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Vitamin C and quercetin combination activity on aspartate aminotransferase and alanine aminotransferase of mice with ethanol induced hepatotoxicity

Anggita Rahma Fedyasasti[1], Nabil Hajar[2], Maya Dian Rakhmawatie[3]

[1]Fakultas Kedokteran Universitas Muhammadiyah Semarang
[2]Bagian Fisiologi, Departemen Ilmu Biomedik, Fakultas Kedokteran Universitas Muhammadiyah Semarang
[3]Bagian Farmakologi, Departemen Ilmu Biomedik, Fakultas Kedokteran Universitas Muhammadiyah Semarang
Email: mayadianr@unimus.ac.id

Abstrak

Hepar merupakan organ utama dalam proses metabolisme etanol. Jumlah intake etanol berlebihan dapat meningkatkan resiko hepatotoksisitas. Pemberian antioksidan vitamin C atau quercetin tunggal diketahui dapat bersifat hepatoprotektor dengan memperbaiki kadar AST dan ALT hepar yang terpapar etanol. Tujuan dari penelitian ini adalah mencari dosis efektif kombinasi vitamin C dan quercetin untuk pencegahan kerusakan hepar akibat etanol dan mengetahui toksisitasnya. Penelitian ini menggunakan rancangan *Post-test with control group design* dengan 30 ekor mencit *swiss-webster* jantan yang dibagi dalam lima kelompok: Non-treatment group (NTG), kontrol negatif (CG) diberi etanol 20% (4 gr/KgBB), perlakuain 1 (T1) kombinasi vitamin C 50 mg/KgBB dan quercetin 20 mg/KgBB serta induksi etanol 20% (4 gr/KgBB), (T2) kombinasi vitamin C 100 mg/KgBB dan quercetin 40 mg/KgBB serta induksi etanol 20% (4 gr/KgBB), dan (T3) kombinasi vitamin C 200 mg/KgBB dan quercetin 80 mg/KgBB serta induksi etanol 20% (4 gr/KgBB). Setelah 28 hari perlakuan, perbedaan kadar AST dan ALT antar kelompok diuji statistik *Kruskal Wallis* yang dilanjutkan dengan uji post-hoc *Games-Howell.* Toksisitas diamati dari kematian 24 jam awal perlakuan, perubahan perilaku, dan perubahan fisik mencit saat perlakuan. Kadar AST dan ALT kelompok kontrol negatif (CG) meningkat signifikan dibanding kelompok tanpa perlakuan (NGT) ($p<0,05$). Semua dosis kombinasi vitamin C dan quercetin dapat menurunkan kadar AST signifikan pada mencit yang diinduksi etanol, dan tidak ada perbedaan aktivitas diantara ketiga kelompok dosis tersebut. Namun ketiga dosis kombinasi vitamin C dan quercetin tidak mampu menurunkan kadar ALT mencit yang diinduksi etanol. Tidak terdapat kematian mencit pada 24 jam awal perlakuan, selain itu tidak ada perubahan perilaku dan fisik mencit selama perlakuan. Dosis efektif kombinasi untuk menurunkan kadar AST adalah dosis vitamin C 50 mg/kgBB dan quercetin 20 mg/KgBB. Dosis tersebut tidak menyebabkan efek toksik terhadap mencit.
Kata Kunci: Antioksidan, hepatoprotektor, quercetin, toksisitas etanol, vitamin C.

Abstract

The liver is the main organ in the process of ethanol metabolism. Excessive amounts of ethanol intake can increase the risk of hepatotoxicity. Administration of the antioxidant vitamin C or quercetin alone is known to act as a hepatoprotector by improving AST and ALT levels in livers exposed to ethanol. The purpose of this study was to find an effective dose combination of vitamin C and quercetin to prevent ethanol-induced liver damage and to determine its toxicity. This study used a post-test with control group design with 30 male swiss-webster mice divided into five groups: Non-treatment group (NTG), negative control group (CG) were given 20% ethanol (4 gr/KgBW), treatment 1 (T1) a combination of vitamin C 50 mg/KgBW and quercetin 20 mg/KgBW and 20% ethanol (4 gr/KgBW), (T2) a combination of 100 mg/KgBW vitamin C and 40 mg/KgBW quercetin and 20% ethanol (4 gr/KgBW), and (T3) a combination of 200 mg/KgBW vitamin C and 80 mg/KgBW quercetin and 20% (4 gr/kgBW). After 28 days of treatment, the differences in AST and ALT levels between groups were statistically tested by Kruskal Wallis, followed by the Games-Howell post-hoc test. Toxicity was observed from death 24 hours early in the treatment, changes in behavior, and physical changes in the mice during treatment. AST and ALT levels in the negative control group (CG) increased significantly compared to the non-treatment group (NGT) ($p<0.05$). All doses of the combination of vitamin C and quercetin significantly reduced AST levels in ethanol-induced mice, and there was no difference in activity between the three dose combinations. However, the three combination doses of vitamin C and quercetin were unable to reduce the ALT levels of ethanol-induced mice. There were no mice deaths in the first 24 hours of treatment, and also no behavioral and physical changes in mice during treatment. The effective combination dose to reduce AST levels is a dose of 50 mg/kgBB of vitamin C and 20 mg/KgBB of quercetin. These doses did not cause toxic effects on mice.
Keywords: Antioxidants, ethanol toxicity, hepatoprotector, quercetin, vitamin C.

1. INTRODUCTION

Excessive alcohol consumption is a global health problem. According to a survey by the World Health Organization (WHO), it has been reported that as many as 320,000 people (aged 15-29 years) in the world die from alcohol every year. Total alcohol consumption is expected to increase until 2025. The highest incidence of alcohol-related deaths occurs in the Southeast Asia region, especially in Indonesia. The proportion of consumption of alcoholic beverages in the Indonesian population aged over 10 years is 3.3% of the percentage of world alcohol consumption.[1]

The amount of alcohol that enters the body greatly affects liver tissue injury, which can cause decreased liver function. This is because the liver is the main organ in the process of alcohol metabolism.[2] To determine the presence of liver damage, several blood tests can be done. The most commonly blood test parameters are serum glutamic oxaloacetic transaminase/aspartate transaminase (SGOT/AST) and serum glutamic piruvic transaminase/alanine transaminase (SGPT/ALT) contained in liver cells. Damage of liver function will cause liver cells to excrete these enzymes in the blood, and causes an increases of both enzymes in the blood.[3]

Ethanol is an alcoholic beverage that is commonly consumed daily.[4] For alcohol intoxication research, animal models can be used as substitutes for human subjects. Rodents are one of the suitable animal models because alcohol intake can increase blood alcohol levels and signs of intoxication.[5] For example, a study with ethanol induction of 100 mg/kgBW for 14 days could significantly increase the levels of AST and ALT in mice.[6]

To reduce the risk of hepatotoxicity due to ethanol use, antioxidants can be used. Antioxidants vitamin C and quercetin are known to improve AST and ALT levels in livers exposed to hepatotoxic compounds.[7] This was proven in a study that each vitamin C doses of 200 mg or quercetin doses of 50 mg could reduce the AST and ALT values of mice induced by ethanol.[8] The hepatoprotective effect of vitamin C is reported to increase synergistically when given together with other antioxidants.[9] Therefore, combination therapy of vitamin C with the antioxidant quercetin can be analyzed for its benefits as an agent to prevent ethanol-induced hepatotoxicity.

Vitamin C and quercetin are drugs that are considered safe when used alone. Mega doses of vitamin C (5-20 g per day) in rats are declared safe for the liver and kidneys.[10] Meanwhile, single use of quercetin is stated to be safe for mice at doses up to 250 mg/kgBB.[11] However, the safety level of using vitamin C in combination with quercetin is not known. Therefore, this study aims to prove the effect of a combination of vitamin C and quercetin on the prevention of liver damage due to ethanol and find the effective combination dose. In addition, we observed the toxic effects of using a combination of vitamin C and quercetin, seen from acute death after 24 hours of treatment, as well as behavioral and physical changes in mice during treatment.

2. METHOD

This research is a true experimental research with a post-test only control group design. Determination of the sample using simple random sampling method, with a total sample of 30 swiss-webster mice. Samples must meet the inclusion criteria for healthy males and no morphological abnormalities, 4-8 weeks of age, and approximately 20 grams of body weight. Exclusion criteria were mice that died during treatment. The sample was then divided into 5 groups with each group consisting of 6 mice, namely the Non Treatment Group (NTG), the negative control group (CG) which was only given 4 g/KgBB of 20% ethanol per day, the treatment group 1 (T1) which was given a combination dose of 50 mg/KgBB vitamin C and 20 mg/KgBB quercetin and 4 gr/KgBW of 20% ethanol per day, treatment group 2 (T2) which was given a combination dose of vitamin C 100 mg/KgBW

and quercetin 40 mg/KgBW and 4 gr/KgBW of 20% ethanol per day, and the group treatment 3 (T3) given a combination dose of vitamin C 200 mg/KgBW and quercetin 80 mg/KgBW and 4 g/KgBW of 20% ethanol per day.

The research has received research ethics approval from Politeknik Kesehatan Negeri Semarang with number 046/EA/KEPK/2023. Prior to treatment, the test animals were adapted for 3 days. Administration of 20% ethanol, vitamin C, and quercetin was carried out orally using a sonde. After calculating the doses of vitamin C and quercetin with reference to the mice's body weight, the doses were dissolved with 0.1 ml of distilled water. Administration of a combination of vitamin C and quercetin was given 45-60 minutes after the ethanol induction. During the treatment, behavioral and physical observations were made to identify any toxic effects due to the treatment given. In addition, to see the effects of acute toxicity, mortality was observed after 24 hours of treatment.

For the assessment of hepatotoprotective effect of a combination of vitamin C and quercetin, all groups were treated for 28 days. Blood was taken from the mice through the orbital sinus, then be prepared into serum. Serum was analyzed for examination of AST and ALT levels by enzymatic kinetic method according to the recommendations of the International Federation of Clinical Chemistry.[12]

AST and ALT levels in all treatment groups were tested for normality and homogeneity tests. Because the data were normal but not homogeneous, the differences in the values of AST and ALT levels in all treatment groups were analyzed using the Kruskall-Walis test with a result of $p<0.05$. Then the Post-Hoc Games-Howell test was carried out to find out the significant differences in each treatment group.

3. RESULTS

Toxicity effects were observed for 28 days of treatment using the criteria stated in the Regulation of the Food and Drug Supervisory Agency Number 10 of 2022.[13] Based on behavioral observations, none of the rats experienced seizures, tremors, weakness, and hypersalivation during the treatment. However, in some mice that died in the NTG, T1, and T3 groups, their activity decreased 12 hours before death (Table 1). All mice deaths did not occur within the first 24 hours after treatment. While based on physical observations, in general there were no abnormalities except on the skin. In all groups there was at least 1 mouse that had an injury to the skin of the back (Table 2).

Table 1. The results of toxicity observations on the behavior of mice in all treatment groups

	NTG	CG	T1	T2	T3
a	100% active	100% active*	100% active*	100% active	100% active*
b	0%	0%	0%	0%	0%
c	0%	0%	0%	0%	0%
d	0%	0%	0%	0%	0%
e	0%	0%	0%	0%	0%

Notes: (a) liveliness; (b) seizure; (c) tremor; (d) weakness; (e) hipersalivation

* Decreased activity occurred 12 hours before death occurred in mice that died.

Table 2. The results of the observation of the physical toxicity of mice in all treatment groups

	NTG	CG	T1	T2	T3
1	83% normal (wound in 1 mice)	50% normal (wound in 3 mice)	83% normal (wound in 1 mice)	83% normal (wound in 1 mice)	66% normal (wound in 2 mice)
2	100% normal	100% normal	100% normal	100% normal	100% normal
3	100% clear	100% clear	100% clear	100% clear	100% clear
4	100% normal	100% normal	100% normal	100% normal	100% normal
5	100% normal	100% normal	100% normal	100% normal	100% normal
6	100% normal	100% normal	100% normal	100% normal	100% normal
7	100% normal	100% normal	100% normal	100% normal	100% normal

Notes: (1) wound in skin; (2) hair loss; (3) eye abnormalities; (4) faeces consistency; (5) mucous membrane; (6) motoric function; (7) sensory function

For the results of the analysis of AST and ALT levels using the Kruskal-Wallis statistical test, it was seen that there was a significant difference in the AST and ALT values in all treatment groups ($p < 0.05$). In the analysis of AST levels, significant results were obtained between the untreated group (NTG) (171.06 U/L) and the negative control group (CG) (334.69 U/L). All treatments T1, T2, and T3 were able to significantly reduce AST levels compared to the average AST levels in the CG group. Statistically, there was no significant difference in reducing AST levels between all treatment groups, both T1, T2 and T3 (Figure 1).

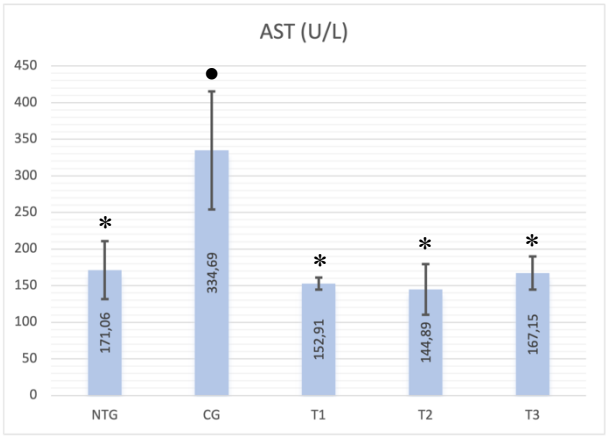

Figure 1. The results of the Post-Hoc Games-Howell analysis of AST levels in all treatment groups. Notes: •Significance of AST levels to NTG ($p<0.05$); *Significance of AST levels to CG ($p<0.05$)

For the analysis of ALT levels, it was found that there was a significant increase in ALT levels in the CG group (124.65 U/L) compared to the non-treatment group (NTG) (29.54 IU/L) ($p<0.05$). In all treatment groups, both T1, T2, and T3 could not reduce ALT levels back to normal. Even though there was a significant decrease in the T2 group compared to the T1 and T3 groups, the decrease in ALT had not been able to restore normal ALT values (Figure 2).

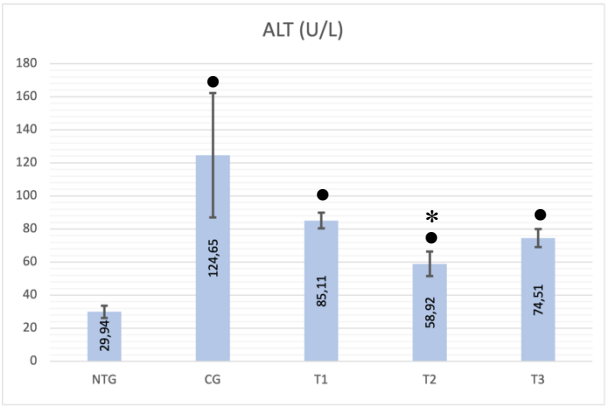

Figure 2. The results of the Post-Hoc Games-Howell analysis of ALT levels in all treatment groups. Notes: •Significance of ALT levels to NTG ($p<0.05$); *Significance of ALT level to T1 and T3 ($p<0.05$)

4. DISCUSSION

During the 28 day treatment there was a death of 1 mice each from the CG, T1, and T3 groups. Mice from the three groups probably died due to infection from deep wounds found on the backs of each mouse (Figure 3). The wound on the back was caused by the activity of mice trying to mate. The mice used in this study were adult male mice that were entering the mating period, which due to alcohol induction caused changes in mood, memory and behavior that tended to be aggressive.[14]

The death of mice in the CG, T1, and T3 groups did not occur during the initial 24 hours of treatment. Information on mortality in the first 24 hours of treatment can be used for acute toxicity analysis to determine LD_{50} (Median Lethal Oral Dose).[13] So it can be concluded that the combined dose of vitamin C and quercetin at T1, T2, and T3 as well as the 20% ethanol induction dose of 4 g/kgBW were not LD_{50}. This is in line with the results of the study that rats that had been given natural ethanol only showed clinical signs of toxicity but without mortality in the subjects.[15]

Figure 3. Description of wounds on the back skin of dead mice (group T1)

From the results of physical toxicity observations during the 28 days of treatment, no other abnormalities were found, such as changes in skin color and texture, hair loss, changes in eye color clarity, faeces consistency, lesions and color of mucous membranes (Table 2). During behavioral observations, no changes in sensory and motoric activity, tremors, seizures, salivation or weakness were found (Table 1). Other signs of behavioral abnormalities indicating toxicity include walking backwards, tummy tuck walking, and tail tension (*straub*)[16], also not found. Therefore, it can be interpreted that either giving only 4 g/kgBW of 20% ethanol or a combination of vitamin C dose of 50 mg/KgBW-20 mg/kgBW quercetin, combination of vitamin C dose of 100 mg/KgBW-quercetin 40 mg/KgBW, and combination vitamin C dose of 200 mg/kgBW-quercetin 80 mg/KgBW did not cause toxic effects on mice. For information, vitamin C which is used as a single drug has an LD_{50} value of more than 5.2 gram/KgBW.[17] Meanwhile, single use of quercetin doses of 2, 4, 8, and 16 gram/KgBW is known not causing the death of mice within 24 hours, but known to affect liver histopathological changes including lysis, necrosis, dilation, and bleeding in the sinusoids.[18] The combination doses of vitamin C-quercetin in the T1, T2, and T3 treatments were lower than the known toxic doses of vitamin C and quercetin.

To evaluate the toxic effects of ethanol induction, organ analysis can be performed. The liver is the main metabolic organ which also has the ability to eliminate toxins. To determine the presence of liver damage, several blood enzyme analyzes can be done. The most commonly used test is the Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) enzyme level which is synthesized by liver cells.

Based on the results of the Games-Howell Post-Hoc statistical test, it was found that there were significant differences in the levels of AST and ALT in NTG to CG (Figures 1 and 2). This can be interpreted that induction of ethanol for 28 days can affect the occurrence of liver inflammation as indicated by increased levels of AST and ALT mice. Consumed ethanol undergoes oxidation in the hepatic cell microsomes. The oxidation process produces the acetaldehyde.[19] Acetaldehyde is a highly reactive and toxic metabolic product that can cause damage to liver cells (hepatocytes) which can lead to liver inflammation.[20] This condition causes changes in permeability which can affect the increase in both AST and ALT enzymes.[21]

Meanwhile, for the analysis of AST levels, there were significant differences between CG to all treatment groups (T1, T2, T3), which means that the administration of vitamin C and quercetin in various dose combinations had a significant effect on reducing mice AST after ethanol induction. There was no significant difference in the AST levels between T1 to T2 group, T1 to T3 and T2 to T3 group. It can be concluded that statistically all combination doses of the treatment groups had the same effect in significantly reducing AST. Therefore, the smallest dose in T1 group (a combination of 50 mg/KgBB vitamin C and 20 mg/KgBB quercetin) was considered the most effective in preventing alcohol-induced liver damage. Aspartate aminotransferase is a non-specific enzyme found in liver cells. This enzyme can also be found in heart cells, liver cells, skeletal muscles, kidneys, brain, pancreas, spleen and

lungs. The high activity of this enzyme is closely related to the amount of damage that occurs in organ cells.[22]

Meanwhile, the ALT enzyme can be found in liver, heart, muscle and kidney cells. Among these organs, the greatest amount of ALT enzymes is found in liver cells which are located in the cytoplasm of liver cells or hepatocytes.[23] ALT level is a marker of acute liver damage, while AST is a marker of chronic liver damage.[24] In inflammatory liver conditions, ALT levels are a more specific indicator than AST. This could be due to the longer half-life for AST to respond due to its release from the mitochondria of the cell. While the release of ALT originates from the cytoplasm of cells that appear as a response to damage to liver cells due to alcohol. Stimulation of ALT release is due to the co-existence of pyridoxal-6-phosphate deficiency in alcohol consumers, which is the main cofactor of ALT enzymatic activity.[25]

In this study, there was no statistically significant difference between NTG in all treatment groups (T1, T2, and T3). In addition, even though there was a decrease in ALT levels in all treatment groups, ALT levels were not able to return to normal. So it can be interpreted that giving various doses of a combination of vitamin C and quercetin does not provide benefits for reducing ALT.

This study is not in line with other studies which state that vitamin C and quercetin, which are flavonoids with antioxidant properties, can improve liver ALT levels exposed to hepatotoxic substances.[26] Antioxidants vitamin C and quercetin should be effective in reacting to cell damage due to superoxide anions, hydroxyl radicals, singlet oxygen and lipid peroxides, which can directly repair and enhance the integrity of cell membranes.[27] The decrease in ALT levels due to ethanol tends to be slower than ALT levels in cases of hepatic cell disorders.[28] After the liver cell membrane is damaged due to alcohol, the cytoplasm containing ALT as the main enzyme in the liver cells exits profusely, caused the amount of ALT contained in the blood counted more.[29] The combination of vitamin C and quercetin is not enough to break the autocatalytic process of lipid peroxidation due to ethanol on cell membranes, so that the integrity of the liver cell membrane cannot return to its initial normal function.

5. CONCLUSION

As a hepatoprotective agent, the combination of vitamin C and quercetin can reduce AST levels but not ALT levels in ethanol-induced mice. The most effective dose for decreasing AST levels in mice after ethanol induction was a combination of 50 mg/KgBB vitamin C and 20 mg/KgBB quercetin. This dose is safe because it does not cause death of mice in the first 24 hours of treatment, and also behavioral and physical changes in mice during treatment.

REFERENCES

[1]. Rekve P. Global status report on alcohol and health 2018. Geneva: World Health Organization. 2018. Avalaible at http://apps.who.int/iris/

[2]. Sijid SA, Muthiadin C, Zulkarnain Z, Hidayat AS. Pengaruh pemberian tuak terhadap gambaran histopatologi hati mencit (*Mus musculus*) ICR jantan. Jurnal Pendidikan Matematika dan IPA. 2020;11(2):193–205.

[3]. Sudatri NW, Setyawati I, Suartini NM, Yulihastuti DA. High doses injection of white vitamin C in long term decreased the liver function of female rat (*Rattus norvegicus L.*) based on SGPT and SGOT levels and histology of the liver. Metamorfosa Journal of Biological Sciences. 2016;51(1):44–51.

[4]. Kementrian Perindustrian Indonesia. Peraturan Menteri Perindustrian Republik Iindonesia Nomor 17 tahun 2019. Jakarta: Kementerian Perindustrian. 2019.

[5]. Becker HC and Ron D. Animal models of excessive alcohol consumption: Recent advances and future challenges.

Alcohol. 2016;48(3):205–8.
[6]. Ounjaijean S and Somsak V. Preliminary study on hepatoprotective activity of aqueous crude extract of *Allium ascalonicum* against ethanol-induced liver injury in mice. Walailak Journal of Science and Technology. 2020;17(10):1088–94.
[7]. Khalish M and Wulandari LY. Vitamin C berpengaruh dalam memperbaiki kerusakan hepar akibat pemberian monosodium glutamat. Jurnal Penelitian Perawat Profesional. 2020;2(2):125–30.
[8]. Ambadath V, Venu RG, Madambath I. Comparative study of the efficacy of ascorbic acid, quercetin, and thiamine for reversing ethanol-induced toxicity. Journal of medicinal food. 2010;13(6):1485–9.
[9]. Adikwu E and Deo O. Hepatoprotective effect of vitamin C (ascorbic acid). Pharmacology and Pharmacy. 2013;04(01):84–92.
[10]. Lee SW, Lee YJ, Baek SM, Kang KK, Kim TU, Yim JH, et al. Mega-dose vitamin C ameliorates nonalcoholic fatty liver disease in a mouse fast-food diet model. Nutrients. 2022;14(11):2195-205
[11]. Cunningham P, Patton E, Vanderveen BN, Unger C, Aladhami A, Enos RT, et al. Sub - chronic oral toxicity screening of quercetin in mice. BMC Complementary Medicine and Therapies. 2022;1–12.
[12]. Huang XJ, Choi YK, Im HS, Yarimaga O, Yoon E, Kim HS. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. Journal Sensors. 2006;6(7):756–82.
[13]. BPOM. Peraturan Badan Pengawas Obat Dan Makanan Nomor 10 Tahun 2022 Tentang Pedoman Uji Toksisitas Praklinik Secara In Vivo. Jakarta: Badan Pengawas Obat dan Makanan Republik Indonesia. 2022;1–220.
[14]. Sher L. Alcohol consumption and suicide. Q J Med. 2006;99:57–61.
[15]. Sulastra CS, Khaerati KKK, Ihwan. Toksisitas akut dan lethal dosis (LD_{50}) ekstrak etanol uwi banggai ungu (*Dioscorea alata L.*) pada tikus putih (*Rattus norvegicus*). Jurnal Ilmiah Medicamento. 2020;6(1):10–4.
[16]. Sundari D and Budi Nuratmi MWW. Toksisitas akut (LD_{50}) dan uji gelagat ekstrak daun teh (*Camellia sinensis*) pada mencit. Media Penelitian dan Pengembangan Kesehatan. 2009;19(4):198–203.
[17]. Andersen FA. Final report of the safety assessment of L-ascorbic acid, calcium ascorbate, magnesium ascorbate, magnesium ascorbyl phosphate, sodium ascorbate, and sodium ascorbyl phosphate as used in cosmetics. International Journal of Toxicology. 2005;24(SUPPL. 2):51–111.
[18]. Lucida H, Primadini Y, Suhatri. A study on the acute toxicity of quercetin solid dispersion as a potential nephron-protector. Rasayan Journal of Chemistry. 2019;12(2):727–32.
[19]. Maliangkay OJ, Assa Y, Rompas DG. Kadar serum glutamate oxaloacetic transaminase (SGOT) pada peminum minuman beralkohol di kelurahan Tosuraya Selatan. Jurnal e-Biomedik. 2020;8(1):132–7.
[20]. Zakhari S. Alcohol metabolism and the body. Journal of the National Institute on Alcohol Abuse and Alcoholism. 2014;29(4).
[21]. Ardiaria M. Pengaruh vitamin E terhadap kadar SGOT dan SGPT pada tikus yang diberi parasetamol. Journal of Nutrition and Health. 2017;5(2):50–68.
[22]. Adelia Novitasari. Aktivitas serum glutamic pyruvic transaminase (SGPT) pada pengkosumsi minuman beralkohol. Jurnal Kesehatan. 2019;8(1):40–6.
[23]. I Kendran AAS, Arjana AAG, Pradnyantari AASI. Aktivitas Enzim Alanine-Aminotransferase dan Aspartate Aminotransferase pada Tikus Putih Jantan yang Diberi Ekstrak Buah Pinang.

Buletin Veteriner Udayana. 2017;9(2):132–8.
[24]. Rompas DG, Kaligis SHM, Assa Y. Kadar Serum Glutamate Piruvate Transaminase Pada Peminum Minuman Beralkohol di Kelurahan Tosuraya Selatan. Jurnal e-Biomedik. 2020;8(1):132–7.
[25]. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology. 2008;47(4):1363–70.
[26]. Arifin. B and Ibrahim S. Flavonoid structure, bioactivity, and antioxidan of flavonoid. Zarah. 2018;6(1):21–9.
[27]. Yanardag R, Ozsoy-Sacan O, Ozdil S, Bolkent S. Combined effects of vitamin C, vitamin E, and sodium selenate supplementation on absolute ethanol-induced injury in various organs of rats. International Journal of Toxicology. 2007;26(6):513–23.
[28]. Widarti W and Nurqaidah N. Analisis kadar serum glutamic pyruvic transaminase (SGPT) dan serum glutamic oxaloacetic transaminase (SGOT) pada petani yang menggunakan pestisida. Jurnal Media Analis Kesehatan. 2019;10(1):35.
[29]. Nurrahma HA, Meliala A, Narwidina P, Herwiyanti S. Hepatoprotective effect of banana peel flour on histological and liver function in diabetic rats. Indonesian Journal of Pharmacy. 2021;32(4):529–38.