The Effect of Ethanol Extract *Mymercodia pendans* on Paracetamol-Induced Hepatotoxicity in White Rats

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Abstract. The present study was investigate the hepatoprotective effect of ethanol extract *Mymercodia pendans* (EEMP) in white rats. Hepatotoxicity was induced in rats by oral administration of Paracetamol (PCM) 250 mg/kg/d for ten days. Effect of concurrent administration of EEMP at a dose of 250 mg/kg/d given by oral route was determined using aspartate transaminase (AST), alanine transaminase (ALT) and histological parameter as indicators of hepatic damage. The results showed that ethanol extract of *M. pendans* resulted in significant (p<0.05) decrease in paracetamol-induced by increasing levels of liver enzymes. Histopathologic examination revealed that PCM administration produced liver cell necrosis, degeneration, hemorrhage and congestion, but the degree of induced hepatotoxicity-paracetamol was reduced by treatment with EEMP. This can be concluded that ethanolic extract of *M. pendans* possess hepatoprotective activity.

1. Introduction
Paracetamol also known as acetaminophen, in therapeutic doses is very safe to use as a non-narcotic analgesic and antipyretic drugs. This drug works by inhibiting the synthesis of prostaglandins in the central nervous system [1]. It is widely used to treat muscle pains, arthritis and acute headaches. Liver injury due to overdose of paracetamol is the most common cause of acute liver failure resulting in hepatotoxicity [2]. This is a global issue, particularly in developing countries.

Liver is a vital organ that plays important role in metabolism, the process of secretion and excretion, synthesis and detoxification [3]. In normal pathways, metabolism of paracetamol partly is conjugated in the liver to form inactive glucuronide and sulfate metabolites, and the other is oxidized to reactive N-acetyl-p-benzoquinoneimine (NAPQI) [4]. Then, NAPQI reacts with sulfhydryl glutathione to form a non-toxic substance [5]. High doses of paracetamol, on the other hand, reduced the availability of liver glutathione and the NAPQI reacts with liver protein sulfhydryl groups to form covalent bonds, cause oxidative stress on hepatocytes [2], [4], [5].

The existing synthetic drugs to treat liver diseases have not given much pronounced outcomes. Conventional herbal plants have become progressively more popular and their utilization is more prevalent. Empirical use of herbal medicine is believed can cure several deseases [6]. Administration of...
Antioxidants can reduce liver damage caused by induced hepatotoxicity-paracetamol [7], [8]. Mymercodia pendans is a plant produce an active compounds that can act as antioxidants, such as phenols, flavonoids, and terpenoids [9], [10]. These compounds are safe to use as herbal medicines without causing toxicity to body cells [11]. Toxicity test of M. pendans extracted in ethanol, up to a dose of 300 mg/kg/d given orally in white rats for 21 days showed no symptoms of toxicity. This hepatoprotector potential is very interesting to study, and induction of hepatotoxicity-paracetamol dose of 250 mg/kg was reported to cause severe damage to the liver [12], [13].

This study aimed to investigate the protector effect of the EEMP to hepatotoxicity induced by PCM in male white rats. Liver function test as a predictor of damage hepatocyte can be predicted by measuring liver enzyme activities, i.e. AST and ALT, and the change of histopathology structure [2], [3], [12]–[14]. Other parameters observed were body weight, liver weight and the ratio of liver weight [15].

2. Methods

2.1. Prepare of ethanol extract of M. pendans

Air dried of M. pendans originaly from Papua, Indonesia was blended into powder. The powder was processed by cold masedated method using ethanol 90% and mixed in a shaker for 2-3 hours. Then stored for 72 hours before further processing. The masedated poweder, then filtered with filter papers. The supernatant was evaporated in a rotary evavorator. The extract was stored in refrigerater before used.

2.2. Animals experimental

Healthy male wistar rats at 2-3 month of age weighing 250-280 g were used for the study. Animal use has been approved by Animal Ethics Committee University of Udayana. They were housed with allowed free access to a good feed and drinking water ad libitum. The animals were acclimatized on laboratory conditions for two weeks before the research begins. All the experiment was done with minimize environmental stress and handling stress.

2.3. Experimental design

Induction of animal hepatotoxicity using PCM at dose 250 mg/kg/day orally for ten days. The effect of concurrent of EEMP at dose 250mg/kg/day by oral. Assessment of hepatotoxicity was determined by liver enzyme levels and histological parameters was measured as an indicator of liver damage. A total of 24 wistar rats were randomly divided into 4 groups as follows:

- Group I as a control, received normal saline with the same volume as PCM orally.
- Group II received PCM at dose 250 mg/kg/day for ten day.
- Group III received PCM 250 mg/kg/day and EEMP 250 mg/kg/day for ten day.
- Group IV received EEMP at dose 250 mg/kg/day for 7 day, followed by administration PCM 250 mg/kg/day and EEMP 250 mg/kg/day for ten day

All of the animals on the day eleventh were weighed. Blood sample for biochemical investigation were taken by cardiac puncture under diethyl ether anesthesia. Then, the animals are terminated from cervical dislocation and the liver was removed for weighed and histopathological studies.

2.4. Measurement of liver enzymes activity

The blood sample is processed to take the serum. Aspartate transaminase (AST) and alanine aminotransferase (ALT) were measured using a standard clinical automatic instrument (Icubio, Ichem-535Vet). The results of AST and ALT activity are expressed in international units per liter (u/L).

2.5. Histopathological examination

All off the animals are necropsy according to the procedure. Then the liver were removed and stored in neutral buffer formalin (NBF) 10%. Processed and cut sections were stained with haematoxyline and eosine stains and examined under light microscope, each in five different fields of view. The degree of histopathological assessment like congestion, haemorrhage, degeneration and necrosis was done by a
score given i.e. Score 0 - normal, Score + - change of focal area, Score ++ - multifocal area, Score +++ - diffuse area.

2.6. Statistic analysis
All information is conferred as Means (SD) i.e. body weight, liver weight, ratio of liver, AST and ALT was subjected to parametric test. Statistic alalysis was performed using ANOVA followed by Duncan test between groups. Non parametric data (histopathological score) was subjected to non-parametric Kruskall Wallis followed by Mann-Whitney U-test. Variations between experimental animal groups were p<0.05 was considered as statistically significant

3. Results

3.1. Body and liver weight, ratio of liver weight after treatment
The body weights of wistar rats induced hepatotoxicity-paracetamol compare to control group and group followed by treatment with EEMP were not significantly affected, but it there has a similar significant effect (P<0.05) on liver weight and ratio of liver to body weight of white rats (Table 1).

| Group Animal (n=24) | Body Weight (g)  | Liver Weight (g) | LW/BW (%) |
|---------------------|------------------|------------------|-----------|
| Control             | 266.66 ± 7.94ª   | 7.57 ± 0.14ª     | 2.84 ± 0.08ª |
| PCM                 | 257.67 ± 6.18ª   | 7.98 ± 0.22ª     | 3.11 ± 0.15ª |
| PCM + EEMP          | 265.50 ± 5.54ª   | 7.75 ± 0.14ª     | 2.92 ± 0.01ª |
| 7 day EEMP + (PCM + EEMP) | 266.16 ± 10.36ª | 7.68 ± 0.11ª     | 2.89 ± 0.09ª |

ªSignificantly different as compared to control group, P<0.05.

3.2. Liver enzymes level
The mean activity of liver enzymes in animal groups can be seen in Table 2.

| Group Animal (n=24) | ALT (u/L) | AST (u/L) |
|---------------------|-----------|-----------|
| Control             | 55.4 ± 18.87ª | 65.70 ± 15.73ª |
| PCM                 | 222.9 ± 48.07ª | 241.41 ± 46.13ª |
| PCM + EEMP          | 73.06 ± 20.34ª | 84.98 ± 20.60ª |
| 7 day EEMP + (PCM + EEMP) | 66.85 ± 15.06ª | 72.66 ± 20.45ª |

ªSignificantly different as compared to control group, P<0.05.

Animals group with induced hepatotoxicity-paracetamol resulted significant increase (p<0.05) in plasma ALT and AST levels in PCM group compared to control group, which is suggestive of severe liver injury. The effect of concurrent administration of EEMP by oral kept the ALT-AST to significantly lower level (p<0.05) when compared to PCM.

3.3. Histopathological evaluation
Histopathological changes of white rats with hepatotoxicity includes congestion, hemorrhage, degeneration, and necrosis. The results of the observation showed a significant difference when compared to the control group and the rat group that received EEMP treatment (Table 3).
Table 3. Scoring histopathological change of animal groups.

| Group         | Animal (n=24) | Degeneration | Congestion | Haemorrhage | Necroses |
|---------------|---------------|--------------|------------|-------------|----------|
| Control       | -             | -            | -          | -           | -        |
| PCM           | +++           | ++           | +          | +           | +        |
| PCM + EEMP    | -             | +            | +          | -           |          |
| EEMP + (PCM + EEMP) | -             | +            | -          | -           |          |

4. Discussion
In developing countries the use of herbs as traditional medicine is very popular and has been around for a long time. Herbal medicine is believed to be a safe treatment, by taking it directly from nature or ingredients that have been in a simple processed [6], [16]. Air dried M. pendans has been traded and promoted to overcome a various diseases, can increase the metabolism and improve blood circulation [17]. That the M. pendans contained active compounds such as flavonoids, tocopherols, phenolics and terpenoids [10], [11] and has antioxidant activity [18], anti-bacterial and anti-cancer [19]. The flavonoid compounds were identified are kaempferol, lutelin, routine, quercetin and apigenin [9]. This compound can be used to overcome oxidative stress due to free radicals [20].

Paracetamol is a strong analgesic and antipyretic drug, it works by inhibiting prostaglandin synthesis in the central nervous system [1], [2]. This drug can be purchased freely at a low price, so its use is often not according to the rules. At high doses exceeding therapeutic doses can cause toxicity, with side effects of liver and kidney injuries [14]. The absorption of PCM through the gastrointestinal tract is very fast, the first cross-metabolism occurs in the cells of the intestinal lumen and is conjugated in the liver [21]. In therapeutic dose, PCM is converted by drug metabolizing enzymes to water-soluble metabolites and secreted in the urine. The toxic dose of PCM caused the depletion of GSH resulting in accumulation of NAPQI which then covalently binds to the cysteinyl sulphydryl groups of cellular proteins forming NAPQI-protein adducts [1], [5]. This event results liver cell injuries [4].

In our study, the animals group with induced an oral multiple doses administration of PCM 250 mg/kg/d for ten day was hepatotoxic in rats as shown by the significant (P<0.05) increase in plasma ALT and AST activities levels [12]. The high concentrations of serum liver enzymes indicates hepatocyte damage because these enzymes are located in the cell cytoplasm and are released into the bloodstream following hepatic cell damage [22]. This is causing the leaking of cellular enzymes and can be measured in the serum [13]. And than, the effect of concurrent administration of EEMP by oral kept the ALT-AST to significantly lower level (p<0.05) in EEMP treated group when compared to PCM group. This indicates that the EEMP shown to protect liver cells from damage caused by oxidative stress [23].

Liver injury is a common histopathologic feature of many hepatic diseases. In our present study, the histopathologic findings in PCM group induced hepatocyte damage [15]. The histopathological change including congestion, hemorrhage, degeneration and necrosis (Table 3). Severe congestion occurs in the central vein and obstruction occurs in capillaries. This obstruction causes blood to accumulate in the vessels and sinusoids, this is explain why in the PCM group has a liver edema so that the size becomes bigger and heavier (Table 1). All of parameters, in PCM group shows a significant difference when compared to the control group. The most characteristic histopathological changes found in hepatotoxicity-paracetamol are necrotic centrolobular [24]. Severe was seen in the PCM group, and there was mild hemorrhage in the EEMP group treated [25]. This proves that the active compounds contained in M. pendans act as antioxidants in protecting endothelial cells from NAPQI as a free radical [4].

5. Conclusion
The conclusion of our present study that ethanolic extract of M. pendans possess hepatoprotective activity.
6. References

[1] Twycross R, Pace V, Mihalyo M and Wilcock A 2013 Acetaminophen (Paracetamol) J. Pain Symptom Manage 46 747–55

[2] Yoon E, Babar A, Choudhary M, Kutner M and Pyrsopoulos N 2016 Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update J. Clin. Transl. Hepatol. 4 131–42

[3] El-Kott A F and Bin-Meferrj M M 2015 Use of Arctium lappa Extract Against Acetaminophen-Induced Hepatotoxicity in Rats Curr. Ther. Res. Clin. Exp. 77 73–78

[4] Jaeschke H, McGill M R and Ramachandran A 2012 Oxidant Stress, Mitochondria and Cell Death Mechanisms in Drug-Induced Liver Injury: Lessons Learned from Acetaminophen Hepatotoxicity Drug Metab. Rev. 44 88–106

[5] McGill M R, Sharpe M R, Williams C D, Taha M, Curry S C and Jaeschke H 2012 The Mechanism Underlying Acetaminophen-Induced Hepatotoxicity in Humans and Mice Involves Mitochondrial Damage and Nuclear DNA Fragmentation J. Clin. Invest. 122 1574–83

[6] Saleem M and Naseer F 2014 Medicinal Plants in the Protection and Treatment of Liver Diseases Bangladesh J. Pharmacol. 9 511–26

[7] Bill R L 2016 Clinical Pharmacology and Therapeutics for Veterinary Technicians - E-Book. (Maryland Heights: Mosby Elsevier Health Sciences)

[8] Dash D K et al 2007 Evaluation of Hepatoprotective and Antioxidant Activity of Ichnocarpus frutescens (Linn.) R.Br. on Paracetamol-Induced Hepatotoxicity in Rats Trop. J. Pharm. Res. 6 755–65

[9] Engida A M, Faika S, Nguyen-Thi B T and Ju Y–H 2015 Analysis of Major Antioxidants from Extracts of Myrmecodia pendans by UV/Visible Spectrophotometer, Liquid Chromatography/Tandem Mass Spectrometry, and High-Performance Liquid Chromatography/UV Techniques J. Food Drug Anal. 23 303–09

[10] Gartika M, Pramesti H T, Kurnia D and Satari M H 2018 A Terpenoid Isolated from Sarang Semut (Myrmecodia pendans) Bulb and its Potential for the Inhibition and Eradication of Streptococcus Mutans Biofilm BMC Complement. Altern. Med. 18 151

[11] Sudiono J, Oka C and Trisfilha P 2015 The Scientific Base of Myrmecodia pendans as Herbal Remedies Br. J. Med. Med. Res. 8 230–37

[12] Utami A R, Berata I K, Samsuri S and Merdana I M 2017 Efek Pemberian Propolis terhadap Gambaran Histopatologi Hepar Tikus yang diberi Parasetamol Bul. Vet. Udayana 9 87–93

[13] Mahmood N D et al 2014 Amelioration of Paracetamol-Induced Hepatotoxicity in Rat by the Administration of Methanol of Muntingia calabura L. Leaves BioMed Research Int. 2014 1–11 Retrieved from https://www.hindawi.com/journals/bmri/2014/695678/ [Accessed on October 13th, 2018].

[14] Robin S, Sunil K, Rana A C, Nidhi S 2012 Different Models of Hepatotoxicity and Related Liver Diseases: A Review Int. Researc J. of Pharm 3 86–95

[15] El-Kott A F and Bin-Meferrj M M 2015 Use of Arctium lappa Extract Against Acetaminophen-Induced Hepatotoxicity in Rats Curr. Ther. Res. 77 73–78

[16] Sreshta B and Babu S R 2018 Hepatoprotective Effect of Poly Herbal Formulation Containing Indigenous Medicinal Plants Against Various Hepatotoxic Agents in Rats Asian J. Pharm. Pharmacol. 4 232–37

[17] Hertiani 2010 Preliminary Study on Immunomodulatory Effect of Sarang-Semut Tubers Myrmecodia tuberosa and Myrmecodia pendens Online J. Biol. Sci. 10 136–41

[18] Agatonovic-Kustrin S, Morton D W, Mizaton H H and Zakaria H 2018 The Relationship between Major Polyphenolic Acids and Stigmasterol to Antioxidant Activity in Different Extracts of Myrmecodia platytyrea South Afr. J. Bot. 115 94–99
[19] Yap L -S, Lee W -L and Ting A –S –Y 2017 Endophytes from Malaysian Medicinal Plants as Sources for Discovering Anticancer Agents, in *Medicinal Plants and Fungi: Recent Advances in Research and Development*, Agrawal D C, Tsay H -S, Shyur L -F, Wu Y -C and Wang S – Y 2017 (Singapore: Springer Singapore) pp. 313–335.

[20] Khalid M, Siddiqui H H and Freed S 2011 Free Radical Scavenging and Total Phenolic Content of *Saccharum spontaneum* L. Root Extracts *Int. J. of Research in Pharm. and Chem.* 1 1160–66

[21] Athersuch T J, Antoine D J, Boobis A R, Coen M, Daly A K, Possamai L, Nicholsone J K and Wilsona I D 2018 Paracetamol Metabolism, Hepatotoxicity, Biomarkers and Therapeutic Interventions: A Perspective *Toxicology Research (RSC Publishing)* 7 347–57 Retrieved from https://pubs.rsc.org/en/content/articlelanding/2018/tx/c7tx00340d/unauth#!divAbstract [Accessed on October 12th, 2018].

[22] Uchida N S et al 2017 Hepatoprotective Effect of Citral on Acetaminophen-Induced Liver Toxicity in Mice *Evidence-Based Compl. and Altern. Med.* 2017 1–9 Retrieved from https://www.hindawi.com/journals/ecom/2017/1796209/abs/ [Accessed on October 13th, 2018].

[23] M. S. N. Hohmann et al 2015 *Hypericum perforatum* Reduces Paracetamol-Induced Hepatotoxicity and Lethality in Mice by Modulating Inflammation and Oxidative Stress *Phytother. Res.* 29 1097–1101

[24] Takahashi Y and Fukusato T 2014 Histopathology of Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis *World J. Gastroenterol.* 20 15539–48

[25] Alarami A M J 2015 Histopathological Changes in the Liver and Kidney of Albino Mice on Exposure to Insecticide, Dimethoate *Int.J.Curr.Microbiol.App.Sci.* 4 287–300

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