## The Effect of Pineapple (Ananas comosus L.) Fruit Extract On The Liver White Rats (Rattus norvegicus) Induced By Soft Drinks

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#### Abstract

Sparkling-soft drinks are calorie-carbonated drinks that contain a mixture of water, flavorings, and sweeteners. Sparkling drinks contain a lot of artificial sugars which can burden the liver's work to filter toxins. Consuming too many soft drinks can lead to fat buildup that is harmful to the liver. Pineapple (Ananas comosus L.) is a plant rich in calcium, phosphorus, sodium and bromelin enzymes. Pineapple (<u>A.</u> comosus L.) also contains vitamin C which functions to stimulate the production of liver enzymes and absorb toxins in the blood circulation that are excreted through the urine. In conditions of impaired liver function, it can be indicated by changes in the levels of Serum Glutamic Oxaloacetic Transminase (SGOT) and Serum Glutamic Pyruvic Transminase (SGPT). The research procedures include plant determination, making pineapple fruit extract, phytochemical screening tests, animal preparation, blood collection and surgery on animals, measuring SGPT and SBOT levels, and data analysis. The purpose of this study was to determine the effect of administration of pineapple fruit extract (Ananas comosus L.) on morphology, SGPT and SGOT levels in the liver of male white rats induced by soft drinks. The study used a complete randomized design method (RAL) with 25 male white rats from 5 groups and 5 replicates, conducted for 30 days, and 7 days of acclimatization. The results of administration of pineapple fruit extract had no significant effect on liver morphology and the dose of 400 mg/kg BB was a good enough dose in reducing SGPT and SGOT levels

Keywords: <u>Ananas comosus</u>; Liver; Soft drinks; SGOT; SGPT



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## INTRODUCTION

Soda (Sparkling-soft drinks) contains a lot of artificial sugars that can burden the liver's work to filter out toxins. Consuming too much soda can cause fat accumulation that is harmful to the liver (Kendran et al., 2017). The liver is an organ that has various kinds of metabolic activities. The liver is also the main organ for detoxification of drugs in the body. Disturbances in liver function caused by fatty liver, fibrosis, cirrhosis, and

necrosis can lead to death (Tsani et al., 2017). In conditions of impaired liver function, it can be indicated by changes in the levels of *Serum Glutamic Oxaloacetic Transminase* (SGOT) and *Serum Glutamic Pyruvic Transminase* (SGPT).

Serum Glutamic Oxaloacetic Transminase (SGOT) is an enzyme found in the body. This SGOT is generally found in the heart, kidneys, brain, and liver. This enzyme is in charge of helping to digest proteins in the body. Serum Glutamic Pyruvic Transminase (SGPT) is an enzyme that is often found in the liver. The level of damage to the liver can be observed through biochemical parameters, including aminotranferase enzymes, namely SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Pyruvic Transaminase) (Novelia et al., 2016). SGOT and SGPT examination is a test to find out whether there is an increase in the activity of SGOT and SGPT enzymes in blood fluids. If there is destruction of organs such as liver cells, these two biocatalysts come out of the liver into the blood vessels so that the number will increase (Widigdo, 2014).

The plant that can repair liver damage in white rats (*Rattus norvegicus*) is the pineapple plant. Pineapple is a plant that has antioxidants contained in pineapple that will stimulate liver function to work better, this can prevent difficulties in the process of removing toxins in the body. So, pineapple water can be an effective detoxification drink. The content of vitamin C, niacin, calcium, phosphorus, magnesium, iron, sodium, potassium, polyphenols and bromelain enzymes stored in pineapples is a tough bullet that can defeat the invasion of serious diseases (Sabella, 2010).

Based on the explanation of the description above, soft drinks can cause changes in the structure and physiological function of the constituent tissues in the liver, therefore it is necessary to conduct research to find out the changes in the liver after consuming soft drinks, and can also see improvements in the liver organs that have consumed soft drinks with improvements by pineapples. Therefore, the researcher is interested in conducting a study on the effect of giving pineapple fruit extract (*Ananas comosus*) on the liver of white rats (*Rattus norvegicus*) induced by soft drinks. The benefits of this study are expected to be able to provide information about the prediction of the dosage of consuming soft drinks and morphological changes in the liver and changes in SGPT and SGPT levels in the blood.

#### METHOD

#### **Time and Place of Research**

The research was carried out in June to August 2023 in several laboratories, such as:

- 1. Ellio Science Laboratory Medan as a place for the maintenance and treatment of experimental animals
- 2. Medan Regional Health Laboratory as a place to check SGPT and SGOT levels of white rats (*Rattus norvegicus*)
- 3. FMIPA USU Organic Chemistry Laboratory as a Phytochemical Screening Site for Pineapple Fruit Extract (*Ananas comosus*)
- 4. USU Pharmacy Laboratory as a place to make pineapple fruit extract (Ananas comosus)

5. Medanense Herbarium Laboratory FMIPA-USU as a place for identification of pineapple plants (*Ananas comosus*).

## **Tools and Materials**

The tools and materials used in this study include saucers, containers, ovens, blenders, black cloths, strainers, spatula, measuring cups, analytical scales, freezers, photometers, 96% ethanol, aquaades, methanol, syringes, capillary pipes, centrifuges, and *vortex* mixers.

### **Research Procedure**

This study included experimental research, namely by using a Complete Random Design (RAL) by means of 5 different treatment groups and 5 replicates (See in table 1).

| Treatment | Description  |
|-----------|--|
| К-        | Negative control is given feed and drink for 30 days (morning and evening)   |
| K+        | Positive controls were fed and drunk after induction with 4 ml of soft drink in the morning for 23 days and acclimatized for 7 days                                      |
| P1        | Treatment 1, fed and drunk after induction with 4 ml soft drink in the morning given pineapple extract 300 mg/kg BB in the afternoon for 23 days                         |
| P2        | Treatment 2, fed and drunk after that induced with 4 ml soft drink in the morning and then given pineapple extract 400 mg/kg BB in the afternoon carried out for 23 days |
| Р3        | Treatment 3, given feed and drink after that is induced with 4 ml soft drink in the morning and then given pineapple extract 500 mg/kg BB in the afternoon for 23 days.  |

Table 1. Treatment design for this research

The procedures of this research include plant determination, production of pineapple fruit extract, phytochemical screening tests, preparation of experimental animals, induction and determination of the dosage of soft drinks, induction and determination of the dosage of pineapple fruit extract, blood sampling and dissection of experimental animals, and measurement of SGPT and SGOT levels.

## **Data Analysis**

The results of the data obtained from the measurement of SGOT levels, SGPT were comparatively tested using the ANOVA (*Analysis of Variance*) test with a significant value of P<0.05. The test began with a normality distribution test with *the Saphiro wilk test*, a variant homogeneity test and continued to the ANOVA test.

### **RESULTS AND DISCUSSION**

#### Screening of Phytochemical Spectrum of Pineapple Fruit (Ananas comosus)

The results of Phytochemical Screening that have been carried out at the FMIPA USU Organic Chemistry Laboratory can be seen in the following table 2.

| It | Secondary Metabolite<br>Compounds | Reagent                            | Screening Result |
|----|-----------------------------------|------------------------------------|------------------|
| 1  | Flavonoid                         | FeCl3(aq) 5%                       | +                |
|    |                                   | H2SO4(p)                           | +                |
|    |                                   | Mg(s) + HCl(P)                     | +                |
| 2  | Alkaloid                          | Bouchardart                        | -                |
|    |                                   | Maeyer                             | -                |
| 3  | Terpenoid                         | Salkowsky                          | +                |
|    |                                   | Liebermann Bourchard               | -                |
| 4  | Steroid                           | Salkowsky                          | +                |
|    |                                   | Liebermann Bourchard               | -                |
| 5  | Tannin                            | FeC13 (aq) 5%                      | +                |
| 6  | Saponin                           | Aquadest + Alkohol 96% + HCl<br>2N | +                |

Based on table 2, it is stated that pineapple fruit extracts contain sacalin metabolite compounds, namely flavonoids, saponins, tannins, and those that use salkowsky reagents are part of the compounds of terpenoids and steroids. Meanwhile, those that do not contain metabolite compounds, namely alkaloids and reagents that used Liebermann bourchard, part of steroid compounds and terpenoids. Phytochemical screening of pineapple fruit ethanol extract aims to identify the content of satopic metabolite compounds derived from the plant (Putri et.al., 2013).

Flavonoids are secondary metabolites of polyphenols, found widely in plants. Flavonoids are compounds consisting of 15 carbon atoms that are generally scattered in the plant world. Flavonoid compounds are also the largest group of phenolic compounds found in nature, these compounds are red, purple, and blue dyes and are yellow dyes found in plants. The content in these flavonoids can treat various diseases (Hu et al., 2016).

Tannins are food compounds that fall under the category of polyphenol compounds (Duraisamy et al., 2019). This compound is found in various parts of the plant (Nofita & Dewangga, 2021). The content of tannin compounds acts as a place to inhibit the growth of microorganisms (Makatamba et al., 2020). Tannins have water-soluble properties and can also be soluble in organic solvents (ethanol, methanol, acetone, etc.). In addition, the main property of tannins is that they can precipitate proteins. The parts of plants that usually contain tannin compounds are the parts of the root tissue, shoots, seeds, leaves, and stems (Wiartini, 2023).

Terpenoids are the largest group of secondary metabolite compounds that have a wide variety of compound types. Terpenoid compounds are secondary metabolites of plants that have high antioxidant activity. The diverse structure of terpenoids can be linear to polycyclic molecules, ranging in size from five-carbon hemiterpenes to rubber that has thousands of isoprene units (Hartati et al., 2016).

Steroids are lipid terpenoids known as four fused carbon base skeleton rings. The compound structure is also quite diverse. The difference is due to the presence of an oxidized functional group bound to the ring and the oxidation of the carbon ring. This compound is important as fat in the body which plays a role in the synthesis and recovery process of body cells (Samejo et al., 2013).

Saponins are natural glycosides that have amphiphilic surface activity, have a large molecular weight and their molecular structure consists of steroid aglicones or triterpenes called sapogenin and glycons that contain one or more sugar chains. Saponins have an antimicrobial effect, as well as inhibiting fungi. Has properties as an antioxidant, and antiviral. Saponins are also a diverse group of compounds in their structure, physicochemical properties, and biological effects (Assefa & Addisu, 2016).

#### Total Antioxidant Level Test (IC50) of Pineapple Fruit Extract

The results of the total antioxidant level test (IC50) in pineapple fruit have a concentration of 69.6761 ppm/IC50, it can be stated that the concentration is strong and can be seen from table 3. The antioxidant level test using DPPH solution is a method of antioxidant test that will neutralize free radicals. The DPPH free radical reduction method is based on the reduction of DPPH free radical methanol solution. In this examination, a UV-Vis spectrophotometer was used which was seen as a result of measuring a certain wave length (Fatmawati, 2023).

Table 3. Antioxidant Test Examination Results

| Sample Name  | Test Type               | Concentration                |
|--|-------------------------|------------------------------|
| Pineapple Fruit Extract                                      | Antioxidant             | 69.6761 ppm/IC50             |
| <b>Note</b> : Very Strong = <50 (ppm), Stro<br>151-200 (ppm) | png = 50-100 (ppm), Mec | lium = 101-150 (ppm), Weak = |

The content of chemical compounds in pineapples, namely flavonoids, tannins, steroids, terpenoids, saponins, are chemical compounds that play a role in antioxidant activity. The results of the test of the total antioxidant level (IC50) in the pineapple fruit content there is a concentration of 69.6761 ppm/IC50, it can be stated that the concentration is strong. This certainly supports this research, where antioxidant activity is needed to ward off free radicals.

#### Flavonoid Level Test of Pineapple Fruit Extract (Ananas comosus L.)

Based on the results of the examination of flavonoid levels in the laboratory, it can be seen in table 4. Based on table 4. There were pineapple fruit extract results in the flavonoid test with a concentration result of 9.1728 mg/gram. Flavonoids are one of the phenols that have an effect as antioxidants that can protect and reduce or overcome damage caused by reactive oxygen species. Antioxidants are indispensable as a protection of the liver organ to prevent continued oxidative damage. Damage

caused by free radicals in the body can be overcome with antioxidants. Antioxidants are defined as a substance that can prevent free radicals (Halliwell & Gutteridge, 2007).

 Table 4. Flavonoid Test Results

| Sample                  | Test Type | Concentration  |
|-------------------------|-----------|----------------|
| Pineapple Fruit Extract | Flavonoid | 9.1728 mg/gram |

## Effect of Pineapple Fruit Extract on Liver Morphology of White Rats Induced by Soft Drinks

Based on the results of the research that has been carried out, morphological changes in the rat liver can be seen in figure 1. Based on Figure 1, the morphological description of the liver of rats that had been given pineapple fruit extract and induced with soft drinks for 23 days and acclimatized for 7 days. In the positive control given feed and soft drinks at a dose of 4 ml, the color of the heart was brownish-red and the surface was slippery. In the negative control only feed is given, the heart is reddishbrown and the surface is smooth.

Furthermore, in the treatment that has been given feed, soft drinks with a dose of 4 ml and administration of pineapple extract with tiered doses of 300 mg/kg, 400 mg/kg, and 500 mg/kg it looks like there are no changes or abnormalities, the liver does not harden, the surface is smooth and brownish. This is in accordance with the statement Liwandouw (2017) that normal waterhas a smooth and flat surface and is brownish-red. While an abnormal liver has a mottled surface, cysts and changes in color. A normal liver also has a slippery surface. According to Fatmawati et al., (2018) a normal heart has a reddish-brown color. This is due to the large amount of blood flowing that is facilitated by blood vessels. A normal liver will be reddish-brown caused by the blood flow that enters the liver organs. The results of the examination of the liver organ index can be seen in the following table 5.

| Treatment | Elementary ± Liver<br>Index | p=value |
|-----------|-----------------------------|---------|
| К-        | 5.44 ± 1.26b                |         |
| K+        | $3.96 \pm 0.85a$            |         |
| P1        | $3.04 \pm 0.40a$            | 0.000   |
| P2        | 3.43 ± 0.49a                |         |
| P3        | $3.30 \pm 0.32a$            |         |

| Table 5   | Liver | Organ | Index | Value  |
|-----------|-------|-------|-------|--------|
| I ubic D. |       | Orgun | mach  | 1 uruc |

**Description**: SD : Definition standard, K- (Negative control) feeding, K+ (Positive control) 4 ml soft drinks, P1 (Treatment 1) 4 ml soft drinks and pineapple fruit extract 300 mg/kg, P2 (Treatment 2) 4 ml soft drinks and pineapple fruit extract 400 mg/kg, P3 (Treatment 3) 4 ml soft drinks and pineapple fruit extract 500 mg/kg



Figure 1. Morphology of observation results for each treatment
Description: K- (negative control) feeding, K+ (positive control) 4 ml soft drinks, P1 (treatment 1) 4 ml soft drinks and pineapple fruit extract 300 mg/kg, P2 (treatment 2) 4 ml soft drinks and pineapple fruit extract 400mg/kg, P3 (treatment 3) 4 ml soft drinks and pineapple fruit extract 500 mg/kg.

The results of the liver organ index examination can be seen in table 5, that pineapple fruit extract and soft drinks have a real effect on the liver of male white rats. Very different from the results of the examination by macroscopic observation on the liver organ that has been treated, it seems that the results show that the liver organ looks normal, because it does not experience any deformities or color changes. In the journal Liwandow (2017) which states that a normal liver has a flat and smooth surface and is brownish-red.

The organ index is a parameter that can provide a general overview of the effects of the compound whether enlargement or shrinkage occurs in the organ. An overview of organ changes, both enlargement and shrinkage of organs, is the most important thing in examining organ indexes. The purpose of this organ index was to compare the organ index between the control group and the treatment group to observe the effects of exposure after administering a test preparation for 23 consecutive days (Safira et al., 2022).

The liver is a vital metabolic organ that performs various functions, which is very important for survival. The liver regulates the body's energy supply, several important compounds and cleanses substances by several excretion methods, so excessive consumption of these drinks or foods can affect damage to the organs (Liwandow, 2017). to see the functioning of the liver, it can be seen from the results of SGPT and SGOT levels.

# Effect of Pineapple Fruit Extract (*Ananas comosus*) on SGPT of White Rats Induced by Soft Drinks

Based on the results of *the one-way* ANOVA test contained in table 6, the SGPT level of male white rats was obtained with a significant level of p-value, namely (p= 0.000) which means that the administration of soft drinks had a real effect on SGPT and pineapple fruit extract during 23 days of treatment in male white rats had a very significant effect on SGPT levels in male white rats. Result of *one way* ANOVA test in the examination of SGPT levels of male white rats can be seen in table 6.

| Group | SGPT (U/L) ± SD | p=value |
|-------|-----------------|---------|
| К-    | 71.80±3.70c     |         |
| K+    | 44.20±3.89a     |         |
| P1    | 73.00±6.59c     | 0.000   |
| P2    | 46.80±1.09a     |         |
| P3    | 53.80±2.16c     |         |

**Table 6.** Average Value of SGPT Number of Male White Rats

**Description**: SD: Deviation standard, K- (Negative control) feeding, K+ (Positive control) 4 ml soft drinks, P1 (Treatment 1) 4 ml soft drinks and pineapple fruit extract 300 mg/kg, P2 (Treatment 2) 4 ml soft drinks and pineapple fruit extract 400 mg/kg, P3 (Treatment 3) 4 ml soft drinks and pineapple fruit extract 500 mg/kg

The SGPT level of the positive group  $(44.20\pm3.89a)$  was significantly different from treatment 1 (73.00±6.59c), treatment 2 (46.80±1.09a) and treatment 3 (53.80±2.16b). Meanwhile, the negative group (71.80±3.70C) was significantly different from treatment 1, treatment 2, and treatment 3. This shows that treatment with pineapple extract can reduce SGPT levels in mice induced by soft drinks. It can be seen from treatment 2 with a dose of 400 mg/kg is the best dose among other treatments.

The negative control had a higher value compared to the positive control treatment and P2 and P3 which was  $(71.80\pm3.70c)$ . This can be caused by stress due to the influence of the rat environment or a lack of good care in the negative control rat category, causing stress in rats. Stress in rats occurs when the formation of reactive oxygen molecules (free radicals) and their removal of actividan are imbalanced, so that it can cause damage to the hepatocyte membrane that affects its structure, function and fluidity. The damage causes cytosolic enzymes hepatocytes and SGOT to come out so that it can cause an increase in SGPT and SGOT levels. Research by Saputra et al., (2018) explained that SGPT is an enzyme that is widely found in the liver organs, especially in mitochondria. Mitochondria organel will send precepts to lysosomes to release lysosomal enzymes to make apoptosis, where apoptosis can cause an increase in SGPT produced from these cells then enters the bloodstream and causes an increase in SGPT levels in the blood.

# Effect of Pineapple Fruit Extract (Ananas comosus) on SGOT of White Rats Induced by Soft Drinks

Result test *one way* ANOVA in the examination of SGOT levels of male white rats can be seen in table 7. Based on the results of *the one-way* ANOVA test contained in table 7, the SGOT levels of male white rats were obtained with a significant level of p value, which is p = 0.000 which means that soft drinks and pineapple fruit extract during 23 days of administration in male white rats greatly affected the SGOT levels in male white rats.

| Treatment | SGOT (U/L) ± SD | p=value |
|-----------|-----------------|---------|
| K-        | 82.60±0.54A     |         |
| K+        | 95.60±0.54d     |         |
| P1        | 93.60±0.54c     | 0.000   |
| P2        | 84.00±2.44a     |         |
| P3        | 86.40±1.51b     |         |

Table 7. Average Value of the Number of SGOT of Male White Rats

**Description:** SD : Deviation standard, K- (Negative control) feeding, K+ (Positive control) 4 ml soft drinks, P1 (Treatment 1) 4 ml soft drinks and pineapple fruit extract 300 mg/kg, P2 (Treatment 2) 4 ml soft drinks and pineapple fruit extract 400 mg/kg, P3 (Treatment 3) 4 ml soft drinks and pineapple fruit extract 500 mg/kg

Then continued with a follow-up test using the duncan test with a significant level of 5%. It can be seen that the negative control group ( $82.60\pm0.54a$ ) is significantly different from the positive control group ( $95.60\pm0.54d$ ). This showed that giving soft drinks at a dose of 4 ml in the morning for 23 days of treatment could increase SGOT levels in male white rats. In a study by Mukharomah et al., (2023) consuming soft drinks can increase SGOT (*Serum Glutamic Oxaloacetic Transaminase*) levels which are carried out for 21 days.

Based on the results of this study, consuming soft drinks with a dose of 4 ml for 23 days can cause problems in the liver, such as liver function at increased SGOT levels. Sugar consumption, especially in the form of fructose, is suspected to cause liver disease and can make the synthesis of hepatic fatty acids increase, if SGOT levels increase, damage to the liver occurs (Lebda, et al., 2017). The content of soft drinks is carbonated water, acidity regulating sugar (citric acid), artificial sweeteners, dyes, and preservatives. Consuming soft drinks can be a toxin that causes damage to liver function. Indicators of liver function can be known through the examination of the SGOT (*Serum Glutamic Oxaloacetic Transaminase*) enzyme which is an enzyme produced by the parenchyma of liver cells.

Based on table 7, it can be seen that the SGOT level of the positive group  $(95.60\pm0.54d)$  is significantly different from treatment 1  $(93.60\pm0.54c)$ , treatment 2  $(84.00\pm2.44a)$  and treatment 3  $(86.40\pm1.51b)$ . Meanwhile, the negative group  $(82.60\pm0.54a)$  was significantly different from treatment 1, treatment 2, and treatment 3. This shows that treatment with pineapple extract can reduce SGOT levels in mice induced by soft drinks. It can be seen from treatment 2 with a dose of 400 mg/kg is the best dose among other treatments.

Based on the results of phytochemical screening that has been carried out on pineapple fruit extract contains secondary metabolite compounds, one of which is flavonoids, from the content of flavonoids in pineapple fruit extract, it can neutralize SGOT (*Serum Glutamic Oxaloacetic Transaminase*) levels in the blood. Flavonoids are natural plant compounds that have health benefits, including anti-inflammatory properties, and antioxidants that can protect body cells from oxidative damage caused by free radicals. Liver damage can also be prevented through the use of herbal ingredients because they contain hepatoprotectors, which are compounds that protect liver cells from the influence of toxic or toxic substances that can damage the liver (Sindi et al., 2023).

The administration of pineapple fruit extract greatly affects the reduction of SGPT and SGOT levels induced by soft drinks. The most effective treatment in reducing SGPT and SGOT levels was found in the P2 treatment with an average value of SGPT ( $46.80\pm1.09a$ ) and SGOT ( $84.00\pm2.44a$ ) with a dose of pineapple fruit extract of 400 mg/kg BB. This is in accordance with the research of Oktariani et al., (2024), the administration of pineapple extract at a dose of 400 mg/kg BB is effective in reducing SGPT and SGOT levels in rats experiencing hyperglycemia. Pineapple fruit extract is very good at repairing liver damage in male white rats, pineapple fruit extract contains a lot of secondary metabolite compounds, one of which is flavonoids, from the content of flavonoids in pineapple fruit extract, it can neutralize SBOT levels. Flavonoids are secondary metabolite compounds that belong to the group of phenolic compounds that have functions as antioxidants and anti-inflammatory agents that can prevent free radicals (Ichsani et al., 2021).

## CONCLUSION

Based on the results of the study on the effect of pineapple fruit extract (*Ananas comosus* L.) on liver morphology, SGPT and SGOT of white rats (*Rattus norvegicus*) induced by soft drinks, it can be concluded that the treatment of pineapple fruit extract had no real effect on liver morphology because there were no signs of changes or abnormalities in the liver. There was an effect of administration of pineapple fruit extract on SGPT in white rats at P2 (46.80 $\pm$ 1.09a) with a dose of 400 mg/kg BB very significantly reduced SGPT levels in male white rats. There was an effect of administration of pineapple fruit extract on SGOT in white rats at P2 (84.00 $\pm$ 2.44a) with a dose of 400 mg/kg BB very significantly reduced SGOT levels in male white rats.

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