erythrocyte aggregation. Increases in PCV, on the other hand, result in a decrease in blood sedimentation velocity due to increased viscosity. Sorbitol-based osmotic diarrhea, intestinal malabsorption of sorbitol is the primary cause of sorbitol-based osmotic diarrhea, and as a result, frequent water perfusion of the intestine leads to dehydration in the body of the organism. Sorbitol-based osmotic diarrhea can also be referred to as sorbitol-based osmotic diarrhea [Islam, 2006].

White blood cells are also called leucocytes. They are found through the whole body either in blood or lymphatic system. These are produced in bone marrow and form immune system. Hence, WBCs not only protect body from invaders (germs which enter into the body); but also, from different diseases [Maton, 1997]. This study showed that the liver of rats given 50 mg/kg of sorbitol daily displays hazy hepatocyte degeneration, leading to sinusoidal constriction and blood vessel congestion. The structure of the liver of the control group is normal, with hepatocytes radiating from the central vein, sinusoidal space, and bile ducts. A histological slice reveals an early granulomatous lesion composed of inflammatory cell aggregates in the parenchyma around clogged arteries. According to Amirou [2018], WBCs perform Phagocytic action, the process in which WBCs engulf the bacteria and died [LaFleur-Brooks, 2008]. The production of WBCs increases when body gets either injury or diseased [Alberts et al., 2002]. One of the most important types of WBCs is neutrophil which constitute 60-70% of WBCs.

The use of sorbitol may potentially alter glycemic control, as glucose absorption may be reduced when carbs are substituted. According to Chukwuma and Islam [2017], this may not result in improved glucose homeostasis, as sorbitol may alter intestinal glucose transport and absorption and reduce insulin secretory capacity. Nevertheless, Chukwuma and Islam [2017] studied the relationship between sorbitol usage and glucose homeostasis to identify the effect of sorbitol on glucose levels. According to



Bazzaz and Al-Johani [2018], most studies based on prospective cohort studies in healthy individuals revealed no conclusive evidence that sorbitol increases the risk of type-2 diabetes. Another study showed that sorbitol had no significant effect on glucose homeostasis (glucose and insulin levels) in healthy individuals and people with diabetes [Manna and Jain, 2015]. However, this study investigated sorbitol's effect in substituting sugar for sorbitol in healthy individuals. It is unknown if the effects on glucose homeostasis found in animal studies can be detected in people when sorbitol is consumed alongside other dietary contain sugar components [Liauw and Saibil, 2019]. When nutrients are eaten, various sensory cues help the body prepare for metabolic digestion and utilisation. Reducing blood glucose levels begins as soon as you are exposed to sweets, even before you eat [Adkison et al., 2018]. According to Liauw and Saibil [2019], sorbitol does not appear to increase incretin (group of metabolic hormones that stimulate a decrease in blood glucose levels) secretion directly, but natural sugars do so by increasing incretin secretion, stimulating insulin release by cells. This present study suggested that sorbitol has a smaller impact on insulin production than natural sugars [Wölnerhanssen et al., 2016].

## Conclusions

Based on our results, it was discovered that sorbitol had a detrimental impact on RBCs and blood glucose, which led researchers to conclude that dehydration had taken place. Therefore, we advice to extending the scope of following investigations on sorbitol to include all relevant angles, with investigating the impact of sorbitol using of different set of research methods. Also, we strongly propose to doing more extensive research on diabetic people who use sorbitol and investigating whether or whether there is a correlation between sorbitol and gender differences. If possible, cut down on your use of sorbitol or eliminate it altogether, and replace it with products derived from natural sources; this is particularly important for diabetic individuals.

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