

Natural product of gambier (*Uncaria gambier Roxb*) extracts to counter against hepatotoxicity effects due to monosodium glutamate induction in male mice

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ABSTRACT

Monosodium glutamate (MSG) is often added in foods to enhance the flavor. It has adverse effect to body organs. Natural remedies, such as: gambier have been known for generations used to improve health. Substances contained in gambier, i.e.: catechins are believed to reduce the occurrence of hepatotoxicity. The study aims to analyze the effects of gambier in reducing the hepatotoxicity of MSG. This study with a posttest only control group design. Mice amount 25 (5 groups, $n = 5/\text{group}$). All interventions are given orally for 4 weeks. At the end of the study, it carried out euthanasia taken the liver of mice to made histopathology preparations then examine by light microscope, $\times 400$, $\times 5$ view field. Liver damage was found in each group with various levels of histological appearance: (I) 2 normal, 2 minimal, 1 moderate and none severe; (II) none normal, 1 minimal, 3 moderate and 1 severe; (III) 1 normal, 4 minimal, none moderate and severe; (IV) 2 normal, 3 minimal, none moderate and severe; and (V) 4 normal, 1 minimal, none moderate, and severe. The data were analyzed using Kruskal–Wallis test. The level of liver damage among all groups was significantly different ($p = 0.017$). The same on the Dunn test also showed the level of liver damage in Group-II to compare with other groups ($p < 0.05$). The present study proves that Gambier (*Uncaria gambier Roxb*) can reduce occurrence of hepatotoxicity caused by MSG.

Key words: Gambier, hepatotoxicity, levels of liver damage, male mice, monosodium glutamate

INTRODUCTION

Monosodium glutamate (MSG) is trusted by some peoples as a food flavoring that improves the delicacy of cuisine as a

nonessential amino acid, L-glutamic acid. It can affect major brain functions including synapses formation and stabilization, memory, cognition, learning, and cell metabolism.^[1] When MSG dissolves in water or is separated in saliva, it changes to free salt and becomes glutamate anion. Glutamate opens Ca^{2+} channels in neurons, allows Ca^{2+} to enter cells, and causes depolarization and subsequent action potentials.^[2] It can secrete 5-HT that comes from the presynaptic taste cell transmitter. Glutamate increases of release 5-HT that caused inhibition of taste evoked adenosine triphosphate (ATP). Umami taste was elicited by glutamate as a prototypic gustatory stimulus.^[3]

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MSG possesses a certain dose limit because intake is safely recommended by the World Health Organization (WHO) with the Food and Agriculture Organization of United Nations (FAO) for adults in daily intake of less than 2 g sodium (safely one-tenth of a tablespoon) per day.^[4,5]

In a previous study, teratogenicity was shown by administering MSG 4 mg/gBW orally to female mice during pregnancy. This can lead to a decrease in the number of live fetuses compared to the control group ($p = 0.018$).^[6] Another study found that MSG doses 0.04 and 0.08 mg/kgBW given for 42 days to mice showed several signs, such as: dilatation of the central vein contain cytoarchitectural distortion of hepatocytes, lyses red blood cells, atrophic and degenerative changes in animal's ($p < 0.0001$).^[7] Bhivate and Kamble reported that there were histopathological changes in liver mice ranging from mild disturbances in arrangements of hepatocytes, enlarged central vein and numerous vacuolation to severe infiltration, and infiltration blood cells in a central vein, dependent on prolonged exposure by MSG.^[8] The consumption of MSG reported has adverse effects on organ function induced by oxidative stress in an animals experimental study. The use of the herbal product as preventive/therapy to protect against the bad effects that may arise from excessive MSG consumption.^[1] Many attempts to overcome the toxic effects of oxidative stress have been carried out, for instance the consumption of medicinal plants curcumin,^[9] and other herbs such as gambier with efficacies as antioxidants. Gambier is rich in antioxidants such as catechins. Catechin polyphenols have the effects of strong radical-scavenging and antioxidants that contribute to preventing many diseases. Gambier is effective as an antioxidant by reducing MDA and BGL and increasing the work of SOD enzymes in patients T2DM.^[10]

METHODS

The preclinical research with a randomized posttest control group design conducted at Laboratory Pharmacology, Faculty of Medicine – Universitas Sumatera Utara (USU).

Ethics

This research had approval for the methodology and the concerned ethical issues by Animal Research Ethics Committees (ARC), USU – Indonesia (No. 0090/KEPH-FMIPA/2018).

Preparation of study sample

The protocol of the study

Twenty-five mice of strain Swiss Webster, 10–12 weeks old, weight 20–40 g purchased from the Department of Biology-USU. All mice were an adaptation for 1 week. The standard feds (CP551) were bought from PT. Charoen Phokphan®-Indonesia. The food and water are given ad libitum. The conditions such as room temperature and humidity within the normal limits.

Allocation and treatment groups

The sample size according to Federer's formula:^[11]

$$(t - 1)(n - 1) > 15$$

t = number of groups

n = number of samples

All mice divided to: (i) negative controlgroup (aquadest 0.5cc); (ii) MSG 5 mg/20 gBW as a positive control group; (iii) MSG 5 mg/20 gBW + Gambier 1 mg/20 gBW; (iv) MSG 5 mg/20 gBW in the first 2 weeks followed by Gambier 1 mg/20 gBW in the 2 subsequent weeks; and (v) Gambier 1 mg/20 gBW. All interventions were given orally for 4 weeks.

Determination of doses of monosodium glutamate

A preliminary study had been carried out to obtain the most appropriate doses to trigger the occurrence of hepatotoxicity by the administration of MSG. The variation of doses was based on the safety limit on human converted to dose on mice (5 mg/20 gBW) with aims to find the early doses which induce hepatotoxicity in mice.

The right dose were given of 5% MSG stock solution was 0.1cc from 5 mg/20 gBW orally.

The study used the MSG brand of Ajinomoto®-Indonesia (Product number: 300525F).

Determination of doses of gambier extracts

The human doses were 350 mg dried Gambier (*Uncaria gambir* Roxb) extract converted to 0.91 mg/20 gBW (~1 mg/20 gBW) on mice. It was made into a solution with 1% concentration, as much as 0.1 cc. The Gambier Extract was produced by Sari Uncariae®-Indonesia (Batch no. 0133517).

Histopathology assessment of liver's mice

Liver damage was assessed by observing fields of large view ($\times 400$). To be more objective, the assessment of liver damage was carried out on as many as 5 fields of large view in different areas. The result was inferred as an average of the degrees of liver damage from each large view. To take the livers and make histopathological preparations with H and E staining. Observations were carried out to look for any liver damage *using light microscope type Olympus® CX-22* with $\times 400$ magnification.

Determination of liver damage

Distribution of Necroinflammatory Features:^[12]

1. Focal lobular necrosis (0 = less than one necroinflammatory focus per lobule, 1 = at least one necroinflammatory focus per lobule, 2 = several necroinflammatory foci per lobule or confluent or bridging necrosis)
2. Portal inflammation (0 = absent, 1 = presence of mononuclear aggregates in some portal tracts,

- 2 = mononuclear aggregates in all portal tracts, 3 = large, and dense mononuclear aggregates in all portal tracts)
- Piecemeal necrosis (0 = absent, 1 = focal alteration of the periportal plate in some portal tracts, 2 = diffuse alteration of the periportal plate in some portal tracts or focal lesions around all portal tracts, 3 = diffuse alteration of the periportal plate in all portal tracts)
 - Bridging necrosis (0 = absent, 1 = present) was also recorded.

The documentation ended with assessing the intensity of necroinflammatory lesions (histological activity). This was indicated as follows:

A0 = no histological activity (PMN = 0; LN = 0)

A1 = mild activity (PMN = 1; LN = 1)

A2 = moderate activity (PMN = 2; LN = 1)

A3 = severe activity (PMN = 3; LN = 2)

*PMN = piece-meal necrosis

*LN = lobular necrosis

Statistical analysis

Data were analyzed to Stata IC 15 using Kruskal–Wallis test continued by *post hoc* Dunn test to compare data between groups.

RESULTS

I.1. Histopathology data of mice in group I; II; III; IV, and V.

Table 1: Number of liver damage on mice

Groups	Sample				
	Mice 1	Mice 2	Mice 3	Mice 4	Mice 5
I	2	1	0	1	0
II	2	3	2	2	1
III	1	1	1	0	1
IV	1	1	0	0	1
V	0	0	0	0	1

Each groups n=5

Table 2: Percentage of the liver damage severity on mice by groups

Treatment group	Level of liver damage				p*
	Negative, n (%)	Minimal, n (%)	Moderate, n (%)	Severe, n (%)	
I	2 (40)	2 (40)	1 (20)	-	0.017
II	-	1 (20)	3 (60)	1 (20)	
III	1 (20)	4 (80)	-	-	
IV	2 (40)	3 (60)	-	-	
V	4 (80)	1 (20)	-	-	

*Kruskal–Wallis test

Table 1 presents the number of liver damage to the mice. Group-V, given only gambier orally for 4 weeks, shows the fewest liver damage among all groups while group-II possesses the highest number of liver damage.

The percentage of liver damage levels on the mice in each group depends on their liver histological appearances. It showed a significantly different ($p = 0.017$) [Table 2 and Figure 1].

The comparison between the group given only with MSG (group-II) for 4 weeks with the other groups shows a significantly difference in the levels of liver damage ($p < 0.05$) by *post hoc* Dunn test.

Figure 1 shows that the assessment of the degree of liver damage on mice due to MSG exposure and histopathological reduction of hepatotoxicity calculated based on a modification of the METAVIR^[12] criteria, as follows:

- Group-I: A0 = No histological activity: Absent portal inflammation and absent lobular necrosis. Some areas show minimal ballooning degeneration
- Group-II: A2 = Moderate activity (PMN: 2, presence of mononuclear aggregates in many portal tract; LN: 1, several necroinflammatory foci per lobule)
- Group-III: A1 = mild activity (PMN: 1, presence of mononuclear aggregates in some portal tract; LN: 1, much-ballooning degeneration)
- Group-IV: A1 = mild activity (PMN: 1, presence of minimal mononuclear aggregates in some portal tract)
- Group-V: A0 = No histological activity: absent portal inflammation and absent lobular necrosis.

DISCUSSION

The liver damage induced by MSG could be observed after a dose of 5 mg/20 gBW was given and consumed for 4 weeks. It is the minimum MSG dose conversion that can be consumed by humans daily based on the WHO and FAO recommendations.^[4,5]

According to the present study, minimal liver damage also be found on the mice which were not given MSG (group-I). It may be caused many factors besides food additive (such as MSG) exposure. The comparison of group-I to other

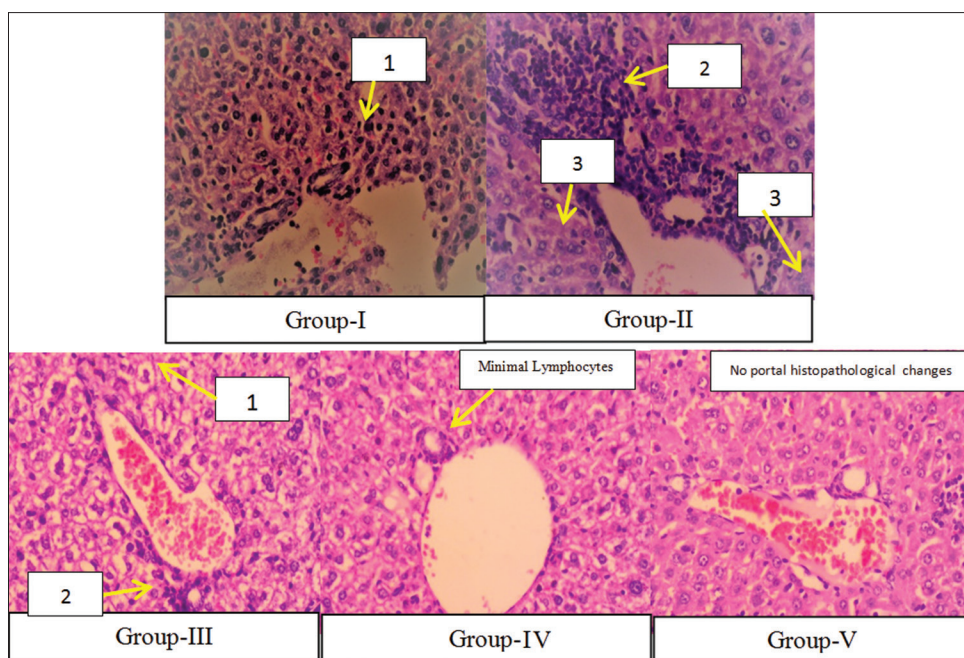


Figure 1: Histopathology of liver damage of male mice in the control group (Group-I) with Group-II, -III, -IV, and -V (H and E, ×400). 1. Balloning degeneration; 2. Inflammatory cells; 3. Necrosis area

groups (III, IV, and V) did not show a significant difference in the levels of liver damage statistically ($p > 0.05$), except on group-II ($p = 0.019$). The group-II was compared to all the treatment groups showed a significant difference in the levels of liver damage ($p < 0.05$). The MSG induced liver damage can be prevented and lessened by administering gambier extract as alternative medicine (on intervention groups III, IV, and V). The most effective dose on group-V (1 mg/20 gBW) of gambier extract daily for 4 weeks [Tables 1, 2 and Figure 1].

The effects of low-dose MSG with 5 mg/kgBW in Albino rats (equivalent to 0.14 mg/20 g on mice) for 28 days. Egbuonu *et al.* discovered that MSG increased MDA levels. The serum MDA concentration increased in the MSG group (48.51 ± 0.15 mg/100 ml) compared to the control group (12.53 ± 0.13 mg/100 ml) ($p < 0.05$).^[13] Malondialdehyde as lipid peroxidation product and antioxidant by measuring from cytosolic and mitochondrial antioxidant proteins and enzymes, namely SOD1, SOD2, glutathione peroxidase, and UCP2 in control of hepatic ROS accumulation. UCP2 is a mitochondrial inner membrane protein that causes protein leakage with an oxidation release mechanism from ATP. This activity causes the liver to be more susceptible to damage.^[14] The increased on hepatic lipids can reduce GSH, CAT, and SOD activity because of MSG intervention.^[15]

The degree of liver damage occurred showed in the histopathological of our study. Although there were differences in doses (Egbuonu used a dose equivalent to 0.14 mg compared to our study using a dose of 5 mg/20 gBW) [Figure 1]. The present study used herbal gambier to treat

hepatic ROS accumulation. Gambier contains many antioxidants, i.e., catechins. It has strong radical effects and antioxidants that contribute to preventing the diseases. Gambier was effective as an antioxidant by reducing the levels of MDA, BGL, and increasing the mechanism of the enzyme SOD in T2DM patients.^[10]

Excessive consumption of MSG can also cause a significant elevation in glutamate and glutamine levels in the blood. The increase in glutamate triggers hyperlipidemia and hyperglycemia.^[15] Hyperglycemia can also trigger auto-oxidation of aldoses and ketoses in forming reactive dicarbonyl sugar (glucose), which reacts with proteins to form ketoimines. The reduced oxygen from the oxidation process will form superoxide and hydrogen peroxide ions, which can precipitate oxidative damage to tissue molecules.^[16] Glycosylation auto-oxidation is a mechanism that generates free radical production. Increased lipid peroxidation in liver microsomes is also caused by escalated levels of glutamate and glutamine, which support the occurrence of lipogenesis. Glutamate can accumulate intracellularly and affect redox reactions in the cells. In addition, an increase in calcium ions due to MSG can promote the occurrence of the lipid peroxidation process. Therefore, MSG is both a hepatotoxic and oxidizing substance, which can cause oxidative stress and liver damage.^[7]

Another study found that MDA levels decreased and SOD levels increased significantly. It was observation implemented in a short period. Although MSG was given in very large doses (equivalent to 56 mg was converted

to mice's dose compared to our study), but there was not show histopathological changes significantly on the organs.^[17] Interestingly, compared to our study, we had found histological changes in the livers by a dose of 5 mg/20 gBW MSG for 28 days.

We suggested for the study further needed to be conducted to re-evaluate the variation of doses of MSG in long-term studies and administration of gambier extract as an alternative medicine to prevent and lessen hepatotoxicity caused by MSG exposure.

CONCLUSION

The gambier extract proved effective in reducing hepatotoxicity induced by MSG.

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Conflicts of interest

There are no conflicts of interest.

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