IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 14, Issue 1 Ver. I (Jan – Feb 2019), PP 01-05
www.Iosrjournals.Org

Hepatoprotective Activities of Girang Leaf Ethanol Extracts (Leea angulata Korth. Ex Miq) on Wistar Rats Induced by Paracetamol

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Abstract: *Paracetamol is an analgesic and antipyretic drug that has a toxic effect when it is used with excessive doses. The toxicity of paracetamol is caused by the formation of NAPQI reactive metabolites and paracetamol inducing the formation of free radicals which can cause liver cell damage. This study aims to test the hepatoprotective activity of ethanol extract of girang leaves (Leea angulata Korth. Ex Miq) in male white rats of the Wistar strain.Acute liver damage was done by inducing paracetamol at a dose of 750 mg / kg BW. Ethanol extract of girang leaves was given at a dose of 50, 100, 200, and 400 mg/kg per oral for 14 days. Liver biochemical parameters (ALT activity) of rat serum were measured by using a spectrophotometer. The results showed that the mean ALT activity of rat given girang leaves ethanol extract of 50 mg/kg BW (82.30 ± 12.06) U/L, 100 mg/kg BW (71.70 ± 2.86) U/L, 200 mg/kg BW (43.50 ± 1.56) U/L, and 400 mg/kg BW (42.90 ± 1.86) U/L significantly ($p<0.05$) lower than the mean of rat of ALT activity which was only induced by paracetamol (109.90 ± 12.06) U/L. The conclusion of this study is that ethanol extract of girang leaves has hepatoprotective activity by reducing the ALT activity of rats given excessive paracetamol with an effective dose of 200 mg/kg BW.*

Keywords*: hepatoprotective effect, paracetamol, ethanol extract of girang leaves*

Date of Submission: 21-12-2018 Date of acceptance: 06-01-2019

I. Introduction

Paracetamol is one of the analgesic-antipyretic drugs that is widely used freely in the community. Paracetamol is declared to be safe at therapeutic doses, but for use with excessive doses or long-term use can induce liver damage ((Nayak, et al., 2011; Dandan and Brunton, 2013).

Excess doses of paracetamol will be biotransformed by the cytochrome P-450 in the liver to form N-acetyl-p-benzoquinoneimine (NAPQI) reactive metabolites which can cause liver cell damage (Subramanian, et al., 2013). Paracetamol also induces the formation of free radicals, which can cause the lipid membrane peroxidation of the cells so that it disrupts the stability of the membrane which ultimately also causes damage to liver cells (Malar and Bai, 2012; Nambiar, 2012).

Liver tissue damage due to the number of dead cells and rupture of the liver cell membrane, causing enzymes contained in the liver to be released into the blood, so that the levels will increase in the blood. The enzyme commonly found in the liver is the enzyme of aminotransferase (transaminase), namely the enzyme of alanine aminotransferase (ALT) or Serum of Glutamate Pyruvate Transaminase (SGPT) and the aspartate aminotransferase enzyme (AST or SGOT). ALT is an enzyme that is only synthesized in liver cells, while AST is produced in liver cells and is also found in the heart, kidneys and brain, so the ALT enzyme is the best indicator of seeing liver damage. In humans, the normal value of ALT enzyme levels ranges from 5 - 25 U/L, whereas in rats the value ranges from 19.3 - 68.9 U/L (Selvam, et al., 2015).

One way to prevent liver damage due to paracetamol toxicity is by giving antioxidants. Due to Concerns about the side effects of synthetic antioxidants, then, natural antioxidants are an alternative choice (Hossny, et al., 2011). One plant material that can be developed as a natural antioxidant, and later is expected to prevent liver damage is the girang plant (Leea angulata Korth Ex Miq).

Girang plants are often used as traditional medicine. Girang leaves are useful as a medicine for headaches, diabetes medications, antidysentery, antidiarrheal, antifungals, malaria drugs, ulcers, and stomach pain medications.

Girang leaves contain useful chemical compounds, including flavonoid compounds. The ability of these flavonoids can capture free radicals that causing liver damage. (Francis, et al., 2011).

This prompted the researchers to be interested in investigating the extent of the potential for girang leaf extract as a hepatoprotector in male white rats Wistar strain induced by paracetamol with measurement parameters of serum of Alanine Amino Transferase (ALT) or Glutamic Piruvic Transaminase (SGPT) activity. The purpose of this study was to determine the effect of hepatoprotector ethanol extract of girang leaves (Leea angulata Korth Ex Miq) on the liver function in white rats induced by paracetamol.

II. Material And Methods

2.1 Materials and Equipments

The materials used in this study were girang leaves (Leea angulata Korth. Ex Miq) obtained from the Dalung area, North Kuta, Badung, Bali, which had been determined in the Botanical Gardens Conservation Center of "Eka Karya" Bali. The chemicals used were ethanol (technical and p.a), paracetamol, 0.1% ketamine solution, ALT reagent which consisted of TRIS buffer pH 7.0, L-alanine, LDH, NADH, and 2-oxoglutarate. The material for the experimental research was male white rats, the Wistar strain (Rattus norvegicus). The equipments used in this study were a set of glassware, blenders, sieves, extractors, rotary vacuum evaporators, centrifuges, scales, and spectrophotometers.

2.2 The Making of Concentrated Ethanol Extract from Girang Leaves.

The girang leaves were washed with distilled water, drained, then cut into small pieces and dried by being placed in the open air and not directly exposed to sunlight. The dried leaves were made into powder by grinding them by using a blender. Dry powder of girang leaves was weighed and ready to be extracted.

As much as 1 kg of dry powder of girang leaves were extracted by maceration using ethanol 96% solvent for 24 hours at room temperature. Extraction was carried out for 5 times. The extract solvent obtained was then evaporated using a rotary vacuum evaporator until a concentrated ethanol extract was obtained and weighed. This ethanol concentrated extract was then used in the treatment of rats.

2.3 Treatment of Experimental Rats

The type of research used was experimental research conducted in the laboratory with the design of Post-test Only Control Group Design with the research location of the Pharmacology Laboratory of the Faculty of Medicine, Udayana University.

All rats aged 8-9 weeks were adapted by being given standard feed and the same drink for seven days, then weighed. Expected body weight ranged from 200-250 g in healthy conditions that can be seen from their activities and no rats' defects. The uniform experimental rats were ready to be applied in the treatment.

A total of 25 healthy rats were grouped into five groups, namely the Control Group (K), Treatment Group One (P1), Treatment Group Two (P2), Treatment Group Three (P3), and Treatment Group Four (P4). Group K was a group of rats which were only given paracetamol of 750 mg/kg body weight for 14 days. The P1 group was a group of rats given an ethanol extract of girang leaves at a dose of 50 mg/kg BB, P2 group was a group of rats given an ethanol extract of girang leaves at a dose of 100 mg/kg BW. The P3 group was a group of rats given an ethanol extract of girang leaves at a dose of 200 mg/kg BW. The P4 group was a group of rats given an ethanol extract of girang leaves at a dose of 400 mg/kg BW. One hour after administration of the extract, all rats in each treatment group were given paracetamol of 750 mg/kg BW. This treatment was carried out for 14 days. On day 15, rats were anesthetized, the blood was taken from the retro orbitalus plexus then the ALT activity was examined.

2.4 Measurement of Alanine Amino Transferase (ALT) Enzyme Activity

Blood samples of rats were collected in tubes, then centrifuged and taken for serum. A total of 0.1 mL of rat serum added with 1 mL of a mixture of four parts reagent one (R1) (100 mmol/L TRIS buffer pH 7.0, 500 mmol/L L-alanine, 1200 U/L LDH) and one part reagent 2 (R2) (0.18 mmol/L NADH and 15 mmol/L 2-oxoglutarate). The mixture was shaken and incubated in a water-bath at 37^0C for one minute, then measured by a spectrophotometer at 340 nm wavelength.

III. Result

Data from the study showed that the mean (± SD) of ALT serum of rats' activities in the 5 experimental rat groups i.e. Control Group (K), Treatment Group One (P1), Treatment Group Two (P2), Treatment Group 3 (P3), and Treatment Group 4 (P4) is as presented in Table 1 below.

Table 1: Mean (± SD) ALT activities of Wistar rat serum

Experimental Rat Group	The mean of ALT activities (U/L) ± SD	Normality (p)	Variant Homogeneity (p)	Kruskal-Wallis Test (p*)
Control	109.90 ± 12,06	0,00	0,00	0,00
Treatment1	82,30 ± 2,21			
Treatment2 Treatment 3 Treatment 4	71,70 ± 2,86 43,50 ± 1,56 42,90 ± 1,86			

Description: p = significant value; * = significant at p <0.05

Based on the results of the analysis of normality with the Shapiro-Wilk Test and variance homogeneity by the Levene's test at a significance level of α = 0.05, the results showed that the serum ALT activities in rat serum were not normally distributed (p<0.05), and the variance was not homogeneous (p<0 , 05) so that Non-Parametric tests can be carried out with the Kruskal-Wallis Test. The results showed that the mean serum of ALT activities of the rats in the 5 experimental groups namely Group K, Group P1, Group P2, Group P3, and Group P4 differed significantly (p<0.05) (Table 1).The Mann-Whitney test results showed that there were significant differences (p<0.05) for ALT activities between Group K and Treatment Group 1; Group K with Treatment Group 2; Group K with Treatment Group 3; Group K with Treatment Group 4; Treatment Group 1 with Treatment Group 2; Treatment Group 1 with Treatment Group 3; Treatment Group 1 with Treatment Group 4; Treatment Group 2 with Treatment Group 3; and between Treatment Group 2 with Treatment Group 4. ALT Activity in Treatment Group 3 was not significantly different from ALT Treatment Group 4 activity (p>0.05). The mean control group ALT activity was significantly higher (p<0.05) compared to Treatment Group 1, Treatment Group 2, Treatment Group 3, and Treatment Group 4. This indicates that excessive administration of paracetamol causes damage to the liver cell membrane so that the enzyme ALT which is found in liver cells flows into the blood so that the activity in the serum is getting higher.Ethanol extract of Girang leaf prevented liver cell damage which was indicated by the serum ALT activity of rats in the lower treatment group compared to the Control Group. The mean of serum ALT activity of rats in the group given ethanol extract of girang leaves at a dose of 200 mg/kg BW (P3 group) was significantly lower (p<0.05) compared to rats given extract at a dose of 100 mg / kg BW (P2 Group), and rats given extract at a dose of 50 mg/kg BW (P1 Group), but not significantly different from rats given extract at a dose of 400 mg/kg BW (P4 Group) (p>0.05). Based on these results, the dose of 200 mg/kg BW is an effective dose in protecting the liver against the toxicity of paracetamol. The ALT activities of each treatment group are presented in Figure 1 below:

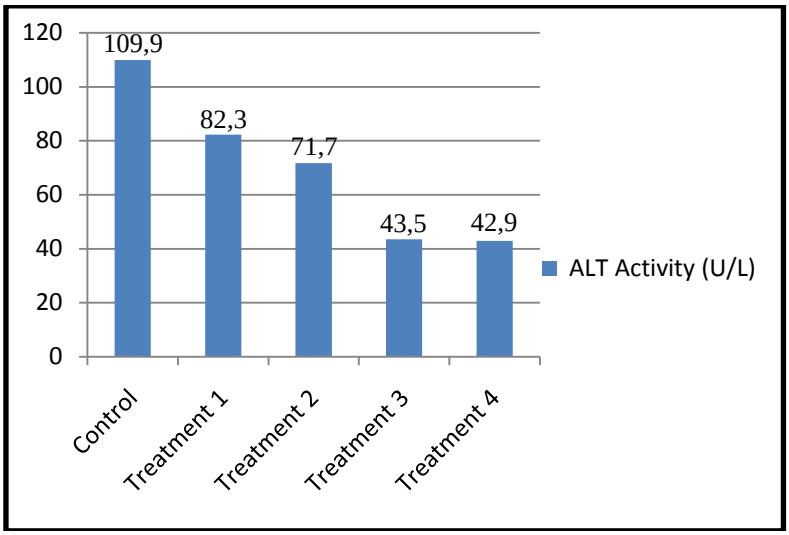

Figure 1: The ALT activities of each treatment group

IV. Discussion

The results showed that the mean (± SD) of ALT serum rat activity in the Control Group (K) was 109.90 ± 12.06 U/L significantly higher (p<0.05) than the P1 treatment group (82.30 ± 2.21 U/L); P2 treatment group (71.70 ± 2.86 U/L); P3 treatment group (43.50 ± 1.56 U/L); and P4 treatment group (42.90 ± 1.86 U/L). According to Selvam (2015), the normal value of ALT activity in humans ranges from 5-25 U/L, whereas in rats the value ranges from 19.3-68.9 U/L.The mean ALT activities of serum in the control group rats, P1 treatment

group, and P2 treatment group were higher than the normal value. Rats in the group that were only given toxic doses of paracetamol (Control Group) suffered liver cell damage, resulting in the ALT enzyme in the liver cells seeping into the serum, so that the levels became high in the serum. Liver cell damage can occur due to the consumption of excessive doses of paracetamol. Excessive doses of paracetamol will be metabolized in liver cells to form N-acetyl-p-benzoquinoneimine (NAPQI) metabolites which are reactive and toxic to liver cells. The NAPQI molecule is detoxified by glutathione. Excessive consumption of paracetamol will increase the number of NAPQI molecules and reduce the amount of glutathione, so the NAPQI molecule will bind macromolecules making up the cell membrane of the liver, resulting in liver cell damage.

Paracetamol also induces the formation of superoxide free radicals (* O_2). Superoxide free radicals turn into hydrogen peroxide (H_2O_2), and hydrogen peroxide changes to hydroxyl radical (*OH). These hydroxyl free radicals will oxidize unsaturated fatty acids (PUFA) that make up liver cell membrane phospholipids. Lipid peroxidation of the liver cell membrane will disrupt membrane stability, causing liver cell damage.

The administration of girang leaf ethanol extract at a dose of 50 mg/kg BW and 100 mg/ kg BW has not been able to prevent liver damage, so the ALT activities were higher than its normal value. This was because the quantity of flavonoid compounds in this has not been able to reduce free radicals that cause liver cell membrane lipid peroxidation.

The mean serum ALT activity of the rats in the P3 treatment group and P4 treatment group was within the limits of normal values, and was not statistically significantly different ($p>0.05$). The content quantity of flavonoid compounds at doses of 200 mg/kg body weight and 400 mg/kg body weight has been able to reduce free radicals causing liver cell membrane lipid peroxidation, so as to prevent liver damage. This is indicated by the ALT value which is within the normal value range. The ALT value of rats in the group given leaf ethanolic extract with a dose of 200 mg/kg BW did not differ significantly from the ALT value of rats in the group given the leaf ethanol extract of the dose 400 mg/kg BW, and both of them were able to protect the liver 200 mg/kg BW; namely an effective dose to prevent liver damage due to the toxicity of paracetamol.

Flavonoid compounds contained in ethanol extract of girang leaves can prevent rats liver damage through several mechanisms, namely (1) by inducing the formation of endogenous antioxidants GSH which functions to detoxify NAPQI molecules, (2) as nucleophilic agents, convert NAPQI reactive metabolites into non-toxic.

Molecules which is soluble in water, so that it can be excreted through urine, (3) chelating the metal so that it can prevent the formation of hydroxyl free radicals, (4) reducing hydroxyl free radicals by donating hydrogen atoms, so as to prevent the occurrence of liver cell membrane lipid peroxidation.

V. Conclusion

The administration of girang leaves ethanol extract (Leea angulata Korth. Ex Miq) can reduce the ALT (SGPT) activity of rat serum, so that it has hepatoprotective activity against male white rats Wistar strain given excessive paracetamol. The effective dose of girang leaf ethanol extract in reducing the serum ALT activity of rats given excessive paracetamol was 200 mg/kg BW.

Acknowledment

Authors would like to thank to the Pharmacology Laboratory, the medical faculty of Udayana University, and the Postgraduate Doctor of Medicine, Udayana University who have helped in completing this research

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Ni Luh Rustini. "Hepatoprotective Activities of Girang Leaf Ethanol Extracts (Leea angulata Korth. Ex Miq) on Wistar Rats Induced by Paracetamol" **IOSR** Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 14.1 (2019): 01-05.