Study of the pineapple peel vinegar potency in restoring the gonadosomatic index of the diabetic rats

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Abstract. The side effect of Diabetes mellitus is the decrease in the reproductive function which could be observed by the Gonadosomatic Index. Gonadosomatic index is a value that expresses the proportion of testis weight to body weight. Pineapple peel vinegar is a diabetic therapy that has been proved in decreasing the blood sugar levels in diabetic rats, but the effect in restoring GSI has not been evaluated yet. This study aimed to evaluate the effect of pineapple peel vinegar in GSI in diabetic rats. Eighteen male rats were divided into 6 groups, i.e., control (C); negative control (Cn), diabetic rats treated with water; positive control (Cp), diabetic rats treated with 0.4 ml apple vinegar; P1, P2, and P3 (diabetic rats treated with 0.2 ml, 0.4 and 0.8 ml of pineapple peel vinegar). They were treated for 21 days. The day after the last treatment, rats were sacrificed, and testes were isolated and weighted. The GSI was determined by calculated the proportion of the gonad mass to the total body mass. The result showed there was a significant difference (p<0.05) in GSI between treated Cn (0.59 ± 0.05), P1 group (0.62 ± 0.06), P3 (0.70 ± 0.04) when compared to C (0.52 ± 0.03) and Cp (0.36 ± 0.24) groups. The P2 group (0.53 ± 0.08) showed has no significant difference (p>0.05) when compared to C (0.52 ± 0.03) and Cp (0.36 ± 0.24). It could be concluded that pineapple peel vinegar has high potency in restoring the GSI on diabetic rats.

1. Introduction

Nowadays, Diabetes is one of the major problems of worldwide health. American Diabetic Association explained that Diabetes is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action [1]. Diabetes leads to chronic hyperglycemia, which is associated with long-term damage, dysfunction, and failure of different organs, including the eyes, kidneys, nerves, heart, and blood vessels [2]. The side effect of Diabetes Mellitus (DM) in male reproductive dysfunction has been explained by Alves et al. (2013), i.e., disorders in erectile and ejaculatory as well as a reduction in semen volume, sperm counts, sperm motility, and abnormal sperm morphology [3]. Kasturi et al. (2008) explained that the impact of metabolic changes on male reproductive health is often associated with dyslipidemia and inflammation [4]. Sitasiwi et al. (2018) stated that the disturbances of male reproductive function could be examined by testicular weight [5]. Further, testicular weight can be used to determine the Gonadosomatic index (GSI) that expressing the reproductive performance of an animal [5, 6].

Therapy for Diabetic patients has been explored by a large number of studies. One kind of Diabetes therapy is by consuming insulin regularly. Insulin is the mainstay of diabetic treatment and is effective...
in controlling hyperglycemia, but they have prominent side effects and fail to significantly alter the course of diabetic complications. They use of insulin as diabetic therapy usually gives the common side effects, i.e. hypoglycemia, weight gain, gastrointestinal disorders, peripheral edema, and liver disease [7]. Johnston et al (2004) explained that is near 25 years that several studies of vinegar showed the effect on glucose metabolism. Those research has been proved not only in vivo and in vitro studies but also in healthy subjects and diabetic subjects [8]. The effect of apple cider vinegar was in the glucose and insulin response to a sucrose or starch load [7]. In that way, the blood glucose level in diabetic mice was decreased when treated by apple cider vinegar.

Jannah et al. (2019) stated that vinegar is commonly used in the preparation of food condiments [9]. Pineapple is one of some fruits that offer a source of vinegar. The previous study showed that pineapple peel vinegar has the same potency with apple vinegar in decreasing the blood glucose level of rats by 41.63%. The effect of pineapple vinegar peel vinegar on the pancreatic structure has been proved [10]. The research showed that the diameter of the Langerhans islet in the group treated by 0.4 ml pineapple peel vinegar/day was close to normal. The antioxidant activity in pineapple vinegar presumed to improve the capability of the Langerhans islet in the binding of free radicals caused by Diabetes Mellitus (DM) [10]. Putra et al. (2020) showed that the administration of 0.4 mL per day pineapple peel vinegar can reduce about 41.64% blood glucose levels in rats [11].

The use of pineapple peel vinegar in decreasing the blood glucose level has been proved, but the effect of it in male reproductive function has not been proved yet. The male reproductive function could be predicted by calculating the Gonadosomatic Index [6]. By that fact, this study was performed to find out the effect of pineapple peel vinegar on male rats GSI. The outcome of this study is offering the advantages of pineapple peel vinegar in restoring the GSI in diabetic rats.

2. Research method
This research was conducted during three months at the Animal Structure and Function Laboratory of Biology Department, Science and Mathematics Faculty, Diponegoro University (UNDIP).

2.1. The laboratory animals
A total of eighteen male adult rats (Rattus norvegicus L) with ± 200 g in weight were used as laboratory animals. All of the rats were in the reproductive phase with 2-3 old months in age. The rats were acclimatized for 2 weeks in an individual cage. The animals were kept under 12:12 light cycle with a room temperature of 24-26 °C and relative humidity of 65-75%. The food and drink water were given ad libitum to the rats during acclimatization time.

2.2. Method
A completely Randomized Design was used in this research. The alloxan monohydrate that was given intraperitoneally was used to induced diabetic rats [12]. The rats were justified as diabetic when the blood glucose level was >200 mg/dL [13]. The rats. were divided into six groups, as follows:
C: Control group, which treated with drinking water only
Cn: Negative control group, diabetic rats treated with drinking water only
Cp: Positive control group, diabetic rats treated with apple vinegar
P1: Diabetic rats, treated by pineapple peel vinegar with 0.2 ml./animal/day
P2: Diabetic rats, treated by pineapple peel vinegar with 0.4 ml./animal/day
P3: Diabetic rats, treated by pineapple peel vinegar with 0.8 ml./animal/day

The rats were treated for 28 consecutive days. The rat's body weight, food, and drink consumption were measured during the treatment period. The blood glucose levels were measured on 0, 7th, 14th, 21st, and 28th treated days.

The day after the last treatment, rats were sacrificed under chloroform anesthetized, and continued by dissected from the abdominal area. The testes were isolated and weighted. The Gonadosomatic Index was calculated by using formula that determined by testis weight divided by bodyweight X 100%[6]. The collected data were analyzed by ANOVA (Analysis of Variance) and continued by
DMRT. Differences at p<0.05 were considered as statistically significant difference. Data were expressed by means ± standard deviation.

3. Result and discussion

3.1. Testes morphology

The morphology of the rat’s testes after 2, 4, and 8 ml of pineapple vinegar administration for 28 days presented in Figure 1. Our result indicating that the largest size of the testes was achieved in the control group and the negative control group was the smallest ones. A comparison of the treated group showed has no differences in testes morphology, indicating that pineapple vinegar at those doses has the same potency with apple vinegar (the positive control group).

![Figure 1. Testis morphology of rats after treated with pineapple vinegar for 28 days](image)

Note: C: control group; Cn: negative control group; Cp: positive control group; P1, P2, and P3: treated groups with pineapple vinegar in 2, 4, and 8 ml/day/animal

3.2. Gonadosomatic Index

The gonadosomatic index was determined on the day after the last day of treatment. Our results presented in Table 1. The analysis of bodyweight by ANOVA continued by DMRT showed that the body weight of rats in the P1 group was not significantly different (p>0.05) from the P3 group. But the P3 group was showed a significant difference (p<0.05) with Cp, Cn, and P2 group. The Cp, Cn, and P2 groups showed no significant difference (p>0.05) when compared to the control group (C).

| Treatment Group | BW (g)       | TW (g)       | GSI (%)     |
|-----------------|--------------|--------------|-------------|
| C               | 276.67± 32.14| 1.44± 0.065  | 0.52± 0.03  |
| Cn              | 230.00bc ± 17.32| 0.83± 0.013 | 0.36± 0.24  |
| Cp              | 233.33bc ± 35.11| 1.37± 0.135 | 0.59± 0.05  |
| P1              | 203.33ab ± 35.11| 1.26ab ± 0.15| 0.62± 0.06 |
| P2              | 233.33bc ± 11.55| 1.23ab ± 0.15| 0.53ab ± 0.08|
| P3              | 180.00a ± 17.32| 1.26b ± 0.17 | 0.70b ± 0.04|

Notes: Values were expressed as Means ± SD. Means in the same column with different superscript letters are significantly different (p<0.05). BW= Body weight; TW= Testis weight; GSI: Gonadosomatic index

The result showed that the rat’s testicular weight of the negative control group (Cn) showed no significantly different (p>0.05) from treated groups P1, P2, and P3. The treated groups (P1, P2, and P3) was showed no significant difference (p>0.05) from the positive control group (Cn) and control group (C).
The gonadosomatic index of the positive control group (Cp), P1 group, and P3) when compared to the control group (C) and the negative control group (Cn). The GSI of the P2 group showed has no significant difference (p>0.05) when compared to the control group (C) and the negative control group (Cn).

We are presenting the GSI in Figure 2 that gives us a clearer explanation about our result. Even though there was no significant difference between the treated group, but the GSI of rats was increased by the pineapple peel vinegar administration. The increase of GSI tends to close with the control group (C). The pineapple vinegar treatment groups (P1, P2, and P3 groups) for 28 days gives a higher potency than apple vinegar (the positive control group, Cp) in increasing the GSI value, as shown in Figure 2.

![Figure 2. Testis weight and GSI of rats after treated with pineapple vinegar for 28 days](image)

Note: C: control group; Cn: negative control group; Cp: positive control group; P1, P2, and P3: treated groups with pineapple vinegar in 2, 4 and 8 ml/day/animal

Alloxan was used as a hyperglycaemic agent in this research Rohilla and Ali (2012) stated that alloxan produces Reactive Oxygen Species (ROS), which accumulated in glucose transporter 2 (GLUT2). Glucose transporter 2 (GLUT2) is a transmembrane carrier protein that enables protein facilitated glucose movement across the cell membrane. The accumulation of ROS will induce cell autophagy, apoptosis, and necrosis [14]. This oxidative stress condition will affect endoplasmic reticulum stress and lysosome destabilization. Further, the necrosis of the cell is stimulated [15] Venegas-Pino et al. (2016) added that chronic hyperglycemia was associated with long-term damage, disfunction, and failure of different organs [16]. The cell damage of the testis tissue is occur in the same mechanism.

The distribution of GLUT2 is in hepatocytes, intestinal absorptive cells, the cells forming the kidney proximal convoluted tubule, the pancreatic beta cells, and in a small number of neurons dispersed in many brain structures and astrocytes [17]. On the other hand, glucose transport is regulated via the hormonal action of the hypothalamic-pituitary-gonadal (HPG) axis [3]. Furthermore, the HPG disorders may cause damage to testis structure and function. At last, the decrease of testis weight and GSI occurs, as seen in this research results.

Saraswaty et al. (2017) stated that pineapple peel contains a high antioxidant that was able to repair the damage of the Langerhans cell structure after treated with alloxan [18]. Hermawati et al. (2020) described that the average inhibition value of antioxidants in pineapple peel vinegar is about 51,46% (unpublished data) [10]. Kalaiselvi et al. (2012) explained that the pineapple peels contain flavonoids, alkaloids, tannins, and steroids [19]. The flavonoid has the oxidation activity of free radicals in the body make it more stable and unreactive radicals [20]. Flavonoids also act as protective of pancreatic β cells and make it able to restore the sensitivity of the insulin receptor of the cell [21]. Prasetyo et al.
(2016) said that flavonoids act as ROS formation and also stimulate the regeneration of pancreatic β cell regeneration [22]. The restoring mechanism of pancreatic β cells by flavonoid from pineapple vinegar was presumed to occur in other cells in the body, including the testicular cell. This recent data showed that testis morphology in diabetic rats (Cn) has the smallest testicular size. It was presumed to be caused by the alloxan administration. When compared to (Cn group), the positive control (Cp) and treated groups (P1, P2, and P3) have a bigger testicular size. It means that the flavonoids of the apple and pineapple vinegar are capable of restoring the structure and function of testicular cells by flavonoids in the pineapple vinegar. Flavonoids have been known can pass the blood-testis barrier and it acts on Sertoli cells in controlling spermatogenesis [23]. On the other hand, the steroid component of apple and pineapple peel vinegar was able to cross the BTB into the luminal space of seminiferous tubules in the testis to build a spermatogenesis microenvironment [24]. Pintus et al. (2015) said that the dynamics of spermatogenesis in mammals contribute to testis weight [25]. Testicular size is the best primary assessment for spermatogenesis since the tubules and germinal elements account for approximately 98% of the testicular mass [26]. Consequently, our previous data showed that the GSI of rats treated with pineapple peel vinegar restore to the normal size.

4. Conclusions
Based on the research results, it can be concluded that the pineapple peel vinegar has high potency in restoring the Gonadosomatic Index on diabetic rats.

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