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Research Article

ANTIOXIDATIVE ACTIVITY OF ALPHA-MANGOSTIN IN ACETALDEHYDE-INDUCED HEPATIC STELLATE CELLS: AN *IN VITRO* STUDY

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ABSTRACT

Objective: Alcohol accumulation in the liver can cause pathological disorders such as liver fibrosis that can develop into hepatocellular carcinoma, one of the main causes of mortality associated with liver disease. The previous studies have shown that a plant compound, alpha-mangostin, has an antioxidant effect in the inhibition of pancreatic tumor growth *in vitro*. This study aimed to analyze the antioxidative properties of alpha-mangostin in acetaldehyde-induced liver fibrosis *in vitro*.

Methods: Immortalized hepatic stellate cells (HSCs), of the LX-2 cell line, were incubated with acetaldehyde in the presence or absence of alpha-mangostin (10 and 20 μM). The cells were then counted and lysed, and LX-2 cell viability was determined with the trypan blue exclusion method. The malondialdehyde levels, superoxide dismutase activity, and glutathione (GSH) levels were also determined using the cell lysates.

Results: Acetaldehyde treatment resulted in a significant increase in HSC cell viability and decreased the production of GSH. Alpha-mangostin treatment resulted in reduced cell viability of the HSCs and prevention of the loss of intracellular GSH.

Conclusion: Alpha-mangostin reduced acetaldehyde-induced cell proliferation, and this was affected at least in part by its antioxidative properties.

Keywords: Acetaldehyde, Alpha-mangostin, Antioxidant.

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INTRODUCTION

Alcohol accumulation in the liver can cause pathological disorders such as liver fibrosis. This disease can develop into hepatocellular carcinoma, which is one of the main causes of mortality associated with liver disease [1,2]. Hepatic stellate cells (HSCs) are central to the development of fibrosis following alcohol accumulation. In the normal liver, HSCs are quiescent cells that differentiate into myofibroblasts when activated. Other than alcohol, HSC can be activated by toxins, disorders of immune systems, or viral and parasitic infections. Active HSCs are characterized by increased proliferation, contractility, and secretion of the fibrogenesis mediator, transforming growth factor β (TGF-β) [3-5].

Alcohol is metabolized in the liver by alcohol dehydrogenase to produce acetaldehyde, a toxic substance, which further metabolized to form acetate, a less active byproduct. When there is a large amount of alcohol, CYP2E1 will also active and metabolize alcohol to acetaldehyde and further produce acetate and reactive oxygen species (ROS) [6,7]. ROS enhance HSC activation and play an important role in the fibrogenesis that occurs in alcohol-induced liver disease. TGF-β increases the production of ROS and suppresses the production of antioxidants. ROS, in turn, activates latent TGF-β and induces its expression. Consequently, TGF-β acts synergistically with alcohol in inducing oxidative stress, thus increasing alcohol-induced liver damage [8,9].

There is no standard therapy for the treatment of liver fibrosis. Research on the treatment of liver fibrosis using active compounds from plants is still ongoing. Alpha-mangostin, one of the main xanthones from *Garcinia mangostana*, is known to have anti-inflammatory, antioxidant, cardioprotective, antidiabetic, and anticancer properties [10,11]. In a previous study by Rahmaniah *et al*., alpha-mangostin was shown to decrease the ratios of pSmad/Smad and pAkt/Akt in an *in vitro* liver fibrosis model induced by TGF-β [12]. Further, investigations of the mechanism of the action of alpha-mangostin as a hepatoprotector are still required. This study aimed to analyze the antioxidant activity of alpha-mangostin in a model of acetaldehyde-induced liver fibrosis.

METHODS

Human HSCs, of the LX-2 cell line, were purchased from Millipore (USA; Cat. No. SCC064). Alpha-mangostin was obtained from Santa Cruz Biotechnology (Texas, USA). Acetaldehyde and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (Singapore). Dulbecco's modified Eagle's Medium (DMEM) high glucose-supplemented media, heat-inactivated fetal bovine serum (FBS), penicillin, streptomycin, and Fungizone were obtained from Biowest (USA). Thermo Scientific Cell Extraction Buffer (Cat No FNN0011) and the Coomassie Plus (Bradford) Assay kit were obtained from Invitrogen (USA).

LX-2 culture

LX-2 cells were cultured and maintained at 37°C in 5% CO_2 in DMEM high glucose supplemented media containing 10% heat-inactivated FBS, 10 U/L penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL Fungizone.

Cell treatments

Cells were divided into five groups and seeded at 2×10^6 each in 10-cm culture dishes. Two groups comprised the untreated cells (medium with DMSO only), whereas the other three groups were subjected to various treatments for 24 h. To the first of the treated groups of cells, acetaldehyde (100 μM) was applied; to the second and third groups of treated cells, acetaldehyde (100 μM) plus alpha-mangostin (10 μM) and acetaldehyde (100 μM) plus alpha-mangostin (20 μM) were applied, respectively. To the fifth group, only alpha-mangostin (10 μM) was

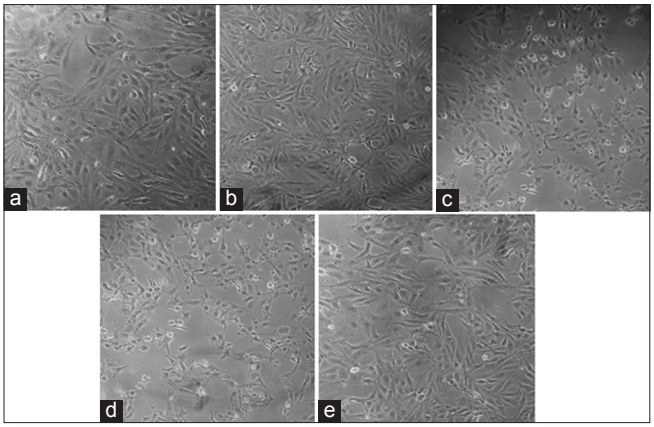

Fig. 1: Treatment of acetaldehyde-induced LX-2 cells with alpha-mangostin. Following 48 h of treatment, morphological observation of the LX-2 cells was performed with an inverted microscope. The treated LX-2 cells did not exhibit any morphological changes (10× magnification). (a) Control group (normal media only), (b) 100 µM acetaldehyde induction, (c) treatment with 10 µM alpha-mangostin and induction with 100 µM acetaldehyde, (d) treatment with 20 µM alpha-mangostin and induction with 100 µM acetaldehyde, and (e) treatment with 10 µM alpha-mangostin (no acetaldehyde induction)

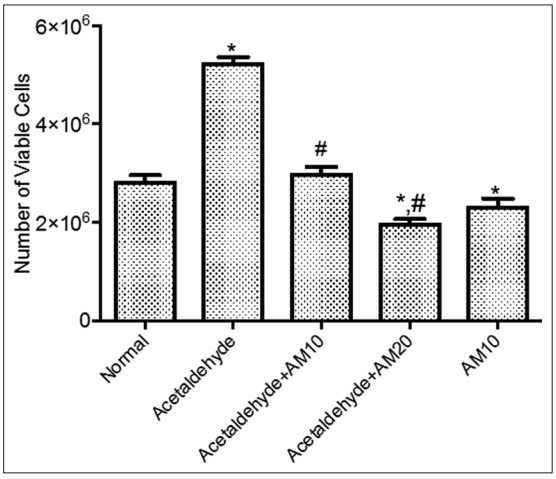

Fig. 2: Effect of alpha-mangostin on hepatic stellate cells proliferation. Cell viability of the LX-2 cells was determined after induction with acetaldehyde and treatment with alpha-mangostin. Normal: Control group; Acetaldehyde: Induced with 100 µM acetaldehyde; Acetaldehyde+AM10: Treated with 10 µM alpha-mangostin and induced with 100 µM acetaldehyde; Acetaldehyde+AM20: Treated with 20 µM alpha-mangostin and induced with 100 µM acetaldehyde; AM 10: Treatment with 10 µM alpha-mangostin only (no induction). Results are presented as the mean ± SE. *p<0.05 versus normal group; #p<0.05 versus acetaldehyde group

applied. After 24 h of treatment, the cells were harvested and subjected to analysis of cell viability, and the proteins were extracted for antioxidant analysis. Cell viability was examined with the trypan blue exclusion method. All experiments were performed in triplicate.

Spectrophotometry analysis

The malondialdehyde (MDA) levels in the cell medium were determined in a microplate reader using colorimetric method described by Siddique *et al.* [13], while glutathione (GSH) levels were measured using Reduced GSH Assay Kit (Elabscience, USA). Activity of superoxide dismutase

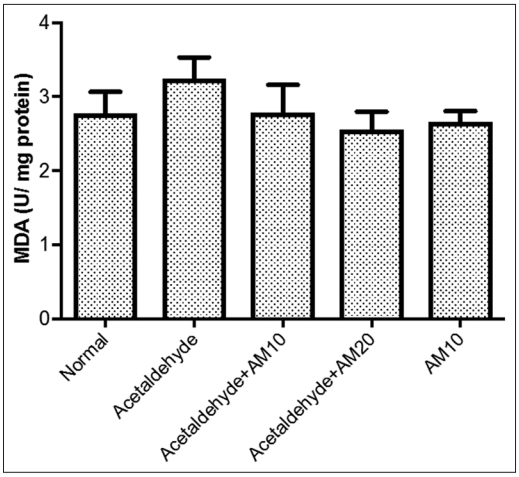

Fig. 3: Effect of alpha-mangostin on malondialdehyde (MDA) levels. The MDA level in LX-2 cells was determined after acetaldehyde induction and treatment with alpha-mangostin. Normal: Control group; Acetaldehyde: Induced with 100 µM acetaldehyde; Acetaldehyde+AM10: Treated with 10 µM alpha-mangostin and induced with 100 µM acetaldehyde; Acetaldehyde+AM20: Treated with 20 µM alpha-mangostin and induced with 100 µM acetaldehyde; AM 10: Treatment with 10 µM alpha-mangostin only (no induction). Results are presented as the mean±Standard error of the mean

(SOD) in cell lysates was measured using the method of Misra and Fridovich [14].

Statistical analysis

Results are presented as the mean±Standard error of the mean. Statistical analysis was performed with one-way analysis of variance. All values with $p<0.05$ were considered statistically significant.

RESULTS

Cell morphology

After 48 h of treatment of the acetaldehyde-induced LX-2 cells with or without alpha-mangostin, no changes in morphology were observed under an inverted microscope (Fig. 1).

Cell viability

Acetaldehyde induction at 100 µM resulted in a significant increase ($p<0.05$) in LX-2 proliferation compared with the control group (Fig. 2). In contrast, alpha-mangostin treatment significantly suppressed ($p<0.05$) acetaldehyde-induced LX-2 proliferation in a dose-dependent manner.

Alpha-mangostin restores MDA levels in the LX-2 culture medium after acetaldehyde induction

Induction with 100 µM acetaldehyde slightly increased MDA levels in the LX-2 culture medium compared with the control group, whereas treatment with alpha-mangostin restores MDA accumulation (Fig. 3).

Alpha-mangostin significantly increased GSH levels

Induction with 100 µM acetaldehyde significantly decreased the GSH levels ($p<0.05$) and SOD activity (not statistically significant) of LX-2 cells compared with the control group, whereas alpha-mangostin significantly enhanced ($p<0.05$) the GSH level (Fig. 4).

DISCUSSION

In this study, no change was observed in the morphology of the LX-2 cells between the control and treatment groups because the cells were already activated with a myofibroblast-like phenotype. Acetaldehyde was, however, observed to enhance HSC proliferation by increasing

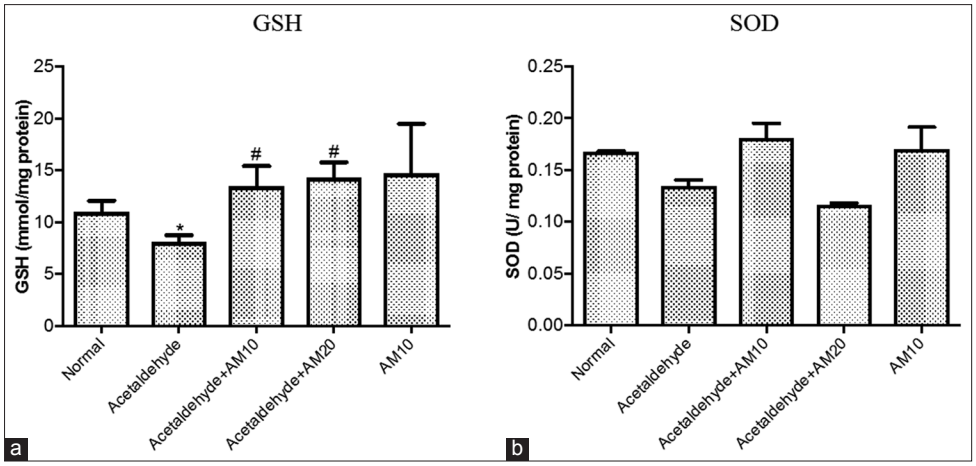

Fig. 4: Effect of alpha-mangostin on antioxidant activity. The glutathione levels (a) and superoxide dismutase activity (b) of LX-2 cells were determined after induction with acetaldehyde and treatment with alpha-mangostin. Normal: Control group; Acetaldehyde: Induced with 100 µM acetaldehyde; Acetaldehyde+AM10: Treated with 10 µM alpha-mangostin and induced with 100 µM acetaldehyde; Acetaldehyde+AM20: Treated with 20 µM alpha-mangostin and induced with 100 µM acetaldehyde; AM 10: Treatment with 10 µM alpha-mangostin only without acetaldehyde induction. Results are presented as the mean ± SE. *p<0.05 versus normal group; #p<0.05 versus acetaldehyde group

cell viability. This result was in line with research conducted by Wang *et al*. and Zhang *et al*., which showed that acetaldehyde significantly increases HSC viability [15,16].

Acetaldehyde was known to elevate intracellular HSC ROS production and oxidative stress which leads to HSC activation promoting fibrogenesis [8,9]. The effects of acetaldehyde on the oxidative stress of HSC cells were studied in this research. Exposure to acetaldehyde caused an increase in the production of ROS in the HSCs, as indicated by a decrease in the concentration of GSH and the activity of SOD, both of which are important in the detoxification of ROS [17]. The effect of alpha-mangostin activity on these processes was then analyzed at two concentrations (10 and 20 µM), and this compound was found to significantly increase the levels of GSH. These concentrations of alpha-mangostin were chosen on the basis of previous research conducted by Rahmaniah *et al*., who reported a 50% cytotoxic concentration for alpha-mangostin in LX-2 cells of 10.77 µM [12].

MDA is a metabolite formed by lipid peroxidation of the cell membrane. Increased levels of MDA indicate the presence of lipid damage to the cell membrane due to oxidative stress [18]. MDA is, therefore, generally considered to be a marker of intracellular oxidative stress and an indicator of lipid peroxidation [17]. In this study, MDA levels were observed to increase in the acetaldehyde-induced group. On the other hand, alpha-mangostin antioxidant activity was expected to prevent this elevation; although there was no significant difference, alpha-mangostin did reduce the MDA levels in a dose-dependent manner.

GSH is an intracellular antioxidant that helps mitigate the oxidative stress caused by various ROS sources. Decreased cellular GSH levels indicate that the cell is experiencing oxidative stress caused by ROS, which requires neutralization by GSH. SOD is an antioxidant enzyme that protects cells from oxidative stress by catalyzing the process of superoxide dismutation into oxygen and hydrogen peroxide. Decreased SOD activity indicates the presence of oxidative stress caused by superoxide [19]. In this study, the antioxidant activity of alpha-mangostin was illustrated by the prevention of cellular GSH loss and increased SOD activity.

The use of antioxidants alone for the treatment of liver disease caused by alcohol is still controversial. The results of clinical trials indicate that antioxidants can be used for prevention but are not effective for the treatment of alcohol-related liver fibrosis [20]. The mechanisms and effects of acetaldehyde induction should be further assessed in future studies by examining changes in specific biomarkers. Further, research is also required to elucidate the mechanisms underlying the activity of alpha-mangostin and to develop its potential as an antifibrotic.

CONCLUSION

Alpha-mangostin was shown to normalize cell viability in HSCs induced by acetaldehyde, which at least in part resulted from protective effect of alpha-mangostin in preventing the loss of intracellular GSH.

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CONFLICTS OF INTEREST

The authors state that there are no conflicts of interest regarding publication of this article.

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