Research Article

Hepatoregenerative effects of honey essence of *paliasa* on the liver of rat induced with carbon tetrachloride

Aliyah Aliyah[1]*, Elly Wahyudin[1], Cahyono Kaelan[2]

ABSTRACT

Background: *Paliasa* (*Kleinhovia hospita* L.) and honey have widely been used to cure hepatitis. This study aimed to determine the effects of honey essence of *paliasa* (HEP) as a hepatoregenerator. **Materials and Methods:** Bees were given an additional feed of mixed syrup and *paliasa stew* with a respective concentration of 0% (HEP-A), 20% (HEP -B), 40% (HEP -C), and 60% (HEP-D), with a ratio of 3:2. 32 rats divided into eight groups were given CCl4 intraperitoneally with a dose of 1 ml/kg of body weight. After 24 h, every group was given the respective treatment of HEP-A, HEP-B, HEP-C, HEP-D, and honey (X) from market, *paliasa stew*, mixture of the same amount of honey solution (X) as *paliasa stew*, and distilled water orally at a dose of 1 g/kg of body weight. 1 day after treatment, blood was taken from the rats and serum was separated for serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase tests, while the livers of rats were taken for histopathological examination. **Results:** The results of the research showed that 2-week treatment with HEP had an ability as hepatoregenerator. HEP produced by bees fed a mixture of syrup and stew of paliasa leaves 20% 3: 2 (HEP-B) was able to return the hepatocyte cell structure of the rats induced with carbon tetrachloride to normal. **Conclusion:** The HEP has better hepatoregenerative effects than *paliasa*, honey from the market, and mixtures of *paliasa* and honey.

KEY WORDS: CCl_4, Hepatoregenerative, Honey essence of paliasa, Serum glutamic oxaloacetic transaminase, Serum glutamic pyruvic transaminase

INTRODUCTION

The liver is an organ that frequently undergoes damage; however, on the contrary, the liver has a very large reserve capacity, as 10–20% of liver tissue still functioning is enough for survival. However, when hepatic disorder (damage) is fairly excessive due to intoxication with phosphor or carbon tetrachloride, or due to virus infection or nutritional diseases, this can cause a hepatic physiological disorder that deteriorates rapidly.[1]

Hepatic damage due to medicines and chemicals occurs when the power reserve of the liver lessens, and the ability to regenerate hepatic cells is lost, meaning that the liver undergoes permanent damage that is fatal.[2]

One of the biochemical tests that are frequently performed to detect the presence of hepatic disorders is serum transaminase, namely, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). When hepatic damage occurs, this enzyme is released into the blood from the cytosol and subcellular organelles, such as mitochondria, lysosomes and the nucleus, so the content in blood increases.[3]

Today, besides modern medical treatments, traditional treatments are also known which use natural or herbal materials.[4,5] Various types of research have succeeded in proving that some natural medicines are quite effective to be used in the condition of hepatic tissue inflammation. One of the natural materials that are largely used, especially by the society of South Sulawesi (Indonesia) to cure hepatitis, is *paliasa* leaf (*K. hospita* Linn.).[6] *Paliasa* leaves contain alkaloids such as scopoletin, quercetin, and kaempferol.[7] Like *paliasa*, honey is also one of the natural materials whose usage is not only limited to flavoring food but has also extended to becoming traditional medicine materials[8] that can cure various diseases, including

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hepatic disease. According to Halawa *et al*.,[9] honey can modulate the damage to hepatic and kidney cells. Honey contains no only fructose and glucose but also antioxidants, and flavonoids such as luteolin, quercetin, and kaempferol.[8]

Utilizing the similarity of active material contents and the efficacy between *paliasa* and honey in curing hepatic damage, a product of honey essence of *paliasa* (HEP) has been produced quickly and naturally; the honey that is obtained from *Apis mellifera* L. honeybees given an additional feed of mixed syrup and *paliasa stew*.[10] The results of the research by Aliyah *et al*.[10] showed that there are components transformed by bees into honey essence of paliasa. Therefore, tests have been performed to determine its effectiveness as a hepatoregenerator rats induced with carbon tetrachloride.

MATERIALS AND METHODS

The materials used included *paliasa* leaves, carbon tetrachloride, honey(X), namely, the honey obtained from factory X, coconut oil, HEP,[10] the SGOT assay kit (Human), the SGPT assay kit (Human), and white male Wistar rats weighting 150–220 g.

Sample

Samples were in the form of:

- HEP (A) = Honey produced by bees fed a mixture of syrup and distilled water 3: 2 (without paliasa)
- HEP (B) = Honey produced by bees fed a mixture of syrup and stew of paliasa leaves 20% 3: 2
- HEP (C) = Honey produced by bees fed a mixture of syrup and stew of paliasa leaves 40% 3: 2
- HEP (D) = Honey produced by bees fed a mixture of syrup and stew of paliasa leaves 60% 3: 2

Honey(X) = The honey from a market produced by factory X

A *paliasa stew* = liquid preparation, cut-sized *paliasa* leaves boiled in 90˚C for 15 minutes using water.

The Making of CCl_4 Solution

Carbon tetrachloride was dissolved in the same amount of coconut oil.

The Making of Sample liquid

Samples in the form of MSP solution, honey (X) in distilled water with a concentration of 10%, *paliasa stew* made with a concentration of 10%b/v in distilled water at a temperature of 90°C for 15 min, a mixture of honey(X) and *paliasa stew* in equal amounts, and distilled water as control.

Selection and Treatment of Test Animal

The test animals used were white male Wistar rats (*Rattus norvegicus*) that were healthy, grown, and weighted 150–220 g. The rats were left to adapt for 1 week. Before given the treatment, the rats were made to fast for 8 h.

Test of Hepatoregenerative Effect

A total of 32 rats were randomly divided into eight groups. Each group consisted of four rats whose blood was taken from the initial sample. The next day, the rats were given CCl_4 intraperitoneally at a single dose of 1 ml/kg of body weight. After 24 h, Groups I–IV were given treatments of HEP (A), HEP (B), HEP (C), HEP (D), respectively; Groups V–VII were given honey (X), *paliasa stew*, and a mixture of honey (X), and *paliasa stew* in equal amounts, respectively, and Group VIII was given distilled water. The samples were given orally, every day, for 1 week at a dose of 1 g/kg of body weight. After 24 h, rats were anesthetized with ether and their blood was taken again for the examination of SGOT and SGPT, after which their livers were taken for histopathological examination. The same procedure was applied to the other groups of rats for a 2-week period of treatment.

Determination of SGOT and SGPT contents

Blood was stored in centrifuge tubes and then rotated at a speed of 3000 rpm for 15 min, which separated the serum. For the determination of SGOT, serum was taken in an amount of 100 µl, and then 1000 µl of GOT buffer solution was added; the sample was left for 5 min, and then 250 µl of GOT substrate was added, left for 1 min, and its content was measured using Humalyzer. For the determination of SGPT content, the same thing was done as for the determination of SGOT content, except that buffer and GPT substrate was used in this determination.

Histopathological Examination

Rat liver was washed clean with physiological NaCl solution and was then put into 10% formalin buffer, embedded and made into slides, before being colored with eosin-hematoxylin. Next, the liver was visualized under a microscope and given a value (score) based on the degree of hepatocyte tissue damage. The values given were 0–4. Value 0 was given to normal cells, one indicated cells with very light damage, two denoted cells with light damage, three were used for cells with medium damage, and four indicated cells with heavy damage. Therefore, the more damage parameters present, the higher the value of the degree of damage.

RESULTS

In the hepatoregenerative tests, the test animals were first induced with CCl_4 and then given the treatments. This means that rat livers were damaged first, and then treated for a cure, with the intention of determining whether HEP could be used as a medicine to cure hepatic disease.

Table 1: Results of average measurements of SGOT, SGPT contents, and scores of hepatic damage degree of the rat given a 2-week treatment

Treatment	SGOT			SGPT			Score of hepatic damage degree
	Start (UI/L)	After induction (UI/L)	After treatment (UI/L)	Start (UI/L)	After induction (UI/L)	After treatment (UI/L)	
A	114.90	175.70	66.75	35.16	62.23	34.40	2.75
B	171.78	322.75	78.56	40.71	186.12	33.74	2.75
C	108.66	286.78	85.87	49.37	166.80	39.54	3.00
D	85.73	212.65	119.33	57.91	159.19	72.05	3.25
M	69.74	144.40	70.27	20.90	70.16	28.97	2.75
P	99.87	184.75	62.71	23.36	56.13	28.59	2.50
MP	127.13	155.22	57.33	37.92	46.85	34.67	2.50
AS	96.83	234.88	66.46	30.32	111.20	30.20	3.75

Remarks: A, B, C, D: HEP (A), HEP (B), HEP (C), and HEP (D), M: honey (X), i.e., the honey obtained from market, P: *Paliasa* stew 10%. MP: Mixture of honey (X) and paliasa stew in equal amounts, AS: Distilled water. SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase

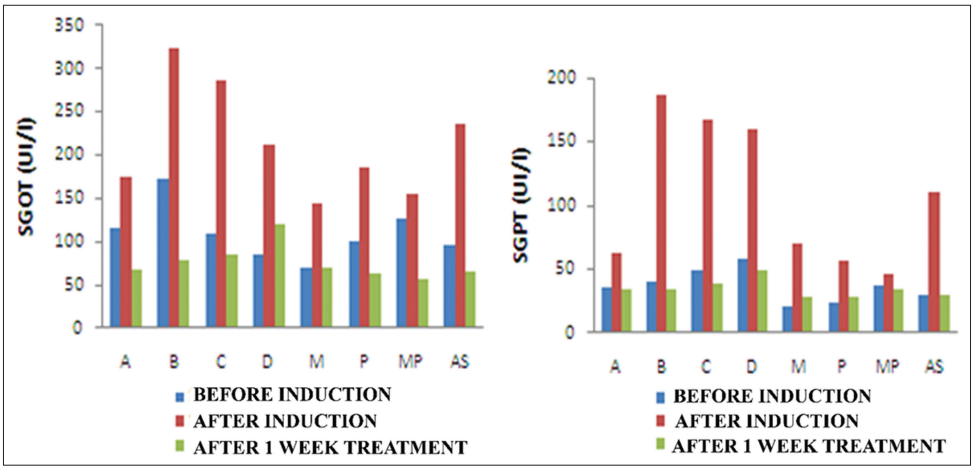

Figure 1: Diagrams of SGOT and SGPT of 1-week treatment in hepatoregenerative test

The results of average measurements of SGOT and SGPT and also the histology of the liver of rats given a 1-week treatment in hepatoregenerative tests are presented in Table 1 and Figures 1 and 2.

The histopathology results and scores of the degree of hepatic damage in the hepatoregenerative test after 1-week of treatment are shown in Table 1 and Figure 2.

From the average scores of hepatic damage, the provision of distilled water produced the heaviest damage to the liver, with the marked occurrence of proliferation of the bile ducts, fibrin, congestion, and inflammation cells [Figure 2]; this group was followed by those given HEP (D) and (C), which produced medium-to-heavy damage. Other treatments produced relatively lighter damage, even though scores of liver damage degree were still higher than 2.50 on average [Table 1, Figure 2]. Meanwhile, the results of the Kruskal–Wallis test with regard to scores of rat hepatic damage showed some differences between the effects of treatments on the degree of liver damage and real degree at 0.055. To determine the difference between the effects of the treatment, the Mann–Whitney U-test was done. The results of such tests showed that the distilled water treatment for 1 week produced hepatic damage that was markedly different from the other treatments, except for treatments of HEP (C) and (D). Treatment with HEP (A) was only really different from distilled water and was similar to that for treatment with HEP (B). In other words, treatments HEP (A) and (B) had effects on the degree of liver damage which were relatively similar to those of other treatments, except for distilled water. Meanwhile, treatments of HEP (C) and (D) gave effects that were relatively the same as for other treatments. The treatment with honey(X), *paliasa stew*, and the mixture of honey(X) with *paliasa* had relatively the same effects on hepatic damage. On average, the values of hepatic damage in hepatogenerative tests with 1-week treatment were still fairly high [Table 1 and Figure 2]. Therefore, the tests were continued for a longer time, namely, 2 weeks. The results of the tests are shown in Table 2 and Figure 3.

As in the 1-week hepatoregenerative test, in the 2-week hepatoregenerative test, it was shown that values of SGOT increased after the induction of CCl_4, namely, 2–3 times the initial values (before induction), while for SGPT, the initial values increased by 4–8 times. However, after a 2-week induction, values of SGOT decreased again, yet, were still larger than the initial

values, except for HEP (C). Meanwhile, for SGPT, the decrease approached the initial values for HEP (A), (C), and distilled water, while HEP (B) and the *paliasa stew* gave decreases lower than the initial values. Meanwhile, HEP (D), honey (X), and the mixture of honey (X) and a *paliasa stew* gave decreased values that were still larger than the initial values [Figure 3].

The results of histopathology and scores of hepatic damage degrees in hepatoregenerative tests of 2-week treatments are presented in Table 2, Figures 4 and 5. In Figure 4, it can be seen that there were four groups of scores related to hepatic damage after the provision of

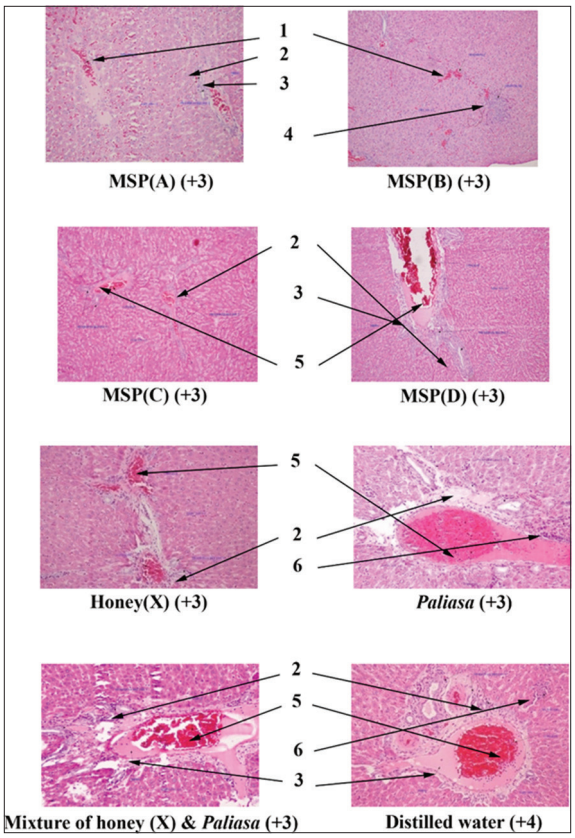

Figure 2: Examples of rat liver microscopic photos enhanced 20× in a 1-week hepatoregenerative test. Remarks: 1= hemorrhagic 2= proliferation of bile ductus 3= fibrin 4= cell necrotic 5= congestion 6= inflammation cell

treatments for 2 weeks. The lowest scores for hepatic damage were produced by the provision of treatments HEP (A) and (B), with scores of hepatic damage that were below one and tending to approach zero (normal). Next, groups with scores of hepatic damage between one and two were produced by treatments HEP (C)and (D), which also still showed necrosis on histological examination [Figure 5]. Scores of hepatic damage between two and three were produced by treatments with honey(X), *paliasa*, and mixed honey(X) and *paliasa*, where there was hemorrhage, congestion, and proliferation of bile ducts found on histological examination [Figure 5]. Meanwhile, the groups with the highest scores of hepatic damage (scores higher than three) were produced by treatment with distilled water, resulting in the occurrence of diffuse hemorrhage on histological observation [Figure 5].

DISCUSSION

Table 1 and Figure 1 show that after the rats were induced with CCl_4, values of SGOT increased the initial values or those before induction by 2–3 times, while the values of SGPT increased by 2–6 times. Biochemically, this indicated the occurrence of acute damage to the rat liver, because, according to Dalimartha,[3] the increase in SGPT enzyme content to a level 3 times as high as the normal content indicated hepatic cell damage.

Nevertheless, after induction for 1 week, SGOT values decreased again. The decrease showed values lower than the initial ones (before induction), except for HEP (D) whose values were still higher than the initial ones, while values of honey(X) were almost the same as the initial values. Meanwhile, for SGPT, values lower than the initial ones were shown by HEP (B),(C), (D), and the mixture of honey(X) with a *paliasa stew*. Meanwhile, HEP (A) and distilled water led to decreased values to levels that were almost the same as the initial values, while honey(X) and values of *paliasa stew* were still higher than the initial values [Figure 1]. This indicated that there has been an improvement in liver function biochemically, or,

Table 2: Results of average measurements of SGOT, SGPT contents, and score of damage degree of the liver of rat given 2-week treatment

Treatment	SGOT			SGPT			Score of hepatic damage degree
	Start (UI/L)	After induction (UI/L)	After treatment (UI/L)	Start (UI/L)	After induction (UI/L)	After treatment (UI/L)	
A	93.96	186.85	114.48	32.01	137.11	34.07	0.50
B	124.35	348.33	137.08	45.18	207.35	39.97	0.25
C	90.65	196.65	68.98	32.12	208.23	31.56	1.25
D	122.21	289.98	160.01	30.12	194.60	34.84	1.50
M	117.82	362.50	147.66	32.03	245.18	43.98	2.25
P	101.92	314.43	115.85	38.53	314.58	35.80	2.25
MP	158.23	337.88	133.75	30.27	184.09	38.06	2.50
AS	102.09	263.48	108.28	32.95	263.74	30.79	3.50

SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase

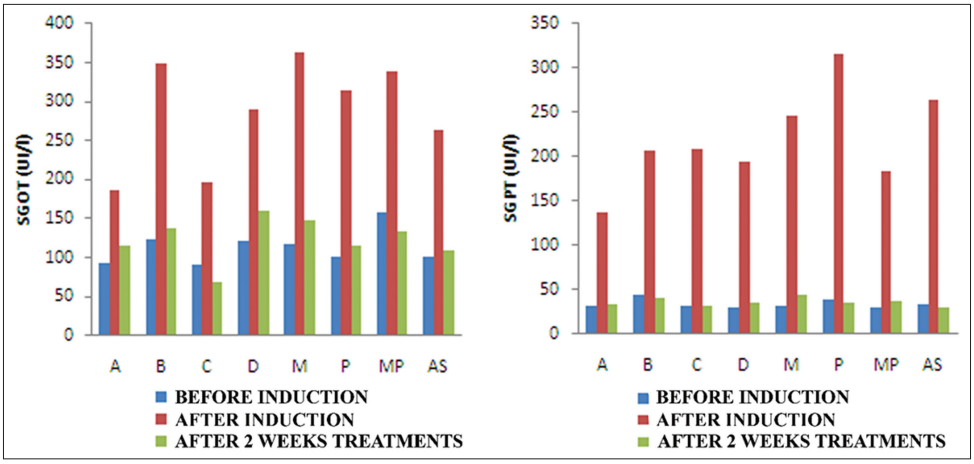

Figure 3: Diagrams of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase of 2-week treatment in hepatoregenerative test

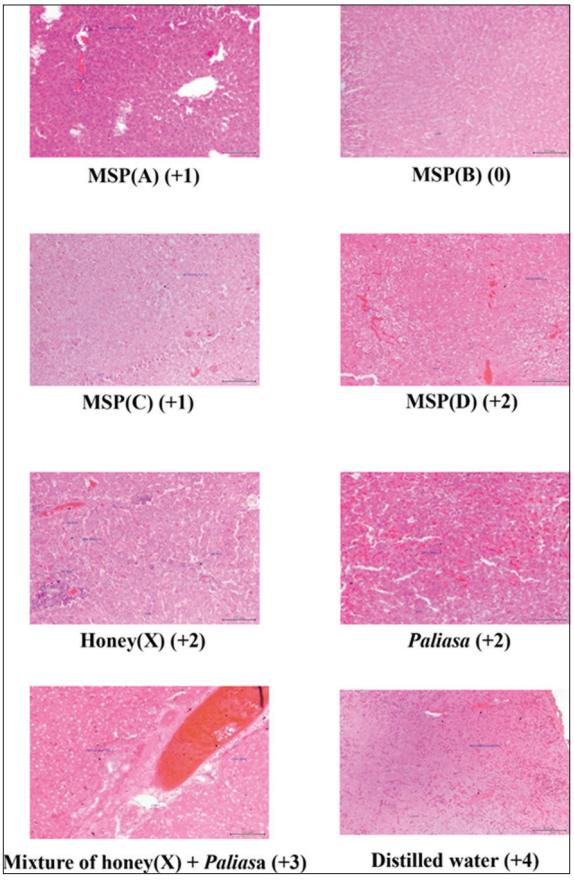

Figure 4: Examples of rat liver microscopic photos enhanced 20× in a 2-week hepatoregenerative test. Remarks: 1=focal necrotic 3=congestion 2=hemorrhagic 4=proliferation of bile duktus

in other words, there were hepatoregenerative effects following acute hepatic damage.

The results of the Kruskal–Wallis test toward the scores of rat liver damage degrees in 2-week hepatoregenerative tests showed the occurrence of different effects of treatments on the degree of hepatic damage with a real degree of 0.018. To determine the

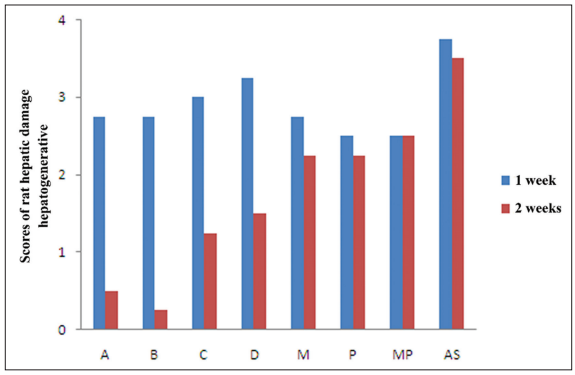

Figure 5: Diagram of the scores of rat hepatic damage in hepatoregerative test of 1-week and 2-week treatments

difference between the effects of treatments, the Mann–Whitney U-test was done. The results of the tests showed that giving the treatment with distilled water alone for 2 weeks produced hepatic damage which was different from that seen with other treatments. The HEP (A) and (B) treatments produced hepatic damage which was relatively the same and both have different effects from the other treatments with regard to hepatic damage. This was similar to the treatments HEP (C) and (D), and treatment with honey(X), *paliasa* and mixtures of honey(X) with *paliasa*.

The results of the research indicated that *paliasa*-honey, 10% *paliasa stew*, honey from the market, and the mixture of honey with the *paliasa stew* were biochemically able to decrease the contents of SGOT and SGPT of rats after being induced with CCl_4 both in 1-week and 2-week treatments [Tables 1 and 2]. Nevertheless, from the histopathology results, it could be seen that the 2-week treatment was already able to repair the acute damage to hepatocyte cells caused by CCl_4, so it can be said that to have hepatoregenerative properties. All HEP resulted in better repair of the liver function than *paliasa*, honey from the market, or the mixture of *paliasa* and honey.

CONCLUSIONS

The HEP has better hepatoregenerative effects than *paliasa*, honey from the market, and mixtures of *paliasa* and honey. HEP produced by bees fed a mixture of syrup and stew of *paliasa* leaves 20% 3: 2 (HEP-B) is the best HEP as a hepatoregeneratorin acute hepatic damage because it tends to be capable of returning the hepatocyte cell structure to normal.

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REFERENCES

1. Darmawan G, Hamijoyo L, Hasan I. Association between serum uric acid and non-alcoholic fatty liver disease: A meta-analysis. Acta Med Indones 2017;49:136-47.
2. Raflizar R, Sihombing M. Dekokdaun Paliasa (*Kleinhovia hospita* Linn) sebagai obat radang hati akut. J Ekol Kesehatan 2009;8:984-93.
3. Dalimartha S. Ramuan Tradisional Untuk Pengobatan Hepatitis Traditional Formulation for Hepatitis Treatment. Jakarta: Penebar Swadaya; 2005.
4. Modi AA, Wright EC, Seeff LB. Complementary and alternative medicine (CAM) for the treatment of chronic hepatitis B and C: A review. Antivir Ther 2007;12:285-95.
5. Luper S. A review of plants used in the treatment of liver disease: Part 1. Altern Med Rev 1998;3:410-21.
6. Djabir YY, Arsyad MA, Sartini S, Lallo S. Potential roles of *Kleinhovia hospita* L. Leaf extract in reducing doxorubicin acute hepatic, cardiac and renal toxicities in rats. Pharmacognosy Res 2017;9:168-73.
7. Tan-ag/*Kleinhovia hospita* Linn. Guest Tree. Philippine: Philippine Medicinal Plants; 2016. Available from: http://www.stuartxchange.com/Tan-ag.html. [Last accessed on 2010 Aug 20].
8. Erguder BI, Kilicoglu SS, Namuslu M, Kilicoglu B, Devrim E, Kismet K, *et al.* Honey prevents hepatic damage induced by obstruction of the common bile duct. World J Gastroenterol 2008;14:3729-32.
9. Halawa HM, El-Nefiawy NE, Makhloul NA, Mady AA. Evaluation of honey protective on lead induced oxidative stress in rats. J Arab Soc Med Res 2009;4:197-209.
10. Wahyudin EA, Kaelan C, Sila AM. Preference level of bees *Apis mellifera* L. to the supplementary feed of mixed syrup and *Paliasa* leaf decoction and physico-chemical characteristics of produced honey. Int J Sci Technol Res 2013;2:4-8.

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