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Gastroprotective effect of ethanol extract of carica papaya seed on rats induced rifampicin and isoniazid

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ABSTRACT

The first-line therapy for tuberculosis is Rifampicin, in combination with Isoniazid (INH), Ethambutol (ETB), and Pyrazinamide (PZA). The side effects of TB drugs that may arise if the drug use for a long time include hepatotoxicity and gastrointestinal disturbances. Carica papaya seeds has a potential effect to prevent the gastrointestinal distrubances caused by rifampicin and isoniazid. This study aimed to evaluate the gastroprotective effect of carica papaya seeds on rats induced rifampicin and isoniazid. Experimental design group was divided into 8 group, including normal, negative 1 (rif 50 mg/kgbb), negative 2 (IN 50 mg/kgbw), negative 3 (rif and inh 50 mg/kgbw), positive 1 (rif and inh 50 mg/kgbw + cimetidtine 18 mg/kgbw), group 1(rif and inh 50 mg/kgbw + EECP 100 mg/kgbb) +, group 2 (rif and inh 50 mg/kgbw + EECP 300 mg/kgbb), group 3 (rif and inh 50 mg/kgbw + EECP 500 mg/kgbb). Rifampicin, isoniazid, and EECP was given 20 days. The result showed that rifampicin and isoniazid induced alteration gastric organ, histopathology of the negative group also showed alteration and also decreased pH level.

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INTRODUCTION

Tuberculosis (TB) is an airborne disease caused by Mycobacterium tuberculosis. This bacterial infection begins in the lungs, causing nodules known as tubercles, or Ghon focii, which are spots left by infected dead tissue. Over time, the disease can spread to areas outside the lungs, and larger areas of lung tissue can die, causing cavities. Bacteria can also spread to other organs, including the kidneys, brain, spine (Koo and Miller, 2019). The most common form of this infection is called active pulmonary TB disease, which means the infection is in the lungs. A person with active pulmonary TB disease often has a cough that is accompanied by constant pain, night sweats, weight loss, and fever. If left untreated, the disease can be fatal. Various environmental toxins and clinically useful drugs can cause severe organ poisoning through metabolic activation to highly reactive free radicals, including superoxides and reactive oxygen species (Naggayi *et al.*, 2015). The first-line therapy for tuberculosis is Rifampin, in combination with Isoniazid (INH), Ethambutol (ETB), and Pyrazinamide (PZA). The side effects of TB drugs that may arise if you have to take the drug for a long time include; hepatotoxicity (van der Meulen *et al.*, 2009), disseminated intravascular coagulation (DIC), nephrotoxicity such as acute tubular necrosis and intersitritial nephritis (IN), (van der Meulen *et al.*, 2009), gastrointestinal disorders such as anorexia, nausea, vomiting, stomach discomfort and diarrhea as well as upper gastrointestinal bleeding. The cause-and-effect relationship between the development of hemorrhagic gastric erosions and TB drug administration was

confirmed by rechallenge with rifampin (Zargar *et al.*, 1990). Gastric ulcer is a digestive tract disease that can cause death.

Based on data from the Indonesian Health Profile (2008), deaths caused by digestive tract diseases in 2007 were 6,590 people and in 2008, there were an increase to 6,825 people (Ahmad *et al.*, 2009). Gastric ulcer disease is characterized by complaints of the painful stomach in the epigastrium, where the common people are familiar with the term ulcer. (Hanafi *et al.*, 2016). Currently, gastric ulcers are a disease that affects many people and in severe conditions can be a cause of death. A gastric ulcer is a form of the peptic ulcer which is characterized by the destruction of the mucosal layer, even to the muscularis mucosa. An imbalance between aggressive and protective factors is the beginning of gastric ulcers. Hypersecretion of gastric acid as an aggressive factor is a pathological condition that occurs due to uncontrolled secretion of HCl from the parietal cells of the gastric mucosa through the H + / K + -ATPase proton pump, while damage to the mucus layer, which functions as a protective factor on the surface of the gastric mucosa can exacerbate the above conditions. Acute gastrointestinal (GI) bleeding can cause everything from mild to life-threatening. It has an incidence of 100 cases per 100,000 population per year and remains a common cause of hospitalization and consultation among acute care surgeons. GI bleeding is defined as up or down, based on the connection with the Treitz ligament. The source of upper GI bleeding is proximal to the Treitz ligament and is associated with a 6% to 10% mortality. Death is often based on the underlying cause and patient comorbidities (Feinman and Haut, 2014).

Papaya seeds are rich in polyphenols, flavonoids, triterpenoids, tannins, saponins, alkaloids (Purwaningdyah *et al.*, 2014), glycosides, reducing sugars, steroids, protein (Kumar and Sreeja, 2017), fat (Naggayi *et al.*, 2015), anthraquinones and anthocyanosides (Adeneye *et al.*, 2009). The ethyl acetate fraction from papaya seed extract had the strongest antioxidant activity, and the n-butanol fraction had the second strongest antioxidant activity. DPPH activity and hydroxyl free radical activity of the ethyl acetate fraction were stronger than that of ascorbic acid and sodium benzoate, respectively, which indicates that the antioxidant components in papaya seeds are mainly concentrated in the ethyl acetate fraction and n-butanol fraction. The total number of phenolics and total flavonoids in the ethyl acetate fraction was the highest among all fractions, and in the n-butanol fraction took the second position. The results of (Zhou *et al.*, 2011) show that p-hydroxybenzoic acid and vanilla acid are the main constituents of the ethyl acetate fraction, accounting for 75% of the total so that the two compounds contribute to the antioxidant activity of the ethyl acetate fraction from papaya seeds. Therefore, papaya seeds and these compounds can be used as natural antioxidants (Zhou *et al.*, 2011), neproprotective (Naggayi *et al.*, 2015) and are rich in ingredients.

In this study, I will evaluate the comparison test of EECP with cimetidine tablets by conducting laboratory tests of blood Hb, examining the pH of gastric juices, and conducting histological tests of gastric tissue in all experimental groups to see changes in the stomach due to TB drugs.

METHODOLOGY

Reagents and chemicals

Razors, alcohol, ethanol, xylol, NaCl, hematoxylin and eosin dyes, soften. Alat-alat bedah, alat-alat gelas laboratorium, *alumunium foil*, blender, cawan porselin, desikator, inkubator, kaca objek, kaca penutup, krus porselin, lemari pengering, *microtube*, mikroskop cahaya, neraca analitik, oral sonde, oven listrik, penangas air, penjepit tabung, rak tabung reaksi, *rotary evaporator*, sentrifugator, seperangkat alat penetapan kadar air, spektrofotometer UV (Microlet 3000), spuit injeksi, tanur, tabung reaksi, timbangan hewan. Akuades, α-naftol, asam nitrat pekat, asam asetat anhidrida, asam sulfat pekat, Etanol (destilasi), merkuri (II) klorida, kalium iodida, iodium, bismut (III) nitrat, asam klorida pekat, timbal (II) asetat, besi (III) klorida , buffer formalin 10%, isopropanol, kloroform, metanol, *n*-heksana, Rifampisin, INH, tab Curcuma FCT, Na CMC 0,5%, reagen kit ureum, kreatinin, asam urat (Sigma Aldrich), serbuk seng, toluen, zat warna (hematoksilin dan eosin).

Methods

The animals used are healthy adult rat, which are 27 males weighing 250-300 grams, in a healthy condition. Before the treatment, rats adapted for one week, and ethical clearance was approved by the commission ethics of Universitas Prima Indonesia.

500 g of papaya seed powder was put into a reagent bottle and macerated using 96% ethanol solvent with a volume ratio between powder and solvent that is 1: 3 w/v. This mixture is shaken using a shaker for $\pm$ 48 hours at a speed of $\pm$ 200-250 rpm. Furthermore, the papaya seed ethanol extract solution is evaporated using a rotary evaporator at temperatures ranging from 45-50 $^\circ$ C. After the rotary evaporator, and the solution is placed in a water bath to evaporate the remaining solvent that

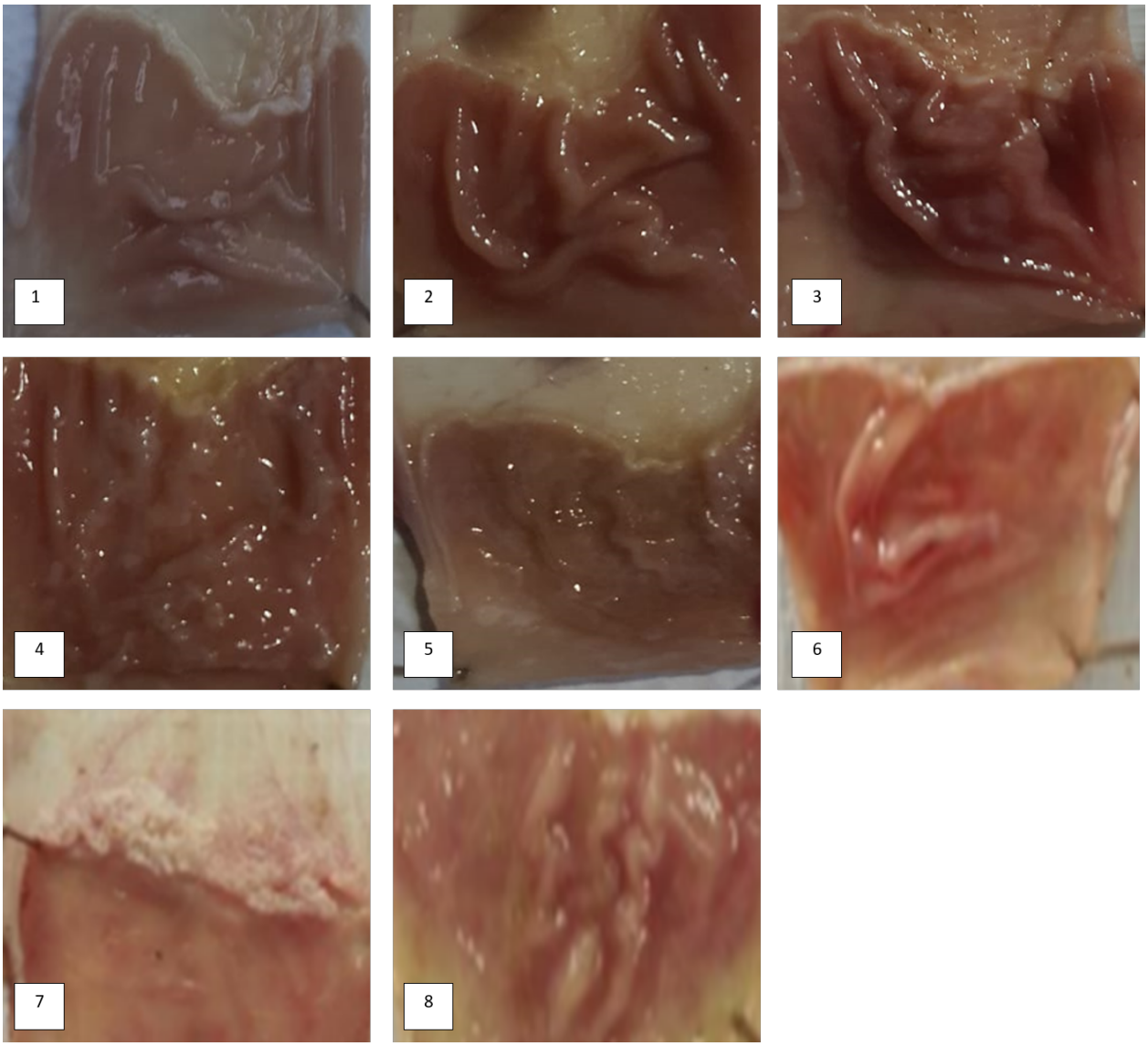

Figure 1: Macroscopicof Gastric [Information:1 (control), 2 (negative 1), 3 (negative 2), 4 (negative 3), 5 (positive), 6(Group 1), 7 (Group 2), 8 (Group 3)]

Table 1: Experimental Design

Group	Inducer	Treatment
Normal	(-)	Na-CMC 0,5%/oral
Negative-1	Rif 50 mg/kgBW/oral	Na-CMC 0,5%/oral
Negative-2	INH 50 mg/kgBW/oral	Na-CMC 0,5%/oral
Negative-3	Rif +INH @50mg/kgBW/oral	Na-CMC 0,5%/oral
Positive-1	Rif +INH @50mg/kgBW/oral	Cimetidine 18 mg/kgbb/oral
Group-1	Rif +INH @50mg/kgBW/oral	EECP 100 mg/kgBW/oral
Group-2	Rif +INH @50mg/kgBW/oral	EECP 300 mg/kgBW/oral
Group-3	Rif +INH @50mg/kgBW/oral	EECP 500 mg/kgBW/oral

information: EECP (Ethanol extract of carica papaya seed), Rif (Rifampicin), INH (Isoniazid)

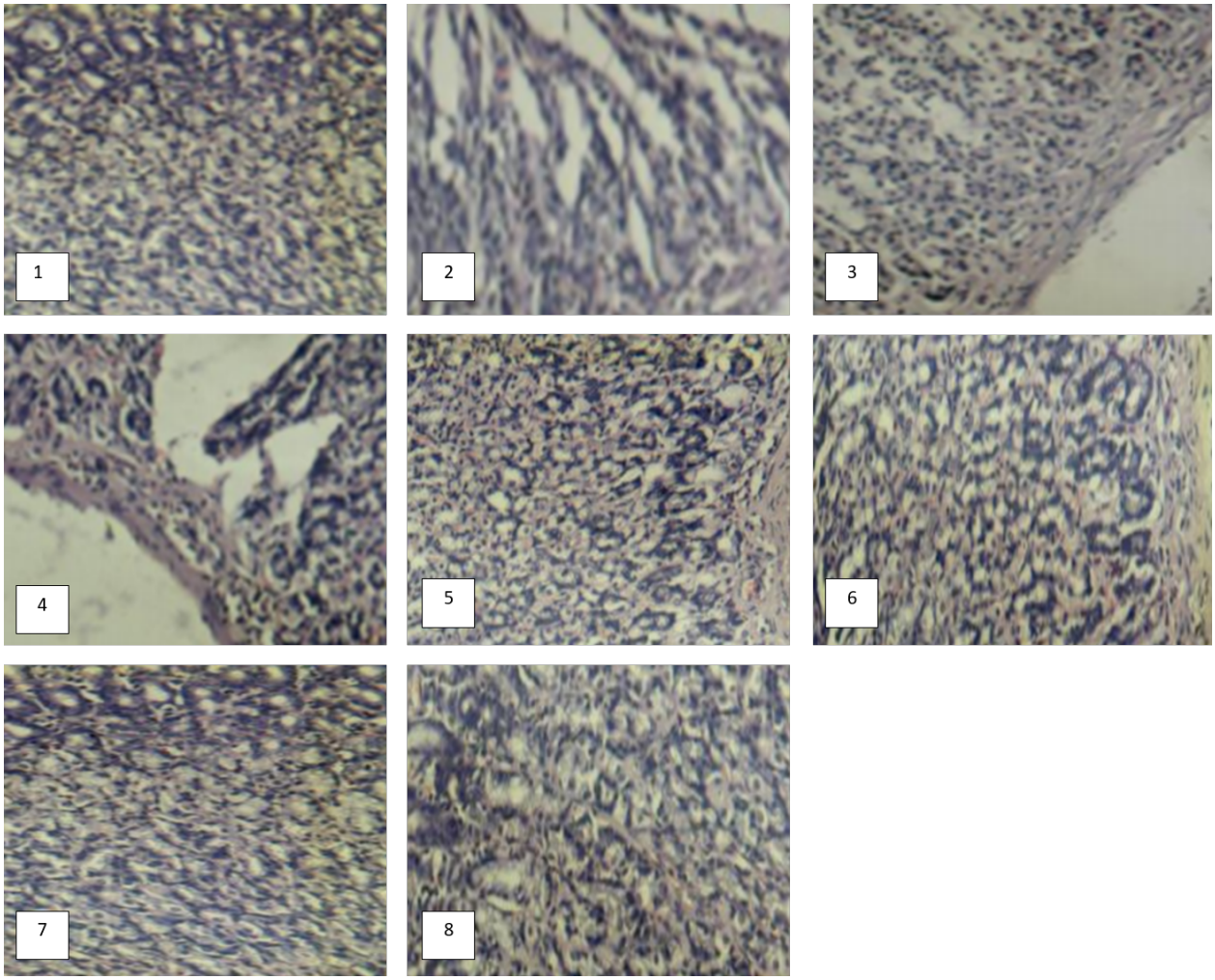

Figure 2: Histophatology of Gastric [Information:1 (control), 2 (negative 1), 3 (negative 2), 4 (negative 3), 5 (positive), 6(Group 1), 7 (Group 2), 8 (Group 3)]

is still in the extract. Phytochemical screening of the ethanol extract of Carica papaya seed (EECP) includes an examination of alkaloids, saponins, flavonoids, tannins, triterpenoids/steroids and glycosides.

Rats Wistar as subjects, with a length of study for 28 days. The in vivo test in the experiment used 27 healthy rats weighing about 200 g ± 10%, divided into 8 groups and each group consisting of 3 rats. The experimental design can be seen in Table 1.

Induction of liver damage using the maximum dose of Rifampin + INH dose 50 mg/kg/oral/day respectively. EECP in experimental rats, group 1 was 100 mg/kg, group 2 was given 300 mg/kg, and Group 3 was given 500 mg/kg body weight every day for 28 days. On the 29th day, the rats were operated on, and blood was taken from the heart and then measured the levels of hemoglobin, gastric was taken and measured for pH and histopatology.

RESULTS AND DISCUSSION

Phytochemical screening result

The results of the phytochemical screening extract obtained showed the presence of flavonoids, saponins, tannins, glycosides. The results of the phytochemical screening of ethanolic extract can be seen in Table 2.

Hemoglobin level

Rifampicin and isoniazid cause a decrease in blood hemoglobin levels, and the ethanol extract of papaya seeds increases blood hemoglobin levels. The hemoglobin level can be seen on Table 3.

Based on the table, it is known that the mean serum hemoglobin value for the normal group is still within the normal range, namely 18.41 ± 0.41 (g / dL). The negative control group 3 had mean serum hemoglobin of 9.36 ± 0.02 (g / dL). The positive control group had mean serum hemoglobin of 17.56 ± 0.67 (g / dL). Treatment group, I had a serum hemoglobin value of 15.09 ± 2.59 (g / dL).

Table 2: Result of phytochemical screening of the extract

No.	Screening contents	Result
1.	Flavonoid	+
2.	Alkaloid	+
3.	Saponin	+
4.	Tanin	+
5.	Glikosida	-
6.	Steroid/triterpenoid	_

Information (+) = positive result
(-) = negative result

Table 3: Hemoglobin

No.	Group	Mean ± SD (g/dl)
1	Normal	18,41 ± 0,41
2	Negative-1	10,21 ± 0,77
3	Negative-2	10,89 ± 0,17
4	Negative-3	9,36 ± 0,02
5	Positive-1	17,56 ± 0,67
6.	Group-1	15,09 ± 2,59
7.	Group-2	15,71 ± 1,05
8.	Group-3	17,81 ± 1,90

Table 4: pH

No.	Group	Mean ± SD
1	Normal	5,1 ± 0,71
2	Negative-1	1,67 ± 0,42
3	Negative-2	1,69 ± 0,23
4	Negative-3	1,63 ± 0,56
5	Positive-1	4,98 ± 2,31
6.	Group-1	3,98 ± 0,98
7.	Group-2	4,12 ± 0,85
8.	Group-3	4,52 ± 0,93

Table 5: Gastric volume

No.	Group	Mean ± SD (ml)
1	Normal	2,8 ± 0,71
2	Negative-1	9,4 ± 0,42
3	Negative-2	10,12 ± 0,23
4	Negative-3	10,31 ± 0,56
5	Positive-1	3,1 ± 0,50
6.	Group-1	5,21 ± 1,09
7.	Group-2	4,98 ± 1,77
8.	Group-3	3,98 ± 0,17

Treatment group II had a serum hemoglobin value of 15.71 ± 1.05 (g / dL). The treatment group III had a serum value of 17.81 ± 1.90 (g / dL). Based on this table, it is known that the largest mean serum hemoglobin in the treatment group is 17.81 ± 1.90 (g / dL) in treatment group III. And the mean serum hemoglobin in the smallest treatment group is 15.09 ± 2.59 (g / dL) in treatment group I.

Gastric pH

The pH levels for each group can be seen in Table 4.

Based on the table, it is known that the average gastric pH value for the normal group is still within the normal value range, namely 5.1 ± 0.71. The negative control group 3 had an average gastric pH value of 1.63 ± 0.56. The positive control group had an average gastric pH value of 4.98 ± 2.31. Treatment group, I had a gastric pH value of 3.98 ± 0.98. Treatment group II had a gastric pH value of 4.12 ± 0.85. Treatment group III had a gastric pH value of 4.52 ± 0.93.

Gastric volume

Rifampicin and isoniazid cause an increase in the volume of gastric fluid due to increased acid production, which causes gastric ulcers. The gastric volume can be seen on Table 5.

Based on the table, it is known that the average value of gastric fluid volume for the normal group is still within the normal range, namely 2.8 ± 0.71. The negative control group 3 had an average gastric fluid volume value of 10.31 ± 0.56. The positive control group had an average gastric fluid volume value of 3.1 ± 0.50. Treatment group, I had a gastric pH value of 5.21 ± 1.09. Treatment group II had a gastric pH value of 4.98 ± 1.77. Treatment group III had a gastric pH value of 2.8 ± 0.71.

Result of Macroscopic organ and histopathology

The results observed in the histopathology of the gastric organs of rats given rifampicin and isoniazid there were changes in the histopathology of the rats gastric organ. The macroscopic and histophatology of gastric can be seen in Figures 1 and 2.

CONCLUSION

Papaya seed ethanol extract at a dose of 500 mg / kgBB is the most effective dose to protect the stomach due to the administration of isoniazid and rifampicin. The ethanol extract of papaya seeds at doses of 100 mg/kg, 300 mg/kg, and 500 mg/kg of body weight has histological gastric protective activity, as evidenced by the absence of damage to the stomach. The ethanol extract of papaya seeds contains phytochemical alkaloids, flavonoids, steroids/terpenoids, tannins, saponins, glycosides. The group given the ethanol extract of papaya seeds at a dose of 500 mg / kgbb did not have a significant difference with the normal group on changes in pH. The group giving papaya seed ethanol extract at a dose of 500 mg / kgbb did not have a significant difference with the normal group on changes in Hb levels. The group giving the papaya seed ethanol extract at a dose of 500 mg / kgbb did not have a significant difference with the normal group on changes in the amount of gastric juices.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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