Anti-toxicity test of Peperomia pellucida steeping on liver function in diabetic-induced rat

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ABSTRACT. Liver is important for both defense mechanism and protein synthesis in the human body. This study examined the anti-toxicity of Peperomia pellucida on liver function of diabetic-induced rats. It was an experimental study using pre- and post-test control group design. Rats were categorized into five groups, i.e., groups of healthy control (A), negative control (B), and treatment (C-E) with dosages of 150, 300, and 600 mg/kg of P. pellucida, respectively. Each group comprises of 5 rats. The TNF-α, IL-12, and GSH were measured before and after a 14 days administration of P. pellucida. The data were analyzed by using one-way ANOVA test followed by Duncan’s post hoc test with a significance level of 5%. The result showed that P. pellucida steeping can improve liver cell damage, which was shown from the parameters of liver function, inflammation, and antioxidants. The mean of TNF-α and IL-12 levels decreased while the total protein, albumin, and GSH levels increased significantly after administration of P. pellucida steeping. Our study concluded that P. pellucida steeping might reduce TNF-α and IL-12 levels, and increased GSH level in diabetic-induced rat. A 300 mg/kg was the most effective dosage to reduce IL-12 and increase GSH.

Keywords: anti-toxicity, GSH, IL-12, Peperomia pellucida, TNF-α

INTRODUCTION

Liver has a strategic role for protecting the body and maintaining homeostasis. Continuous exposure xenobiotic compounds especially hepatotoxin have the detrimental effect on liver cells. Drugs, food/drink contaminated, or chemical pollution cause liver injury (Andrade & Robles-Diaz, 2020; Teschke, Eickhoff, Brown, Neuman, & Schulze, 2020). The prevalence of liver diseases is very high. About 2000 cases of acute liver failure occur in the United States per year and more than 50% of them are induced by drugs. Study in France showed about 13.9% of Drug Induced Liver Injury (Reuben, Koch, & Lee, 2010). While study in a hospital in Tasikmalaya showed 96% of patients with impaired liver function were caused by drug side effect (Cintha, Pradipta, & Abdulah, 2012).

The use of drugs (pharmacological therapy) to restore liver cells actually is able to lead to toxicity and side effects. On the other hand, herbal therapy is increasingly in demand. It is safe to consume, cheap, affordable, and more likely to have less side effects. One of the natural ingredients that can be utilized is Peperomia pellucida (P. pellucida) plants. The previous research showed that P. pellucida contained flavonoids, tannins, steroids, alkaloids, and saponins (Idris, Olatunji, & Madufo, 2016; and Ogunmoye, Oladosu, Olubomehin, Ojajobi, & Tijani, 2018).

P. pellucida steeping has been widely used as antioxidant, anti-hyperglycemia, and anti-dyslipidemia (Hamzah, Odetola, Erukainure, & Oyagbemi, 2012; Rahmawati et al., 2020). The methanol extract of P. pellucida at dose of 400 mg/kg can increase antioxidant activity in white rats. Other research showed that P. pellucida extract at a dose of 300 mg/kg was able to reduce blood glucose levels in diabetic rats (Sheik et al., 2013). Even, at a dose of 4000 mg/kg, P. pellucida did not cause acute liver toxicity (Waty, Saputri, & Mun’im, 2017). However, research on P. pellucida steeping as antitoxicity has never been done. This study aimed to examine the antitoxicity of P. pellucida on liver function in diabetic-induced rats.
**EXPERIMENTAL SECTION**

This was a true experiment study with a pre- and post-test with control group design. A total of 30 simple randomly selected rats were categorized into 5 groups, i.e., groups of healthy control (A), negative control (B), treatments (C-E) with dosages of 150, 300, and 600 mg/kg. The study was approved under the ethical clearance from the Faculty of Medicine, Padjadjaran University No. 1424/UN6.KEP/EC/2018.

**Preparation of experimental animals**

Wistar strain healthy and non deformed male rats aged 1.5-2 months, 150-200 grams were selected and adapted for a new environment for one week. Both AD II standard pellet and drinks were provided ad libitum. Rats are placed in cages made of plastic boxes with lids made of woven wire. At the base of the cage was coated with ± 2 cm thick of husk. It was replaced every 2 days. Hence, the comfort and health of rats were maintained. Air circulation, temperature, and room lighting are regulated controlled as best as possible.

**Making a Peperomia pellucida steeping**

Fresh *P. pellucida* plants (leaves and stems) were washed under running water. Following withering process for 1 day, the washed plants were then dried directly under the sunlight for 7 days to obtain 1 kg dry plants (0% water). Dried plants were stored in aluminum foil to keep it dry. The plant was brewed with boiling water making the dosage of 150, 300, and 600 mg/kg, respectively. These dosages were dissolved to 3.6 ml/200 grams of rat body weight. Brewed *P. pellucida* was dissolved with 200 ml of warm water to ease the brewing process. Because of the conversion factor for 70 kg of human to 200 g of rat is 0.018, the dosages 150, 300 and 600 mg/kg of human body weight were converted to 1.665, 3.333, and 6.666 g/kg rat body weight, respectively. Afterwards, these steeping were stirred and taken as many as 3.6 ml/200g of rat body weight.

**Phytochemical screening**

The phytochemical test was performed to identify polyphenols, tannins, saponins, and terpenoids. Flavonoid compound was tested quantitatively and qualitatively. The test was carried out by using standard procedures to identify the constituents as described in the previous study (Saryono & Proverawati, 2018).

**Treatment of experimental animals**

Rats were injected with a single dose of streptozotocin (STZ) of 50 mg/kg intraperitoneally on the 8th day to induce diabetic. Prior injection, rats were fasted for 6-8 hours. Streptozotocin was dissolved in 0.5M buffer citrate before hand (Sarie, Budirahardjo, & Yuwono, 2016). Prior to and following the treatment of *P. pellucida* steeping, blood was taken for examination of total protein, globulin, albumin, TNF-α, IL-12, and GSH. As many as 3 ml of blood from the orbitalis plexus was drawn. It was then stored in a vacutainer. Treatment was given orally for 14 consecutive days every morning through a gastric tube.

A quantitative TNF-α and IL-12 plasma measurements were done with enzyme-linked immunosorbent assays using commercially available kits (BT Laboratories, Shanghai) according to the instructions available from the suppliers. Total protein, GSH, and albumin were measured by Biuret method (Dyasis kit, Germany), spectrophotometer (Biovision kit), Brom Cresol Green (BCG) method (Dyasis kit, Germany), respectively, while globulin was measured indirectly by subtracting total protein to albumin.

**Statistical Data Analyses**

Normality of the data was tested by using the Sapiro-Wilk test. To find out the significant difference of normally distributed data for all groups, one-way ANOVA tests were done. The Duncan's Post-hoc analysis was then performed to determine the differences between each group and the most effective dose. Data was presented as the mean ± standard deviation. p-Value <0.05 was considered significance.

**RESULTS AND DISCUSSION**

It was the first study to improving liver function of *P. pellucida*. Based on the phytochemical test, *P. pellucida* steeping contained polyphenols (++), tannins (+), saponins (+), and terpenoids (+). The main polyphenols were flavonoids. *P. pellucida* contained polyphenol with flavonoid compounds by 17.2 mg/mL and 15.6 mg/mL, respectively. Previous study also showed that *P. pellucida* contained active substances such as flavonoids, alkaloids, saponins, and tannins (Majumder, 2012). Our study was in accordance with previous studies which showed that ethanol extracts of *P. pellucida* contains the main compounds, i.e., flavonoids, tannins and saponins (Sheikh et al., 2013). However, water extracts of *P. pellucida* contains steroids, alkaloids, and phenolics (Kalaiarasing, Johnson, Janakiraman, & Sivaraman, 2016).

Statistical analysis showed that our data was normally distributed (p> 0.05) and homogeneous (p> 0.05). ANOVA test showed that total plasma protein levels between the healthy control group and the diabetic-induced group (p <0.05) were significant differences, but not between the diabetic-induced groups. There was a significant increase in total protein content (p <0.05) after administration of *P. pellucida* (Figure 1). Post hoc LSD test showed that treatment group dose of 150 mg/kg was not significantly different to dose of 600 mg/kg. It indicates that the
dose of 150 mg/kg is a minimal dose of P. pellucida to increase total protein level.

The mean albumin level in the healthy control group was 3.41±0.14 mg/dL. It was significantly different from diabetic-induced group (p<0.05). After administration of P. pellucida steeping, the mean albumin level increased significantly (Figure 2). Post hoc LSD test demonstrated that there was no significant difference after treatment in the healthy control group and the treatment group doses of 300 and 600 (p>0.05). However, there was a significant difference after treatment between NC, T1 and the other groups (p<0.05). The 300 and 600 mg/kg dose groups had similar effect.

There were significant differences in the decrease of globulin levels between the study groups, the healthy control group, and the diabetic-induced group (p<0.05). The biggest pre-post deviation was found in the T3 group by 0.30 mg/dL (Figure 3). The post hoc LSD test on deviation data showed there was no significant difference in the decrease in globulin levels between the T1, T2 and T3 groups, but it was significantly different between the HC and NC groups (p<0.05).

Figure 1. Total protein before and after administration of P. pellucida steeping. Before treatment, there was no difference in total protein levels between treatment groups, but significantly different from the healthy control group (HC). After treatment, there were significant differences in total protein levels (p <0.05). HC: healthy control; NC: negative control. T1-T3: groups treated with P. pellucida with doses of 150, 300 and 600 mg/kg, respectively. **p<0.01. (least significant difference (LSD) test).

Figure 2. Albumin levels before and after the treatment of P. pellucida steeping. HC: healthy control; NC: negative control. T1-T3: groups treated with P. pellucida dose of 150, 300 and 600 mg/kg respectively. **p<0.01. (least significant difference (LSD) test).
Lipid peroxidation in the liver cell membrane damages cell membrane integrity and worsen cell injury (Saryono, Sumeru, Proverawati, & Efendi, 2018). Prolonged liver cell injury causes cell damage to death, leading to liver failure. Liver function disruption causes various complications such as total protein, albumin and globulin deficiency, oedema, disruption of fluid, and electrolyte regulation. Reactive oxygen species will cause free radical chain reactions. Superoxide radicals are toxic because they are able to form hydroxyl radicals and react to nitric oxide (NO) to form peroxynitrite radicals (ONOO-). In addition, the STZ induction triggers oxidative stress, due to an imbalance between free radicals and antioxidants (Mondal, Das, Junejo, Borah, & Zaman, 2016).

P. pellucida contains phenolic compounds, such as flavonoids with antioxidant activity (Ayu, Catur, Muhamad, Eljabbar, & Ketut, 2018). This will repair liver cell damage. Phenolic compounds work as free radical scavengers, through the H⁺ donor from hydroxyl groups (OH) in flavonoids against free radical compounds to form stable molecules (Ferraz et al., 2020; Mutee et al., 2010; Saryono et al., 2017). Previous studies showed that flavonoids in herb plants are useful for chelating free radicals by increasing endogenous antioxidant enzymes, reducing free radicals, and donating atoms. Hence free radical chain reactions are disrupted (Beltran-Benjamin, Co, Gaspi, Matibag, & Sia Su, 2013). Recent studies also showed that the methanol extract of P. pellucida has a potent antioxidant activity (Rhaman, Islam, & Shoeb, 2019).

Initial data (prior to P. pellucida administration) showed a significant difference in TNF-α levels between the diabetic-induced and the healthy control group (p<0.05). TNF-α levels experienced a significant increase after a STZ induction (Figure 4). While, after administering P. pellucida steeping treatment, TNF-α levels significantly decrease. There was a significant difference in TNF-α levels between treatment groups (p<0.05). The post hoc LSD test results from post test data showed that there were significant differences between the T1, T2, and T3 groups (p<0.05), except for T1 to T2.

Streptozotocin-exposed hepatocyte cell membrane experiences lipid peroxidation. Streptozotocin produces more free radical compounds due to an increase of reactive oxygen species (ROS) (Ruiz et al., 2019). High levels of free radicals will reduce endogenous antioxidant enzymes, such as GSH, catalase, and superoxide dismutase. As a source of free radicals, STZ will cause damage resulting in inflammation that increases inflammatory indicators, such as TNF-α and IL-12 (Saryono, Warsinah, & Isworo, 2018). There were significant differences of the reduction of IL-12 levels between groups (p<0.05) (Figure 5). Post hoc test on post test data showed that there were no differences between the T2 and T3 groups, but there were differences between others. The greatest reduction of IL-12 was found in the T3 group. This shows that a dose of 600 mg/dL was able to reduce IL-12 optimally.

P. pellucida treatment are also able to inhibit the activity of NF-kB (Nuclear Factor Kappa B), COX2, and prostaglandin synthesis leading to the decrease of pro-inflammatory mediators (Van De Wier, Koek, Bast, & Haenen, 2017). Previous studies also showed that the P. pellucida extract reduce TNF-α and IL-12 (Finato et al., 2018). P. pellucida extract in doses of 100 and 200 mg/kg significantly increase white blood cells (WBCs) (Florence et al., 2017) as well the inflammatory rate in the liver cells. Other study showed that flavonoids reduce TNF-α NF-kB and IL-1β expression in liver tissue (Liu et al., 2020).
Figure 4. TNF-α levels before and after treatment with *P. pellucida* steeping. HC: healthy control; NC: negative control. T1-T3: groups treated with *P. pellucida* dose of 150, 300 and 600 mg/kg respectively. *p<0.05, **<0.01. {least significant difference (LSD) test}.

Figure 5. IL-12 levels before and after treatment with *P. pellucida* steeping. HC: healthy control; NC: negative control. T1-T3: groups treated with *P. pellucida* dose of 150, 300 and 600 mg/kg respectively. *p<0.05, **<0.01. {least significant difference (LSD) test}.

Figure 6. GSH levels before and after treatment with *P. pellucida* steeping. HC: healthy control; NC: negative control. T1-T3: groups treated with *P. pellucida* dose of 150, 300 and 600 mg/kg respectively. *p<0.05, **<0.01. {least significant difference (LSD) test}.
There were significant differences in GSH deviation level between groups (p<0.05). The highest increment of GSH level occurred in the T3 group at 1.40 mg/dL (Figure 6). The post hoc LSD test results showed that there were no differences of the GSH deviation between T1 and T2, but were significantly different from the T3, HC, and NC groups (p < 0.05).

Flavonoids increase endogenous antioxidant defenses such as GSH (glutathione reductase) by donating H⁺. It reduces inflammation processes through the inhibition of ONOO⁻ (peroxynitrite) (Chen, Lü, Yao, & Chen, 2016). GSH eliminates free radicals by converting various hydrogen peroxide and lipid peroxide into water. Flavonoids work by donating hydrogen atoms to peroxyl radicals to form flavonoid radicals, which easily react to free radicals. Hence, the radical chain reaction is completely disrupted. Recent studies have shown that flavonoids act as anti-inflammatory (Ahmed et al., 2020; Kartika, Insanu, Safitri, Putri, & Adnyana, 2016; Zubair, Samiya, Jalal, & Mostafizur, 2015).

Many studies have also shown that *P. pellucida* of 10% w/w and 20% w/w of *P. pellucida* mixed with 900 g and 800 g of standard rat feed combined with glibenclamide, respectively causes an increase in SOD, catalase and GSH. This confirms that the 20% w/w supplement is more effective than the 10% w/w supplement in increasing SOD levels (Hamzah et al., 2012). Flavonoids and tannins have hydroxyl groups (-OH) to be donated to O₂⁻. Thus, they become neutral. In addition, the consumption of water-based supplements can increase GSH, which serves as a defense against oxidative stress by means of GSH oxidized to GSSG, and then GSSG is reduced again to GSH assisted by glutathione reductase. The component of flavonoids is able to become fat-soluble chain breaking antioxidants working on cell membranes to break the lipid peroxidation chain (Saryono, Taufik, Proverawati, & Efendi, 2019). The effects of flavonoids on ROS occur through two mechanisms, i.e. 1) by capturing free radicals/neutralizing and 2) by increasing endogenous antioxidants such as SOD (Panich, Ananta, Onkokoosong, & Jaemsak, 2007).

This study did not examine the differences in temperature during the making of *P. pellucida* steeping. Temperature differences in the process of drying and storing affect *P. pellucida* contents such as vitamin C and flavonoids as proven in other studies (Minh, 2019).

**CONCLUSIONS**

Our findings showed that *P. pellucida* steeping was able to improve liver cells function by reducing TNF-α, IL-12, and GSH levels in diabetic-induced rats. Our study also showed that the most effective dose of *P. pellucida* steeping was the 300 mg/kg with the highest reduction in TNF-α, IL-12, and GSH. Hepatic histopathology testing is necessary to ensure the improved hepatic cells.

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