Hepatoprotective activity of ethyl acetate fraction from Lygodium microphyllum leaves in CCl4 induced damage rats

by Herman Herman

Submission date: 30-Sep-2021 09:23PM (UTC+1000)
Submission ID: 1661480652
File name: 5.0059476.pdf (528.92K)
Word count: 3653
Character count: 18545

Hepatoprotective activity of ethyl acetate fraction from *Lygodium microphyllum* leaves in CCl_4 induced damage rats

Cite as: AIP Conference Proceedings **2360**, 040002 (2021); https://doi.org/10.1063/5.0059476
Published Online: 28 September 2021

M. R. Hidayat S. Herman, S. Nurul Annisa and Hadi Kuncoro

Hepatoprotective Activity of Ethyl Acetate Fraction From *Lygodium microphyllum* Leaves in CCl_4 Induced Damage Rats

Herman, M R Hidayat S, Nurul Annisa and Hadi Kuncoro[a)]

Laboratory of Pharmaceutical Research and Development farmaka Tropical, Faculty of Pharmacy, Mulawarman University, Samarinda 75117, Indonesia

[a)]Corresponding author: *hadikuncoro@farmasi.unmul.ac.id*

Abstract. *Lygodium microphyllum* is one of the plants that have the potential as an agent hepatoprotective because known of these plants have been used as the chinese traditional medicine. They use it to treat hepatitis diseases with drink a stew of the plant. The purpose of this study is to study the effects of a ethyl acetate fraction as hepatoprotective from *L. microphyllum* leaves in CCl_4 induced damaged rats. This study uses animals of about 36 rats divided into six groups for 14 days. On the 15th -day, animal testing sacrificed and extracted the blood and surely to know the activity of the SGOT and SGPT and hold the histological structure of the liver to know the liver damage. The result showed the idea of a fraction of ethyl acetate leaves *L. microphyllum* may lower levels of SGOT significantly ($p<0,05$) but cannot be lower the levels SGPT significantly. The highest SGPT activity indicated by the ethyl acetate leaves *L. microphyllum* 200 doses of mg/kg b.w that measure being 202.50±88.459 U/L compared with the negative of 2362.50±1213.592 U/L. Examination histopathology scoring liver damage done by counting the number of cells from normal hepatocytes and suffered from damage (degeneration and necrosis). The administration of *L. microphyllum* leaf ethyl acetate fraction did not affect mice weight. Faction doses of ethyl acetate leave *L.microphyllum* 200 mg/kg b.w show that there was a protective effect against the hearts. Based on the results, it can be concluded that ethyl acetate faction leaves *L. microphyllum* having the effect of hepatoprotective against CCL_4 induced damage rats

INTRODUCTION

The liver is the largest metabolic organ and the main biochemical factory in the body. The liver plays a role in maintaining metabolic balance in the body, such as the metabolism of proteins, carbohydrates, lipids, vitamins, serum protein synthesis, bile secretion, and detoxification [1]. As an organ that has important functions in a complex body, the risk of damage to the liver increases. Testing the hepatoprotector effect made from nature is considered important to find natural ingredients that have the potential to protect the liver because currently both drugs and natural-based liver supplements are still limited in number. One of the natural ingredients that can be used as a hepatoprotective agent is *L. microphyllum*. This plant has been widely used as a traditional medicine by the Chinese community. They use this plant to treat hepatitis by drinking boiled water from the plant. In addition, based on the research of Gnanaraj et al. [5] proves the existence of a strong hepatoprotector effect of the water extract of *L. microphyllum* in white rats induced by carbon tetrachloride.

Lygodium microphyllum, including genus Lygodium from Polypodiaceae family. The plant is known as "krokot" in Indonesia, which typically grows in rain forests [2]. This plant is used in Indonesian traditional medicines in Dayak Tribe, such as Fever and kidney stones [3]. In previous studies, we reported the isolation and structure elucidation from *Lygodium microphyllum*. Steroid compound, stigmast-5-(6)-en-3-ß-ol and stigmast-4-en-3-one [4]. Six flavonoid compounds kaempferol, quercetin, acacetin, quercetin-3-O-ß-D-glucopyranoside, kaempferol-3-O-ß-D-glucopyranoside, and isorhamnetin-3-O-ß-D-glucopyranoside with their antioxidant activity against DPPH [3]. *L. microphyllum*. In this study, we used Carbon tetrachloride (CCL_4) as an inducer. CCl_4 is a well-knownt hat know can inflict cellular injuries similar to acute viral hepatitis. The aqueous extract of *L. microphyllum* showed strong

hepatoprotective activities against CCL_4-induced oxidative stress. This study aims to reported hepatoprotective activities from ethyl acetate fraction from *L. microphyllum* in CCl_4 induced damage rats.

EXPERIMENT

Chemical

Hematoxylin-Eosin (Sigma-Aldrich), Hepa-Q®, CCl_4 (Merck), Corn oil, SGPT-SGOT Reagent, TMP, Methanol (Merck), Ethyl Acetate (Merck), Aqua.

Plant Materials

L.microphyllum leaves were collected from Samarinda, East Kalimantan, in June 2019. The plant was identified by staff at the Faculty Of Forestry, Mulawarman University, Samarinda.

Plant Extraction

Powder of *L. microphyllum* leaves was extracted with methanol at room temperature for four days. The methanol extract was evaporated using a rotary evaporator. Methanol extract dissolved in 520 mL of aqua (4:1) and partitioned successively with *n*-hexane and ethyl acetate and evaporation using Rotary evaporator.

Animals

Thirty-six rats aged ten weeks ± two weeks with a bodyweight of 150 grams ± 20 grams were randomly divided into six groups. Each treatment group consisted of 6 individuals. Group I as a normal control which was not given treatment; group II was given carbon tetrachloride (1 mL/kg BW) orally (p.o); while groups III, IV and V were given L. microphyllum leaves fraction with a concentration of 100 mg / kg, 200 mg/kg, 400 mg/kg BW for 14 days followed by giving carbon tetrachloride (1 mL/kg BW) orally on day 7-13 and 14th day. The ethyl acetate fraction of L. microphyllum leaves was administered through the treatment. The group VI was given Hepa-Q® as a positive control.

Animal Blood Sampling

Blood samples were taken from rats on the 15th day. 1-3 mL of blood from rats was taken through the vena cava. The blood is then collected in an eppendorf tube to take serum which is then tested for SGPT and SGOT activity. Serum was obtained by centrifuging a blood sample at 3000 rpm for 10 minutes.

SGOT and SGPT level

The SGOT measurement was started by inserting 800 µL of SGOT reagent I and 200 µL SGOT reagent II into the vacutainer. Then 100 µL of centrifuged serum was added to the vacutainer. After all the ingredients are mixed then incubated for 60 seconds. Read the absorbance for 60 seconds and calculate the SGOT level. The SGPT measurement was started by inserting 800 µL of SGPT reagent I and 200 µL SGPT reagent II into the vacutainer. Then 100 µL of centrifuged serum was added to the vacutainer. After all the ingredients are mixed then incubated for 60 seconds. Read the absorbance for 60 seconds and calculate the SGPT level.

Liver Histological

Each group was sacrificed by using inhalation using chloroform, then the liver was taken and then fixed using 10% neutral buffered formalin for 1 week and then stained with Hematoxylin-Eosin (HE). The results of histological staining were observed under a light microscope, then based on the changes that appeared, a score was given.

Statistical Analysis

In statistical analysis using SPSS software. For the results of body weight and levels of SGOT and SGPT as well as liver histopathology, the homogeneity of variance data was analyzed. If the diversity is proven to be homogeneous, a one-way analysis of variance (ANOVA) is performed. If the data proves to be not homogeneous, a non-parametric Kruskal-Wallis test is performed. Meanwhile, for weight data, paired T-test was performed.

RESULTS AND DISCUSSION

The Effect of *Lygodium microphyllum* Leaf Ethyl Acetate Fraction on Rat Body Weight

One of the supporting parameters in observing the occurrence of liver damage is the weight loss of experimental animals. The liver is known to play an important role in metabolic processes and metabolic balance in the body, such as the metabolism of proteins, carbohydrates, lipids, vitamins, bile secretion, and detofication. Hepatocytes contain glycogen which consists of glucose deposits so that they are mobilized when blood glucose levels fall. Thus hepatocytes will play a role in maintaining blood glucose levels. Based on this approach, if there is liver damage, the mobilization process will be disrupted. Body cells that cannot use glucose will send signals to the brain to command glucose for the breakdown of muscle tissue and fat. This breakdown of muscle and fat will lead to weight loss. Muscle and fat contribute ± 40% of body weight. Weight loss in liver damage is caused by disturbed liver metabolic processes. The liver is an organ that plays a important role in the metabolism of glucose and fat and plays a role in regulating energy balance and body weight.

TABLE 1. Rat Body Weight

Group	Rat body weight (gram) ± SD		
	Day-1	Day-13	Day-14
Normal control	128.5 ± 14.20	145.25 ± 18.99	155 ± 19.30
CCl_4 (1,0 ml/kgBB)	129.75 ± 16.5	157 ± 23.95	152.25 ± 23.47
L.microphyllum (100 mg/kgBB)	122.5 ± 13.30	137.75 ± 23.89	127.75 ± 16.31
L.microphyllum (200 mg/kgBB)	139.75± 17.05	153.75 ± 14.24	148 ± 15.29
L.microphyllum (400 mg/kgBB)	135.25± 10.30	144 ± 15.53	134.5 ± 13.72
Hepa-Q®	121 ± 5.22	131.5 ± 11.12	130.5 ± 10.37

TABLE 2. Rat Body weight Differences

Group	Day 1 – Day 13		Day 13 – Day 14	
	Mean ± SD	*P-Value*	Mean ± SD	*P-Value*
Normal control	-16.75 ± 10.90	0.054	-9.75 ± 3.59	0.012
CCl_4 (1,0 ml/kgBB)	-27.25 ± 23.37	0.102	4.75 ± 5.90	0.206
L. microphyllum (100 mg/kgBB)	-15.25 ± 11.52	0.077	10.00 ± 8.75	0.107
L. microphyllum (200 mg/kgBB)	-14.00 ± 8.83	0.050	5.75 ± 3.30	0.040
L. microphyllum (400 mg/kgBB)	-8.75 ± 8.18	0.122	9.50 ± 3.69	0014
Hepa-Q®	-10.50 ± 8.10	0.081	1.00 ± 2.00	0.391

Information:
SPSS 25 Windows, Paired T-Test assay

Based on the results obtained, all test groups experienced an increase on day 1 to day 13, this can be seen in table 1 showing the mean of each group is negative, this means an increase. This negative result is obtained from the difference between the mean on day 1 minus the mean on day 13. The body weight of the mice increased but not significantly ($p > 0.05$), this could be due to the activity of each rat and different food intake. All experimental animal groups induced with CCl_4 (1.0 ml / kgBW) experienced a decrease in body weight induced on the 13th day (See table 1). These results can be seen in Table 1, the difference between body weight on day 13 and day 14, except in the normal group experiencing an increase in body weight with a mean value of -9.75 ± 3.59. This weight loss is due to the administration of CCl_4 inducers, in the liver CCl_4 will be metabolized by cytrochrome P450 2E1 (CYP2E1) to become CCl_3 * free radicals. CCl_3 * with oxygen will form another radical, namely CCl_3O_2 * which attacks the lipid membrane in the endoplasmic reticulum at a rate that exceeds the free radical CCl_3 *. These CCl_3O_2 * radicals interfere with lipid metabolism in the liver followed by weight loss.

The test group experienced an insignificant increase in body weight ($p > 0.05$). This shows that the ethyl acetate fraction of *Lygodium microphyllum* leaves does not affect the weight loss of rats which in this study was used as one of the supporting parameters in the occurrence of liver damage (see table 2).

SGOT and SGPT Serum Level

In this study, SGOTand SGPT enzyme levels were measured in test animals as one of the test parameters to determine the effect of L. microphyllum leaf ethyl acetate fraction which is thought to have activity as a hepatoprotector after being given a toxic agent, namely CCl4. SGOT levels obtained from the test group were compared with the normal group and assessed whether there was a hepatoprotector effect from the administration of the ethyl acetate fraction of L. microphyllum leaves.

TABLE 3. The effect of *L. microphyllum* leaves ethyl acetate fraction on SGOT levels

Group	Average±SD (U/L)	*P value*	Information
Normal control	139.50±21.687		
CCl_4 (1,0 ml/kgBB)	2362.50±2427185		
L.microphyllum (100 mg/kgBB)	255.00±68.069	Sig.=	significantly
L.microphyllum (200 mg/kgBB)	202.50±88.459	0.025 < 0.05	different
L.microphyllum (400 mg/kgBB)	242.50±57.373		
Hepa-Q®	1925.5±1333.272		

Information:
SPSS 25 Windows, Kruskall-Wallis test

TABLE 4. The effect of *L. microphyllum* leaves ethyl acetate fraction on SGPT levels

Group	Average ± SD (U/L)	*P value*	Information
Normal Control	79.00±15.188		
CCl_4 (1,0 ml/kgBB)	205.50±224.127		
L.microphyllum (100 mg/kgBB)	305.00±187.705	Sig.=	Not significantly
L.microphyllum (200 mg/kgBB)	180.00±131.149	0.503 >0.05	different
L.microphyllum (400 mg/kgBB)	185.00±59.722		
Hepa-Q®	351.00±287.748		

Information:
SPSS 25 Windows, Kruskall-Wallis test

From Table 3, The average value of SGOT rats of normal group, negative control, positive control, ethyl acetate fraction *L. microphyllum* leaves 100 mg/kg, 200 mg/kg, and 400 mg/kg respectively sequentially is 139.50; 2362.50; 1925.50; 255.00; 202.50; and 242.50 U/L. The negative group had the highest SGOT levels with an average of 2362.50 U/L, this is in accordance with previous research conducted by Gnanaraj (2017) where the use of CCl_4 dose of 1.0 ml/kgBW orally as a hepatotoxic agent caused an increase in levels. SGOT was significant when compared with the normal group and caused liver damage from the histopathology of the liver [5].

The decrease in SGOT levels is thought to be due to the presence of secondary metabolites from the leaves of *L. microphyllum* which act as antioxidants or scavenger of free radicals exposed by CCl_4 so as to prevent oxidative

stress. The secondary metabolite of *L. microphyllum* leaves which is thought to play a role in hepatoprotector activity is quarsetin. This is supported by previous research by Kuncoro (2017) who conducted research on the isolation of the ethyl acetate fraction of *L. microphyllum* leaves, obtained by secondary metabolites of the flavonoids, namely quarsetin and quercetin-3-O-β-D-glucopiranoside [4].

Based on research conducted by Chen (2013), quarsetin can reduce fat deposits in liver cells, this can prevent fat accumulation (steatosis) in liver cells. According to Myhrstad (2002) quarsetin can increase endogenous antioxidant activity in the liver, namely Glutathione (GSH), so that when administering CCl_4 (1.0 ml / kgBB) a toxic dose will be anticipated by GSH in the free radical scavenger exposed by CCl_4 [8].

The average value of SGPT levels in normal, negative control, positive control groups, ethyl acetate fraction of *L. microphyllum* leaves 100 mg/kg, 200 mg/kg, 400 mg/kg, respectively 79.00; 205.50; 351.00; 305.00; 180.00 and 185.00 U/L (See table 4). The positive group had the highest SGPT levels with an average of 351.00 U/L. Giving ethyl acetate fraction of *L. microphyllum* leaves at a dose of 100 mg/kg, 200 mg/kg, and 400 mg / kg of body weight can reduce SGPT levels in rats, although not significantly.

Gnanaraj (2017) reported that giving CCl_4 1.0 ml/kgBW sustained for 2 days can significantly reduce the levels of glutathione or endogenous antioxidants in the body. The decrease in glutathione levels has an impact on the incidence of liver cell damage due to the presence of free radicals from the CCl_4 metabolites, namely CCl_3 * and CCl_3O_2 *[8]. This is called oxidative stress, which is a condition where an imbalance between the amount of free radicals and antioxidants in the body [6]. The decrease in SGPT levels is thought to be due to the presence of secondary metabolites in the form of quarsetin from Lygodium microphyllum leaves which act as antioxidants or free radical scavengers exposed by the metabolites of CCl_4, namely CCl_3 * and CCl_3O_2 * so as to prevent oxidative stress. The secondary metabolite of *Lygodium microphyllum* leaves which is thought to play a role in hepatoprotector activity is quarsetin. This is supported by previous research by Kuncoro (2017) who conducted research on the isolation of the ethyl acetate fraction of *Lygodium microphyllum* leaves, obtained by secondary metabolites of the flavonoid group, namely quarsetin and quercetin-3-O-β-D-glucopiranoside [3].

The results of the histopathological picture observation can be seen in Figure 1 and the scoring results using the Manja Roenigk histopathological scoring model in Table 5. After observing the liver organ preparations of mice using the Olympus CX40 microscope.

TABLE 5. Rat Liver Hispatology Scoring Data

Group	Average±SD	*P Value*	Information
Normal Control	100.20±0.447*		
CCl_4 (1,0 ml/kgBB)	183.00±20.676		
L.microphyllum (100 mg/kgBB)	149.60±16.652*	Sig.=	significantly different
L.microphyllum (200 mg/kgBB)	128.40±7.436*	0.001<0.05	
L.microphyllum (400 mg/kgBB)	160.40±5.413		
Hepa-Q®	153.20±12.786*		

Information:
SPSS 25 Windows, Kruskall-Wallis test followed by the Mann-Whitney test
* = Value is significantly different from negative control ($p < 0.05$)

In the experimental animal group given the ethyl acetate fraction of *L. microphyllum* leaves with doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg of body weight, normal hepatocytes and kuffer cells were evenly distributed. Cells undergo parenchymal regeneration and necrosis and sinusoidal dilation can also be observed. Sinusoid dilation or dilation occurs due to pressure on the sinusoid wall due to toxins. In addition to observing the histopathological picture of the liver, the number of hepatocyte cells with conditions and those experiencing damage was also observed, such as parenchymal degeneration, hydropic degeneration, and hepatocyte cells experiencing necrosis (see Figure 1). The liver condition was then scored using the Manja Roenigk method. Based on the statistical analysis that has been carried out using the Kruskal-Wallis method which is shown in Table 5. The average value of the scoring results using the Manja Roenigk method of each group of rats from the normal group, negative control, positive control, the ethyl acetate fraction of *L. microphyllum* leaves 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. 100.20; 183.00; 153.20; 149.60; 128.40; 160.40. The difference in hepatocyte cell damage in each group showed a significant difference between groups with a significance value of 0.001 ($p < 0.05$). This significant difference occurred between the negative group with the normal group, the positive control, and the group given the ethyl acetate fraction of leaves of Lygodium microphyllum at a dose of 100 mg/kg and 200 mg/kg. Whereas in the

group giving the ethyl acetate fraction of *L. microphyllum* leaves at a dose of 400 mg/kgBW did not show a significant difference, this is presumably because of the number of cells experiencing degeneration.

From this study, it was found that the ethyl acetate fraction of *L. microphyllum* leaves was able to repair liver cell damage due to exposure to free radicals from CCl_3* and CCl_3 O_2*. Quarsetin, which is one of the ingredients contained in the ethyl acetate fraction of *L. microphyllum* leaves, functions as an antioxidant in lipid peroxidation. So that it does not interfere with Ca^{2+} hemeostasis and does not cause fat in the liver.

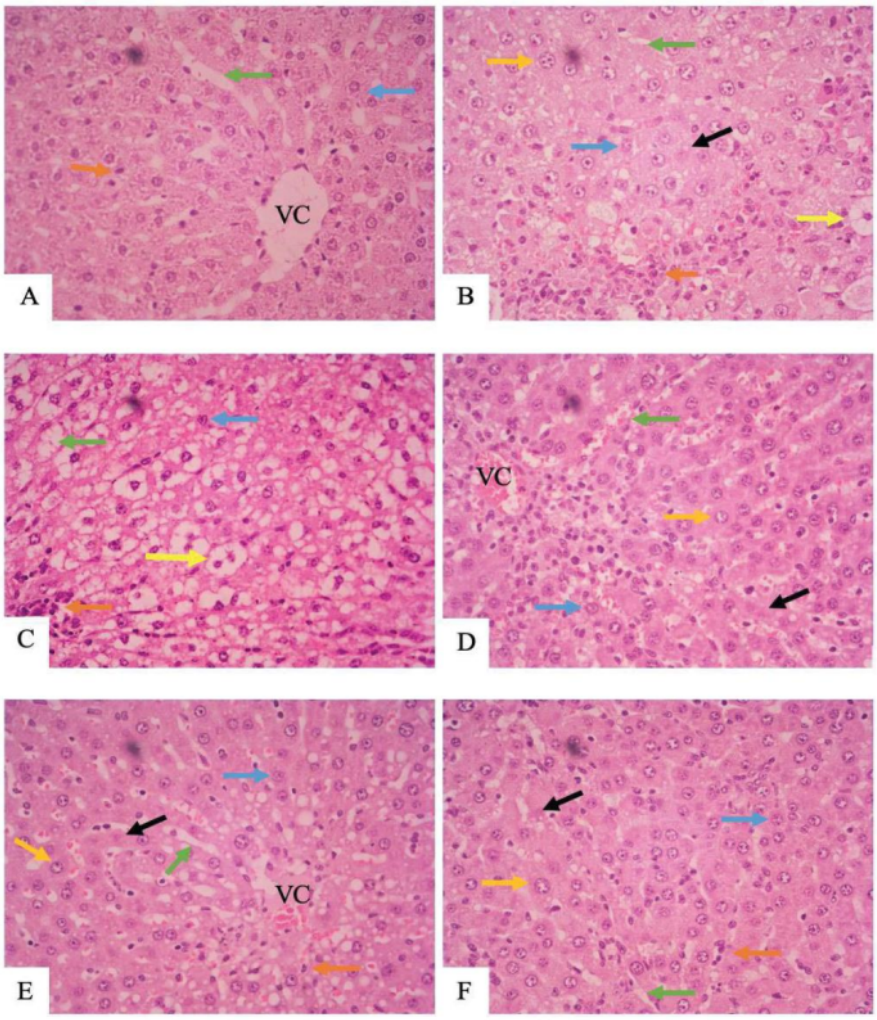

FIGURE 1. Histopathology of rat liver in each group with a magnification of 40x
A: Normal Control; B: CCl4 (1.0 ml / kg); C: Hepa-Q®; D: Ethyl acetate fraction of Lygodium microphyllum leaves 100 mg / kg BW; E: Ethyl acetate fraction of Lygodium microphyllum leaves 200 mg / kgBW; F: Ethyl acetate fraction of Lygodium microphyllum leaves 400 mg / kgBW.

: Normal hepatocyte cells
: Kupffer cells
: Sinusoid
: Parenchymal degeneration
: Hydropic degeneration
: Necrosis
VC : Vena Centralis

The protective effect of the liver or hepatoprotector is thought to be due to the presence of a secondary metabolite content in the form of quarsetin from the leaves of *L. microphyllum* which acts as an antioxidant or antidote to free radicals exposed by CCl_4 to prevent oxidative stress. The mechanism of the antioxidant effect of quarsetin is the ability to scavenge free radicals through hydrogen proton donors from hydroxyl groups. The antioxidant activity of quarsetin is mainly influenced by the substitution of hydroxyl groups in the ortho and para positions against the OH group. Based on research conducted by Chen (2013), quarsetin can reduce fat deposits in liver cells [6]. Through this mechanism, quarsetin can reduce the effects of oxidative stress and prevent oxidative damage such as lipid peroxidation, by acting as a neutralizer for free radicals produced by CCl_3* and CCl_3O_2* which are reactive metabolites of CCl_4 [5].

CONCLUSION

The ethyl acetate fraction of *L. microphyllum* leaves given to rat induced with CCl_4 showed a hepatoprotector effect at a dose of 200 mg/kg bw. The ethyl acetate fraction of *L. microphyllum* leaves did not affect rat body weight and was able to reduce SGOT and SGPT levels as well as provide protection against the liver as seen from the rat liver histopathology.

REFERENCES

1. R.S. Cotran, S.L. Robbins, V. Kumar. Robbins Pathology I and II Teacing Book. ***4th Edition***. Translate ed. Publisher B.U. Jakarta: EGC (1999).
2. H. Kuncoro, K. Farabi, L. Rijai, E. Julaeha, Y. Shiono and U. Supratman. Makara Journal of Sciences. **22**, (4), 4, (2018).
3. H. Kuncoro, K. Farabi, L. Rijai, E. Julaeha, U. Supratman and Y. Shiono. Journal of Mathematical and Fundamental Sciences. **50**, (2), 192-202, (2018).
4. H. Kuncoro, K. Farabi, L. Rijai. J. Appl. Pharm. Sci. **7**, (11) ,136-141, (2017) .
5. C. Gnanaraj, M.D. Shah, T.T. Song, and M. Iqbal, Biomedicine & Pharmacotherapy. **92**, 1010-1022, (2017).
6. C. Xiaowei, X. Ying, W. Zhang, Y. Chena, C. Shi, Y. Hou, Y. Zhang. International Immunopharmacology **17**, 543–547, (2013)
7. E. Sanmugapriya, S. Venkataraman. Journal of Ethnopharmacology. **105**(1-2):154-160, (2006).
8. M.C. Myhrstad, H. Carlsen, O. Nordström, R. Blomhoff, and J. Ø.Moskaug. Free Radical Biology and Medicine, **32**(5), 386-393, (2002).

Hepatoprotective activity of ethyl acetate fraction from Lygodium microphyllum leaves in CCl4 induced damage rats

ORIGINALITY REPORT

10%	6%	7%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

3%

★ www.hindawi.com

Internet Source

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		

Hepatoprotective activity of ethyl acetate fraction from Lygodium microphyllum leaves in CCl4 induced damage rats

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8