RESEARCH ARTICLE

Soursop Leaf Extract Reduces AST, ALT, Bilirubin Levels, and Liver Damage Scores in Sorafenib-treated Wistar Rats with Hepatocellular Carcinoma

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Abstract

BACKGROUND: Sorafenib, the standard therapeutic agent for advanced hepatocellular carcinoma (HCC), may induce hepatic dysfunction, thereby necessitating adjunctive therapy to mitigate this adverse effect. While preliminary research has suggested that Soursop (*Annona muricata*) leaves exhibit anti-tumor and hepatoprotective properties, their efficacy in mitigating liver damage associated with sorafenib treatment remains unexplored. This study was conducted to assess the liver-protective effects of soursop leaf extract in Wistar rats receiving sorafenib for HCC treatment.

METHODS: Ethanol extract of soursop leaves was prepared using the maceration method. Twenty-nine Wistar rats were divided into five groups: healthy control (HC) group, HCC groups receiving no treatment, sorafenib only, sorafenib + 50 mg/kgBW/day soursop extract, and sorafenib + 100 mg/kgBW/day soursop extract. All groups, except the HC group, were given Diethyl Nitrosamine (DEN) to cause HCC. Following a two-week treatment period, serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed using colorimetric methods, while total bilirubin was assessed using diazo with sulphanilic acid method. From histopathological specimen, relative liver weight was measured and liver damage score was assessed using Hematoxylin and Eosin.

RESULTS: Administration of sorafenib resulted in a reduction of AST, ALT, total bilirubin, relative liver weight, and liver damage scores. Furthermore, the combined administration of sorafenib with soursop leaf extract at dosages of 50 and 100 mg/kgBW/day led to a dose-dependent amelioration of these indicators. The most pronounced improvement was observed with the highest dose of soursop extract, which significantly reduced AST, ALT, total bilirubin, relative liver weight, and liver damage scores compared to the sorafenib-only group.

CONCLUSION: Soursop leaf extract at 100 mg/kgBW/day effectively reduced AST, ALT, bilirubin levels, and liver damage score in sorafenib-treated Wistar rats with HCC, indicating its hepatoprotective effects. These findings suggest that soursop leaf extract may be a promising adjuvant therapy for mitigating sorafenib-induced hepatotoxicity in HCC treatment.

KEYWORDS: *Annona muricata*, hepatocellular carcinoma, sorafenib, AST, ALT, bilirubin, hepatoprotective

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Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent and lethal cancers globally, contributing significantly to cancer-related mortality.(1) HCC is the fourth leading cause of cancer-related deaths worldwide.(2) Risk factors for HCC include alcohol addiction, metabolic liver disease (particularly non-alcoholic fatty liver disease), exposure to aflatoxins and aristolochic acid in food, and chronic hepatitis

B and C.(2) Unfortunately, most patients are diagnosed at an advanced stage when the tumor is incurable, despite the fact that prompt identification is crucial for therapeutic procedures such liver resection or transplantation.(3) Systemic treatment for advanced-stage HCC is progressing slowly, and current options remain limited.(4) Over the past five years, molecular targeted therapy has emerged as the predominant treatment for advanced HCC.(4)

Sorafenib is the first FDA-approved systemic therapy for patients with advanced HCC who are not candidates for surgical resection or liver transplantation.(5) It is a synthetic compound that targets growth signaling and angiogenesis. (5) The Sorafenib HCC Assessment Randomized Protocol (SHARP) trial demonstrated that sorafenib was superior to placebo in patients with advanced-stage HCC, indicating a significant advancement in HCC treatment.(6) For many years, sorafenib was the sole accepted standard of care for advanced HCC.(6) However, sorafenib has been reported to cause liver dysfunction, as evidenced by a case report of severe liver dysfunction characterized by elevated levels of total bilirubin, aspartate aminotransferase (AST), and alanine transaminase (ALT).(7) Sorafenib continues to be an effective initial treatment, but the occurrence of resistance to this drug is increasingly observed.(8,9) Though sorafenib is approved to treat HCC, it can cause serious liver damage and hepatic failure, especially in people with cirrhosis.(10)

The combination of hesperetin with sorafenib shown enhancements in behavioral modifications, hepatic injury, cerebral mitochondrial dysfunction, and hepatic apoptosis relative to the sorafenib-only group in murine models.(11) Sorafenib and epigallocatechin gallate (EGCG) together provide superior chemoprotection and work well against hepatocellular cancer.(12) The two studies indicate that the addition of an adjuvant to sorafenib is necessary to prevent liver dysfunction as a side effect of sorafenib. In addition to hesperitin and EGCG, soursop leaves have also been reported to have hepatoprotective properties.

Various herbal medicines have been known for its medicinal benefits.(13,14) Soursop, or *Annona muricata*, is a tropical plant species belonging to the Annonaceae family, renowned for its numerous medicinal applications. The leaves, fruit, bark, and seeds of soursop have traditionally been employed as empirical remedies for various disorders, including cancer.(15) The active compounds in soursop leaves, known as acetogenins, include annocatacin, annomuricatin, annomuricin, and annomutacin, among others, and are reported to possess antitumor, anti-inflammatory, antihyperlipidemic, antihyperglycemic, and antioxidant properties.(16)

Sorafenib therapy for HCC may induce liver damage; and up to date there is no guideline for adding hepatoprotective agents for sorafenib therapy. Therefore, additional therapy is necessary to mitigate the hepatic adverse effects associated with sorafenib. Furthermore, no *in vivo* studies have been conducted that combine soursop leaves with sorafenib treatment to prevent liver damage caused by diethylnitrosamine (DEN)-induced hepatocellular carcinoma. Soursop leaves is also known for its hepatoprotective, antioxidant and antitumor effects. The hepatoprotective properties of soursop leaves have been demonstrated in studies involving rats administered carbon tetrachloride and acetaminophen.(8,16,17) Soursop leaves have not been studied to reduce liver damage during sorafenib therapy. Previous studies indicate that ALT and bilirubin levels are elevated in individuals with HCC. (18) Total bilirubin, AST, and ALT are often utilized as measures for evaluating liver function.(19,20) This study aimed to investigate the hepatoprotective effects of soursop leaf extract supplementation in HCC-induced Wistar rats receiving standard sorafenib therapy, as assessed by AST, ALT, bilirubin levels, liver damage score, also relative weigh of the liver.

Methods

Extract Preparation

The extract was a concentrated ethanol extract obtained from soursop leaves, prepared by PT Sidomuncul Tbk (Semarang, Indonesia), using the maceration technique. The dried leaves were ground into fine particles. Percolation was carried out with 90% ethanol (solvent, 1:10), and the solvent was evaporated at 60°C under vacuum conditions. In a prior study, the median lethal dose (LD_{50}) of the soursop leaves extract exceeds 2000 mg/kgBW, indicating that the extract was essentially non-toxic.(21) The concentrated soursop leaf extract was subsequently stored at 4ºC. A portion of the crude extract was weighed and dissolved in 10 mg/mL (for dosage 50 mg/kg) or 20 mg/mL (for dosage 100 mg/kg) distilled water, followed by the addition of 1% carboxy methyl cellulose (CMC), to be administered to the experimental rats.

Animal Model Preparation

Male Wistar rats were obtained from the Integrated Laboratory for Research and Testing (LPPT) Universitas Gadjah Mada. The rats underwent a one-week acclimatization period that was provided with food and water *ad libitum.*

The management of Wistar rats adhered to animal welfare regulations. Twenty-three male Wistar rats, aged 6-8 weeks and weighing 160-180 grams, were used in this study. In accordance with the Guidelines for Pharmacodynamics and Pharmacology of Herbs, each group comprised a minimum of five rats. All rats except healthy control were induced intraperitonially with 100 mg/kgBW DEN, once weekly for a duration of six weeks since week-2 to week-7. And the tumor proliferation was later observed until week-16.(22)

Animals Model Intervention

Total 29 Wistar rats were included in this study and were allocated to five groups, where one group as the healthy control, and the four others were induced with HCC. DEN (Sigma Aldrich, St. Louis, MO USA) was used in this study for the for HCC induction. After an induction period (commencing in week-17), the treatment protocol was initiated as follows: 1) The HC group was not HCC-induced, but was only given food and drink *ad libitum* (n=6); 2) The HCC group did not receive either soursop leaf extract or sorafenib (n=6); 3) The sorafenib group did not receive soursop leaf extract but was administered sorafenib at 200 mg/kgBW/day (Nexavar®; PT Bayer Healthcare, Jakarta, Indonesia) orally from week-17, continuing for two weeks (n=6); 4) The AM50 group received soursop leaf extract at a dose of 50 mg/kgBW/day orally, along with sorafenib at 200 mg/kgBW/day (PT Bayer Healthcare), starting from week-17 for a duration of two weeks (n=6); 5) The AM100 group was orally administered soursop leaf extract at a dose of 100 mg/kgBW/day, in conjunction with sorafenib at 200 mg/kgBW/day (PT Bayer Healthcare), commencing from week-17 and continuing for two weeks (n=5) (Figure 1).

Following the treatment period, blood samples were collected from the retro-orbital vein of each rat under general anesthesia with ketamine hydrochloride. After completing the treatment, Wistar rats were euthanized using ketamine, followed by cervical dislocation and liver organ sample was conducted to assess relative liver weight and liver damage score. All procedure in this study was conducted in accordance with the Declaration of Helsinki for animal experiment. Ethical approval was obtained from the Medical Research Ethics Commission of the Faculty of Medicine, Universitas Diponegoro, and Dr. Kariadi Hospital Semarang (Ethical Clearance No. 62/EC/H/FK-RSDK/VIII/2017).

Measurement of serum ALT, AST, Bilirubin and AFP

Serum levels of alanine transaminase (ALT) and aspartate aminotransferase (AST) were assessed using colorimetric methods, with a DiaSys Kit (Diagnostic Systems GmbH, Holzheim, Germany). The total bilirubin levels were measured using the diazo with sulphanilic acid method. Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid, bilirubin reacts with diazotized sulfanilic acid to form azobilirubin (bilirubin total).(20,22) The evaluation of alpha-fetoprotein (AFP) as a diagnostic marker for HCC was conducted using the enzyme-linked immunosorbent assay (ELISA) technique, utilizing Rat Afp(Alpha-fetoprotein) ELISA Kit (Cat. No. ER0074; FineTest, Boulder, CO, USA)

Measurement of Relative Liver Weight

Excised liver, of sacrificed rats were washed in phosphate buffered saline (PBS) and weighed to obtain the absolute

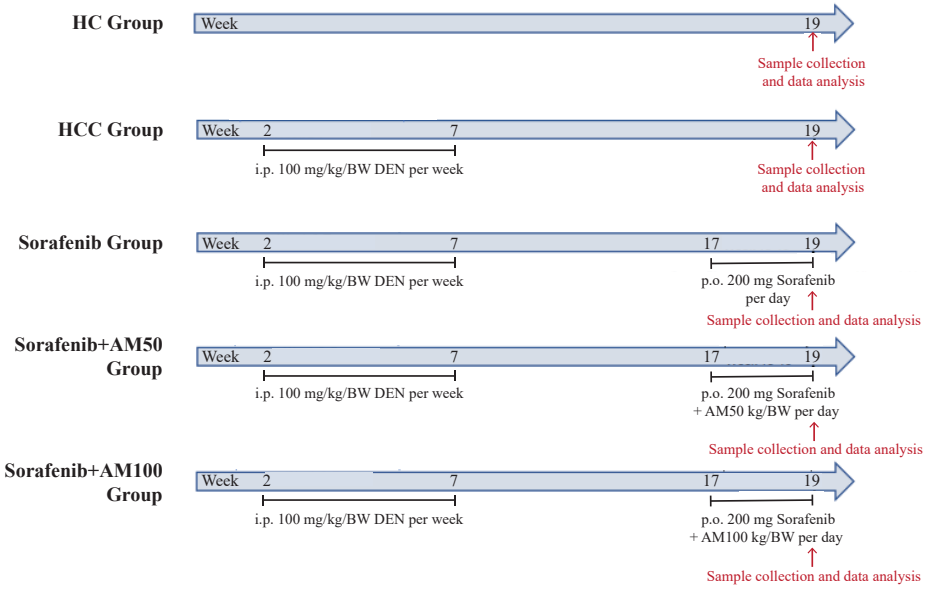

Figure 1. Timeline of research for all experiment groups.

liver weights, were calculated using the formula: Relative Organ Weight = Absolute liver weight / Body weight at sacrified x 100%.(23)

Measurement of Liver Damage Score

Histopathological specimen of Wistar rat livers were stained using Hematoxylin and Eosin (HE), then examined under a light microscope to assess their histopathological features. The evaluation of the histopathological specimen was performed by two independent pathologists in a blinded manner. Initial observation was conducted under 40× magnification to identify any significant abnormalities, followed by detailed examination at higher magnifications of 100× and 400×. Microscopic assessment of liver abnormalities was carried out using a scoring system adapted from the Metavir scoring system and the scoring system for NASH, evaluating three parameters: ballooning cells, inflammation, and fibrosis.

Ballooning cell criteria were divided into four categories: 0 = no ballooning cells, 1 = presence of ballooning cells in a small number, 2 = presence of ballooning cells in a moderate number, and 3 = presence of ballooning cells in a large number. Inflammation criteria were also divided into four categories: 0 = no lymphoplasmacytic inflammatory cell infiltration, 1 = scattered lymphoplasmacytic inflammatory cells in a small number, 2 = scattered lymphoplasmacytic inflammatory cells in a moderate number, and 3 = scattered lymphoplasmacytic inflammatory cells in a large number. Fibrosis/Necrosis criteria were divided into five categories: 0 = no fibrosis/necrosis in the liver, 1 = fibrosis/necrosis present in the perisinusoidal area, 2 = fibrosis/necrosis present in the periportal area, 3 = presence of bridging fibrosis/necrosis, and 4 = formation of nodules in the liver. (24,25)

Statistical Analysis

The data collection process involved several stages, including editing, coding, entry, and cleaning, before tabulation and analysis using a computer program. Data analysis began with univariate analysis to derive descriptive statistics for all variables, specifically the mean or median, standard deviation, and maximum and minimum values. The results of univariate analysis are depicted in a boxplot diagram. Subsequently, the normality of the data was evaluated using the Shapiro-Wilk test. The analysis was then advanced to bivariate analysis to test the hypothesis using the Anova test followed by the Post hoc LSD test. Decisions were made at a significance level of $p<0.05$, with a 95% confidence interval.

Results

Based on data in Table 1, the HC group showed the lowest enzyme levels and liver damage score, indicating normal liver function. In contrast, the HCC group exhibited significantly elevated AST, ALT, and total bilirubin levels, increased relative liver weight, and has highest liver damage score, reflecting liver injury of HCC condition. The levels of serum AFP as a biomarker for HCC were significantly elevated compared to those in healthy control subjects. Treatment with sorafenib alone further increased AST and ALT levels and reduced relative liver weight and liver damage compared to HCC group, while co-administration of sorafenib with soursop leaf extract at doses of 50 mg/kg (Sorafenib+AM50) and 100 mg/kg (Sorafenib+AM100) resulted in a dose-dependent reduction in these markers. Notably, the highest dose of soursop leaves extract (Sorafenib+AM100) showed the greatest improvement, with significantly lower AST, ALT, total bilirubin, relative liver weight, and liver damage scores compared to sorafenib alone. These differences were significant ($p=0.001$), demonstrating the protective effect of soursop leaf extract against sorafenib-induced liver damage.

Reduction of Serum AST Levels After Soursop Leaf Extract Administration

The serum AST levels were lowest in the HC group (56.3±2.15 U/L), indicating normal liver function. The HCC group exhibited significantly elevated AST, reflecting liver injury compared to HC group ($p=0.005$). The administration of sorafenib at a dosage of 200 mg/kg body weight resulted in a statistically significant elevation in serum AST levels when compared to the HCC group ($p<0.001$), thereby indicating hepatocellular damage. Conversely, treatment with ethanolic leaf extract of soursop leaves led to a significant reduction in AST levels in a dose-dependent manner. Specifically, the sorafenib+AM50 and Sorafenib+AM100 group exhibited a notable decrease in AST levels relative to the sorafenib group ($p=0.044$ and $p=0.011$). However, no significant difference was observed between the Sorafenib+AM50 and Sorafenib+AM100 groups ($p=0.462$), suggesting that both doses exert comparable effects.

Reduction of Serum ALT Levels After Soursop Leaf Extract Administration

The ALT levels show a pattern similar to AST, with the HC group exhibiting the lowest value (15.7±2.28 U/L), indicative of normal liver function. The HCC group

Table 1. Effect of different treatment on Serum AFP, AST, ALT, total bilirubin level also relative liver weight and liver damage score.

Groups	AFP (ng/mL)	AST (U/L)	ALT (U/L)	Total Bilirubin (mg/dL)	Relative Liver Weight (%)	Liver Damages Scores
HC	140.0±20.07	56.3±2.15	15.7±2.28	0.09±0.47	2.9±0.08	0
HCC	878.0±16.85	127.7±20.24	73.8±15.68	0.3±0.11	4.0±0.11	37.5±2.74
Sorafenib	252.2±23.65	303.3±48.98	236.9±39.10	0.31±0.16	3.5±0.23	15.83±5.85
Sorafenib+AM50	226.7±16.36	249.8±74.93	183.3±50.28	0.2±0.10	3.7±0.19	17.5±8.80
Sorafenib+AM100	216.3±9.17	230.1±31.24	153.0±27.89	0.08±0.03	3.5±0.29	7.8±2.86
p -value*	0.001	0.001	0.001	0.001	0.001	0.001

Values are presented as mean±SD. *Significance is tested with One-Way Anova. AFP: Alpha Feto-Protein; AST: aspartate aminotransferase; ALT: alanine transaminase.

exhibited significantly elevated AST, reflecting liver injury compared to healthy control (p=0.004).

Serum ALT levels were significantly elevated in the Sorafenib group compared to the HCC group (p<0.001). The administration of Soursop extract resulted in a marked reduction in ALT activity in both the Sorafenib+AM50 and Sorafenib+AM100 groups. Notably, the Sorafenib+AM50 and Sorafenib+AM100 group demonstrated a significant decrease in ALT levels compared to the sorafenib group (p=0.008 and p=0.001), whereas the difference between the Sorafenib+AM50 and Sorafenib+AM100 groups was not statistically significant (p=0.133), suggesting comparable hepatoprotective efficacy.

Reduction of Total Bilirubin Levels After Soursop Leaf Extract Administration

The total bilirubin levels in Table 1 showed a significant increase in the HCC (0.3±0.11 mg/dL) compared to the HC group (0.09±0.47 mg/dL), indicating impaired liver function or bile flow associated with liver damage in the HCC group. Treatment with Sorafenib alone maintain a similarity elevated bilirubin level (0.31±0.16 mg/dL) suggesting limited improvement in bilirubin clearance. However, the combination treatment with Sorafenib and soursop leaves extract (Sorafenib+AM50 and Sorafenib+AM100) show a dose-dependent reduction in total bilirubin levels, with Sorafenib+AM100 restoring bilirubin close to the healthy control level (0.08±0.03 mg/dL). This suggest that soursop leaves extract particularly at 100 mg/kgBW may enhance liver function or bile metabolism when combined with Sorafenib, mitigating liver damage effect more effectively than Sorafenib alone. The Anova test identified significant differences among all groups (p=0.001). Subsequent post hoc LSD test revealed significant differences between the Sorafenib+AM100 and Sorafenib groups (p=0.001), as well as between the Sorafenib+AM50 and HCC groups (p=0.048), while no significant difference was observed between the Sorafenib+AM50 and Sorafenib+AM100 groups (p=0.056).

Improvement of Liver Damages Score After Soursop Leaf Extract Administration

The histopathological assessment aligned with the biochemical results. The HCC group exhibited significantly elevated liver damages score, reflecting liver injury compared to HC group (p=0.001). The HCC group presented the most pronounced hepatic lesions, including hepatocellular degeneration, necrosis, inflammatory infiltration, and fibrosis with a mean score of 37.5. In contrast, the Sorafenib group showed moderate liver damage, scoring 15.8. The Sorafenib+AM50 group had a score of 17.5, while the Sorafenib+AM100 group demonstrated marked histological improvement, achieving a score of 7.8, indicative of nearly normal liver architecture. The Anova test confirmed a significant difference among the groups (p=0.01). Post hoc LSD test highlighted significant differences between the Sorafenib+AM100 and Sorafenib (p=0.033), the Sorafenib+AM100 and HCC (p=0.001), Sorafenib and HCC (p=0.001), the Sorafenib+AM50 and the Sorafenib+AM100 (p=0.012). However, the differences between the Sorafenib+AM50 and Sorafenib group (p=0.622) were not statistically significant. Figure 2 provided representative liver micrographs.

No Significant Difference of The Relative Liver Weight

The mean relative liver weight was presented in Table 1, accompanied by standard deviations for each group. The HCC group exhibited significantly elevated liver damages score, reflecting liver injury compared to HC group (p<0.001). The HCC group presented the most relative liver weight, with a mean score of 4.0. The Anova test confirmed a significant difference among the groups (p<0.001).

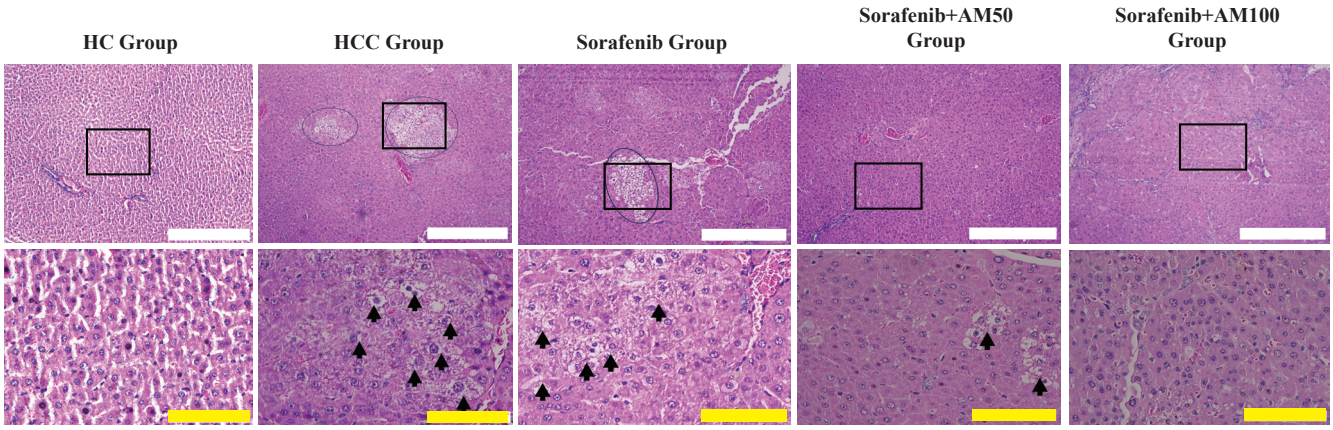

Figure 2. Liver histopathology results across experiment groups. Black arrow: ballooning; Circles: nodule/fibrosis; White bar: 500 µm; Yellow bar: 100 µm. The bottom figures are the zoomed version of the top figures, with the zoom parts marked in black square.

The Sorafenib group showed relative liver weight with score 3.5 lowering than HCC group (p<0.001). Post hoc LSD test highlighted significant differences between HCC and Sorafenib/ the Sorafenib+AM50/ the Sorafenib+AM100 (p<0.001). However, the differences between Sorafenib and Sorafenib+AM50/AM100 groups were not statistically significant (p=0.998 and p=0.169), as well as between Sorafenib+AM50 and Sorafenib+AM100 (p=0.169).

Discussion

This study shows that liver damage caused by DEN leads to problems in liver cells, changing enzyme levels in the blood. The study found that the HCC group had much higher AST, ALT, and bilirubin levels, a bigger liver, and more liver damage than the healthy group. Previous study showed liver damage through high levels of AST, ALT, lactate dehydrogenase (LDH), and alkaline phosphatase (ALP).(22) The level of AFP, a marker for HCC, was also much higher than in the healthy control group. Serum AFP is widely recognized as a key indicator in the progression of HCC.(12)

The findings of this study revealed no significant differences in AST levels between Sorafenib+AM50 and the Sorafenib groups, which were notably higher than those in the HCC group. The AST levels in the Sorafenib+AM50 and Sorafenib+AM100 group exhibited a notable decrease to the Sorafenib group. However, no significant difference was observed between the Sorafenib+AM50 and Sorafenib+AM100 groups, suggesting that both doses exert comparable effect. This suggests that sorafenib administration may elevate AST levels, whereas the combination of 50 and 100 mg/kgBW/day soursop leaf extract with sorafenib may reduce AST levels relative to sorafenib alone. Sorafenib is globally utilized for the treatment of advanced or metastatic HCC.(7) However, other studies have also reported that sorafenib administration can induce severe hepatotoxicity, necessitating close monitoring of patients receiving this treatment, as evidenced by elevated AST, ALT, and total bilirubin levels.(7,26)

`This study highlights the dose-dependent liver-protective effects of soursop leaf extract in rats receiving sorafenib treatment for liver cancer, with the 100 mg/kgBW/day dose showing the greatest benefit. These findings suggest that administering soursop leaf extract at a dose of 100 mg/kgBW/day may lead to a decrease in AST levels in a Wistar rat model of liver cancer undergoing sorafenib treatment. Soursop leaf extract has shown promising hepatoprotective effects in various studies, and previous research has indicated that it may exhibit dose-dependent liver-protective properties.(27)

In this study, ALT levels were much higher in the Sorafenib group than in the HCC group. This matches the findings for AST levels, showing that sorafenib can harm liver function. This was also seen in a case report where sorafenib caused severe liver problems, with high levels of total bilirubin, AST, and ALT.(6,22) Lower ALT levels in the Sorafenib+AM100 group compared to the Sorafenib group means that a daily dose of 100 mg/kgBW/day of soursop leaf extract can mitigate ALT levels in Wistar rat models of hepatocellular carcinoma undergoing sorafenib therapy. This effect may be attributed to the hepatoprotective properties of soursop leaf extract. These findings match earlier studies that showed soursop leaf extract protects the liver in rats affected by DEN, as seen by the changes in ALT,

total cholesterol, total protein, albumin, as well as globulin levels.(28)

Furthermore, soursop extract at 100 mg/kgBW/day for four weeks has been reported to exhibit hepatoprotective and antioxidant effects in streptozotocin-induced diabetic rats, reducing low-density lipoprotein (LDL) and triglyceride levels while enhancing high-density lipoprotein (HDL) and insulin production.(29) Previous study reported that the chemotherapeutic drug (sorafenib), when administered together with an antioxidant (EGCG) in a specific dosage could definitely enhance the functioning of the drug under treatment rather than administering the chemotherapeutic drug alone.(12) ALT and AST values were significantly increased in the sorafenib-treated group relative to the other trial groups. The findings highlighted the effect of including hesperetin into sorafenib therapy on decreasing liver enzyme levels.(11)

Total bilirubin levels did not differ significantly between the Sorafenib and HCC groups, although there was a trend toward higher levels in the Sorafenib group. In contrast, total bilirubin levels in the Sorafenib+AM100 group were significantly lower than those in the Sorafenib+AM50 and Sorafenib groups. Administration of 100 mg/kgBW/day Soursop extract can mitigate liver function disorders, as evidenced by total bilirubin levels in hepatocellular carcinoma rat models treated with sorafenib. These results are consistent with a study reporting that the ethanol extract of soursop leaves has a protective effect on the liver of DEN.(28) *In vivo* studies demonstrated a hepatoprotective effect from the administration of soursop at doses of 50 and 400 mg/kgBW/day orally, which reduced bilirubin levels in phenylhydrazine-induced rats.(30) Hepatoprotective effects have also been reported in studies involving chloroform (CCl_4) and acetaminophen-induced rats, where extracts were administered at doses of 50, 100, 300, and 400 mg/kg for 7 days.(8) Previous studies have reported that the aqueous extract of soursop has a bilirubin-lowering potential.(23)

Elevated serum liver biomarkers indicate hepatocellular injury resulting from sorafenib administration. However, these values were significantly lower in the group receiving Sorafenib+AM100. This study aligns with previous research, which has shown that serum levels of liver biomarkers can decrease to near-normal levels following Soursop extract administration. A prior investigation revealed that administering the ethanolic seed extract of soursop effectively mitigated these anomalies by restoring serum enzyme activities to normal levels.(16,31)
In this current study, the HCC group exhibited the highest hepatic damage score, indicating more severe liver damage than the Sorafenib, Sorafenib+AM50, and Sorafenib+AM100 groups. Sorafenib treatment resulted in improved liver histopathology compared with that in the HCC group. This suggests that both dosage of the extract administration can mitigate the liver damage in HCC with sorafenib treatment. This was consistent with previous research indicating that soursop extract functions as a hepatoprotective agent against hepatotoxic substances. (2,4,8,15,17,29,32)

According to a prior study, sorafenib-treated mice's liver sections stained with H&E showed areas of diffuse lobular necrosis, characterized by severely degenerated ballooned hepatocytes, congested central veins, significant inflammatory infiltrates, pyknotic and karyorrhectic nuclei, and sparse necrotic areas. Hesperetin coadministration, on the other hand, greatly enhanced the liver's histological structure in the combination group, which showed well-organized hepatocyte cords, reduced vascular congestion, and few inflammatory infiltrates.(11)

The result of this study also showed that the HCC group presented the most relative liver weight, with a mean score of 4.0. The Sorafenib group showed relative liver weight with score 3.5 lowering than HCC group ($p<0.001$). However, there was no statistically significant difference between Sorafenib and Sorafenib+AM50/AM100 or between Sorafenib+AM50 and Sorafenib+AM100. This observation aligns with several experimental studies indicating that soursop leaf preparations are generally hepatoprotective or at least not overtly hepatotoxic at moderate doses. Both ethanolic and aqueous leaf extracts have been documented to mitigate histological liver damage and enhance biochemical markers in models of toxin-induced liver injury, without causing significant changes in absolute liver weight.(33) Toxicological studies further suggest that leaf extracts are tolerated at relatively high doses (acute LD_{50} > 2000 mg/kg, with sub chronic administration deemed safe up to approximately 800 mg/kg).(34) This is particularly relevant if the underlying model involves hepatocellular injury or tumor burden, as both sorafenib and soursop have demonstrated efficacy in reducing liver tumor mass or cirrhotic remodeling in experimental systems. This is consistent with the stable relative liver weight observed in the present experiment.(35)

To obtained more detailed mechanism related to the effect of soursop extract as a hepatoprotective agent, the bioactive compounds of the soursop extract should be further analysed to make sure which ones that might be effective as hepatoprotective agents. Unfortunately, in this current study, the analysis was not performed.

Conclusion

The research findings indicate that administering soursop leaf extract, particularly at a dose of 100 mg/kgBW for two weeks to HCC rats treated with sorafenib exhibited hepatoprotective effects. This was evident when compared to the group that received sorafenib only, as assessed by AST, ALT, and bilirubin levels, as well as liver damage score and relative liver weight. This finding suggests that soursop leaf extract is a promising agent for protection against hepatic damage of HCC with sorafenib treatment.

Acknowledgments

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Authors Contribution

NS, YWP, SB, EP and IR were involved in concepting and planning the research; NS, EP, HI and IR performed the data acquisition/collection; NS, SB and MM calculated the experimental data and performed the analysis; NS, HI and MM drafted the manuscript and designed the figures, MM and IR aided in interpreting the results. All authors took parts in giving critical revision of the manuscript. NS and MM approved the final version of the manuscript.

Conflict of Interest

The authors affirm that they have no conflicts of interest or competing interests concerning the content of this manuscript.

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