The Correlation of Pegagan and Beluntas Leaf Extract Co-Treatment on Liver Histological Alteration and Circulating Transaminase Enzyme Level

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Abstract. Currently, people prefer to use herbal treatment because it is considered relatively cheaper, efficient, and smaller in its side effects compared to synthetic drugs. However, it does not mean that herbal medicine has no adverse side effects if it is used in less precise. Some plants that have properties as antifertility, as well as hepatoprotective, are pegagan (*Centella asiatica*) and beluntas (*Pluchea indica*). This study aimed to find a combination dosage of pegagan and beluntas extract that was safe to the liver. The research design used a completely randomized design with six treatments and four replications. The leaf extract of pegagan and beluntas with doses of 0, 25+25, 50+50, 75+75, 125+125, and 200+200 mg/kg BW was administered to 24 female Wistar strains. The data of glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), and the level of liver histological damage were analyzed using ANOVA (α 5%) and followed by DMRT (α 5%). The results revealed that the administration of the leaf extract of pegagan and beluntas up to doses of 200+200 mg/kg BW did not affect GPT-GOT levels but began to show histological liver damage. In the conclusion, the use of a combination of pegagan and beluntas extract each up to a dose of 125+125 mg/kg BW was safe for the liver.

Keywords: Pegagan, beluntas, co-treatment, liver, transaminase enzyme

1. Introduction

Contraceptive hormones have mark effect on liver function [1]. This effect could occur in long-term oral contraceptive use or in those who have experienced jaundice during pregnancy. Changes in transaminase enzyme levels are an indicator of liver damage. The presence of liver parenchymal cell damage or membrane permeability would cause glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT), arginase, lactate dehydrogenase, and gamma-glutamyl transaminase outed of cells, so that blood enzymes exceeded normal levels. Both GPT and GOT enzymes would rise first with the higher increase among other proteins [2].

It is necessary to seek herbal treatment with possible ingredients to minimize the negative impact of drug use. Among the types of plants, those that have efficacy as an ingredient in oral contraceptives are Pegagan (*Centella asiatica*) and Beluntas (*Pluchea indica*). These plants in specific dose are allegedly capable of causing negative feedback on the release of gonadotropin hormones and also have potent antioxidant activity [3].

Research by Muchtaromah *et al.* reported that the antifertility of *C. asiatica* extract in mice for 30 days, doses of 125, 200 and 275 mg/kg BW could reduce the number of ovarian follicles. The treatment of 275 mg/kg BW could significantly decrease the total number of follicles up to 49% and ovulation number up to 30% but the treatment did not affect the levels of GPT and GOT in the serum or liver histology [4].

Further research of Muchtaromah, *et al*. reported that the combination of *C. asiatica* and *P. indica* leaf extract in shorter periods (15 days) exhibited antifertility effects on the reproductive system of the female rats. Group 4 (T4) with the combination dose of 125 mg/kg BW *C. asiatica* and 125 mg/kg BW of *P. indica* revealed the highest antifertility effect on the ovulation number (corpus luteum), total of ovarian follicles, and level of estrogen, respectively. This dose reduced the total number of follicles up to 40% and decreased the ovulation number up to 65% within 15 days, the half of the duration of the previous study [3].

Asiaticoside contained in pegagan can stimulate collagen synthesis (tissue enhancement). The mechanism of action involves the proliferation of fibroblasts, increasing collagen synthesis mucopolysaccharide acid, increasing intracellular fibronectin content, and mitotic activity in the germinal layer. Madecassoside is a triterpenoid compound that stimulates the formation of proteins and lipids needed by the body. Alkaloids suppress reproductive hormones; flavonoids can inhibit the aromatase enzyme, an enzyme that functions to catalyze the conversion of androgens to estrogen [5]. Besides, the content of flavonoids and tannins in beluntas proved to be used as anticancer, antioxidant, hepatoprotective, anti-inflammatory, and antinociceptive [6]. This study aimed to find out whether the combination of pegagan and beluntas extract in several doses are safe for the liver, given that the liver is the largest gland in the body which occupies the first place to get the toxic effects of foreign compounds. Transaminase enzyme levels and liver histology profile represent liver health status.

2. Methods

2.1. Materials

This study used 24 female rats, Wistar strain, pegagan and beluntas (Balai Materia Medika, Batu Malang, Indonesia), Prostaglandins (Pfizer), Na CMC, aquades, chloroform, 10% formalin, ethanol, paraffin, running tap water, xylene, Meyer hematoxylin and eosin stain, Giemsa dyes, Phosphate Buffer Saline, NaCl, reagent 1 solution (Tris pH 7.5 140 mmol/L, L-Alanine 700 mmol/L, Lactate dehydrogenase 2300 U/L) and reagent 2 (2-Oxoglutarate 85 mmol/L), Nicotinamide adenine dinucleotide hydrogen (NADH) 1 mmol/L. All chemicals in this study were purchased from Merck Specialties Ltd.

2.2. Procedures

2.2.1. Animals Preparation

Animal handling was carried out with ethical approval by the Ethics Committee of the Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim Malang with the approval number 001/EC/KEP-FST/2018. Before the experiment, the rats adapted for one week, each group was placed in a cage and fed with standard feed ad libitum. The rats weighing between 180 and 220g were in natural photoperiod conditions, twelve hours of light alternating with twelve dark hours at room temperature.

2.2.2. Extract Preparation

Simplicia of pegagan and beluntas were macerated with 70% ethanol for 24 hours while being stirred occasionally, filtration of macerated powder using a Buchner funnel, the filtrate obtained was concentrated using a rotary evaporator temperature of 40 ºC, the resulted thick extract was stored and used for treatment [4].

2.2.3. Estrous Cycle Synchronization and Vaginal Smear Preparation

Synchronization of the estrus cycle in rats using an intramuscular injection of 0.1 mL of prostaglandin (PGF2α). The estrus cycle was determined by observing at the results of vaginal smears, and Giemsa staining then saw under a microscope with 400x magnification [7].

2.2.4. Experimental Procedures

This study used six treatment groups, each group consisting of four rats as replications. The treatment groups were divided as follows: a) Group I (control): the rats were given 2.5 ml of 0.5% Na CMC, b) Group II: the rats were given pegagan extract dose of 25 mg/kg BW + beluntas extract dose of 25 mg/kg BW + 2.5 ml Na CMC 0.5%, c) Group III: the rats were given pegagan extract dose of 50 mg/kg BW + beluntas extract dose of 50 mg/kg BW + 2.5 ml Na CMC 0.5%, d) Group IV: the rats were given pegagan extract dose of 75 mg/kg BW+ beluntas extract dose of 75 mg/kg BW + 2.5 ml Na CMC 0.5%, e) Group V: the rats were given pegagan extract dose of 125 mg/kg BW + beluntas extract dose 125 mg/kg BW + 2.5 ml Na CMC 0.5%, f) Group VI: the rats were given pegagan extract dose of 200 mg/kg BW + beluntas extract dose of 200 mg/kg BW + 2.5 ml of Na CMC 0.5%.

2.2.5. Transaminase Enzyme and Histopathological Analysis of The Liver

The combination of pegagan and a beluntas extract was given orally for 15 days, three days after prostaglandin injection according to the dose and time specified. At the end of the study, the rats were sacrificed. The liver was removed at the time of surgery, washed with PBS 10 mM and weighed as much as 0.5 g, crushed with the mortal. Next, the liver was added 0.9% NaCl 10 times and homogenized. After homogeneous, the liver was then centrifuged at a speed of 8000 rpm for 10 minutes. The supernatant was separated from the pellet and placed in an Eppendorf tube for measurement of transaminase enzyme levels using a blood analyzer with a wavelength of 517 nm at a temperature of 37 °C [2].

Next, the remaining liver was put into 10% formalin solution. The preparation of histology used the paraffin method with the hematoxylin-eosin staining, which included the stages of fixation, dehydration, cleaning, infiltration, embedding, slicing, and staining. The histological features of the liver were observed under a light microscope with 400x magnification in 5 visual fields and an assessment of liver tissue conditions which included the requirement of hepatocyte cells, sinusoid and central vein in each area of view. The histological observations of scoring liver used a reference in Table 1. The data were summed and calculated for the mean; thus, the values for one replication in each treatment were obtained.

2.3. Data Analysis

The data of transaminase enzyme (GPT and GOT) and the level of histological liver damage were analyzed using a One-Way Analysis of Varian Test (α 5%) if F count > F table carried out a further test with Duncan's Multiple Range Test (α 5%).

Table 1. Liver histological scoring

Liver Tissue	Score
Normal	1
Hepatocyte cell damage at the stages of pycnosis, caryoecsis, and karyolysis widening of the sinusoid and central vein reached ≤ 1⁄2 the area of the visual field.	2
Hepatocyte cell damage at the stages of pycnosis, caryoecsis, and karyolysis widening of the sinusoid and central vein reached ≥ 1⁄2 the area of the visual field.	3
Loss of hepatocyte cells, widening of the sinusoid and central vein reached ≤ 1⁄2 area of the visual field.	4
Loss of hepatocyte cells, swelling of the sinusoid and central vein reached ≥ 1⁄2 area of the visual field.	5

3. Results and Discussion

3.1. Profile of Transaminase Enzyme

The average GPT levels of each treatment due to the combination of pegagan and beluntas were Control (340.345 ± 1.541 U/I), T1 (256.412 ± 2.564 U/I), T2 (149.080 ± 1.390 U/I), T3 (224.452 ± 1.699 U/I), T4 (465.742 ± 0.776 U/I), and T5 (514.617 ± 3.187 U/I). The results of the statistical analysis revealed that the administration of several combination doses of pegagan and beluntas extract did not produce significant differences (P> 0.05) on levels of Glutamate Pyruvate Transaminase (GPT) in rats' liver. The average GOT levels of each treatment were Control (478.682 ± 3.235), T1 (212.980 ± 2.864 U/I), T2 (156.615 ± 1.157 U/I), T3 (209.472 ± 1.246 U/I), T4 (452.887 ± 1.198 U/I), and T5 (646.732 ± 3.458 U/I). The analysis of variance resulted that Glutamate Oxaloacetate Transaminase (GOT) levels in this study did not show significant differences (P> 0.05) between treatments (Table 2). It means that the administration of pegagan and beluntas extract up to a dose of 200+200 mg/kg BW for 15 days did not cause changes in liver GPT and GOT levels.

Table 2. GPT and GOT Level

Treatment	GPT (U/I)	GOT (U/I)	*P value*
Control	340.345±1.541	478.682±3.235	*P > 0.05*
T1 (25 mg/kg BW)	256.412±2.564	212.980±2.864	*P > 0.05*
T2 (50 mg/kg BW)	149.080±1.390	156.615±1.157	*P > 0.05*
T3 (75 mg/kg BW)	224.452±1.699	209.472±1.246	*P > 0.05*
T4 (125 mg/kg BW)	465.742±0.776	452.887±1.198	*P > 0.05*
T5 (200 mg/kg BW)	514.617±3.187	646.732±3.458	*P > 0.05*

Liver transaminase enzyme levels in the study were still within reasonable limits. It indicated that the use of a combination of pegagan and beluntas as an antifertility drug to a dose of 200 + 200 mg/kg BW did not interfere with the presence of the transaminase enzyme. Triterpenoids contained in pegagan and beluntas acted like estrogen. In high concentrations, they would give negative feedback to the hypothalamus-pituitary-ovarian axis and thus inhibited the release of Luteinizing Hormones (LH) and Follicle Stimulating Hormones (FSH). The decline in LH and FSH also would decrease the number of follicles and ovulation [3].

Muchtaromah, *et al.* reported that the administration of *C. asiatica* leaf extract in mice at the doses of 125mg/kg BW, 200mg/kg BW, 275mg/kg BW, and 350 mg/kg BW for 30 days could reduce the number of primary follicles, secondary follicles, tertiary follicles, ovulation number, theca cell thickness, granulosa cell thickness and ovarian weight, where the most effective dose was 275 mg/kg BW [9].

The use of natural or synthetic estrogens with the large doses over a long period can affect the physiological processes and functions of the liver because the liver is a vital organ in the metabolic process. Impaired bile secretion, bilirubin excretion, and bromsulphthalein metabolism because oral contraception is a very complicated process and can represent the result of the effects of sex hormones on the metabolism of the liver parenchymal cell [1]. Sergio *et al*. reported that individual doses of flavonoids in pegagan and beluntas could repair damage liver cells and maintain normal transaminase enzyme levels, through Fe chelation activity, inhibition of protein kinase C (PKC), inhibition of lipoxygenase and inhibition ROS activity. In the human's body, they showed many biological properties such as antioxidants, antiallergenic, antibacterial, antifungal, antiviral, and anti-cancer agents [9].

Triterpenoid saponins include asiaticoside, centellaside, madekossida, and asiatic acid contained in pegagan and beluntas as hepatoprotectors. Pegagan also contains genin triterpenoids, essential oils, flavonoids, phytosterols, and other ingredients, while beluntas contains lignans, a sesquiterpene, phenylpropanoid, benzoid, monoterpenes, triterpenes, sterols, and alkanes. Beluntas leaves contain hydroquinone, tannins, alkaloids and sterols, flavonols, such as myricetin, quercetin, and kaempferol

which is productive for antifertility, overcoming vaginal fluids and fertility drugs [10,11]. Beluntas leaves contains vitamin C (98.25 mg/100g), carotene (2.55 mg/100 g) and protein (17.78-19.02%) with the types of amino acids, including leucine, isoleucine, tryptophan, and threonine [12,13].

GPT often also called ALT (alanine aminotransferase) is an enzyme found in liver cells and useful for diagnosing hepatocellular destruction. The heart, kidneys and skeletal muscles contain this small amount of enzyme. GOT or also called AST (aspartate aminotransferase) is an enzyme found in the heart muscle, liver, skeletal muscle, kidney, and pancreas. The low concentrations are in the blood, unless there is a cellular injury in the liver, so large amounts leak into the circulation. GPT is considered to be far more specific to assess liver damage than GOT [14].

The combination of pegagan extract dose of 125 mg/kg BW and beluntas dose of 125 mg/kg BW for 15 days in rats produced a more effective antifertility effect compared to a single dose of pegagan extract (275 mg/kg BW) for 30 days in mice. The combined treatment decreased the number of follicles by 40% and ovulation by 65% while the single dose reduced the number of follicles by 49% and ovulation by 30%. The levels of GPT and GOT enzymes in both studies were still within reasonable limits [4].

Following Besung, the society uses pegaganused as a traditional medicine both in the form of fresh, dry and in the way of herbs (*jamu*) [15]. Asiaticoside as the main content of triterpenoids could enhance the antioxidant effect to protect liver damage due to hepatotoxins. Madekasoside and madekasat acid helped to cure liver damage due to their anti-inflammatory and immunomodulatory activities. In addition to the content, the total glucoside from pegagan also improved damaged liver function [16].

Jatayu *et al.* reported that *C. asiatica* extract had some bioactive compounds such as a flavonoid, alkaloid, terpenoid, tannin, saponin, and DPPH scavenging activity with an IC_{50} value of 125 μg mL^{-1}. C. *asiatica* extracts also enhanced the level of SOD in fish liver [17]. A community of China, Southeast Asia, India, Sri Lanka, Oceania, and Africa has used pegagan as a vegetable and medicine. Traditionally, Southeast Asian societies have used pegagan to treat health problems such as skin diseases, rheumatism, inflammation, syphilis, mental illness, epilepsy, hysteria, dehydration, and diarrhea. Pegagan is used to treat skin problems, decreased memory and psychiatric disorders in the Indian, Indonesian and Chinese medicine systems [5].

Beluntas leaves are usually used by the community to eliminate body odor, increase appetite, improve digestion, overcome joint pain, muscle aches, pain during menstruation, reduce fever, sweat, treat scabies, tuberculosis (TB), lymph nodes, overcome vaginal discharge, improve fertility and contraception. Several studies have shown that beluntas plants have some effects as antioxidants, anti-inflammatory, antimicrobial, anti-cholesterol and antifibrosis of the liver [18]. These various medicinal plants have hepatoprotective effects on various liver injuries may be due to the following factors: (i) inhibition of cytochrome P-450 activity; (ii) prevention of lipid peroxidation; (iii) stabilization of the hepatocellular membrane, and (iv) enhancement of protein synthesis [19].

3.2. Histopathological Profile of Liver

The average scoring data on the level of liver tissue damage of female rats obtained the following results of Control (1.7 ± 0.2), T1 (1.5 ± 0.2), T2 (1.6 ± 0.3), T3 (1.8 ± 0.1), T4 (1.9 ± 0.3), and T5 (2.2 ± 0.2). The data were then analyzed using ANOVA (α 5%), and the results showed that T1, T2, T3, and T4 were not significantly different from the controls while T5 showed differences. This case indicated that the combination of pegagan and beluntas to a dose of 125+125 mg/kg bw did not reveal any liver damage, while the treatment of 200+200 mg/kg bw had begun to show damage (Table 3).

Figure 1 and Figure 2 describe the histological profile of rat's liver after administering several doses of the combination of pegagan and beluntas leaf extract. T1, T2, T3, and T4 exhibited normal central veins, as well as sinusoids and hepatocyte cells. In T5, there were dilatations of the primary and sinusoid veins and some hepatocyte cells that experienced picnotic, but on the other hand, cell division (mitosis) occurred. It is possible because the liver is an organ that has excellent regeneration ability and can remain functioning within normal limits even though 80% of hepatocytes are damaged.

Therefore, the incidence of drug side effects in the liver was relatively low (± 2%), but the fatality rate was relatively large (10-50%). Each active compound or drug at the cellular level would be bound to the cell receptor, which then responds positively (benefits or efficacy) or responds negatively (side effects or poisoning) depending on the type of drug and the dose [20].

Table 3. The average scoring data on the level of liver tissue damage

Treatment	Hepar Scoring (U/I)	P Value
Control	1.7 ± 0.2^{ab}	P > 0.05
T1 (25 mg/kg BW)	1.5 ± 0.2^{a}	P > 0.05
T2 (50 mg/kg BW)	1.6 ± 0.3^{ab}	P > 0.05
T3 (75 mg/kg BW)	1.8 ± 0.1^{bc}	P > 0.05
T4 (125 mg/kg BW)	1.9 ± 0.3^{bc}	P > 0.05
T5 (200 mg/kg BW)	2.2 ± 0.2^{c}	P < 0.05

Hepatoprotector is a nutritious substance protecting liver cells from the effects of destructive toxic substances. These compounds can repair disrupted liver tissue. The mechanism of action is by detoxifying the toxic compounds that enter from outside as well as those formed in the body in the metabolic process, improve liver cell regeneration, anti-inflammation, and as an immunostimulator. Usually, hepatoprotector is an ingredient that has antioxidant properties that can reduce oxidation reactions in liver damage [21].

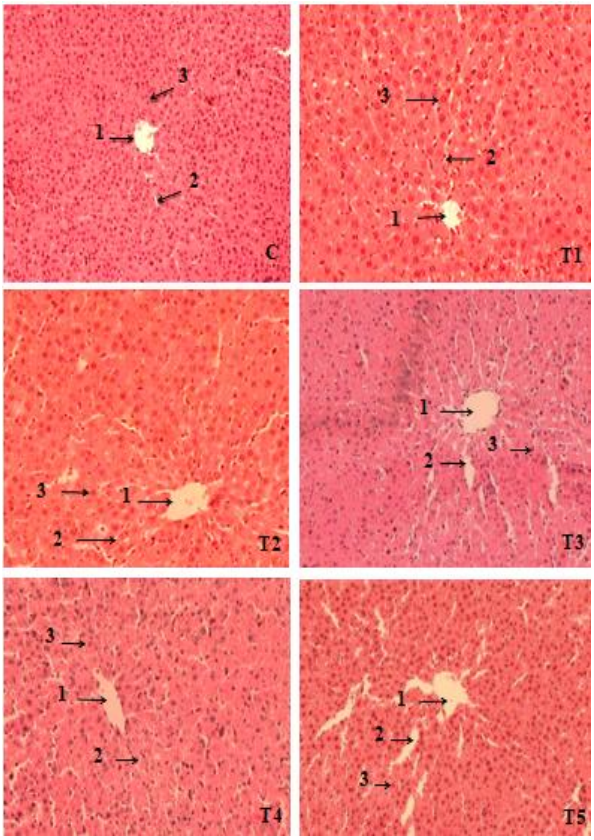

Figure 1. Histology of rat's liver tissue (100x).
C = Control, T1 (25+25 mg/kg BW), T2 (50+50 mg/kg BW), T3 (75+75 mg/kg BW), T4 (125+125 mg/kg BW), T5 (200+200 mg/kg BW).
1 = central vein, 2 = sinusoid, 3 = hepatocyte cell

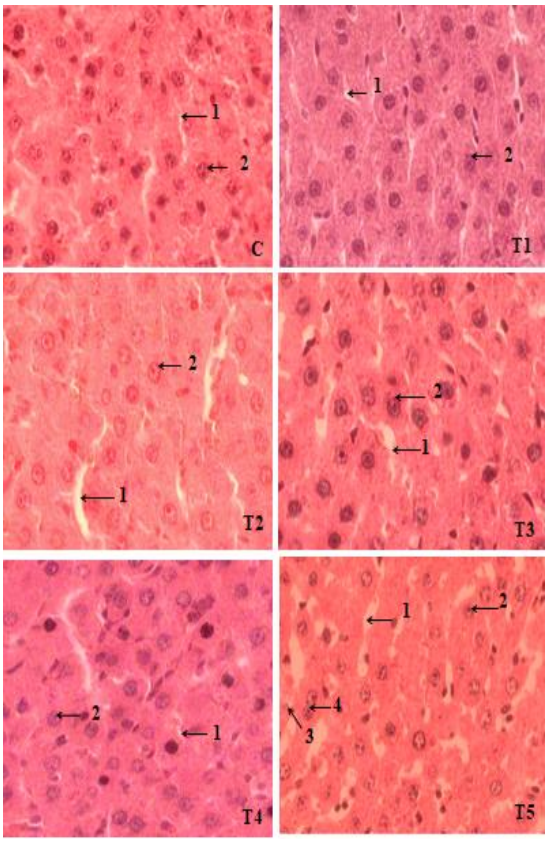

Figure 2. Histology of rat's liver tissue (400x).
1 = sinusoid,
2 = normal hepatocyte,
3 = picnotic hepatocyte,

One compound that has a hepatoprotector effect on a combination of pegagan and beluntas is flavonoid. The mechanism of action is as a natural antioxidant by inhibiting lipid peroxidase and can protect the defense mechanism of antioxidants by increasing absorption of vitamin C so that it can prevent liver damage or necrosis, [20] while essential oils are effective in accelerating hepatocyte cell regeneration and reducing the activity of cyclooxygenase enzymes. The disorders of ion balance, liquids or metabolic products such as free fat and decomposition of phospholipid membranes can generally cause liver damage. These conditions can disrupt the fluid balance in the form of cell swelling and cellular degeneration. In severe cases such conditions can cause cell death, which can be known by the changes in the cytoplasm and cell nucleus. Acute poisoning will cause liver failure which can cause death within 12-24 hours.

Daryoush *et al.* stated that the body has a set of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione to prevent and neutralize damage caused by free radicals. Thus, these results indicated that Pegagan and Beluntas contain free radical scavenging activities, which can provide beneficial actions against pathophysiological changes reported by not finding liver histological abnormalities up to a dose of 125 + 125 mg/kg BW [22].

Backed by research Gupta and Flora (2006) that administration of *Centella asiatica* (100, 200 or 300 mg kg^{-1} simultaneously showed a significant protective measures against the activity of delta-aminolevulinic acid dehydratase (-ALAD) and restored blood levels of glutathione (GSH) blood, whereas most other blood biochemical parameters remained unchanged in supplementation of Centella asiatica.

Interestingly, most liver biochemical variables showed protection against oxidative stress especially at the level of renal oxidized glutathione (GSSG) and the levels of hepatic and brain thiobarbiturate (TBARS) especially at the doses of 300 mg kg (-1) [23]. Nevertheless, the liver is a very great organ in maintaining its function; thus, it can still keep its normal function even with only 10-12% standard functional units [21].

4. Conclusion

This research concluded that the administration of leaf extract of pegagan and beluntas up to the doses of 125+125 mg/kg BW revealed normal transaminase enzyme (GPT and GOT) levels and type of the histological profile, while the treatment of 200+200 mg/kg BW exhibited histological liver damage.

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References

[1] Brynhildsen J 2014 Combined Hormonal Contraceptives: Prescribing Patterns, Compliance, and Benefits *Versus* Risks Ther Adv Drug Saf **5(5)** 201–213

[2] Pathak S, Ghosh K and Thakur M K 2016 Liver Problems and Natural Cure *International Journal of Science and Research* **5 (10)** 2319-7064

[3] Muchtaromah B Mukholifah I S Nasiroh and M Ahmad R **2018** 1st International Conference in One Health (ICOH 2017) 1st International Conference in One Health (ICOH 2017) *Advances in Health Sciences Research (AHSR)* **5** 235-240

[4] Muchtaromah B Kiptiyah and Adi T K 2011 Transaminase Enzyme and Liver Histological Profile of Mice Administered Extract of Pegagan (*Centella asiatica* (L.) Urban) *Media Peternakan* 88-92

[5] Bylka W Znajdek-Awiżeń P Studzińska-Sroka E and Brzezińska M 2013 *Centella asiatica in* Cosmetology *Postepy Dermatol Alergol* **30(1)** 46–49

[6] Li Kao C Cho J Zhe Lee Y Bin Cheng Y Yen Chien C Feng Hwang C Ren Hong Y Neng Tseng C and Lung Cho C 2015 Ethanolic Extracts of Pluchea *indica* Induce Apoptosis and

Antiproliferation Effects in Human Nasopharyngeal Carcinoma Cells *Molecules* **20** 11508-11523
[7] Cora M, Kooistra L, and G Travlos 2015 G. Vaginal Cytology of the Laboratory Rat and Mouse: Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears. *Toxicologic Pathology* **43(6)** 776-793
[8] Muchtaromah B Romaidi Tyas P Griani and Y Hasfita 2015 Potential Antifertility of Centella asiatica Leaf Extract *Australian Journal of Basic and Applied Sciences* **9(7)** 102-105
[9] Sergio A Payan-Gomez Norma Flores-Holguin Antonino Perez-Hernandez Manuel Pinon-Miramontes & Daniel Glossman-Mitnik 2010 Computational Molecular Characterization of The Flavonoid Rutin *Chem Cent J.* **4** 12
[10] Seevaratnam Vasantharuba P Banumathi M R Premalatha Sp Sundara, and T Arumugam 2012 Functional Properties of *Centella asiatica* (L.) *A Review. International Journal of Pharmacy and Pharmaceutical Sciences* **4(5)** 8-14
[11] Sa'roni dan Yun Astuti Nugroho 2012 Ramuan Obat Tradisional di Sumatra Barat dan Nusa Tenggara Barat Untuk Keluhan pada Sistem Reproduksi.*Media Litbang Kesehatan* **22(3)** 144-151
[12] Rukmiasih 2011 *Penurunan Bau Amis Off-odor) Daging Itik Lokal dengan Pemberian Daun Beluntas (Pluchea indica Less) Dalam Pakan dan Dampaknya terhadap Performa* [*disertasi*] *(Institut Pertanian Bogor)*
[13] Sudjaroen Y 2012 Evaluation of Ethnobotanical Vegetables and Herbs in Samut Songkram Province *Procedia Engineering* **(32)** 160-165
[14] Brian T Palmisano Lin Zhu and John M Stafford 2017 Estrogens in the Regulation of Liver Lipid Metabolism *Adv Exp Med Biol* **1043** 227–256
[15] Besung K I 2009 Pegagan (*Centella asiatica*) sebagai Alternative Pencegahan Infeksi Pada Ternak *Jurnal Penelitian* **2(1)** (Universitas Udayana)
[16] Vohra K Pal G, Gupta V K Singh S Bansal Y 2011 An Insight on *Centella asiatica* Linn: A Review on Recent Research *J Pharmacol* **2** 440-462
[17] Jatayu D., Nursyam, H., Hertika, A. M. S. (2018). Antioxidant Effect of *Centella asiatica* Ethanolic Extracts to Superoxide Dismutase (SOD) level on *Cyprinus carpio* Liver. *Research Journal of Life Science* **5** (3) 163-172
[18] Widyawati PS, C Hanny W, Peni S H, dan Dondin S 2010 Evaluasi Aktivitas Antioksidatif Ekstrak Daun Beluntas (*Pluchea indica*) Berdasarkan Perbedaan Ruas Daun. *Jurnal Penelitian* **1(1)**
[19] Al-Howiriny TA, Al-Sohaibani M.D, El-Tahir KH and Rafatullah S 2003 Preliminary Evaluation of The Anti-Inflammatory and Anti Hepatotoxic Activities of "Parsley" *Petroselinum crispum* in rats *J Nat Remed.* **3(1)** 54
[20] Cheedella H K, Alluri R and Mohan KG 2014 Hepatoprotective Effect of Ethanolic Extract of *Swertia Chirayata* on Paracetamol Induced Liver Damage in Albino Rats *World Journal of Pharmacy and Pharmaceutical Sciences* **3(8)** 612-621
[21] Balasubramanian T and TP Chatterjee 2010 Hepatoprotective and Antioxidant Effects of *Stereospermum suaveolens* on Carbon Tetrachloride-Induced Hepatic Damage in Rats *Journal of Complementary and Integrative Medicine* **7** 22-38
[22] Daryoush M Bahram AT Yousef D Mehrdad N 2011 Protective effect of turnip root (*Brassica rapa*. L) ethanolic extract on early hepatic injury in alloxanized diabetic rats. *Austr J Basic and Applied Sci* **5(7)** 748–756
[23] Gupta R and Flora SJ 2006 Effect of Centella asiatica on arsenic-induced oxidative stress and metal distribution in rats *J Appl Toxicol* **26(3)** 213-22