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Impact of Stigmasterol from Beluntas Leaves (*Pluchea indica*) on SGOT and SGPT Levels in Male Rats (*Rattus norvegicus*)

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ABSTRACT

Stigmasterol compounds have the potential as natural male antifertility drugs; based on the results of multi-year research and bioinformatics, further research is needed on the safety of stigmasterol preclinically. The study aimed to examine selected stigmasterol from blunt leaves that are safe for blood SGOT and SGPT levels the type of experimental research, the Post-test control design group. The study was conducted at the Biomedical Laboratory of the University of Muhammadiyah Malang, the Hufaka Bandung Laboratory, and the FKH Laboratory of Brawijaya University. The study was conducted from June to August 2024. The research sample of stigmasterol from blunt leaves came from the UPT materia medica of Batu City. The sampling technique was purposive random sampling. There were three dose treatments (0.125 mg/kgbb, 0.25 mg/kgbb, 0.5 mg/kgbb and control) with seven replications. Data analysis used one-way ANOVA and Duncan's further test. The results showed that giving various doses of stigmasterol could reduce SGPT in the blood but not SGOT. Duncan's test results of SGOT levels showed no significant difference between treatment categories; SGPT testing showed that the 0.125 mg/kgbb and 0.25 mg/kgbb treatments were not significantly different from the 0.5 mg/kg bb treatment but were substantially different from the control treatment. At the same time, the 0.5 mg/kgbb treatment was not significantly different from the other treatments. The conclusion and findings of the stigmasterol compound are in the safe category for use because it does not cause an increase in blood SGOT and SGPT and potentially tends to decrease.
Keywords: Antifertility, Beluntas, Men, Safety Test, Stigmasterol.

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INTRODUCTION

Male antifertility has not been widely practiced, especially oral antifertility. The limited contraceptive methods in men mean that the family planning process in men is still very little in demand[1]. Good contraception must meet certain criteria and requirements to be safe to use. The requirements that must be met by a good contraception are that it is not dangerous, reliable, cheap, widely accepted, safe, easily available[2], reversible and has few side effects[1,3]. However, until now there has been no completely ideal contraceptive method. The use of contraception that has fewer side effects really needs to be studied. Another reason behind this research is that the potential of traditional materials derived from plants has not yet been scientifically proven for its bioactivity[4]. Many plants contain active compounds that have the potential to be antifertility.

Beluntas is one of the medicinal plants that has been developed. Previous research data stated that the active compounds of beluntas leaves in the form of tannins, alkaloids and flavonoids affect the quality of spermatozoa: spermatozoa motility, spermatozoa concentration, testosterone levels, male white mice and the number of female white mouse offspring[5,6,7]. Tannins can reduce the fertilization potential of mouse spermatozoa. There is a difference in tannin levels from fresh and dry beluntas leaves, namely tannin in fresh beluntas leaves is 0.61% and in dry beluntas leaves is 1.885%[8]. Tannins can inhibit protein synthesis, resulting in male reproduction. Inhibited protein synthesis will affect the concentration of spermatozoa which plays a role in the fertilization process. Inhibition of protein synthesis does not affect the composition and amino acid levels of spermatozoa[9], will result in spermatozoa motility[10].

Tannins from crude extracts affect amino acid levels but do not affect mt DNA profiles in spermatozoa of male white mice[11,12,13]. The results of in-silico research or bioinformatics studies (in-silico) in 2021 and 2023 to complement previous research findings, namely by molecular docking analysis which can see the apoptosis value. Male contraceptive studies generally focus on sperm quality. Apoptosis is one of the useful indexes for seeing sperm quality. Apoptosis can affect sperm concentration and have an effect on fertility[14]. The results of the study showed that there was a potential stigmasterol compound. Stigmasterol is a sterol compound in plants that cannot be produced naturally by the human body, so it is only available through food and other types of vegetables, grains, medicinal plants, which are common sources of phytosterols[15].

Based on the results of Pass Stigmasterol is a compound that has the potential as a contraceptive candidate for men, it is predicted that stigmasterol can attach to the same active site (from Methytrienolone) as the control to affect AR. Stigmasterol has a binding affinity value close to the inhibitor control, which is -5.4 kcal/mol while the inhibitor control has a binding affinity value of -4.6 kcal/mol. The ideal value of a control is -7 kcal/mol[16]. The findings of this study are that stigmasterol in beluntas leaves molecularly has the potential to be an antifertility agent in men because it has a binding affinity value close to the control binding affinity value. The

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chemical structure of stigmasterol $C_{29}H_{48}O$[17], if identified the structure of stigmasterol is similar to tannin. More specific research is needed on the safety test of stigmasterol compounds from beluntas leaf extracts in preclinical tests. Safety tests in the form of acute and subchronic toxicity and effectiveness tests of stigmasterol preparations from beluntas leaf extracts. Further studies are needed on SGOT, SGPT, kidney histology, liver, LD_{50}/behavior of male white mice after exposure to stigmasterol from beluntas leaves. The aim of this study was to study the selected stigmasterol preparations from beluntas leaves that are safe for SGOT and SGPT levels in the blood of male white mice.

METHODS

The research used experimental research "The Post test control design group". The population in this study were male white rats. The sample used was 28 adult male white rats, and in healthy condition. Sample size (n-)(t-1) ≥ 15, n = number of replications, t = number of treatments. (n-)X4-1) ≥ 15, (3n -3) ≥ 18, n = 6. Converted 7. The sampling technique was purposive random sampling. The material used was beluntas leaves number 5 from the shoots obtained from Metria Medica Batu. The manufacture of stigmasterol extract was carried out in the Hufaqa Bandung laboratory. Testing of stigmasterol extract quality standards. Efficacy testing is an observation of safety tests with SGOT, SGPT parameters and acute toxicity by looking at LD_{50} (rat behavior). The study was conducted from June to August 2024. The location of the study was conducted at the Biomedical Laboratory of the University of Muhammadiyah Malang for animal treatment and data collection activities, the Hufaka Laboratory of Bandung for isolation of stigmasterol compounds from beluntas leaves, and the FKH Laboratory of Brawijaya University for analysis of blood SGOT and SGPT levels. Research stages: Isolating stigmasterol from beluntas leaves.

Extract for stigmasterol of beluntas leaves using the soxhletation method, Fractionation using TLC[18]. SGPT/SGOT testing after 28 days of treatment, male white mice were killed by anesthesia. Then the white mice were dissected using a section tool, then their blood was taken using a 2.5 cc syringe from the heart aorta. The blood serum was centrifuged at a speed of 6000 rpm then the SGPT and SGOT levels were calculated, using the Bergmeyer method. To ensure that this research is ethically feasible, the proposal was submitted to the Ethics Committee (Animal Care and Use Committee) to obtain an assessment and approval of ethical feasibility, and has received an ethical statement for the quality and safety test proposal (2023-2024). Data analysis for SGOT, SGPT, LD50 using a one-way ANOVA test followed by a Duncan test with a significance level (α) = 0.05%.

RESULTS AND DISCUSSION

A summary of the results of the ANOVA test of various doses of stigmasterol from beluntas leaves for the levels of SGOT and SGPT in the blood of male white mice is presented in Table 1.

The test results obtained sig values of 0.180 and 0.006, from these values only SGPT is <0.05, so it can be said that the treatment given

Table 1. Summary of ANOVA Results.

		Sum of Squares	df	Mean Square	F	Sig.
SGOT	Between Groups	9431.259	3	3143.753	1.771	.180
	Within Groups	42599.640	24	1774.985		
	Total	52030.899	27			
SGPT	Between Groups	18000.090	3	6000.030	5.284	.006
	Within Groups	27251.471	24	1135.478		
	Total	45251.561	27			

Table 2. Duncan SGOT Advanced Test Results.

Treatment	Average	Notation
0,5 mg/kgbb	139.829	a
0,25 mg/kgbb	154.743	a
0,125 mg/kgbb	170.071	a
kontrol	189.329	a

Table 3. Duncan's Advanced Test Results SGPT.

Treatment	Average	Notation
0,25 mg/kgbb	56.414	a
0,125 mg/kgbb	80.429	a
0,5 mg/kgbb	93.000	ab
kontrol	126.686	b

significantly affects SGPT, while on SGOT it does not have a significant effect, giving various doses of stigmasterol from beluntas leaves can reduce SGPT in the blood but not for SGOT.

Duncan's test results on SGOT levels showed that there was no significant difference between treatment categories. This result can be seen from the same notation for all, with the highest average being the control treatment and the lowest average being the 0.5 mg/kgbb treatment.

The test results on SGPT levels showed that the 0.125 mg/kgbb and 0.25 mg/kgbb treatments were not significantly different from the 0.5 mg/kgbb treatment, but were significantly different from the control treatment. Meanwhile, the 0.5 mg/kgbb treatment was not significantly different from the other treatments. The highest average value was the control treatment, while the lowest was the 0.25 mg/kgbb treatment. Although the control treatment had the highest average, the 0.5 mg/kgbb treatment would not give results that were significantly different from the control treatment. This is because the two treatments were not significantly different.

The treatment of various doses of stigmasterol from beluntas leaves is still in the safe category if given to experimental animals because the highest dose treatment has the greatest effect on reducing SGOT levels, the amount of stigmasterol from beluntas leaves given is inversely proportional to the control group. The administration of various doses of stigmasterol from beluntas leaves is still in the safe category because it does not cause an increase in SGPT levels in the blood of white mice as experimental animals.

Stigmasterol has diverse pharmacological properties, so it is called a unique phytosterol compound and has been the subject of much research because of its potential health benefits[19,20]. Stigmasterol compounds are often used in various chemical manufacturing processes intended to produce various semi-synthetic components and synthetic components for the pharmaceutical industry[21,22]. The many potential benefits of stigmasterol open up opportunities for various researchers to make many developments. Stigmasterol compounds in the European Union are registered as food additives with registration number E499, which can be used to increase phytosterol levels in food production as emulsifiers and stabilizers, thereby helping to improve low-soluble lipoprotein cholesterol levels (LDL cholesterol)[23].

The main mechanism behind the cholesterol-lowering effect of stigmasterol is its ability to interfere with cholesterol absorption in the intestine. Stigmasterol competes with cholesterol for absorption in the small intestine. As a result, less cholesterol is absorbed into the bloodstream, which in turn can result in decreased LDL cholesterol levels. In addition, stigmasterol is also believed to increase LDL receptor activity in the liver, which helps in the removal of LDL cholesterol from the blood[24]. However, it is important to remember that stigmasterol's cholesterol-lowering effects may not be as great as those of prescription

cholesterol-lowering medications, and study results have not been consistent overall.

The safety of stigmasterol given to experimental animals strengthens the potential of stigmasterol from beluntas leaves as a potential compound for male antifertility, in addition stigmasterol also has many health benefits. The stigmasterol compound has anti-diabetic properties from phytosterols, where previous studies have shown that administration of stigmasterol can increase glucose transporter type 4 (GLUT4) translocation and insulin resistance, reduce fasting glucose, and induce β-cell regeneration[25]. A study showed that stigmasterol extracted from soybean oil can significantly increase GLUT4 translocation and glucose uptake in L6 cells, after supplementation of 50 and 100 mg/kg of this substance in KK-Ay diabetic mice for 4 weeks, indicating that the hyperglycemic phenotype in diabetic mice improved significantly after stigmasterol treatment[26]. In addition, the same experiment showed a marked hypoglycemic effect through decreased fasting blood glucose, serum insulin levels, and oral glucose tolerance in the treatment group. Another study reported that diabetic rats supplemented with stigmasterol 0.25 and 0.50 mg/kg for 21 days, showed a significant decrease in fasting blood glucose levels associated with an increase in serum insulin[25].

Stigmasterol is also efficacious to increase body immunity because it has the potential as an immunomodulator. Research conducted to highlight the immunomodulatory activity of stigmasterol and plant extracts containing stigmasterol compounds has been shown to increase the regulation of the intestinal mucosal immune response involved in inflammatory bowel disease (IBD) by activating the butyrate-PPAR section in colitis with the aim of exploring the possible role of stigmasterol against IBD and providing more detailed information on its mechanism of action[27,28]. This process is also stimulated (In vivo) by specific and non-specific immune responses[29]. In addition, plant extracts containing stigmasterol show strong immunomodulatory activity in vitro, so that it is able to reduce the release of pro-inflammatory mediators (TNF-α, NO, IL-1β, and IL-6), as well as COX-2 activity[30,31]. The findings and research reports on stigmasterol strengthen further research and follow-up plans in applying it as a drug ingredient and making it a research product to be utilized by the community in the pharmaceutical and health fields.

CONCLUSION

The conclusion and findings of this study are that stigmasterol is safe to use because it does not cause an increase in blood SGOT and SGPT and has the potential to decrease, meaning that the administration of various doses of stigmasterol from beluntas leaves is still in the safe category because it does not cause an increase in SGPT levels in the blood. Recommendations for further research are more specific studies on the doses given to determine the doses that have the potential to decrease blood SGPT levels. The recommended dose is higher than the previous dose.

DISCLOSURE STATEMENT

The authors have declared that no competing interests exist.

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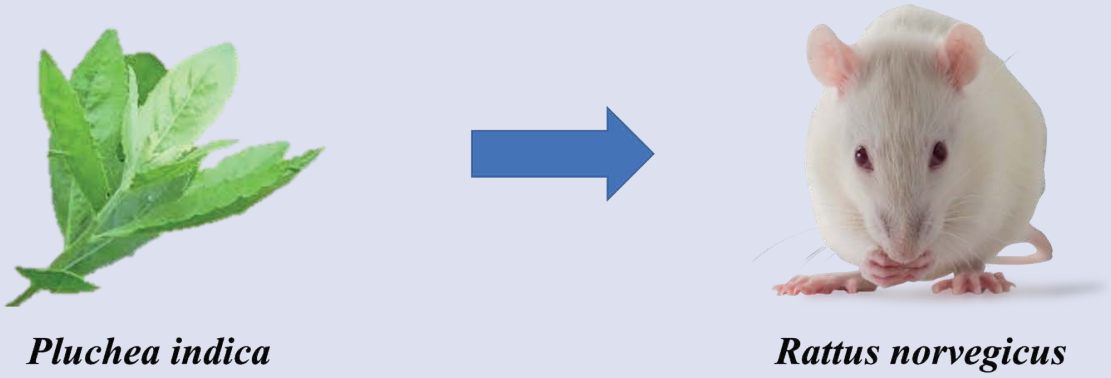
GRAPHICAL ABSTRACT



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