Pharmaceutical and Biomedical Sciences Journal
Volume 8(1), June 2026, 33-40

The Effect of Purified Gambier (Uncaria gambir Roxb.) and Vitamin C on Aspartate Aminotransherase (AST) Levels of Male White Mice

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Received: 12 November 2025; Accepted : 29 June 2026

Abstract: This study aimed to evaluate the effects of purified gambir (Uncaria gambir Roxb.), vitamin C, and their combination on AST levels in male mice subjected to maximal physical activity. Twenty-five male albino mice were randomly divided into five groups (n=5/group): normal control, maximal physical activity control, purified gambir 200 mg/kg body weight + maximal physical activity, vitamin C 65 mg/kg body weight + maximal physical activity, and a combination of purified gambir 100 mg/kg body weight and vitamin C 32.5 mg/kg body weight + maximal physical activity. Treatments were administered orally for 7 days. On day 8, maximal physical activity was induced by forced swimming for approximately 15 min. Serum AST levels were measured photometrically at 340 nm. Data were analyzed using the Shapiro-Wilk test, Levene's test, Kruskal-Wallis test, and exploratory Mann-Whitney U test. The mean serum AST levels in Groups I, II, III, IV, and V were 141.4 ± 5.98, 146.6 ± 6.88, 122.6 ± 14.26, 124.8 ± 9.96, and 118.8 ± 6.46 IU/L, respectively. The Kruskal-Wallis test showed a significant overall difference among groups (p=0.007). Descriptively, the purified gambir, vitamin C, and combination groups showed lower AST levels than the maximal physical activity group. The combination group showed the lowest mean AST level; however, no significant difference was found between the combination group and the single-treatment groups in exploratory pairwise analysis. Purified gambir, vitamin C, and their combination were associated with lower serum AST levels in male mice subjected to maximal physical activity compared with the physical activity-only group. Although the combination treatment showed the lowest mean AST level, its statistical superiority over purified gambir alone or vitamin C alone was not demonstrated. These findings suggest a potential protective effect against exercise-associated tissue stress, although further studies using larger sample sizes and more specific biomarkers are needed.

Keywords: antioxidant activity; catechin; flavonoid; exercise-induced oxidative stress; forced swimming; hepatocellular injury

DOI: https://doi.org/10.15408/pbsj.v8i1.43581

1. INTRODUCTION

Antioxidants are molecules that can counteract the harmful effects of free radicals (Wibawa, Arifin and Herawati, 2020). Based on their sources, antioxidants are generally classified into synthetic and natural antioxidants (Karim, Jura and Sabang, 2015). Among natural antioxidants, purified gambir and vitamin C have attracted attention because of their antioxidant potential (Marlinda, 2018; Hani and Milanda, 2021). Purified gambir, derived from Uncaria gambir Roxb., is rich in catechin compounds that have been reported to reduce oxidative damage in experimental studies.

Previous studies have shown that gambir possesses antioxidant and protective effects. Purified gambir improved gastric ulcers and reduced serum malondialdehyde (MDA) levels in ethanol-induced rats (Irramah, Julizar and Irawati, 2017). Gambir catechin also reduced serum MDA in a d-galactose-induced mouse model (Situmorang, Zulham and Feryawati, 2021). In addition, gambir extract showed hepatoprotective activity by lowering liver enzyme levels, reducing MDA, maintaining glutathione, and improving histopathological changes in CCl4-induced rats (Fahrudin et al., 2015). Vitamin C is also a well-known natural antioxidant. Previous studies reported that vitamin C supplementation

reduced oxidative stress markers, and its combination with other antioxidants produced greater effects than single treatment alone (Listyawan, 2016; Sinaga and Sinaga, 2015).

Oxidative stress caused by free radicals can induce lipid peroxidation, damage cell membranes, and contribute to various pathological conditions, including liver injury (Hardiningtyas, Purwaningsih and Handharyani, 2014; Siswanto et al., 2014; Nurdyansyah, 2017). When hepatocytes are damaged, intracellular enzymes such as aspartate aminotransferase (AST) leak into the bloodstream, resulting in increased serum AST levels (Prasetyawan, Suarsana and Kendran, 2021). Therefore, AST may be used as a biochemical marker of oxidative stress-related tissue injury.

One trigger of free radical formation is maximal physical activity. During strenuous exercise, oxygen consumption increases markedly, leading to greater free radical production (Harahap and Marpaung, 2021). Since both purified gambir and vitamin C have antioxidant activity, their administration may help reduce oxidative damage. Therefore, this study aimed to evaluate the effects of purified gambir, vitamin C, and their combination on serum AST levels in male mice subjected to maximal physical activity.

2. MATERIAL AND METHODS

2.1 Study Design and Ethical Approval

This study was conducted at the Pharmacology Laboratory and Animal House, Faculty of Pharmacy, Universitas Andalas, over a period of approximately 3 months. The overall workflow of the study is presented in Appendix 1. The experimental protocol was approved by the Ethics Committee of the Faculty of Pharmacy, Universitas Andalas, under approval number 07/UN.16.10.D.KEPK-FF/2023, and the ethics clearance document is provided in Appendix 2.

2.2 Experimental Animals

A total of 25 healthy male albino mice weighing 20–35 g were used in this study. The animals were maintained under standard laboratory conditions and were given standard feed and water ad libitum. Before treatment, all animals were acclimatized for 7 days. During acclimatization, the animals were observed daily and excluded from the study if they became ill, died, or lost more than 10% of their body weight.

2.3 Materials and Instruments

The materials used in this study were purified gambir obtained from PT Andalas Sitawa Fitolab, pure vitamin C, distilled water, 0.5% sodium carboxymethyl cellulose (Na-CMC), TruCal U, and an AST reagent kit (Proline®). The certificate of analysis for purified gambir is provided in Appendix 3, while the composition of the AST reagent kit is shown in Table 3.1. The instruments used included animal cages, an animal balance, an analytical balance (Pioneer®), microtubes (Monotes®), a micropipette (Eppendorf®), tips (Nesco®), a centrifuge (Thermo Scientific®), a 5010 V5+ photometer, test tubes, a tube rack, an oral gavage needle, syringes, a razor blade, a swimming container, and a mortar and pestle.

2.4 Preparation of Test Materials and Dose Determination

Purified gambir was administered at a dose of 200 mg/kg body weight. The administration volume was determined using the formula:

$$\text{VAO} = (\text{BW} \times \text{dose/kg BW}) / \text{concentration (mg/mL)}$$

Vitamin C was administered at a dose of 65 mg/kg body weight, and the dose conversion is presented in Appendix 4. Purified gambir was suspended in 0.5% Na-CMC, whereas vitamin C was dissolved in

distilled water. The 0.5% Na-CMC suspension was prepared by dispersing 500 mg of Na-CMC in hot water, stirring until a homogeneous gel formed, and then adding distilled water to a final volume of 100 mL. All test preparations were administered orally using a gavage needle.

2.5 Experimental Groups and Treatment

The animals were divided into five groups, with five mice in each group. Group I received a standard diet and served as the normal control. Group II received a standard diet and was subjected to maximal physical activity. Group III received a standard diet, purified gambir at 200 mg/kg body weight, and maximal physical activity. Group IV received a standard diet, vitamin C at 65 mg/kg body weight, and maximal physical activity. Group V received a standard diet, a combination of purified gambir at 100 mg/kg body weight and vitamin C at 32.5 mg/kg body weight, and maximal physical activity.

2.6 Induction of Maximal Physical Activity

Maximal physical activity was induced by forced swimming for approximately 15 min. After swimming, the animals were dried for approximately 10 min before blood collection.

2.7 Blood Collection and Serum Preparation

For serum collection, blood samples were collected terminally from the carotid artery under inhalational ether anesthesia in accordance with the approved experimental protocol. The blood was transferred into centrifuge tubes and centrifuged at 3000 rpm for 10 min to separate the serum. The serum was then transferred into microtubes for AST analysis.

2.8 Measurement of Serum AST

Serum AST activity was measured using a 5010 V5+ photometer at a wavelength of 340 nm. The working reagent was prepared by mixing AST Reagent I and AST Reagent II in a ratio of 4:2. For the assay, 100 µL of serum was mixed with 1000 µL of the working reagent, incubated for 1 min, and then measured photometrically.

2.9 Statistical Analysis

Data normality was assessed using the Shapiro-Wilk test, and homogeneity was evaluated using Levene's test. Since not all groups were normally distributed, differences among groups were analyzed using the Kruskal-Wallis test. Pairwise comparisons were subsequently explored using the Mann-Whitney U test. Because these pairwise comparisons were not adjusted for multiple testing, the results were interpreted cautiously. A p-value of less than 0.05 was considered statistically significant for the overall analysis.

3. RESULTS AND DISCUSSION

Purified gambir used in this study contained 91.8% catechin and was obtained from PT Andalas Sitawa Fitolab. A total of 25 male albino mice aged 2–3 months and weighing 20–35 g were used in this study. During the 7-day acclimatization period, no animals showed a body weight loss of more than 10%, and all animals met the inclusion criteria for the experiment, as shown in Table 1. After acclimatization, the animals received treatment according to their respective groups for 7 days, followed by maximal physical activity on day 8 in Groups II–V.

The mean serum AST levels in Groups I, II, III, IV, and V were 141.4 ± 5.98331, 146.6 ± 6.87750, 122.6 ± 14.25833, 124.8 ± 9.95992, and 118.8 ± 6.45755 IU/L, respectively, as presented in Table 2. The highest mean AST level was observed in Group II, which received maximal physical activity only, whereas the lowest mean AST level was observed in Group V, which received the combination

of purified gambir and vitamin C. Groups III, IV, and V showed lower AST levels than both Group I and Group II. However, all AST values remained within the reported normal range for mice (BPOM, 2021).

Table 1: Body weight of mice before and after acclimatization

Treatment group	*Animal*	*Before (g)*	*After (g)*	*Difference (g)*	*% change*	*Mean ± SD**
Standard diet	1	28.1	28.7	0.6	↑ 2.14	29.34285714 ±
	2	28.3	32.1	3.8	↑ 13.43	1.275628038
	3	28.2	30.5	2.3	↑ 8.17	
	4	27.7	28.3	0.6	↑ 2.17	
	5	29.1	30.3	1.2	↑ 4.12	
Standard diet + maximal physical activity	1	28.4	30.0	1.6	↑ 5.63	28.82285714 ±
	2	27.5	29.0	1.5	↑ 5.45	1.064697061
	3	27.5	29.9	2.4	↑ 8.72	
	4	29.4	30.8	1.4	↑ 4.76	
	5	27.0	29.0	2.0	↑ 7.41	
Purified gambir 200 mg/kg BW + maximal physical activity	1	23.4	25.8	2.4	↑ 10.26	28.28285714 ±
	2	26.8	29.0	2.2	↑ 8.21	1.934438011
	3	28.2	29.7	1.5	↑ 5.32	
	4	28.9	31.9	3.0	↑ 10.38	
	5	29.7	31.5	1.8	↑ 6.06	
Vitamin C 65 mg/kg BW + maximal physical activity	1	27.1	25.7	1.4	↓ 5.17	29.07428571 ±
	2	29.2	28.6	0.6	↓ 2.05	1.722744537
	3	27.4	26.5	0.9	↓ 3.28	
	4	27.6	27.4	0.2	↓ 0.72	
	5	31.0	31.6	0.6	↑ 1.93	
Combination (purified gambir 100 mg/kg BW + vitamin C 32.5 mg/kg BW) + maximal physical activity	1	29.6	30.6	1.0	↑ 3.38	29.75428571 ±
	2	27.8	30.5	2.7	↑ 9.71	1.016725672
	3	28.4	30.0	1.6	↑ 5.63	
	4	29.0	29.3	0.3	↑ 1.03	
	5	29.4	29.5	0.1	↑ 0.34	

* Mean value of all experimental animals in one group during 7 days.

Normality testing using the Shapiro-Wilk test showed that AST data in Group I ($p = 0.930$), Group II ($p = 0.172$), and Group III ($p = 0.114$) were normally distributed, whereas Group IV ($p = 0.039$) and Group V ($p = 0.048$) were not normally distributed, as shown in Table 3. Homogeneity testing using Levene's test showed that the data were homogeneous ($p = 0.558$), as shown in Table 4. Since not all groups were normally distributed, differences among groups were analyzed using the Kruskal-Wallis test, which showed a statistically significant overall difference in AST levels among groups ($p = 0.007$), as presented in Table 5.

Table 2. Serum AST measurement results in mice

Animal	*Group I*	*Group II*	*Group III*	*Group IV*	*Group V*
1	140	140	119	118	118
2	134	142	121	118	115
3	139	155	116	124	117
4	150	153	110	142	114
5	144	143	147	122	130
Mean	141.4	146.6	122.6	124.8	118.8
SD	5.98331	6.87750	14.25833	9.95992	6.457554

Group description

- Group I: Standard diet
- Group II: Standard diet + maximal physical activity
- Group III: Standard diet + purified gambir 200 mg/kg BW + maximal physical activity
- Group IV: Standard diet + vitamin C 65 mg/kg BW + maximal physical activity
- Group V: Standard diet + combination of purified gambir 100 mg/kg BW and vitamin C 32.5 mg/kg BW + maximal physical activity

Exploratory pairwise comparisons using the Mann-Whitney U test suggested lower AST levels in the treatment groups than in the maximal physical activity group. No significant difference was observed

between Group I and Group II or Group III, whereas nominally significant differences were observed between Group I and Group IV and between Group I and Group V. Group II also showed nominally significant differences compared with Groups III, IV, and V. No significant differences were found among the three treatment groups. Detailed results of the pairwise comparisons are presented in Supplementary Tables S1–S10. Because these pairwise comparisons were not adjusted for multiple testing, they should be interpreted with caution.

Table 3. Normality test results for AST levels in all treatment groups

Group	***Kolmogorov-Smirnov Statistic[a]***	***df***	***Sig.***	***Shapiro-Wilk Statistic***	***df***	***Sig.***
Standard diet	0.193	5	0.200*	0.979	5	0.930
Standard diet + maximal physical activity	0.300	5	0.162	0.842	5	0.172
Purified gambir 200 mg/kg BW + maximal physical activity	0.345	5	0.052	0.818	5	0.114
Vitamin C 65 mg/kg BW + maximal physical activity	0.332	5	0.075	0.763	5	0.039
Combination of purified gambir 100 mg/kg BW and vitamin C 32.5 mg/kg BW + maximal physical activity	0.349	5	0.046	0.773	5	0.048

***** Lower bound of the true significance.
a. Lilliefors significance correction.

The present study showed that maximal physical activity was associated with a higher mean AST level, as observed in Group II in Table 2, while the groups receiving purified gambir, vitamin C, or their combination showed lower AST values. This finding is biologically plausible because strenuous physical activity markedly increases oxygen consumption and may enhance reactive oxygen species production, resulting in oxidative stress (Sinaga, 2016). Oxidative stress can disrupt cell membrane integrity and increase membrane permeability, allowing intracellular enzymes such as AST to leak into the circulation (Siswanto et al., 2014; Prasetyawan, Suarsana and Kendran, 2021).

Table 4. Homogeneity test results for AST levels in all treatment groups

Variable	***Method***	***Levene statistic***	***df1***	***df2***	***Sig.***
AST level (U/L)	Based on mean	0.769	4	20	0.558
	Based on median	0.310	4	20	0.868
	Based on median and with adjusted df	0.310	4	12.441	0.866
	Based on trimmed mean	0.607	4	20	0.662

The lower AST levels observed in Groups III, IV, and V in Table 2 may be related to the antioxidant properties of purified gambir and vitamin C. Gambir contains catechin compounds and other flavonoid constituents that have been reported to contribute to antioxidant activity (Marlinda, 2018; Hani and Milanda, 2021). Vitamin C is a water-soluble antioxidant that reacts with superoxide anions, hydroxyl radicals, and lipid peroxides, thereby helping preserve membrane integrity (Prasetyawan, Suarsana and Kendran, 2021). These mechanisms may explain the lower AST levels in the treated groups compared with the maximal physical activity group.

Table 5. Kruskal-Wallis analysis of AST levels in all treatment groups

Statistic [a,b]	***AST***
Kruskal-Wallis H	14.054
df	4
Asymp. Sig.	0.007

a. Kruskal Wallis Test
b. Grouping variable: Group

The statistical analysis supports this pattern at the overall group level. As shown in Table 5, the Kruskal-Wallis test demonstrated a significant overall difference in AST levels among groups. Descriptively, the purified gambir, vitamin C, and combination groups showed lower mean AST levels than the maximal physical activity group (Table 2). Exploratory pairwise comparisons presented in Supplementary Tables S1–S10 also suggested lower AST levels in the treatment groups; however, these findings should be interpreted cautiously because the pairwise analyses were not

adjusted for multiple comparisons. Therefore, the most robust interpretation of the present results is that antioxidant treatment was associated with a lower AST profile than maximal physical activity alone, while the relative superiority among treatment groups remains uncertain.

Although the combination group showed the lowest mean AST value in Table 2, this finding should be interpreted descriptively rather than as evidence of statistical superiority. As shown in Table 14 and Table 15, no significant difference was observed between the combination group and the purified gambir-only group ($p = 0.602$) or the vitamin C-only group ($p = 0.113$). Therefore, the combination treatment may be described as having the lowest mean AST level, but not as having the best statistically proven effect.

AST interpretation in this model should be made cautiously. Although AST is commonly used as a marker of hepatocellular injury, it is not liver-specific. Compared with AST, ALT is generally considered more specific for liver injury because AST is also present in extrahepatic tissues, including skeletal muscle, heart, brain, and kidneys (Prasetyawan, Suarsana and Kendran, 2021; American Association for the Study of Liver Diseases, 2025). Therefore, an increase in AST alone cannot be interpreted as definitive evidence of liver injury.

This point is particularly important in the present study because oxidative stress was induced by forced swimming, which imposes substantial muscular workload. Strenuous exercise itself may increase AST through skeletal muscle injury or increased membrane permeability, even in the absence of primary liver damage (Pettersson et al., 2008; Kim and Wu, 2020). Previous studies have shown that intense exercise can cause elevations in aminotransferases in healthy individuals, and these changes may persist for at least 7 days after exercise (Pettersson et al., 2008). Aminotransferase elevation has also been reported in skeletal muscle disorders, further supporting that AST may originate from muscle tissue rather than the liver alone (Nathwani et al., 2005). Thus, in a swimming-based animal model, serum AST alone cannot clearly distinguish hepatic injury from exercise-related muscle effects.

Accordingly, the lower AST levels observed in the treatment groups in Table 2 should be interpreted as evidence of a protective effect against exercise-associated tissue stress rather than definitive proof of hepatoprotection based on AST alone. Additional parameters such as ALT, creatine kinase, lactate dehydrogenase, oxidative stress biomarkers, or liver histopathology would be needed to confirm a specific hepatoprotective effect (Prasetyawan, Suarsana and Kendran, 2021; American Association for the Study of Liver Diseases, 2025).

This study has several limitations. First, AST values in all groups remained within the reported normal range for mice, as shown in Table 2, which may reduce the biological contrast among groups (BPOM, 2021). Second, AST is a non-specific biomarker in an exercise-based model and may reflect both hepatic and muscular sources (American Association for the Study of Liver Diseases, 2025; Pettersson et al., 2008; Kim and Wu, 2020; Nathwani et al., 2005). Third, the sample size was relatively small, with five animals per group. Another limitation is that multiple pairwise comparisons were performed without correction for multiplicity, as presented in Supplementary Tables S1–S10, which may increase the risk of type I error. Therefore, the pairwise findings should be considered exploratory and interpreted with caution. Further studies using larger sample sizes and additional biochemical or histopathological markers are needed to better define the protective effects of purified gambir and vitamin C on oxidative stress-related tissue injury.

4. CONCLUSION

This study has several limitations. First, AST values in all groups remained within the reported normal range for mice, as shown in Table 2, which may reduce the biological contrast among groups (BPOM, 2021). Second, AST is a non-specific biomarker in an exercise-based model and may reflect both hepatic and muscular sources (American Association for the Study of Liver Diseases, 2025; Pettersson et al., 2008; Kim and Wu, 2020; Nathwani et al., 2005). Third, the sample size was

relatively small, with five animals per group. Another limitation is that multiple pairwise comparisons were performed without correction for multiplicity, as presented in Tables 6–15, which may increase the risk of type I error. Therefore, the pairwise findings should be considered exploratory and interpreted with caution. Further studies using larger sample sizes and additional biochemical or histopathological markers are needed to better define the protective effects of purified gambir and vitamin C on oxidative stress-related tissue injury.

5. ACKNOWLEDGMENT

The authors would like to thank the Faculty of Pharmacy, Universitas Andalas, for providing laboratory facilities and research support. Appreciation is also extended to all staff of the Pharmacology Laboratory and Animal House, Faculty of Pharmacy, Universitas Andalas, for their technical assistance during the study.

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