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The Effect of Combination Psophocarpus Tetragonolobus and Pisum sativum Extract on The Histological Structure of The Liver, Kidney and Stomach of Rattus norvegicus

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ABSTRACT

This research aims to determine the effect of the extract of winged beans (*Psophocarpus tetragonolobus*) and peas (*Pisum sativum*) on the histological structure of the liver, kidneys, and stomach of white rats (Rattus norvegicus).

The objects of this research are twenty (20) white rats female Wistar strain with ±100-200 grams of weight which had never been pregnant. White rat samples are divided into 1 group as a control (P0) and 3 treatment groups with P1 25 mg/kgBW of winged bean extract + 75 mg / kgBW of peas extract, P2 50 mg / kgBW of winged bean extract + peas extract 50 mg / kgBW, and P3 winged bean extract 75 mg / kgBW + peas extract 25 mg / kgBW given for 21 days. Each group consists of 5 repetitions. The analysis used the One Way Anova test, he results were significant, and then proceeded with Duncan's Multiple Range Test (DMRT) test. To determine the trend of the effect of winged bean seed extract and pea on the histological structure of the liver, kidneys, and stomach, descriptive analysis was performed.

The results showed that the extract of winged bean and pea had no significant effect ($p > 0.05$) on the histological structural damage of the liver and kidneys of white rats. Giving winged bean extract and peas extract on the histological structure of the stomach of white rats in all treatment groups was under normal conditions.

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1. INTRODUCTION

Peas (*Pisum sativum*) is one type of local bean with a low consumption rate, because it is less optimal in terms of utilization. In peas there are phytoesterogen content that serves to improve fertility profiles. According to Khan et al. (2017), peas contain 20-25% starch, 4-10% sugar, 0.6-1.5% fat, and 2-4% minerals which can play an important role in meeting nutritional deficiencies. In addition, in pea seeds according to Saragih et al (2018), there are bioactive compounds or phytochemical properties, namely as *anti-Helicobacter pylori*, anti-bacterial, anti-diabetic, anti-cancer, anti-fungal, anti-inflammatory, anti-lipidemic and antioxidant activities.

Chipper (Psophocarpus tetragonolobus) is a plant that also has a utilization rate that is not optimal. Based on research conducted by Wahyuni, S. (2010), raw bean has an isoflavone content of 0.212 g where the isoflavone content in the bean is higher than raw soybeans (0.179 g) and raw beans (0.148 g). Chipper fruit contains flavonoids, saponins, polyphenols, steroids and terpenoids known

from phytochemical test results. In the leaves and seeds of bean there are active compounds namely saponins, flavonoids and tannins. In addition, chipper contains various important minerals such as calcium, zinc, sodium, potassium, magnesium, phosphorus, and iron (Kaihena, M., & Samson, E., 2019).

Peas and chippers have a fairly high nutrient content, but both plants also contain potentially toxic substances in the body. The chemical content in chippers that are potentially toxic in the body is phytic acid (0.897 g), tannins (0.368 g) (Almasyhuri et al, 1990), saponins (Kaihena, M., &; Samson, E., 2019). While in peas there are tannins and phytic acid (IdnMedical, 2020). If these substances are consumed in very large quantities, of course, it will affect the work function of organs.

The liver is the largest parenchyma organ which has many and complex functions. One of the functions of the liver is as a detoxification center for toxic substances in the body. Toxic substances enter the body through gastrointestial, after being absorbed, toxicants will be carried by the portal vein to the liver (Frank C. Lu, 1995). So that if the liver is disturbed, of course it will reduce the body's work function (degeneration) and can cause death (Harada et al., 1996). In addition to the liver, the kidneys are also one of the organs that have a large role in metabolism. The kidneys as one of the organs for toxicity testing of a drug material play an important role in the sustainability of compounds / drugs in the body system, because most of the toxicants are excreted through the kidneys. The liver and kidneys will undergo cell death. The stages of cell death include pycnose, cariorexis, and karyolysis (Harisson, 1999).

The stomach organ is a digestive organ that often experiences problems when consuming too much of a food that does not match the proper dose content. Materials that enter the stomach organs can damage the glandular mucosa and tight epithelial junctions between stomach lining cells to often cause severe acute or chronic gastritis (Guyton, 1997).

Based on the background above, the utilization of phytoestrogen content in the combination of bean and pea extract needs further testing. In this study, white rats (Rattus norvegicus) were used as test animals, because the working system of anatomical and physiological organs is almost the same as human organs. Therefore, this study was conducted to examine the effect of giving pea seed extract and bean on the histological structure of the kidneys, liver and stomach of white rats (Rattus norvegicus).

2. RESEARCH METHOD

2.1. Types of research

This research was conducted in December 2020 – March 2021. This research was conducted at the Pharmaceutical Laboratory Unit II UGM for the manufacture of chipper and pea seed extract, the maintenance of female white rats was carried out at the Biological Garden Animal Management Unit FMIPA UNY, the manufacture of histological preparations of liver, kidney, and stomach organs was carried out at the AMC Anatomical Pathology Laboratory, Observation of histological preparations of liver, kidney, and stomach organs was carried out at the Biological Microscopy Laboratory FMIPA UNY.

2.2. Population and Research Sample

The population of this study was female white rats (*Rattus norvegicus*) with a body weight of ± 100 – 200 grams and had never been pregnant. The research samples used were 20 female white rats (*Rattus norvegicus*), wistar strains, aged 2 months with a body weight of approximately 100-200 grams obtained from the Faculty of Pharmacy UGM.

2.3. Procedure

The rats were divided into 4 groups, namely the control group (P0), treatment 1 (P1), treatment 2 (P2), and treatment 3 (P3). The control group was not given a combination of bean seed extract and peas, P1 was given bean seed extract 25 mg/kgBW + pea seed extract 75 mg/kgBW, P2 bean seed extract 50 mg/kgBW + pea seed extract 50 mg/kgBW, and P3 bean seed extract 75 mg/kgBW + pea seed extract 25 mg/kgBW. The rats were fed and watered during the acclimatization period of 7 days. Vaginal review was done on the first day before giving a combination of bean and pea extract as a sign of whether the rat had estrus or not. Rats were given seed extracts of bean and peas for 21 days once every day in the morning before being fed. After

21 days of strangulation, surgery was performed on day 22 of the rat's estrus. Then the collection of liver, kidney, and stomach organs is carried out. After that, preparations were made at AMC.

2.4. Data Collection Techniques

Data collection was carried out through observations on each preparation of liver, kidney and stomach of white rats that had been made and taken pictures as documentation, then calculated the number of kidney and liver cells that experienced pycnosesis, karyorexia and karyolysis in 4 fields of view to reach 100 cells in each field of view. For gastric organs, histological structures are observed, whether they have damage to the mucosana layer or are still normal. Damage to the gastric organs is characterized by loss of epithelium in the mucous lining of the gastric organs. The results obtained are then analyzed.

2.5. Data Analysis Techniques

The data obtained were analyzed using *One Way Anova*. If there is a noticeable effect, then proceed with *Duncan's Multiple Range Test* (DMRT). To determine the tendency of the influence of winged seed extract (*Psophocarpus tetragonolobus*) and pea seed extract (*Pisum sativum*) on the histological structure of the liver, kidneys, and stomach, descriptive analysis was carried out.

3. RESULTS AND ANALYSIS

3.1. The Effect of Giving Winged Grape Extract and Pea Extract on the Histological Structure of White Rat Liver

This data is taken by observing the preparation and then counting the number of liver cells that experience pycnosesis, karyorexia and karyolysis in 4 fields of view to reach 100 cells in each field of view. The calculation results of the number of histological structures of the liver that experience pycnosesis, cariorexis, and karyolysis can be seen in table 1 below:

Table 1. Average Number of Picnosis, Caryorexia, and Caryolysis in White Rat Liver after Treatment of Winged Grape Extract and Pea Extract.

	P0	P1	P2	P3
Picnosis				
Average number	4	5	4	5
Standard deviation	0,49	0,74	0,66	1,85
Karyorexia				
Average number	3	4	4	3
Standard deviation	0,49	0,4	0,48	0,4
Karyolysis				
Average number	1	1	1	1
Standard deviation	0,49	0	0,4	0,4

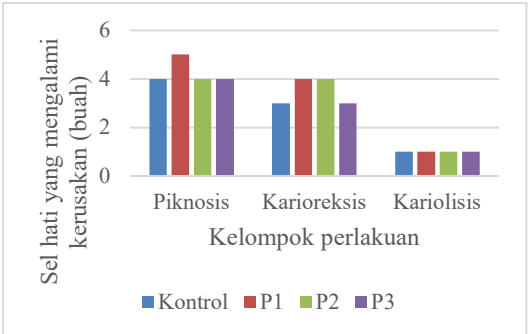

Figure 1. Bar chart of the number of pycnose, karyorexia and liver karyolysis

Based on the results of observing the number of liver cells of white rats (*Rattus norvegicus*) that experienced pycnosesis, karyorexia and karyolysis after administration of bean extract (*Psophocarpus tetragonolobus*) and pea extract (*Pisum sativum*) it can be seen that by comparing the value of the average number of pycnose, cariorexis, and karyolysis between the control group and the treatment group showed the results that the administration of bean extract (*Psophocarpus tetragonolobus*) and pea extract (*Pisum sativum*) had no effect on changes in the histological structure of white rat livers at the pycnose, cariorexis, and karyolysis stages.

To determine the effect of treatment on changes in the liver structure of white rats at the stages of pycnose, cariorexis, and karyolysis, an analysis was carried out using the anova test. The results of the anova test as in table 2 are as follows:

Table 2. *One Way Anova* Analysis of Giving Winged Seed Extract and Pea Extract to Changes in Liver Histological Structure at the Stage of Pricknose, Caryorexia, and Karyolysis of White Rats

Variable	Sig.
Picnosis	.683
Karyorexia	.214
Karyolysis	.224

* No noticeable difference (p>0.05)

Based on the results of data analysis using the one way anova test in table 2 showed insignificant results ($p > 0.05$) at the pycnose, cariorexis, and karyolysis stages. This means that giving winged seed extract (*Psophocarpus tetragonolobus*) and pea seed extract (*Pisum sativum*) to white rats (*Rattus norvegicus*) had no effect on changes in the histological structure of white rat livers. Some possibilities that cause the ineffect of giving test treatment to white rats, namely the absence of preliminary tests for the determination of treatment doses, so that the number of treatment doses given is still within the limits of liver tolerant doses to survive potentially toxic substances contained in bean seeds and peas. If the number of treatment doses given is increased, it is likely to affect the histological structure of the white rat liver. In addition, the intensity of time in giving treatment is also likely to affect in experiments so that if the intensity of time in giving treatment is increased it is likely to affect changes in the histological structure of white rat livers. A substance that can cause changes in the structure of liver cells, due to exposure to compounds that have too long an intensity of administration. Frank C. Lu, (1995) said that although toxicity is an innate property of a substance, but the real factors that affect the toxicity of a substance are dosing and exposure.

Esrefuglo, 2013 in Safithri F., (2018) said that the liver has the ability to regenerate itself by increasing the speed of mitosis of hepatocytes and also increasing the differentiation of stem cells into hepatocytes or cholangiocytes. Stem cells are the main *cell lineage* for liver regeneration. There is more than one population of stem cells in the liver, namely resident stem cells, progenitor cells and extrahepatic stem cells, which can differentiate into hepatocytes and biliary epithelial cells. So it can be concluded that, the dose and duration of treatment of bean and pea extracts are still within the limits of liver tolerant doses to survive potentially toxic substances contained in bean and pea extracts so that there is no change in the histological structure of white rat livers.

3.2. The Effect of Giving Winged Beans Extract and Pea Extract on the Histological Structure of White Rat Kidneys

Data on the histological structure of damaged kidneys are taken by observing the preparation and then counting the number of kidney cells that experience pycnosesis, karyorexia and karyolysis in 4 fields of view to reach 100 cells in each field of view. The calculation results of the number of histological structures of the kidneys that experience pycnosesis, cariorexis, and karyolysis can be seen in table 3 below:

Table 3. Average Number of Picnosis, Caryorexia, and Carolysis in White Rat Kidneys after Treatment of Winged Grape Extract and Pea Extract.

	P0	P1	P2	P3

Picnosis				
Average number	2	1	2	2
Standard deviation	0,49	0,49	0,4	0,4
Karyorexia				
Average number	4	4	3	3
Standard deviation	1,02	0,4	0,49	0,49
Karyolysis				
Average number	1	1	1	1
Standard deviation	0	0	0	0

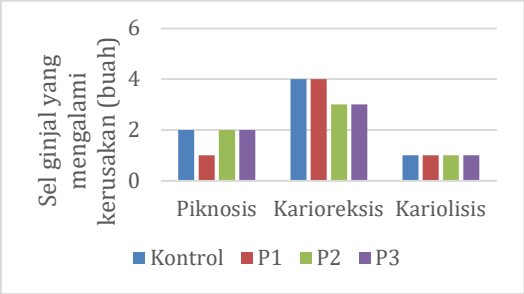

Figure 2. Bar chart of the number of pycnosesis, karyorexia and renal cariolysis

Based on the results of observations on the number of kidney cells of white rats (*Rattus norvegicus*) that experienced pycnosesis, karyorexia and karyolysis after administration of bean extract (*Psophocarpus tetragonolobus*) and pea extract (*Pisum sativum*) it can be seen that by comparing the value of the average number of pycnose, cariorexis, and karyolysis between the control group and the treatment group showed the results that the administration of bean extract (*Psophocarpus tetragonolobus*) and pea extract (*Pisum sativum*) had no effect on changes in the histological structure of white rat kidneys at the stages of pycnosesis, karyorexia, and karyolysis.

To determine the effect of treatment on changes in the liver structure of white rats at the stages of pycnose, cariorexis, and karyolysis, an analysis was carried out using the anova test. The results of the anova test as in table 4 are as follows:

Table 4. *One Way Anova* Analysis of Giving Winged Seed Extract and Pea Extract to Changes in Kidney Histology Structure at the Stage of Pricknose, Caryorexia, and Caryolysis of White Rats

Variable	Sig.
Picnosis	.936
Karyorexia	.918
Karyolysis	.978

* No noticeable difference (p>0.05)

Based on the results of data analysis using *the one way anova test* in table 4 showed insignificant results (p > 0.05) at the pycnose, cariorexis, and karyolysis stages. This means that giving winged seed extract (*Psophocarpus tetragonolobus*) and pea seed extract (*Pisum sativum*) to white rats (*Rattus norvegicus*) had no effect on changes in the histological structure of white rat kidneys. There are several possibilities that cause the absence of the effect of treatment of winged seeds and peas on white rats, namely at the beginning of the experiment no preliminary tests were carried out to determine the dose of treatment, so that the number of treatment doses given was still within the limits of the tolerant dose of kidney organs to survive potentially toxic substances contained in winged seeds and peas. If the number of treatment doses given is in accordance with the results of preliminary trials, it is likely to have an influence on the histological structure of the kidneys of white rats. In addition, the treatment dose interval is also likely to

affect during the experiment so that if the intensity of time in treatment is increased it is likely to affect changes in the histological structure of the kidneys of white rats.

The kidney organ has tremendous compensation, even after some fairly important changes to the function and morphology of the kidney, the kidney can compensate and function normally. Marfuati et al (2014) in Yuliana (2019) said that tubule epithelial cells that experience damage in the form of necrosis can regenerate normally as a form of mitotic activity in existing tubular epithelial cells. Total epithelial cell regeneration can occur if the damage does not reach the basement membrane.

So, it can be concluded that the treatment dose in this study is still within the limits of the tolerant dose of kidney organs with the intensity of administration time that is still within reasonable limits, so it does not affect changes in the histological structure of the kidney organs of white rats.

3.3. The Effect of Giving Winged Grape Extract and Pea Extract on the Histological Structure of White Rat Stomach

Based on the results of the study, it can be seen that the histological structure of the stomach of white rats in all treatment groups is normal. This means, the administration of pea seed extract and bean bean did not affect the histological structure of the stomach of white rats. The treatment dose of white rats is still within the limits of the tolerant dose of gastric organs to survive toxic substances. According to Tarigan 2006 in Eiffellia, A. R. (2010), the defense system of the gastroduodenal mucosa under normal circumstances is a system that can recover and is able to defend against damaging materials such as stomach acid, pepsin, bile acids, pancreatic enzymes, drugs and bacteria. The gastroduodenal mucosal defense system consists of 3 obstacles, namely the pre-epithelial layer, surface epithelial cells and sub-epithelium.

Bintara, 2013 in Erlinda, D. (2017), said that damage to the gastric mucosa can occur due to inhibition of prostaglandin synthesis. Prostaglandins are cytoprotective, decreased prostaglandin levels will cause an imbalance between aggressive factors (stomach acid and pepsin) with protective factors (mucus, bicarbonate, blood flow, epithelial regeneration) so that it can trigger inflammatory processes that will increase the formation of free radicals. These free radicals can cause damage to the gastric mucosa. Seeds and peas contain flavonoid compounds which act as antioxidants to capture free radicals so that there is no inhibition of prostaglandin synthesis.

There are several things that are important in the defense of the gastric mucosa, namely mucus, cell regeneration power, mucosal blood flow and prostaglandins. Gastric mucus is a mechanical and chemical irritant protector which can protect the gastric mucosa from acid and pepsin. The defense of the hull is also aided by the rapid regeneration power of the hull. This high regeneration power makes the damaged gastric epithelium always replaced, so it does not produce erosion of the epithelium. In addition, mucosal blood flow also helps maintain cell integrity by removing excessive acid in the cell by maintaining tissue oxygenation so as to buffer acid diffusion back. In gastric defense, prostaglandins improve blood and mucosal flow and inhibit acid secretion (Amirudin &; Usman, 1991 in Eiffellia, A. R., 2010). So it can be concluded that, the dose of treatment and the intensity of administration are still within the limits of the dose and tolerant time of the gastric organs in defense.

4. CONCLUSION

From the existing research, the following decisions were made:

1. Giving winged seed extract (*Psophocarpus tetragonolobus*) and pea seed extract (Pisum sativum) had no effect on changes in the histological structure of white rat liver (*Rattus norvegicus*) in the form of pycnose, karyorexia and karyolysis.
2. The administration of winged seed extract (*Psophocarpus tetragonolobus*) and pea seed extract (*Pisum sativum*) had no effect on changes in the histological structure of white rat kidneys (*Rattus norvegicus*) in the form of pycnose, karyorexia and karyolysis.
3. Administration of winged seed extract (*Psophocarpus tetragonolobus*) and pea seed extract (*Pisum sativum*) to the histological structure of the stomach of white rats (*Rattus norvegicus*) in all treatment groups under normal circumstances.

Suggestion

1. It is necessary to conduct preliminary tests first to determine the dose to be used in the study, so as to obtain the desired research results.
2. We recommend that the white rat to be used is tested first so that it is homogeneous.

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