Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
NLM (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2022: 83.058
Volume 13, Issue 2; 2024, 39-50

ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

Original Research Article

Hepatoprotective Activity of *Piper crocatum* (Ruiz &Pav.) Leaves Against Monosodium Glutamate -Induced Hepatotoxicity in Rats

Sri Endarti Rahayu[1], Bayu Kharisma[1], and Suprihatin[1]

Faculty of Biology and Agriculture, Universitas Nasional Jakarta, Jl. Sawo Manila No. 61, Pejaten, Pasar Minggu, Jakarta Selatan, Indonesia.

Article Info: Received: 14-01-2024 / Revised: 18-02-2024 / Accepted: 28-03-2024
Address for correspondence: Sri Endarti Rahayu
DOI: https://doi.org/10.32553/jbpr.v13i2.1070
Conflict of interest statement: No conflict of interest

Abstract:
Monosodium glutamate (MSG) is widely used as additive in food. Excess consumption of MSG was reported to cause oxidative stress on liver resulted increased production of reactive oxygen species (ROS). Without proper treatment, it can induce liver injury and fatal hepatic diseases, including cirrhosis. Red betel (*Piper crocatum* Ruis and Pav.) is one of Indonesia's medicinal plants that has been known to exhibit antioxidant activity. The current study evaluated hepatoprotective effect of red betel leaves extract (RBLE) towards liver injury. Animals (n=5 per group) were randomly divided into five groups as follows: Group 1, control (distilled water); group 2, negative control, receive 7 mg/kg bw of MSG; group 3, receive oral 7g/kg b.w of MSG plus 200 mg/kg bw RBLE; group 4, receive oral 7g/kg b.w of MSG plus 400 mg/kg b.w RBLE and group 5, receive oral 7g/kg b.w of MSG plus 600 mg/kg b.w RBLE. The rats were sacrificed on twenty-ninth day of the experiment. Measuring serum levels of aspartate aminotransferase (AST) and alanin aminotransferase (ALT) was done. Histopathological examination of liver was performed by calculated necrosis cell percentage. RBLE treatment (200 mg/kg b.w) decrease alanin aminotransferase and aspartate aminotransferase demonstrated increased compare to negative control. The histological findings revealed RBLE treatment were also able to decrease necrosis cells percentage. The study was able to demonstrate that RBLE treatment could decrease necrotic cell percentage. Low concentration of RBLE (200 mg/kg b.w) exhibit best anti-inflammatory properties and had the lowest ALT enzyme and increased live cell percentage by shifting cell death pathway from necrosis to apoptosis, and these indicating its antioxidant properties. These results could be used as a baseline in the purification of red betel bioactive components to be used for liver injury medication.
Key words: Antioxidant, hepatoprotective, histopathology, liver enzymes, MSG, red betel.

Introduction

Food additives have been used to keep the quality, texture, consistency, taste, colour, alkalinity or acidity of foods to make them more acceptable to the users. Their use has reached alarming proportions and humans are daily exposed to these chemical substances in their foods without defining the exact and safe limit. Glutamate is found in wide variety of foods and as a result of its flavour enhancing effects, glutamate is often deliberately added to foods usually as purified monosodium salt called as

monosodium glutamate or MSG (Zia *et al*., 2014).

Monosodium L-Glutamate (MSG) is a white crystal-like substance that contains 78% of glutamic acid, 22% of sodium and water (Alalwani, 2014). The Majority, MSG used as a food additive in everyday life. MSG can increase the taste of umami in food and stimulated of appetite (Henry, 2017). Monosodium glutamate (MSG) is common marketed under such trade name as Ajinomoto, Vetsin (Beyreuther *et al.*, 2007).

Glutamate, when bound to proteins, is tasteless. When free glutamate dissociates from proteins; during the processes of fermentation, ripening and cooking, the sweet umami taste and flavor becomes perceptible. Large quantity of glutamate are present in cheese, mushrooms and tomatoes and thus used to enrich the flavor and taste of foods (Oyetunji, 2013).

Commercially MSG is produced by molasses fermentation and it is used in home cooking, restaurants and for industrial food production (Farombi & Onyema, 2006). In general, the natural glutamic acid in food is not harmful, while the synthetic free glutamic acid is toxic (Geha *et al*., 1998). Although MSG has been classified by FDA that's generally recognized as safe, its use remains controversy (Ortiz *et al*., 2006).

The interest in adverse reaction of MSG is Chinese restaurant syndrome which is characterized by headache, flushing, numbness, muscle tightness, generalized weakness and bronchoconstriction in asthmatics (Cortese & Phan, 2005). In high doses, MSG can produce neuroendocrine abnormalities, neuronal degeneration (Moreno *et al*., 2005) and oxidative damage in different organs (Farombi & Onyema, 2006; Pavlovic *et al*., 2007). Also, retinal degeneration, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis are reported (Samuels, 1999). Despite of all the above reported adverse outcome associated with use of MSG, the safe concentration of MSG in food and its toxicity in human is still controversial issue.

The liver plays a major role in metabolism of xenobiotics including glutamate. The by-product of such metabolism could lead to liver injury and emergence of liver diseases (Ishak, 1991). Prolonged exposure to exogenous substance, such as drugs might causing liver injury. It has been shown that long-term or overdose drugs intake could lead to serious liver problems as a result of increased reactive oxygen species (ROS) in hepatic cells, causing oxidative stress (Yoon *et al*., 2016). ROS have oxygen content and can react easily with other molecules, and ROS can cause damage to DNA, RNA and protein, and even cause cell death. Thus, they can affect the functioning of the liver, especially in biotransformation and eliminating toxic substances (Noh et al., 2015).

Free radicals can trigger a liver metabolism imbalance. The generation of ROS within the hepatocytes causes cell metabolism disorder, cell necrosis and cell death (Hiraganahalli, *et al.*, 2012). Continuous exposure to ROS can cause serious damage to the liver, including cirrhosis (Pareek *et al*., 2013). The World Health Organization (WHO) states that cirrhosis causes about 800,000 of the 2.4 million liver-related deaths each year (Delgado *et al*., 2015]. The incidence of liver disease does not decrease in spite of the many modern drugs available today (Abdallah *et al*., 2013).

Many studies have been done to find antioxidant properties in medicinal plants that could be used as a potent candidate for liver injury medication (Aara *et al*., 2020). Red betel (*Piper crocatum* Ruiz and Pav) is one of Indonesia's medicinal plants has medicinal function and used as medicine since it introduces as medicinal plants producer in Blunyahrejo (Rinanda and Alga, 2012).

Red betel leaves extract (RBLE) has been reported to contain active compounds, mainly flavonoids, steroids, tannins, saponins, alkaloids, polyphenolics, quinones, and essential

oil groups (Wulandari *et al*., 2018). It has been reported RLBE contain eugenol and hydroxychavicol, a phenolic compound that has antioxidant and anti-inflammatory activities (Dervis *et al*., 2017). From previous studies, RLBE shown can exhibit antioxidant activities (Lister *et al*., 2019a); anti-inflammatory and antifungal properties (Misra *et al*., 2009); and have anticancer activity especially cervical cancer (Widowati *et al*., 2013) and breast cancer (Zulharini *et al*., 2018).

Despite the fact that the above species have already been evaluated in various studies abroad, local analysis needs to be conducted because secondary metabolites vary depending on geographical location and environmental conditions (Yang et al. 2018; Labarrere et al. 2019; Recuenco et al. 2020). Thus, the aim of this study is to screen the phytochemicals from red betel extracts collected from Kasomalang District, West Java, Indonesia as well as evaluate their antioxidant activities, and efficacy as hepatoprotective against MSG induced hepatotoxicity in rats.

Previous studies have shown the effect of MSG treatment at high doses (200, 600 and 4000 mg/kg) and at low dose (60 and 120 mg/kg) to cause the increment of AST and ALT enzymes in rats (Onyema *et al*., 2006; Masre *et al*., 2019; Onyema & Farombi, 2006). This study differs from previous studies because it uses a very low doses of MSG, i.e 7mg/kg bw. The study aims to evaluate possible negative effect of MSG (7 mg/kg bw) and to investigate putative healing of red betel leaf extract (at an oral dose of 200, 400 and 600 mg/kg) on liver tissue. The parameters that observed in this study was phytochemical screening, antioxidant activity, biochemical markers of hepatic damage and necrotic cells percentage.

Materials and Methods

Plant sampling and extraction

Red betel plants (*Piper crocatum* Ruiz and Pav.) were obtained from farms in Kasomalang District, West Java, Indonesia. It was then identified and authenticated by the Botanical laboratory of Universitas Nasional Jakarta, Indonesia. Samples were air dried for about two weeks and manually chopped before grinding into a a fine powder using a mechanical grinder. Extraction was carried out using maceration method. 900 g of powdered red betel leaves were macerated with the solvent of ethanol for 5 days. The filtrate was collected every 24 h and maceration process were repeated until the filtrate become colorless. Then the collected filtrate was concentrated using evaporator at 50^{o}C until RBLE was obtained and stored at -20^{o}C (Lister *et al*., 2019).

Qualitative Phytochemical screening

Phytochemical screening was conducted using the tube test, and chemical compounds tested for included flavonoids, tannins, saponins, and alkaloids (Abiodun *et al*., 2011).

DPPH (2,2-diphenyl1-1-picrylhydrazyl)

The capacity of extract to scavenge the free radical 2,2-diphenyl1-1picrylhydrazyl (DPPH) can be used to measure their antioxidant activity. The DPPH assay is acommonly employed method for assessing the ability of natural substances to scavenge radicals. In this investigation, the antioxidant potentials of extracts was evaluated using the DPPH assay, as reported by (Lister et al, 2019). 0.1 mM DPPH solution was prepared in methanol.Throughout the experiment, the solution was kept dark to prevent light from compromising its integrity. To measure the antioxidant activity, standards and extracts at various concentration were prepared 22µl samples with different concentration were incubated with 200µl of 0.1 mM DPPH solution. After 30 minutes of incubation, the reaction mixture was measured at 517 nm. The amount of DPPH radical scavenging was assessed by observing change in the color of the DPPH solution, which suggests that the antioxidants components in the extracts were reducing the free radical.

Animals and treatment

A total of 25 male *Sprague-Dawley* rats weighing between 170 to 200 g were obtained

from Laboratory Animal Resource Unit, Agricultural University, Bogor were used for experiment. Animals were handled and maintained follow research ethics permission from Universitas Muhammadiyah Purwokerto, Indonesia , number KEPK/UMP/60/X/2019. The rats were randomly divided into 5 groups (1-5) of 5 rats each. They were kept in separate cages and left for 1 week to acclimatize. The animals were fed with standard pellet foods, with access to water *ad libitum*.

G 1 (Control): Five mL distilled water was given orally for 28 days – Group I

G 2 (MSG): 7 mg/kg MSG was given daily by oral gavage for 28 days - Group II

G3 (MSG): 7 mg/kg MSG was given and after 8 hours of treatment with ethanolic extract of red betel at a dose of 200 mg/kg daily by oral gavage for 28 days. – Group III

G4 (MSG): 7 mg/kg MSG was given and after 8 hours of treatment with ethanolic extract of red betel at a dose of 400 mg/kg daily by oral gavage for 28 days. – Group IV

G5 (MSG): 7 mg/kg MSG was given and after 8 hours of treatment with ethanolic extract of red betel at a dose of 600 mg/kg daily by oral gavage for 28 days – Group V

During 28 days experiment period, environmental conditions were controlled; the humidity was 55 to 60% and the room temperature was 22 ±2ºC with 12-h light exposure.

The animals were sacrificed 24 h after 28 days of administration by cervical dislocation. The blood was taken by heart puncture in dry tubes without anticoagulant to measure liver enzymes (Alanine Transaminase (ALT) and Aspartate Amino Transaminase (AST). After sacrificing the rats, the liver tissue was dissected, cut into small pieces and dropped in formalin in which they were kept for appropriate time. After fixation, they were subjected to the usual procedure for paraffin embedding. Sections were cut at the thickness of 5 microns and stained with Haematoxyline-Eosin (HE) and then tissue section were investigated using light microscope according to Drury and Wallington (1980). Histological changes including necrosis were evaluated in all samples.

Assessment of serum liver function tests

The biochemical markers of hepatic damage including serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined as reported previously (Reitman & Frankel, 1957). For determination of ALT activity, the serum sample was added to the buffered solution containing DL-alanine and 2-ketoglutarate (pH 7.4) and incubated for 30 min at 37ºC. After incubation, 1.0m*M* DNPH was added, followed by the addition of 0.4 *M* NaOH. The absorbance was read at 500 nm and the ALT activity deduced. For determination of AST activity, L-aspartic acid was used in place of the DL-alanine and the incubation time was 1 hr.

Statistical Analysis

The results of the study are expressed as the mean ± standard error of the mean (S.E.M). Statistical analysis of the data was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc multiple comparison test. The differences in the test were considered significant at $p<0.05$.

RESULT

Phytochemical Screening

A preliminary phytochemical examination of *Piper crocatum* leaves has identified a number of significant phytochemical substances, including alkaloids, saponin, tannins, steroids, terpenoids, and glycosides. These plant components provides a wide variety of bioactive compounds with a promise for the creation of new pharmaceuticals.

Antioxidant Activity

The antioxidant activity of *P. crocatum* extract was assessed by DPPH assay. Significant in vitro DPPH (2,2-diphenyl-1- picrylhydrazyl) radical scavenging activity was demonstrated by the ethanolic extracts obtained from Piper

crocatum in a dose-dependent manner. L-ascorbic acid, a common antioxidant, on the other hand, showed strong DPPH radical scavenging ability. The IC50 values, or the amounts of the examined plant extracts needed to scavenge 50% of the DPPH radicals, was determined as 196.10 ±0.01 mg/ml. These findings underscore the robust DPPH radical scavenging potential of the Piper crocatum extract while highlighting the superior efficacy of L-ascorbic acid as a reference antioxidant in this context as 73.61 mg/ml.

Effect of RBLE on the Serum Level of AST and ALT

The serum marker enzymes (AST and ALT) levels increased significantly in monosodium glutamate treated rats compared to control groups, as presented in Table 3.

Table 1: Effects of P. crocatum leaves extract on serum level AST and ALT of MSG induce rats.

Group	AST (U/L)	ALT (U/L)
rol	43 ± 5,65 [a]	5 ± 26,45 [a]
. Negative control (7g/kg bw of MSG)	69,2 ± 9,54 [b]	4 ± 340,71 [a]
I.Treatment (7g/kg bw of MSG + 200 mg/kg bw of RBLE	80 ± 14,76 [b]	± 30,23 [a]
'. Treatment (7g/kg bw of MSG + 400 mg/kg bw of RBLE	79 ± 3,80 [b]	373,8 ±186,98 [a]
'. Treatment (7g/kg bw of MSG + 600 mg/kg bw of RBLE	109,2 ± 4,20 [c]	721,6 ± 299,03 [b]

The data shown as mean: [a,b] considered as significantly different

Histopathological Examination

The number of necrosis hepatocyte cells increased significantly in monosodium glutamate treated rats than the control group of rats, as presented in Table 4.

Table 2: Effects of RBLE on necrotic of hepatocyte cells of MSG induced rats

Group	Necrosis hepatocyte cells
Control	0,000 ± 0,000 [a]
ative control (7g/kg bw of MSG)	56,82 ± 6,509 [b]
Treatment (7g/kg bw of MSG + 200 mg/kg bw of RBLE	27,72 ± 6,795 [c]
Treatment (7g/kg bw of MSG + 400 mg/kg bw of RBLE	54,82 ± 7,894 [c]
Treatment (7g/kg bw of MSG + 600 mg/kg bw of RBLE	87,12 ± 11,888 [d]

The data shown as mean: [a,b] considered as significantly different

Histopathology observation of the control group in Figure 1 (A) showed normal liver architecture of hepatic cells without histopathological changes. In contrast, the liver section of monosodium glutamate treated rats showed a remarkable alteration, degeneration, and necrosis of hepatic cells (Fig.1B). The rats' liver section treated with monosodium glutamate and 200 mg/kg bw of RBLE revealed hepatocyte cell amelioration and regeneration and almost near-normal liver architecture (Fig. 1C). This indicates the hepatoprotective activity of RBLE while liver section treated with monosodium glutamate and 400mg/kg bw RBLE (Fig. 1D) and liver section treated with monosodium glutamate and 600 mg/kg bw RBLE (Fig. 1E) showing cytoplasmic vacuolation of hepatocyte and necrosis of sporadic hepatocyte.

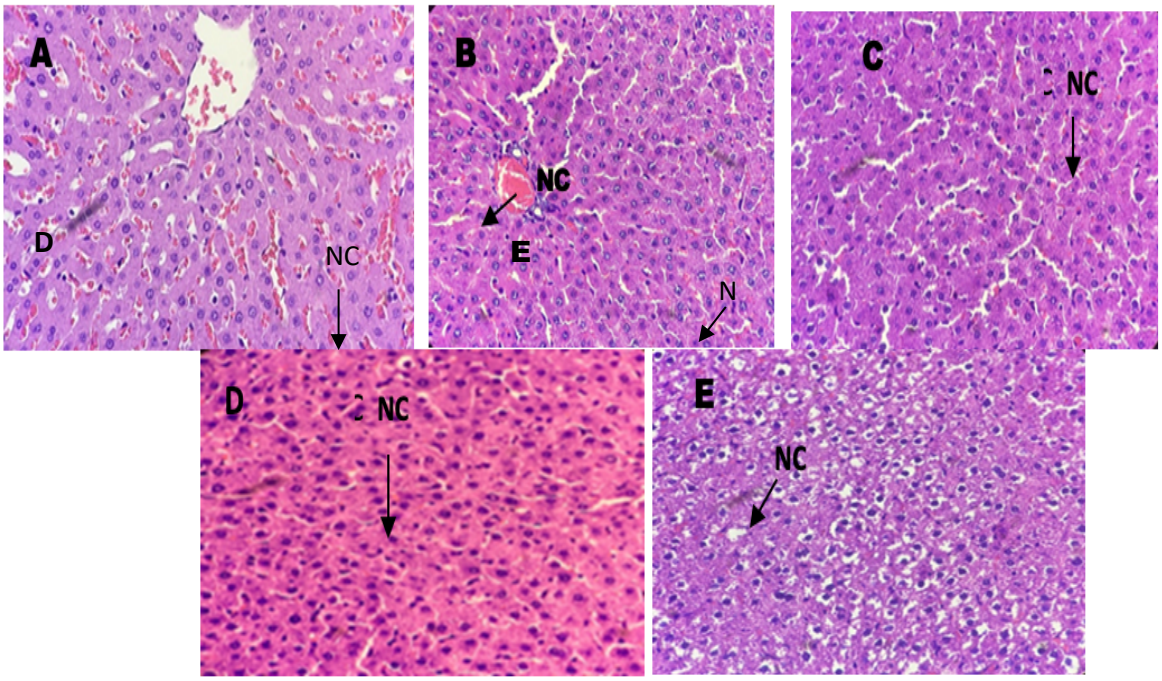

Figure 1: Sections of the livers of monosodium glutamate- treated rats indicating necrotic cells

Discussion

MSG is widely used as a flavor enhancer in the processed food and drinks (Rundlett & Armstrong, 1994). At the time of discovery, MSG was thought to be safe since it was a natural substance (an amino acid). Recently considerable attention has been focused on its unusual side effect (Reis *et al*., 2009). MSG may have acted as toxins to the hepatocytes. One of the ways for estimating of the extent of hepatic damage is through the determination of the serum level of cytoplasmic enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

The current study revealed significant elevation of liver enzymes ALT and AST (in the group 2 and 5) in comparison with control group. These results indicate that consumption of MSG may lead to liver cell damage. These agree with (Onyema *et al*., 2006; Masre *et al*., 2019; Onyema & Farombi, 2006) studies who report that the increase serum ALT in male rats which were fed MSG is probably due to MSG induced oxidative stress in the liver.

ALT is a type of metabolic enzyme that is specifically located in the cytoplasm of hepatocytes. Due to its specific locations, ALT is considered a more valuable and accurate liver damage biomarker compared to others (Novillia *et al*., 2018). AST is another enzyme used in conjunction with ALT to monitor various liver diseases. AST is present not only in LDH-like liver tissue but also in other damaged tissues, such as tissues of heart, kidney, pancreas, brain, skeletal muscles and lungs (Arika *et al*, 2016).

Serum ALT and AST activities were found to be increased significantly in MSG Induced (Table 3), which might be due to oxidative stress and damage to cell membrane. Co-administration of red betel and MSG showed a significantly low level of ALT. This could be attributed to overall protective effect of red betel to hepatocytes. Novilia *et al*. (2018) and Lister *et al* (2022) also reported that RBLE could reduce ALT level significantly. Administratipn of RBLE at 200 mg/kg bw along with MSG could offer a complete protection against the oxidative stress induced acceleration in membrane integrity, which changed the permeability resulting in the leakage of intracellular enzymes.

RBLE contain phytochemicals constituent such as alkaloids, saponins, tannins, steroids, triterpenoids, glycosides, phenols and flavonoids (Table 1). It is probable that the various phytoconstituents of the extracts are involved in scavenging free radicals from tissues, thus reducing oxidative stress. It is reasonable to suggest that the phytochemicals shown in Table 1 may act individually or synergistically to produce the observed hepatoprotective activity of red betel. For example, flavonoids and tannins are phenolic compounds, and plant phenolics are a major group of compound that act as primary antioxidants or free radical scavengers. Similarly, terpenoids act as regulators of metabolism and play a protective role as antioxidants (Soetan, 2008). Possibly, flavonoid and alkaloids present in crude leaf extract exerted hepatoprotective effect by their free radical scavenging activity, prevention of lipid peroxidation and damage to cells as such an action has been suggested for some other plants (Cheedella *et al*., 2013). Besides, alkaloids and flavonoids are known as natural antioxidants by their free radical scavenging activity (Pietta, 2000).

As the liver is continuously exposed to oxidative stress, the release of free radicals is the main hepatotoxicity mechanism of toxicants. Free radicals damage and oxidative stress are the major reasons for liver tissue damage. In oxidative stress, the balance between the formation of reactive oxygen species and the amount of antioxidants is disturbed. Oxidative stress causes damage to cell components, such as proteins, lipids and nuclei acid (Spahis *et al*., 2017). To confirm the antioxidant activity of the plant extract, *in vitro* DPPH radical scavenging assay was carried out. In this free radical scavenging assay, 70% ethanol extract of RBLE were observed (Table 2). The crude ethanol extract of red betel had a calculated IC50 of 196.10 µg/mL, which is nearly similar to the calculated IC50 value of the known antioxidant, ascorbic acid, I,e 73.61 µg/mL (Oleszek and Kozachok, 2018). Thus, the RBLE hepatoprotective mechanism might result from their radical scavenging neutralization of the free radicals and diminishing generation of ROS. Antioxidants are required in the body as a shield against ROS by physiological and pathological processes.

The mechanism of hepatoprotective is by detoxifying toxic compounds, increasing the regeneration of liver cells, 45nti-inflammatory and as an immunomodulator (Balne *et al*., 2013). As the anti-inflammatory agent, flavonoids can restore the permeability and increase the resistance of the capillary of blood vessels (Fitriyani *et al*., 2011).

Result of histological studies provide supported evidence for biochemical analysis. In normal control rats (belong to group I) liver sections showed normal hepatic cells with normal cellular architecture and normal hepatic lobulus (Fig. 1A). In negative control animals (belong to MSG intoxicated, Group II) the liver section showed total loss of cellular architecture with degeneration and necrotic hepatocyte (Fig. 1B). These findings indicate liver cell injury. The rats' liver section treated with monosodium glutamate and 200 mg/kg bw of RBLE which return to about normal in the recovery group as the liver enzymes and lipid peroxidation were decreased with increased antioxidant enzymes (Fig. 1C). This indicates the hepatoprotective activity of RBLE, while liver section treated with monosodium glutamate and 400mg/kg bw RBLE (Fig. 1D) and liver section treated with monosodium glutamate and 600 mg/kg bw RBLE (Fig. 1E) showing cytoplasmic vacuolation of hepatocyte and necrosis of sporadic hepatocyte. Necrosis can be attributed to severe liver cell injury and degeneration. As the centrilobular hepatocytes have more surface receptors, so they are primary sites of toxins. As a defense mechanism against toxic substance, the hepatocytes tend to vacuolate. These vacuoles are responsible for prevention of the toxic substance for interfering with biological activities of these cells (Kumar *et al*, 2015). Improvement of MSG-induced hepatic histopathological changes was observed in rats after adding RBLE at dose 200 mg/kg bw to

MSG-fed groups. This means that RBLE has antioxidant effect and protective role against liver cell injury. Egbuonu *et al*., (2009) indicated that MSG induced hepatic necrosis and degeneration of hepatocytes. Histological changes to the liver in MSG group have similar outcome to the data by Shresta *et al*. (2018), where vacuolated hepatocytes and congested central veins with blood cells were observed after given MSG at 600 mg/kg.

Hepatocellular death is considered as an important process involved in the liver injury progression. Several types of cell deaths have been reported to take place side by side in the development of the injury. Apoptosis and necrosis are the types of cell death that involved in the process (Weng *et al*., 2015). Apoptosis is one of programmed cell death phenomenon known by several characteristic including cell shrinkage and DNA fragmentation. The generation of ROS is one the effective mechanism which leads to apoptosis (Kaur *et al*., 2020).

In this study, treatment with RBLE at dose 200 mg/kg b.w successfully shifted hepatic cell death pathway from necrosis to apoptosis. It was shown that necrotic cells percentage were significantly decreased, while the apoptotic cell percentage of RBLE treatment groups were significantly higher than liver injury model (Table 4). This result demonstrated RBLE act as anti-inflammatory agent, as apoptosis has known to trigger lower inflammation response compared to necrosis (Luedde *et al*., 2014). RBLE treatment were also able to decrease dead cells percentage, and subsequently improve cell condition as shown by significant increase of live cells percentage.

Based on this study, it was evident that RBLE at dose 200 mg/kg b.w demonstrated its hepatoprotective effect through its antioxidant and inflammatory activities. RBLE has been proven to contain many phytochemicals that could be potential to be used for liver injury medication.

Conclusion

Administration of MSG significantly increased the levels of AST, ALT, the markers of hepatotoxicity. Supplementation with RBLE decreased the markers of hepatotoxicity and exerted significant protection against MSG-induced toxicity by its ability to decrease the lipid peroxidation and thus oxidative stress through its free radical scavenging activity, which improved the levels of antioxidant defense system. RBLE at 200 mg/kg B.W improved the hepatic histopathological damages, i.e decrease necrotic cells induced by MSG. This study revealed the hepatoprotective potential of RBLE against MSG-induced liver damage. These results could be used as a baseline in the purification of red betel bioactive components to be used for liver injury medication.

Acknowledgements

The authors are grateful to the Biology Faculty, Universitas Nasional Jakarta, Indonesia, for accommodating space in the animal house.

References

1. Abdallah, H.M, et al (2013). Protective Effect of Echinops galalensis Against Ccl4 Induced Injury on The Human Hepatoma Cell Line (Huh7). Phytochemistry Letters, 6(1): 73-78.
2. Abiodun, F, et al (2011). Phytochemical and Anticancer Studies on Ten Medicinal Plants Used in Nigeria. Bayero Journal of Pure and Applied Sciences, 4(1): 36–39. DOI:10.4314/bajopas.v4i1.7.
3. Alalwani, A.D. (2014). Monosodium glutamate induced testicular lesions in rats (histological study). *Middle East Fertility Society Journal*, 19: 274-280. http://dx.doi.org/10.1016/j.mefs.2013.09.003
4. Al-Mamary, M, (2002). Investigation into the toxicological effects of Catha edulis leaves: a short-term study in animals. Phytotherapy Research,16:127- 132. doi: 10.1002/ptr.835.

5. Aniagu, S.O, et al (2004). Toxicity studies in Rats fed nature cure bitters. African Journal of Biotechnology, 4(1):72-78.
6. Arika, W.M. et al (2016). Biochemical Markers of In Vivo Hepatotoxicity, Journal Clinical toxicology, 6(2):297-305. DOI: 10.4172/2161-0495.1000297.
7. Balne, D., et al (2013). Hepatoprotective effect of whole plant extract fractions of Marsilea minuta Linn. Asian Journal of Pharmaceutical and Clinical Research, 2013;6:100-107.
8. Beyreuther K., Biesalski, H.K. and Fernstrom J.D. (2007). Consensus meeting: monosodium glutamate - an update. *European Journal of Clinical Nutrition,* 61(3): 304–13. DOI: 10.1038/sj.ejcn.1602526
9. Cheedella, H.K., Alluri, R. and Ghanta, K.M. (2013). Hepatoprotective and antioxidant effect of Ecbolium viride (Forssk.) Alston roots against paracetamol-induced hepatotoxicity in albino wistar rats. Journal of Pharmacy Research, 7(6):496–501. doi:10.1016/j.jopr.2013.06.001.
10. Clark, J.D. et al (1997). The 1996 guide for the care and use of laboratory animals. ILAR J, 38(1):41-48. doi: 10.1093/ilar.38.1.41.
11. Cortese, B.M. and Phan, L.K. (2005). The Role of Glutamate in Anxiety and Related Disorders. CNS Spectrum. 10(10): 820-830. DOI: 10.1017/s1092852900010427
12. Delgado-Montemayor, C. et al (2015). Models of Hepatoprotective Activity Assessment. Med. Universitaria, 17(69): 222-228.
13. Dervis, E. et al (2017). In vitro incorporation of radioiodinated eugenol on adenocarcinoma cell lines (Caco2, MCF7, and PC3). Cancer Biotherapy and Radiopharmaceuticals, 32(3):75–81. DOI:10.1089/cbr.2017.2181.
14. Drury, R.A. and Wallington, E.A,(1980). Carleton's Histological Technique. 5th Edition, Oxford University Press, New York.195 pp.
15. Egbuonu, A.C.C. (2010). Some biochemical effects of subacute oral administration of L-arginine on monosodium glutamate-fed wistar albino rat 2: serum alkaline phosphatase, total acid phosphatase and aspartate amino transferase activities. Asian Journal of Biochemistry,5: 89-95.
16. Farombi, E.O. & Onyema, O.O. (2006). Monosodium glutamate induced oxidative damage and genotoxicity in the rat: modulatory role of vitamin C, vitamin E and guercetin. Human Experimental Toxicology, 125: 251-259. http://dx.doi.org/10.1191/0960327106ht621oa
17. Fitriyani, A. et al (2011). Antiinflammatory test of red betel leaf methanol extract (Piper crocatum Ruiz & Pav) in white rats. Traditional Medicine Journal, 16:34-42. https://doi.org/10.22146/tradmedj.8020.
18. Geha, R.S.(2000). Multicenter, double-blind, placebo-controlled, multiple-challenge evaluation of reported reactions to monosodium glutamate. Journal of Allergy and Clinical Immunology,106(5):973-80. doi: 10.1067/mai.2000.110794.
19. Henry, H.N. (2017). Review update on food safety of Monosodium L-Glutamat (MSG). Pathophysiology. 24:243-249. DOI: 10.1016/j.pathophys.2017.08.001.
20. Hiraganahalli,B.D. et al (2012). Hepatoprotective and Antioxidant Activity of Standardized Herbal Extracts. Pharmacognosy Magazine, 8(30): 116-123. doi: 10.4103/0973-1296.96553
21. Ishak, K.G., Zimmerman, H.J. and Ray, M.B. (1991). Alcoholic liver disease: pathologic, pathogenetic and clinical aspects. Alcoholism: Clinical and Experimental Research, 15(1):45-66. doi: 10.1111/j.1530-0277.1991.tb00518.x.
22. Jiang, S.X.,(2005). Chlortetracycline and demeclocycline inhibit calpains and protect mouse neurons against glutamate toxicity and cerebral ischemia. Journal of Biological Chemistry, 280: 33811–33818. doi: 10.1074/jbc.M503113200.
23. Kaur, S., et al (2019). Amelioration of hepatic function, oxidative stress, and histopathologic damages by Cassia fistula L. fraction in thioacetamide-induced liver

toxicity. Environmental Science and Pollution Research (ESPR), 26(29): 29930–29945. doi: 10.1007/s11356-019-06158-y.
24. Kaur, S. et al (2020). Antioxidant, Antiproliferative and Apoptosis-Inducing Efficacy of Fractions from Cassia fistula L. Leaves. Antioxidants, 9(2):173. doi:10.3390/antiox9020173
25. Kumar, U., et al (2010). Phytochemical investigation and comparison of antimicrobial screening of clove and cardamom. International Journal of Pharmaceutical Sciences Rresearch, 1(12):138–147. http://dx.doi.org/10.13040/IJPSR.0975-8232.1(12).138-47
26. Kumar, S., Kumar, N. and Kumar, B. 2015. Evaluation of monosodium glutamate induced hepatotoxicity in adult wistar albino rats. World Journal of Pharmaceutical Research, 4(2): 569-84.
27. Lister, I.N.E. et al (2019). Antioxidant properties of red betel (Piper crocatum) leaf extract and its compounds. Journal of Natural Remedies, 19(4):198–205. https://doi.org/10.18311/jnr/2019/23633
28. Lister, N.E et al (2020). Hepatoprotective properties of red betel (Piper crocatum Ruiz and Pav) leaves extract towards H2O2-induced HepG2 cells via anti-inflammatory, antinecrotic, antioxidant potency. https://doi.org/10.1016/j.jsps.2020.08.007.
29. Luedde, T., Kaplowitz, N. and Schwabe, R.F. 2014. Cell death and cell death responses in liver disease: mechanisms and clinical relevance. Gastroenterology 147 (4): 765–783. doi: 10.1053/j.gastro.2014.07.018.
30. Masre, S.F. et al (2019). Biochemical and Histological Effects of Low Dose of Monosodium glutamate on the liver of adult male Sprague-Dawley rats. Jurnal Sains Kesihatan Malaysia. 17(2): 107-112. DOI:10.17576/JSKM-2019-1702-12.
31. Mattson, M.P. (2008). Glutamate and neurotrophic factors in neuronal plasticity and disease. Annals of the New York Academy of Sciences,144: 97–112. doi:10.1196/annals.1418.005.
32. Bender D.A., and Mayes P.A. 2018. The citric acid cycle: the central pathway of carbohydrate, lipid, & amino acid metabolism. In Harper's Illustrated Biochemistry, 31e. Rodwell V.W., & Bender D.A., & Botham K.M., & Kennelly P.J., & Weil P(Eds.). Mc Grawhill Education.https://accessmedicine.mhmedical.com/content.aspx? bookid=2386
33. &sectionid=187836462
34. Misra, P., et al (2009). Pro-apoptotic effect of the landrace bangla mahoba of Piper betle on Leishmania donovani may be due to the high content of eugenol. Journal of Medical Microbiology. 58(8):1058–1066. doi: 10.1099/jmm.0.009290-0.
35. Moreno, G.et al (2005). Orexin a stimulates hypothalamic-pituitary-adrenal (HPA) axis function, but not food intake, in the absence of full hypothalamic NPY-ergic activity. Endocrine. 26(2):99-106. doi: 10.1385/ENDO:26:2:099.
36. Mujeeb, F., Bajpai, P. and Pathak, N.(2014). Phytochemical Evaluation, Antimicrobial Activity, and Determination of Bioactive Components from Leaves of Aegle marmelos. BioMed Research International 2014: 497606. DOI: 10.1155/2014/497606
37. Nithianantham, K.et al (2011). Hepatoprotective Potential of Clitoria ternatea Leaf Extract against Paracetamol Induced Damage in Mice. Molecules 16: 10134–10145. doi: 10.3390/molecules161210134.
38. Noh, J.R. et al (2015). Sulforaphane protecs afainst aacetaminophen – induced hepatotoxicity. Food and Chemical Toxicology, 80: 193-200. doi: 10.1016/ j.fct.2015.03.020.
39. Novilia, L., Harapap, U., and Hasibuan, P.A.Z.(2018). Evaluation of hepatoprotective effect of ethanolic extract from red betel (Piper crocatum Ruiz and Pav) leaves. Asian Journal of Pharmaceutical and Clinical Research. 11: 248-250. DOI: https://doi.org/10.22159/ajpcr.2018.v11i7.25542

40. Oleszek, M. and Pecio, S. (2018). Antioxidant activity of plant extract and their effect on methane fermentation in bioreactors.I nternational Agrophysics, 32: 395-401. DOI: 10.1515/intag-2017-0031.
41. Onyema, O.O. et al (2006). Effect of vitamin E on monosodium glutamate induced hepatoxicity and oxidative stress in rats. Indian Journal of Biochemistry & Biophysics 43: 20-24.
42. Oyetunji, T.K.(2013). Assessment of the effects of monosodium glutamate on some biochemical and hematological parameters in adult wistar rats. American Journal of BioScience, 1(1): 11-15. DOI: 10.11648/j.ajbio.20130101.13
43. Pareek, A. et al (2013). In Vitro Hepatoprotective Activity of Corchorus Depressus L. Against Ccl4 Induced Toxicity In HepG2 Cell Line. Pharmacognosy Magazine. 5(4): 191-195.
44. Pavlovic, V. et al (2007). Effect of monosodium glutamate on oxidative stress and apoptosis in rat thymus. Molecular and Cellular Biochemistry, 303(1-2):161-6. doi: 10.1007/s11010-007-9469-7
45. Pietta,P.G. (2000). Flavonoids as antioxidants. Journal of Natural Products. 63 (7):1035–1042. doi:10.1021/np9904509.
46. Reis, H.J.et al. (2009). Neurotransmitters in the central nervous system & their implication in learning and memory processes. Current medicinal chemistry. 16:796-840.
47. Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28(1):56–63. DOI: 10.1093/ajcp/28.1.56.
48. Rinanda, T. and Alga, D.M. (2012). Antibacterial activity of red betel (Piper crocatum) leaf methanolic extracts against methicillin resistant Staphylococcus aureus. Proceedings of The 2nd Annual Int. Conference, Syiah Kuala University 2012 & The 8th IMT-GT Uninet Biosciences Conference-Life, 2(1) :270–275.
49. Rundlett, K.L. and Armstrong, D.,W. (1994). Evaluation of free D-glutamate in processed foods. Chirality,6: 277-282. https://doi.org/10.1002/chir.530060410.
50. Samuels, A. (1999). The toxicity/safety of processed free glutamic acid (MSG): a study in suppression of information. Accountability in Research, 6(4):259-310. doi: 10.1080/08989629908573933
51. Sherlock, S. (1997). Assessment of liver function Disease of liver and biliary system: Sheila Sherlock, 10th ed., London; Blackwell science ltd; 17-32.
52. Shih A.Y., et al (2006). Cystine/glutamate exchange modulates glutathione supply for neuroprotection from oxidative stress and cell proliferation Journal of Neuroscience, 26: 10514–10523. DOI: https://doi.org/10.1523/JNEUROSCI.3178-06.2006
53. Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, 191-289.
54. Soetan, K.O. (2008). Pharmacological and other benefits of antinutritional factors in plants. A. Review, African Journal of Biotechnology, 7: 4713-4721. DOI: 10.4314/ajb.v7i25.59660.
55. Spahis, S., et al (2017). Oxidative stress as a critical factor in nonalcoholic fatty liver disease pathogenesis. Antioxidants and Redox Signaling, 26(10):519–541. doi:10.1089/ars.2016.6776.
56. Tojo, A., et al (2002). Angiotensin II and oxidative stress in Dahl saltsensitive rat with heart failure ypertension, 40(6): 834–839. https://doi.org/10.1161/01.HYP.0000039506.43589.
57. Weng, H.L.et al (2015). Two sides of one coin: massive hepatic necrosis and progenitor cell-mediated regeneration in acute liver ailure. Frontiers in Physiology, 6: 178–190. doi: 10.3389/fphys.2015.00178.
58. Widowati, et al (2013). Antioxidant, anticancer and apoptosis-inducing effects of piper extracts in HeLa cells. Journal of Experimental and Integrative Medicine,

3(3):225–230. DOI:10.5455/JEIM.160513.OR.074.
59. Wulandari, N. Et al (2018). Red betel leaves methanolic extract (Piper crocatum Ruiz and Pav.) increases cytotoxic effect of doxorubicin on WiDr colon cancer cells through apoptosis induction. Indonesian Journal of Cancer Chemoprevention, 9(1):1–8.
60. Yaqub, H., et al (2008). Hepatoprotective Effect of N-acetyl Cysteine and/or ßCarotene on Monosodium Glutamate-Induced Toxicity in Rats. Research Journal of Medicine and Medical Sciences, 3(2): 206-215.
61. Yoon, E., et al (2016). Acetaminophen-induced hepatotoxicity: A comprehensive update. Journal of clinical and translational hepatology, 4(2):131–142.
62. Zhang, C., et al (2018). Molecular mechanisms involved in oxidative stress-associated liver injury induced by Chinese herbal medicine: An experimental evidence-based literature review and network pharmacology study. International journal of molecular sciences, 19(9), 2745–2774. DOI: 10.3390/ijms19092745
63. Zia M.S., Qamar K.R.H. and Moazzam K. (2014). Effect of monosodium glutamate on the serum estrogen and progesterone levels in female rat and prevention of this effect with diltiazem. Journal of Ayub Medical College Abbottabad, 26(1):18-20.
64. Zulharini, M. Et al (2018). Methanolic extract of red betel leaves (Piper crocatum Ruiz & Pav) perform cytotoxic effect and antimigration activity toward metastatic breast cancer. Indones. Journal of Cancer Chemoprevention, 8(3): 94–100.