The influence of mountain papaya and bitter melon extract supplementation on aspartate transaminase enzyme in diabetic rats models

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Abstract. The present study aimed to investigate mountain papaya fruit extract (MFE) and bitter melon fruit extract (BFE) supplementation on aspartate transaminase enzyme in diabetic rat models. Aspartate transaminase (AST) is one of the enzymes produced when liver damage occurs by free radicals. MFE and BFE contain flavonoids that have antioxidant activity to neutralize free radicals. Forty rats were divided into 8 groups (n=5) such as normal control, 0.25% CMC-Na as negative control, silymarin at dose 100 mg/kg body weight (b.w) as positive control, and treatment groups by 174 mg/kg b.w MFE, 380 mg/kg b.w BFE, MFE:BFE (75:25)%, MFE:BFE (50:50)%, MFE:BFE (25:75)% orally. Alloxan at dose 150 mg/kg b.w used intraperitoneally for induction. AST level measured before the induction of alloxan (pretest), on day 7th, 14th, and 21st after treatment. The data of AST levels were analyzed statistically using One Way ANOVA and Post Hoc LSD. The results showed that all combined extract and single-dose could significantly reduce AST levels (p<0.05) compared to the negative control group. The effect of a single extract dose was not significantly different (p>0.05) with combination to reduce AST levels.

1. Introduction
Liver damage is a global problem, with a total of 31 million people suffer liver damage. Liver damage caused 2% of deaths worldwide and caused one million deaths in 2010 [1]. Liver damage is mainly caused by toxic chemicals, xenobiotics, immunosuppressants, analgesic anti-inflammatory, anti-tubercular drugs, radiations, heavy metals, mycotoxins, galactosamine, and lipopolysaccharides [2,3]. Free radicals can lead to damage of the liver [4].

The liver is an essential organ as the defense-first line against oxidative damage caused by alloxan [5]. Alloxan and its reduction product, dialuric acid, produce reactive oxygen species (ROS) in a cyclic redox reaction. Dialuric acid autoxidation produces superoxide radicals, hydrogen peroxide, and hydroxyl radicals in a final iron-catalyzed reaction stage. Ultimately, these hydroxyl radicals are responsible for the death of beta cells, which have a feeble antioxidant protection ability, and the subsequent insulin-dependent 'alloxan diabetes' condition. As a thiol reagent, by its capacity to block the beta-cell glucose sensor glucokinase, alloxan also selectively prevents glucose-induced insulin secretion [6]. ROS overproduction causes oxidative stress, which leads to liver damage [7]. The aminotransferase enzymes were investigated to evaluate the biochemical properties of the liver function [8]. AST enzyme
is a sensitive marker in the diagnosis of liver diseases. AST commonly occurs in the cytoplasm. They are released into the blood when an injury to cell membrane damage. AST leak through the liver cell membrane into the blood circulation to the contraction in serum levels increases when liver cells are injured or died [9,10].

Mountain papaya (Vasconcellea pubescens A.DC.) grows in Dieng Plateau. Vasconcellea pubescens A.DC. contains flavonoid and phenolic compounds [11]. Bitter melon (Momordica charantia L.) has many constituents such as cucurbitane, phenolic, flavonoids, alkaloids, and polysaccharides. The phytochemical profile suggests that the main components of the plant may be flavonoids. Flavonoid is known for having an antioxidant activity to scavenge free radicals and decrease oxidative stress in the liver [12].

This study's samples use combination extracts of mountain papaya and bitter melon because both are known as having antioxidant activity [13,14]. The combination extracts produce a more substantial synergistic effect than single extract [15]. The antioxidant effect of mountain papaya and bitter melon combination extracts has not been explored in the hepatotoxic model experimental.

2. Experimentals
2.1. Materials and tools
Mountain papaya fruit (Vasconcellea pubescens A.DC.) obtained from Dieng, Wonosobo, Central Java, Indonesia, and bitter melon fruit (Momordica charantia L.) from Surakarta, Central Java, Indonesia. Wistar rats obtained from Faculty of Medicine Universitas Sebelas Maret, Surakarta, Indonesia. Silymarin (LIV-R-ACTIN®), alloxan monohydrate (Sigma Aldrich®), the reagent of AST (BioSystem®).

2.2. Ethical clearance
All procedures for handling these animals test received approval from The Health Research Ethics Committee of Medicine of Muhammadiyah, ethical clearance number: 1772/A.2/KEPK-FKUMS/I/2019.

2.3. Extract and diabetic rats models
Mountain papaya fruit extract (MFE), bitter melon fruit extract (BFE) preparation, and diabetics rats models are following the research previously published by Sasongko et al. [11].

2.4. The experiment in animal test
The rats were acclimatized in 7 days, then measured the AST levels of pretest data. The dose of alloxan monohydrate 150 mg/kg b.w given intraperitoneally to induce diabetes in rats [17]. After 72 hours of alloxan induction, rats were given the extracts orally once a day. Forty rats were divided into eight groups (n=5): normal (I), 0.25 % CMC-Na as a negative control (II), positive control (III), and five treatment groups. Silymarin 100 mg/kg b.w used as positive control. Treatment groups consist of single extract 174 mg/kg b.w MFE (IV), 380 mg/kg b.w BFE (V), combination extract MFE:BFE (25:75)% (VI), MFE:BFE (50:50)% (VII), MFE:BFE (75:25)% (VIII) orally. Extract dosage based on the total flavonoids value of each extract [18].

2.5. Statistical analysis
Statistical was used for AST levels analysis. The result presented as mean ± standard error of the mean (SEM). Data were checked for normality using Shapiro Wilk and tested homogeneity using Levene’s Test. The difference of investigated parameters between groups was calculated using One Way ANOVA and Post Hoc Test using Least Significant Difference (LSD).

3. Result and discussion
The AST levels test in this research was using alloxan induction, which caused liver damage. Alloxan, as the reduction product of alloxan, establish a redox cycle with the formation of superoxide radicals.
These radicals undergo dismutation into hydrogen peroxide; after that, highly reactive hydroxyl radicals are formed by the Fenton reaction [19]. Reactive oxygen species destroyed liver cells. Increasing of enzyme AST in serum liver indicates hepatocellular destruction. The free hydroxyl group in flavonoids have a radical capturing activity. The presence of more hydroxy groups on ring B will significantly increase the antioxidant activity [20]. The result of the AST levels in diabetic rat models can be seen in table 1.

Table 1. The effect of MFE and BFE on AST levels in diabetic rats models

| Group                  | AST Level ± Standard Error Mean (SEM) (IU/I) |
|------------------------|---------------------------------------------|
|                        | Pretest | Day 7th | Day 14th | Day 21st |                        |
| Normal                 | 135.305±3.832 | 150.333±4.442<sup>ab</sup> | 159.018±1.629<sup>ab</sup> | 157.960±6.890<sup>a</sup> |
| Silymarin 100 mg/kg b.w| 138.266±4.977 | 234.164±3.621<sup>a</sup> | 201.886±2.712<sup>a</sup> | 143.802±2.302<sup>a</sup> |
| 0.25 % CMC-Na          | 136.026±5.567 | 259.113±4.361<sup>b</sup> | 285.883±2.248<sup>b</sup> | 256.434±2.168<sup>b</sup> |
| MFE 174 mg/kg b.w      | 138.199±3.471 | 237.025±2.975<sup>a</sup> | 204.126±1.102<sup>a</sup> | 147.071±3.049<sup>a</sup> |
| BFE 387 mg/kg b.w      | 138.026±5.803 | 237.242±2.896<sup>a</sup> | 204.554±1.248<sup>a</sup> | 145.193±4.095<sup>a</sup> |
| MFE:BFE (25:75)%       | 135.578±9.101 | 231.789±3.096<sup>a</sup> | 203.794±1.602<sup>a</sup> | 170.673±5.064<sup>ab</sup> |
| MFE:BFE (50:50)%       | 112.622±3.732 | 230.219±0.747<sup>a</sup> | 202.816±1.295<sup>a</sup> | 162.535±12.891<sup>ab</sup> |
| MFE:BFE (75:25)%       | 133.772±5.912 | 239.771±5.075<sup>a</sup> | 206.343±1.227<sup>a</sup> | 159.526±2.998<sup>a</sup> |

Description: a: Significant difference between negative control groups (p≤0.05)

b: Significant difference between positive control groups (p≤0.05)

The average AST level of control group pretest data was 112.622 ± 3.732 IU/I to 138.266 ± 4.977 IU/I. Base on another study, the normal AST level of rats 141 ± 67.4 IU/I [21]. On the pretest test, AST levels showed that they were still within the normal range in all experimental groups. The data also showed no significant difference in AST levels between one experimental group and another. Table 1 shows that the normal group's AST level has a considerable difference compared to the negative control group (p≤0.05). That indicates alloxan induce affected the liver function, causing toxicity, characterized by an increase in AST levels. Administration of MFE and BFE in the days 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> were significantly different from the negative control group (p≤0.05). It showed all treatment groups, both in combination and single extracts, reduce AST levels. On days 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup>, MFE and BFE treatment groups were not significantly different from the silymarin treatment. This result indicated that all treatment groups reduce AST levels comparable to the positive control. Among the treatment groups (MFE, BFE, and both extract) also did not differ significantly (p≥0.05); it showed that the combination and single extracts have the same effect of reducing AST levels. On the 21<sup>st</sup> day, MFE: BFE (25:75)% and MFE: BFE (50:50)% were significantly different from the positive control group with a higher AST value. The single extract of MFE and BFE 100% was significantly different from the combination of MFE: BFE (25:75)% and MFE: BFE (50:50)% on the 21<sup>st</sup> day.

Mountain papaya fruit and bitter melon extract have antioxidant activity [13,14]. Antioxidants can neutralize free radicals by donating one proton atom so that free radicals are stable and unreactive. The antioxidant activity of mountain papaya fruit and bitter melon is due to flavonoids that have a hydroxyl group in their structure. The liver injury commonly measured by increased serum liver-bound biomarkers increased activities following glucose increase is a crucial consideration in diabetes mellitus.
The catalysis of amino-transfer reactions includes aminotransferases (AST) [22]. The leakage into the bloodstream of these enzymes from the hepatic cytosol was recognized as a liver damage sign [23]. In this research, the untreated liver injury control rats reported an improvement in these biomarker proteins' serum activities that are likely to signal necrotic liver (Table 1). Based on the research, the single extract of mountain papaya fruit was preferred for reducing AST levels at smaller, which were not significantly different (p≥0.05) from the single extract of bitter melon.

4. Conclusion
The conclusion showed that all of the combined extract and single-dose could significantly reduce AST levels. The effect of a single extract dose (MFE and BFE) was not significantly different (p>0.05) with both combinations to reduce AST levels. Further studies should be undertaken to determine the other biochemical parameters such as Alanine aminotransferase (AST), bilirubin, Alkaline phosphatase (ALT), albumin, and total protein.

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