The effectiveness of addition of noni fruit (*Morinda citrifolia* L) in yoghurt on blood glucose levels of male mice (*Mus musculus*)

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Abstract. Yogurt is a functional fermented drink. To improve its functionality, fortification with noni juice (*Morinda citrifolia* L) is used which can reduce blood glucose levels. This study aims to determine the effectiveness of the addition of noni juice (*Morinda citrifolia* L) in yoghurt to the blood glucose levels of male mice (*Mus musculus*). The research method was experimental using Completely Randomized Design (CRD) with four treatments and three replications. The treatments consisted of P0 (0%), P1 (5%), P2 (10%) and P3 (15%). The data were analyzed using ANOVA and continued by Duncan's multiple range test if there were significantly different results. The result showed that addition of noni fruit in a yogurt did not give significant effect (P>0.05) on body weight gain of male mice but give significant effect (P<0.05) on blood glucose levels of male mice (*Mus musculus*). The result concluded that addition noni fruit (*Morinda citrifolia* L) in yogurt effectively reduced blood glucose levels in male mice with the best results of P3 (15%).

1. Introduction

Yogurt is one of the most popular fermented dairy products which has a wide acceptance worldwide whereas its nutritional and health benefits are well known for centuries [1]. Yogurt is fermented milk of unique sensory, nutritive and dietetic value offered in a variety of types and in different flavours [2]. Yogurt is considered as a probiotic carrier food that can deliver significant amounts of probiotic bacteria into the body which can claim specific health benefits once ingested. According to the Code of Federal Regulations of the United States Food & Drug Administration (FDA), yogurt can be defined as a food produced by culturing one or more of the optional dairy ingredients namely, cream, milk, partially skimmed milk, and skim milk, used alone or in combination with a characteristic bacterial culture that contains lactic acid producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* [3]. Yogurts are now being manufactured in numerous styles and varieties with different fat contents, flavors, and textures or with added pieces of fruit or honey. One of them is noni fruit.

*Morinda citrifolia* L belongs to the family *Rubiaceae*, the genus Morinda comprises some 80 species which all occur exclusively in tropical climate zones [4]. *Morinda citrifolia* L. has been recognized as an important herb for treating various physiological disorders worldwide. *M. citrifolia* is
commonly known as Indian mulberry or Noni in India [5]. *Morinda citrifolia* (Noni) has been used widely as a complementary and alternative therapy in many countries owing to its potent anti-oxidant activity and proven health benefits. Traditionally, it finds used as a therapeutic remedy to various diseases as an antibacterial, antitumor, anthelmintic, analgesic, anti-inflammatory, immunostimulant. Also, it has proved beneficial in conditions like gastritis, skin diseases, respiratory infections, menstrual and urinary tract disorders, fever, diabetes and venereal diseases [6].

The unripe fruit is dark green in color and the ripe fruit releases a strong butyric acid like a decayed smell. The pulp is juicy and bitter, light dull yellowish white, gelatinous when the fruit is ripped; numerous hard triangular reddish brown pits are found [7]. The ripe fruit has an unpleasant odor and bitter taste. *Morinda citrifolia* is used for more than 40 types of ailments worldwide. The crude extract of various parts of plant and fruit juice are reported to contain amino acids, anthraquinones, fatty acids, flavonoids, iridoids, lignans, polysaccharides, sterols, sugars, terpenoids etc. which are therapeutically useful for a broad range of pathological conditions [6]. Fermented Noni juice used to investigate the hypoglycemic activity in diabetes-induced rats. On the 20th day, after treatment in diabetic experimental animals, showed a significant decrease in fasting glucose from an excess of 300 mg/dl (day 3) to 150 mg/dl (day 20). It can be assumed that *M. citrifolia* either potentiates the action of insulin directly or that it increases peripheral tissue sensitivity to the storage hormone [8].

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to insufficiency in insulin numbers and function resulting in abnormal metabolism of carbohydrates, fats, and proteins [9]. Globally, rates of type 2 diabetes were 15.1 million in 2000 [10]. The number of people with diabetes worldwide is projected to increase to 36.6 million by 2030 [11]. Diabetes mellitus (DM) is not a contagious disease in Indonesia that needs attention, because the incidence and prevalence are increasing and can be fatal [12]. The aim of this study was to determine the effectiveness of noni juice extracts (*Morinda citrifolia* L) on body weight gain and glucose levels of mice (*Mus musculus*).

2. Materials and Methods

2.1. Materials

For the preparation of yogurt, fresh milk was obtained from local farm in Enrekang and noni fruit was obtained from the farmer's garden. A set yogurt starter culture named *biokul* containing *Lactobacillus acidophilus* and *Bifidobacteria*. Male mice used were 20 with an average weight of 20-30 gr.

2.1.1. Procedure for making yogurt. The milk was pasteurized with Low-temperature long time (LTLT) method, cooled until 45°C. Next, the milk was inoculated with yogurt culture and incubated at 43-45 °C. After reaching pH 4.5 yogurt was fortified with noni juice with percentage of 5%, 10%, and 15%.

2.1.2. Male mice (*Mus musculus*) to hyperglycemia (DM) procedure. The procedure of male mice into hyperglycemia (DM) conditions was initiated by not feeding 20 male mice for ± 18 hours, then mice were injured in the tail to measure blood glucose levels and weighed their body weight in the next two hours (after the wound dried) the mice were injected with *Alloxan tetrahydrate* solution 70 mg/kg body weight intravenously at the tail, then mice were fed and left in the cage for two days. On the third day, after mice fasted for ± 18 hours, blood glucose levels and body weight were measured again. The results of measurements of blood glucose levels indicate that mice have been conditioned to type 2 DM.

2.1.3. Fortified yogurt to male mice treatment. The fortified yogurt treatment of Noni Juice (*Morinda citrifolia* L) was carried out after mice in hyperglycemia (DM) condition. The treatment applied was 4 treatments with five replications.
2.1.4. Determination of glucose levels. Glucose levels were determined using multi-monitoring system auto check.

2.2. Statistical Analysis. Glucose levels and body weight gain were analyzed statistically using one-way analysis of variance (ANOVA) and the means were compared by the Duncan test at a significant level of 0.05.

3. Result and Discussion

3.1. Weight gain of male mice (Mus musculus)
Weight gain was analyzed and the results are presented in figure 1. Figure 1 showed that the addition of noni fruit (Morinda citrifolia L) did not give significant (P>0.05) effect on the weight gain of male mice.

Figure 1. Weight gain of male mice (Mus musculus)

Alloxan induction aims to condition mice in a state of diabetes with a method of destroying the structure of the pancreas. The experimental condition of diabetes will result in normal mice being diabetic mice marked by one of the features of the clinical diagnosis of weight loss. Based on figure 1 shows that the treatment of P1 (5%) and P2 (10%) body weight of mice (Mus musculus) has increased even though statistically, it does not give a real effect this is because the noni juice extract into yogurt can increase the appetite impact on body weight gain. Further in figure 1, shows that there is a weight loss of mice in P3 (15%), it is suspected that the content of noni fruit compounds, namely tryptophan, affects the appetite of mice. Tryptophan is an amino acid that is converted into 5-Hydroxytryptophan (5-HTP), which is then converted to serotonin in the body. Serotonin is a neurotransmitter that can affect the brain in the process of prolonging satiety after eating and can reduce appetite [13].

3.2 Glucose levels of male mice (Mus musculus)
Addition of noni juice into yogurt significantly affected (P<0.05) to blood glucose levels of male mice (Mus musculus). Before measuring glucose levels after drinking yogurt, mice were injected with alloxan with the aim of making mice in a diabetic condition. In this study, it was found that the addition of noni juice (Morinda citrifolia L) into yogurt gave a significant effect on the decrease in blood glucose levels of mice (Mus musculus) with a decrease in each treatment shown in figure 2.
Figure 2. Glucose levels of male mice (*Mus musculus*)

Figure 2 shows that *alloxan* induction (before treatment) increases glucose levels in mice with an average glucose level of 505.85 mg/dl. After treatment average of glucose levels of 377.45 mg/dl, an increase noni juice (*Morinda citrifolia* L) percentage into yogurt was followed by a decrease in glucose level of male mice (*Mus musculus*). The most decrease was found in P3 (15%) from 557.6 mg/dl decreased to 362.8 mg/dl, decreased by 194.8 mg/dl. This suggests that noni fruit (*Morinda citrifolia* L) had the ability to decrease glucose levels. According to [8] that in noni fruit there is a substance content of *prexeronine* and *proxeroninase* through the formation of xeronine which can regenerate pancreatic beta cells which are damaged so that the pancreatic beta cells can function properly and produce enough insulin to control blood glucose levels. Diabetes mellitus is caused by damage to pancreatic cells where pancreatic beta cells cannot produce enough insulin so that blood sugar levels rise and are not absorbed by cells in the body. Endang et al. [14] reported that Noni leaves contain active substances such as flavonoids, xeronine, and amino acids that have the potential to reduce blood glucose levels. *Proseronine* turns into seronine in the intestine capable of uniting peptides or amino acids into proteins. In the pancreas that is damaged due to alloxan, many proteins are broken down, so that seronine is able to regenerate damaged pancreatic cells. Flavonoids act as antioxidants that can bind free radicals, thereby reducing oxidative stress, and reduced oxidative stress can reduce insulin resistance and prevent the development of dysfunction and damage pancreatic beta cells. *Alanine, glucopyranoside, threonine, cysteine, cysteine, glycine, proline, glucopyranosyl*, are amino acids in noni leaves that work on the glycolysis pathway which can affect blood glucose levels.

4. Conclusions

It can be concluded from the result of this study that addition of noni fruit (*Morinda citrifolia* L) into yoghurt significantly decreased glucose levels of male mice (*Mus musculus*). The best yogurt with addition noni fruit extract (*Morinda citrifolia* L) is 15%.

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