The Effect of Extract Areca Seeds (*Areca catechu* L.) on the Thickness of the Colonic Tunica Muscularis in Mice (*Mus musculus*) Feeded *Trichuris muris* Infective Eggs Peroral

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Abstract. One species of worm Trichuris trichiura that is often used in research on experimental animals is Trichuris muris. Administration of T. muris infective eggs can significantly increase the thickness of the tunica muscularis of the colon. This thickening of the tunica muscularis intestine is associated with increased contractility of smooth muscle cells lining the intestinal wall. Increased contractility is one of the important mechanisms in expulsion of gastrointestinal parasites. In recent years, various researches on the development of natural and traditional ingredients have been developed to see the effect of areca seeds on changes in the thickness of the tunica muscularis in the large intestine. Flavonoids, alkaloids and tannins contained in areca nut have a role in the immune response and the eradication of worm parasites which have an influence on the thickness of the tunica muscularis of the colon. The purpose of this study was to determine the effect of ethanol extract and ethylacetate fraction of areca seeds on changes in the thickness of the tunica muscularis colon in male mice fed T. muris infective eggs orally. This research is a quasi-experimental study with post-test only control group design conducted at the FMIPA, Universitas Sumatra Utara, Medan. A sample of 10 male mice was randomly assigned to 7 group: (1) negative control; (2) induction of 200 *T. muris* infective eggs (positive control); (3) positive control and ethanol extract of areca seeds (EEAS), 100 mg/kgbw; (4) positive control and EEAS, 150 mg/kgbw;(5) positive control and the ethylacetate fraction of areca seeds (EAFAS), 100 mg/kgbw; (6) positive control and EAFAS, 150 mg/kgbw; (7) positive control and albendazole, 1mg/20g. The mean differences in the groups were analyzed by the Kruskal Wallis Test method. The results of statistical analysis showed an increase in the thickness of the tunica muscularis colon in the positive control (Mean = 1008.39) compared to the negative control (Mean = 666.16). The thickness of the tunica muscularis colon in group P1 (Mean = 237.34), group P2 (Mean = 216.33), group P3 (Mean = 373.30), group P4 (Mean = 339.69) and group P5 (Mean = 361.26). Pairwise statistical significance (p value) between all groups was $p < 0.001$. Areca seeds ethanol extract 150 mg/kgbw can effectively reduce the thickness of the tunica muscularis of the colon.

Keywords: A*reca catechu L.* · Colon · Mice · *Mus musculus* · Trichuris muris · Tunica muscularis

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1 Introduction

Trichuris trichiura (*T. trichiura*) is a soil-transmitted worm that is commonly found in humid, tropical and subtropical areas and areas with poor sanitation [1, 2]. Worm infections are classified as neglected diseases, namely infections that are not noticed and are chronic without causing clear clinical symptoms and the effects can only be seen in the long term [3]. The *T. trichiura* worm is the most difficult to treat, where if it is usually infected by 2 other worms (*Ascaris lumbricoides* and hookworm) once taking medicine, it will immediately heal. However, in *T. trichiura* worm infection, you have to take medicine for three days in a row and you can only show improvement [4, 5].

T. muris is a natural pathogen in mice. This worm is biologically and antigenically similar to the species *T. trichiura* that infects humans and livestock. Infective eggs given orally will hatch in the distal intestine and attack the intestinal epithelial cells (IEC) that line the crypta caecum and proximal colon. The mechanism of expulsion of *T. muris* consists of increased epithelial cell turnover, mucin production by goblet cells, and intestinal smooth muscle contractility [8]. The three mechanisms of worm expulsion can potentially cause changes in the structure of the tunica mucosa and tunica muscularis intestine. However, until now there has not been much research on the effect of exposure to *T. muris* on the structure of the tunica mucosa and tunica muscularis intestine of mice [6, 7].

Worm infection will stimulate Antigen Presenting Cell (APC) which will stimulate Th0 so that the immune response develops towards Th2. Activation of Th2 causes an increase in cytokines such as IL-4, IL-5, IL-9 and IL-13 [9–13]. Th2 cytokines promote physiological changes in the microintestinal environment that include rapid IEC turnover, goblet cell differentiation, epithelial retraction and permeability changes, and smooth muscle contraction [6, 7, 14].

IEC (intestinal epithelial cell) secretes several cytokines, such as TSLP (thymic stromal lymphopoietin), IL-25, and IL-33 in response to trichuris infection. TSLP is important for Th2 cell activation via dendritic cells (DC). IL-25 induces MPP type 2 multipotent progenitor cells that secrete IL-4 to help shape the Th2 response. Basophils are activated in the intestine and travel to the mesenteric lymph nodes. Basophils activate Th2 cells at this site. Goblet cells secrete mucus and proteins such as RELM-β that can bind to the secretory structures of the trichuris. IFN-γ induces the production of the chemokine CXCL 10. CXCL 10 induces accelerated IEC turnover and crypt elongation [15].

The use of traditional medicine is a way to study the potential of medicine in the future. Plants are a great source of discovery for medicinal products. Currently, various chemicals from plants are important medicinal ingredients and are used in various countries in the world. One of the medicinal plants that has the potential as an alternative to reduce side effects and treat intestinal worm infections or anthelmintics is *Areca catechu* L. [16, 17].

In an in vitro study conducted by Tiwow et al. (2013), 10% areca seeds (*Areca catechu L.*) ethanol extract was able to paralyze the *Ascaris lumbricoides* (*A. lumbricoides*) worms and at a concentration of 20% it was able to lyse the *Ascardia galli* (*A. galli*) worms. Ethanol extract of areca seeds with a concentration of 30% more effective anthelmintic against *A. lumbricoides* and *A. galli* worms [18].

Based on previous research, with the many benefits of areca nut and the public has not received sufficient scientific information related to the anthelmintic and anti-inflammatory properties of areca nut, it is hoped that it will become an alternative medicine that is easy to obtain and relatively inexpensive. This research has differences and novelties from previous studies. Previous studies only discussed the effect of *T. muris* infective eggs on the tunica muscularis, while this study looked at the effect of areca nut on thickness in the tunica muscularis of the colon.

Areca nut contains arecoline compounds (alkaloid components) which is a methyl tetrahydromethyl-nicotinate compound in the form of hard alkaline oil, this compound is widely used in the form of arecolinum hydrobromicum which functions to eradicate tapeworms in animals such as poultry, cats and dogs, before the discovery of drugs. Synthetic worms, such as piperazine, tetramisole, and pyrantel pamoate. Areca nut also contains flavonoids which function as anti-inflammatory and tannins as anti-parasitic [3, 5].

2 Material and method

This study used a quasi-experimental laboratory research design in mice (*Mus musculus* Strain Balb/c obtained from the biology laboratory, FMIPA USU) trichuriasis model induced by *T. muris* infective eggs orally.

This study was to determine the effect of ethanol extract and ethyl acetate fraction of Areca seeds (*Areca catechu L.*) on the thickness of the colonic tunica muscularis in mice (*Mus musculus*) fed *T. muris* infective eggs orally.

2.1 Production of Areca Seeds Ethanol Extract

The sample used was 10 kg of areca seeds (wet weight) purchased from Petisah Market. The samples were then cleaned of dirt and attached fruit flesh (wet sorting), washed with running water until clean, then drained. Then dried in an oven at 50oC until dry. The next step is to grind the dried simplicia so that it becomes powdered simplicia and is sieved, then stored in a clean and tightly closed container. The powder was macerated using 96% ethanol as solvent. A total of 1000 g of simplicia powder was put into a vessel and then poured with 75 parts of the filter, namely 96% ethanol (4.2 L), covered and left for 3 days protected from light, while stirring repeatedly. After 3 days the juice is sifted, the dregs are squeezed out. The dregs were added with 75 parts of 4.5 L of ethanol (96% ethanol) and then stirred and dispersed, so that 100 parts of the whole juice were obtained. The vessel is closed, left in a cool place and protected from light for 2 days. The precipitate was then separated and a liquid extract was obtained. After that, the extract obtained was evaporated using a rotary evaporator at a temperature of 300 °C-400 °C and then concentrated again using a freeze dryer to obtain a thick areca seeds extract.

2.2 Production of Areca Seeds Ethylacetate Fraction

Fractions were made by liquid-liquid extraction (ECC) using ethyl acetate as solvent. A total of 10 g of ethanol extract was added with 40 ml of ethanol and 100 ml of distilled

water, homogenized and then put into a separating funnel and allowed to stand for a while. Added 50 ml of ethylacetate fraction, shaken, allowed to stand until a layer is formed, namely the ethylacetate fraction. Fractionation was carried out until the ethylacetate layer was clear.

Inclusion Criteria. The inclusion criteria in this study were: 2.5 - 3 months old**,** Weight 180 - 220 g**,** Male gender and Healthy condition (active and not disabled).

Exclusion Criteria. Exclusion criteria in this study are: Male white rats are not active and Male white rats died during the study period.

2.3 Statistical Analysis

The independent variables in this study were the dose of ethanol extract and the ethylacetate fraction of areca nut (*A. catechu L*).

The dependent variable in this study was the histopathological appearance of the large intestine (colon) to assess the thickness of the tunica muscularis in response to *T. muris* worm infection in male mice.

Estimated sample size using G Power 3.1 Software, with the following calculations:

$\alpha = 0{,}05$
Power = 0,80
Sample size = 49

Each treatment group contained at least 7 male mice. In this study, to avoid lost to follow-up, each group was made up of 10 male mice.

The sample of this study amounted to 70 mice divided into 7 groups: (1) negative control (normal); (2) induction of 200 *T. muris* infective eggs (positive control); (3) induction of 200 infective eggs of *T. muris* and ethanol extract of areca seeds p.o. (orally) 100 mg/kgbw (P1);(4) induction of 200 infective eggs of *T. muris* and ethanol extract of areca seeds, p.o. 150 mg/kg body weight (P2);(5) induction of 200 infective eggs of *T. muris* and the ethylacetate fraction of areca seeds p.o. 100 mg/kg body weight (P3);(6) induction of 200 infective eggs of *T. muris* and ethylacetate fraction of areca seeds, p.o. 150 mg/kg body weight (P4); (7) induction of 200 infective eggs of *T. muris* and Albendazole p.o. 1mg/20g (P5). The mean differences in the groups were analyzed by the Kruskal Wallis Test method.

3 Result

Identification or analysis (determination) of the sample material in this study Areca seeds (Table 1).

The results of the analysis of the thickness of the tunica muscularis (1) of the intestine of the colonic tissue (Table 2).

This table shows the thickness of the tunica muscularis of mice in the colon. Based on the results above, there were significant differences in colonic tissue between groups with $p < 0.05$).

Table 1. Determination of *Areca* seeds

Name	Type	Ethnic group
Biji pinang	*Areca seeds*	Arecaceae

Table 2. Thickness of the tunica muscularis intestinum of male mice on day 37 after oral administration of infective *T. muris* eggs

Colonic Thickness of Tunica Muscularis (μm)			
Dependent variable	Group	Mean ± SD	P value
Colon	K(-)	666,16 ± 185,53	< 0,001*
	K(+)	1008,39 ± 2454,35	
	P1	237,34 ± 70,48	
	P2	216,33 ± 63,08	
	P3	373,30 ± 46,36	
	P4 P5	339,69 ± 141,97 361,26 ± 51,77	

* Kruskal Wallis Test (Signifikan $p < 0,05$

4 Discussion

Worm infection is the master of manipulation of the host immune response so that intestinal worm disease is a chronic infection and is generally asymptomatic [19]. Exposure to *T. muris* infection in the intestines of mice can induce an immune response to eliminate worms. The mechanism of elimination of worm expulsion in the form of increased epithelial cell turnover, mucin production by goblet cells, and intestinal smooth muscle contractility has the potential to cause changes in intestinal structure [3, 8].

The mechanism of expulsion of *T. muris* consists of increased epithelial cell turnover, mucin production by goblet cells, and intestinal smooth muscle contractility [8]. The three mechanisms of worm expulsion can potentially cause changes in the tunica muscularis intestine. However, until now there has not been much research on the effect of exposure to *T. muris* on the structure of the tunica muscularis intestine of mice.

In the research of Husairi [20], it was stated that there was an effect of giving *T. muris* infective eggs to changes in the structure of the tunica mucosa and muscularis intestine of mice. One of the medicinal plants that has the potential as an alternative to reduce side effects and treat intestinal worm infections or anthelmintics is *Areca catechu L.* [16, 17, 20].

The results of the analysis of the thickness of the tunica muscularis of male mice, showed a significant difference in the colon between groups with $p < 0.05$ (Table 2). This study showed that administration of *T. muris* infective eggs at a dose of 200 infective eggs could significantly increase the thickness of the tunica muscularis colon in K(+) compared to the K(-) group and P1, P2, P3, P4 and P5 groups.

This thickening of the tunica muscularis intestine is associated with increased contractility of the smooth muscle cells lining the intestinal wall. Increased contractility is one of the important mechanisms in expulsion of gastrointestinal parasites [8].

According to Berlina and Arisandi & Andriani, areca nut contains arekolina compounds (alkaloid components) and tannins, which have anthelmintic properties [21, 22]. This anthelmintic ability is related to the content of tannin compounds from the ethanol extract of areca nut which can inhibit enzymes and damage membranes. The inhibition of enzyme work can cause the digestive metabolic process to be disrupted, which causes less nutrition so that eventually the worms will die due to lack of energy [3].

The administration of areca seeds ethanol extract which has anti-inflammatory and antihelmintic effects on areca nut does not increase the thickness of the tunica muscularis of the colon. This decrease in thickness is due to parasites, namely worms that are in the cecum and colon, so that the process of increasing contractility of smooth muscles in the intestinal wall does not occur. This study shows that ethanol extract 150 mg/kgbw (P2) can effectively reduce the thickness of the tunica mucularis of the colon (216.33 ± 63.08) from other groups.

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Author Contribution. E.J.A designed the experiment, performed the experiments, analysed the data, wrote the first draft of the manuscripts and the final version. Author read and approved the final manuscript.

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