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Role of Harmaline in liver cirrhosis protection via arginase-I activity modulation in liver

Doha M. Beltagy[1], Khloud Gamal Abdelsalam[1*], Tarek M Mohamed[2], Mai M. El-Keey[2]

1 Biochemistry Division, Chemistry Department, Faculty of Science, Damanhour University, Egypt.
2 Biochemistry Division, Chemistry Department, Faculty of Science, Tanta University, Egypt.

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ABSTRACT

Background: Liver cirrhosis, the 11th death common cause, is a severe disorder that includes inflammation, oxidative damage, also immune response. Harmaline shows the antioxidant and anti-inflammatory mechanisms that aid in partial protection of hepatic cirrhosis.

Objective: This work aimed to evaluate the protection effect of harmaline against liver cirrhosis induced by thioacetamide in mice via arginase-1 enzyme activity modulation.

Methods: The study was carried out on forty male mice divided into four groups. Control group (GI), thioacetamide group (GII), harmaline group (GIII), and co-treated group (GIV). By the end of the experiment, arginase-1 activity and liver enzymes were measured in serum and liver tissue.

Results: The results showed that combined harmaline administration can cause significant suppression of liver inflammation by increasing ariginase-1 activity to nearly normal values. Inhibition of activated myofibroblasts cells and extracellular matrix accumulation was also noticed.

Conclusion: Harmaline has protective role against liver cirrhosis induced by thioacetamide in mice. It can be therapeutically used as a safe liver support after further in vivo studies.

INTRODUCTION

Liver is an essential organ in the body. It is central to the regulated metabolism and performs a vital function in endogenous and exogenous compounds detoxification (1). Chronic diseases of various etiology lead to cirrhosis, including oxidative stress, dysfunction of cytochrome P450, inflammation, and mitochondrial dysfunction. Two million deaths every year worldwide account for Liver disease, cirrhosis is within the 20 top cause of death. Biochemical and radiological tests are vital for the diagnosis as patients in the early course of cirrhosis have no clinical signs or symptoms (2). Cirrhosis, the end stage of chronic liver diseases causes an inability of the liver to perform its biochemical functions and also a risk factor for hepatocellular carcinoma (HCC) development.

Liver cirrhosis results from the activation of the liver type of cells called hepatic stellate cells (HSCs) which are found between hepatocytes and sinusoidal endothelial cells, in the space of disse, and account for nearly5%-8% of the healthy liver cell population. HSCs store about 80% of vitamin A in the body in large intracellular lipid droplets also can produce growth factors, cytokines, and other bioactive substances (3). In liver injury and toxicant exposure, HSCs activation occurs and acquires a Pathogenic myofibroblasts phenotype that shows increase ability in proliferation, contractility, and synthesis of ECM collagen and other proteins (1). Thioacetamide (TAA), which causes cirrhosis resembling that in humans, leads to hepatotoxicity by oxidation processes. The metabolites from TAA are more toxic and can result in liver injury (4). Harmaline (HAL) is the abundant β-carboline alkaloids in Peganum harmala L. HAL shows numerous clinical pharmacological effects, including reduction of inflammation, protection from radiation, immunosuppression, antipruritic effects, analgesia, and antitumor effects. Recent evidence has revealed the in vivo and in vitro antitumor effects of Harmaline in human liver carcinoma cells (5). Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) an enzyme in the urea cycle that metabolizes L-arginine to urea and L-ornithine. Arginase has two isoforms which encoded by different nuclear genes: arginase-1 (ARG1) and arginase-2 (ARG2). ARG1 is a cytosolic enzyme and is expressed most abundantly in the liver, most especially in the periportal hepatocytes, where it plays a vital role in the urea cycle, whereas ARG2 is mainly expressed in kidney and prostate. ARG1 is known to regulate oxidative stress in various degenerative diseases by nitric oxides (NO) modulating (6). Arginase involved in the biosynthesis of Polyamines for cell proliferation and regeneration regulation. Arginase converts arginine into ornithine, ornithine in turn, converted by ornithine decarboxylase into polyamines and by ornithine aminotransferase into proline, a parent compound for collagens. Also it competes with nitric oxide synthase for the common substrate, arginine. Based on being a part in so many important biochemical pathways, arginase is considering a key point in the development of various pathological processes (7).

In the present study, the protective effect of harmaline administration against liver cirrhosis induced by thioacetamide (TAA) was studied via arginase-1 (ARG1) activity assessment.

2. *Materials and Methods.*

Chemicals

Harmaline (HAL), thioacetamide (TAA), Phosphate buffer (PBS, pH 7.4), Sodium bicarbonate, MnCl2, Arginine, urea, and all other chemicals used were purchased from Sigma-Aldrich, Co., Ltd. (St.Louis, MO, USA). Kits for assessment of liver function (ALT, AST) also kidney function (urea, creatinine) profiles were purchased from Bio-Diagnostics, Egypt.

Animal grouping

The experiment employed 40 albino mice (C57BL/6J strain) weighing 25-30g, kept in breeding cages, received a similar basic care with a standard diet. Then mice were grouped into four main equal groups; each one contains 10 mice as follow:

Group I (Normal control Group) mice fed with ordinary diet only without any treatment during the entire experimental period of 10 weeks.

Group II (TAA Group) mice injected with Thioacetamide with a dose level (150 mg/kg b. Wt.) intraperitoneally twice weekly for 4 weeks (8).

Group III (HAL Group) mice injected with Harmaline at a dose level of (10 mg/kg b. Wt.) intraperitoneally twice weekly for 6 weeks (9).

Group IV (Co-treated Group) mice injected simultaneously with Thioacetamide and Harmaline at the same dose level twice weekly for 6 weeks.

N.B: During the period of the experiment, all dosage was adjusted continuously, according to changes in body weight to maintain similar dose/kg body weight of mice over the entire period of the study for each group.

Sample collection

By the experiment end, all animals were weighed sacrificed; the blood and liver tissues were collected then, blood samples were centrifuged at 5000 rpm for 10 min to obtain

serum then stored at -20oc for biochemical investigations and liver and kidney tissues were immediately removed and weighed. Liver homogenates were prepared and used in the determination of arginase-1 activity.

Preparation of liver homogenate for Arginase-1 enzyme analysis

One gram of liver tissue was cut into small pieces and immersed in ice-cold 0.1M phosphate buffer (PBS pH 7.4), and the 10% (w/v) homogenates were centrifuged at 4oC at 12,000 rpm for 10 min, and the supernatants were separated and then stored at -80oC until use.

Biochemical assays

Liver function tests in serum

Activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), Urea and Creatinine were determined following the recommended procedures of the commercial kits (Bio-diagnostic, #K AT 10 34 (45), UR 21 10, CR 12 50, Egypt), respectively.

Arginase-1 (E.C. 3.5.3.1) activity determination in liver homogenate

Exactly 0.5 ml of sodium bicarbonate buffer was mixed well with 0.1 ml MnCl2, 0.1 ml Arginine, 0.1 ml Liver homogenate (10%), and 0.2 ml H2O. The mixture was incubated all at 37oC for 1 h then the reaction was stopped by adding 1 ml of 5% of trichloroacetic acid (TCA). The reaction mixture was centrifuged at 5000 rpm for 5 min. Urea (100 µmole/ml concentration) was used as a standard according to Campbell method (10).

Data Analysis and Statistics

The obtained data statistical evaluation was carried out with SPSS version 25.0 software, and the data were expressed as mean ± SD. The data of the biomarkers changes were tested by one-way ANOVA for multiple comparisons. $p \leq 0.05$ were considered significant and for correlation by use of a Spearman rank test.

3. Results:

Results showed normal gain in body weight in mice in the control group (GI), while in the thioacetamide group (GII), the body weight significantly decreased. On the other hand, the liver weight increased significantly in the thioacetamide administered mice. The weights kidney was unaffected by thioacetamide (Table 1).

As shown in table 2, TAA administration induced an increase in all liver specific enzymes as AST and ALT in GII mice (TAA Group), when compared with control group GI. Liver damage also affected kidney functions as serum levels of Urea and Creatinine showed significant increase compared to control group. The activity of Arginase-1 enzyme decreased in the liver tissues of the TAA group by 48.7% of the control group (figure 1). Harmaline administration succeeded to elevate ARG1 enzyme activity and retain it with nearly normal levels.

Relationship between the level of arginase-1 activity and the level of liver and kidney functions.

In this study, the hypothesis that the level of arginase-1 activity is decreased in cirrhotic mice were studied, which might contribute to liver dysfunction and disease progression. First, we tested the levels of arginase-1 activity in the liver tissues for all study groups. As shown in Figure 2(A, B) we found a statistically significant correlation between the levels of arginase activity and the liver enzyme ALT and AST ($r = -0.746$; $P = 0.005$, $r = -0.760$; $P = 0.004$ respectively).

Another significant correlation was observed between the level of arginase-1 activity and the kidney parameters urea, creatinine Figure 2(C, D) ($r = -0.822$; $P = .001$, $r = -0.896$; $P < 0.001$).

4. Discussion

Cirrhosis considers the final outcome of the progressive liver fibrosis. As the most advanced stage of chronic liver disease, decompensated cirrhosis causes more than 1 million deaths per year globally (11).
Liver cirrhosis induced by TAA show cirrhosis resembling that in humans. TAA cause hepatotoxicity by oxidation processes as its metabolites are more toxic than TAA itself and can result in the liver injury (4).

ECM deposition by HSCs is the key mechanism of liver scar tissue formation. Liver dysfunction was evidenced by elevation of AST, ALT, kidney functions and changes in hepatic arginase-1 enzyme also illustrated (12).

This study demonstrated that Harmaline has been shown to suppress and stop TAA damage effect on liver, this anti cirrhotic effect of harmaline clearly showed reduce liver enzymes and increase arginase-1 activity. Serum biomarkers are important criteria for the evaluation of liver damage. AST is found in the liver, kidney, cardiac muscles, skeletal muscles, and brain whereas ALT concentration is highest in the liver and therefore, alanine aminotransferase appears to be a more sensitive test for hepatocellular damage than aspartate aminotransferase. Their elevation is interpreted as a result of liver cell destruction and repressed the synthetic, detoxification functions of the liver and/or changes in the membrane permeability, all suggesting their leakage from injured hepatocytes into the blood. The concentrations of enzymes that leak into the blood stream indicate the severity of hepatic damage (13).

Our results are in line with recent studies showing the impact of TAA on liver damage biochemical markers. Thioacetamide hepatotoxic significantly increased aminotransferase activities. Correlation analysis of liver arginase-1 activity with AST and ALT also, the relationship between the kidney function and the liver arginase activity shown in Fig.2. The analysis revealed a significant negative correlation between these parameters. Arginase-1 activity in cirrhotic livers was significantly lower than in control livers, this is mainly associated with impaired liver function in cirrhosis estimated according to the Child-Pugh classification (14, 15).

Liver type arginase (ARG1) almost exists exclusively in the liver and serves as a more specific marker of liver injury. It has been reported that the urea cycle enzymes leak rapidly from hepatocytes during liver inflammations (16).

A significant ($p < 0.05$) decrease in the arginase-1 activity following TAA administration compared with the control group (Fig. 1), combined injection with HAL (10 mg/kg b.wt) resulted a significant ($p < 0.05$) increase in the enzyme activity when compared to the TAA group.

A shorter half-life (1.5-2.0 h) of arginase-1 in the circulation, give arginase its advantage over many hepatic markers. This property maybe a helpful index for the subsequent treatment of patients with hepatic disorders (17).

Harmaline co-treatment to TAA–intoxicated group caused a significant decrease in elevated serum enzymes activities. Also, findings submitted that alkaloids from seeds of P. harmala exerted an antioxidant effect towards in vitro oxidation. The results lend support to the theory that one of the roles of the harmaline is to offer protection against oxidative stress (9).

Additionally, elevated levels of urea and creatinine parameters in serum suggest a compromised functional integrity of the kidney, which can result from infections or chemical abuse, as observed in TAA-induced mice in this study. Kidney function changes with TAA injection and harmaline displayed key improvement in all functions comparable with that of the thioacetamide group.

In conclusion, the activation of HSC is accompanied by down regulation of ARG-1. Harmaline administration which cause increase in ARG-1 activity can be an anti-fibrotic target for the treatment of liver cirrhosis after further in vivo studies.

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Table1. Weight of mice also Liver and Kidney weight of all Animals groups. Data are shown as means ± S.D. Small letters (a) and (b) indicate a significant P<0.05 change of the corresponding group compared to GI, GII, respectively.

Groups n=10	weight of Body g	Weight of Liver g	liver weight to body weight Ratio	Weight of Kidney g
GI	29±1.6[b]	1.7±0.17[b]	0.03±0.04	0.45+0.02
GII	18.5±3[a]	2.4±0.22[a]	0.14±0.07	0.37+0.06
GIII	31.5±1.2[b]	1.3±0.19[b]	0.03±0.02	0.42+0.03
GIV	26.5±1.9[b]	1.8±0.5[b]	0.07±0.05	0.40+0.01

Table2. Serum levels of liver and kidney functions. Small letters (a) and (b) indicate a significant change of the corresponding group compared to GI, GII, respectively. Mean values of the groups were compared by ANOVA test.

Group	**ALT U/L**	**AST U/L**	**Urea mg/dL**	**Creatinine mg/dL**
GI	100±**2.1**	112±1.4	30±1.1	0.56±0.02
GII	151±**3.2**[a]	254±2.3[a]	56±**1.8**[a]	1.07±0.04[a]
GIII	98±1.6[b]	114±2.2[b]	31±0.99[b]	0.59±0.02[b]
GIV	120±**3.6**[b]	117±4.1[b]	45±2.3[b]	0.63±0.1[b]

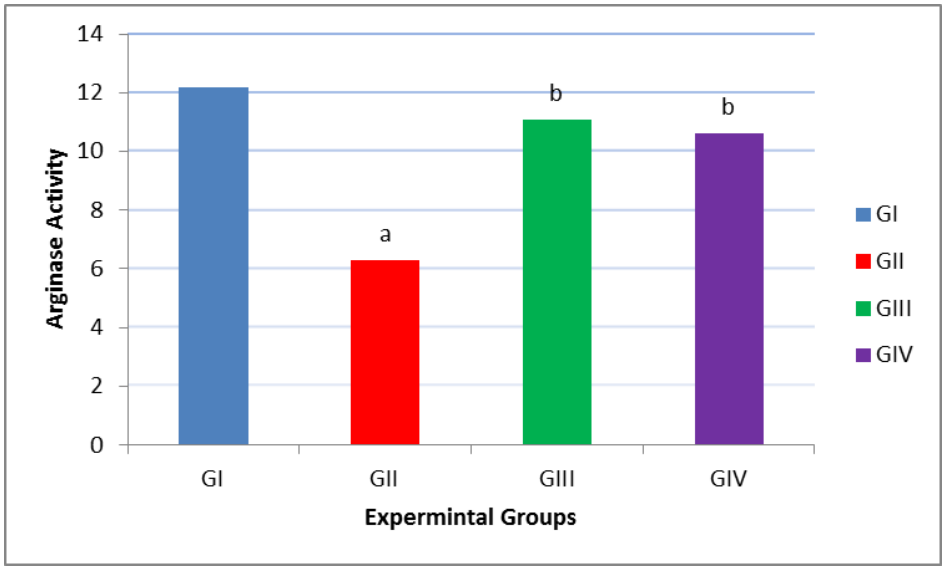

Fig 1: Arginase-1 enzyme activity. Small letters (a) and (b) indicate a significant change *P<0.05, of the corresponding group compared to GI and GII, respectively. Mean values of the groups were compared by ANOVA test.

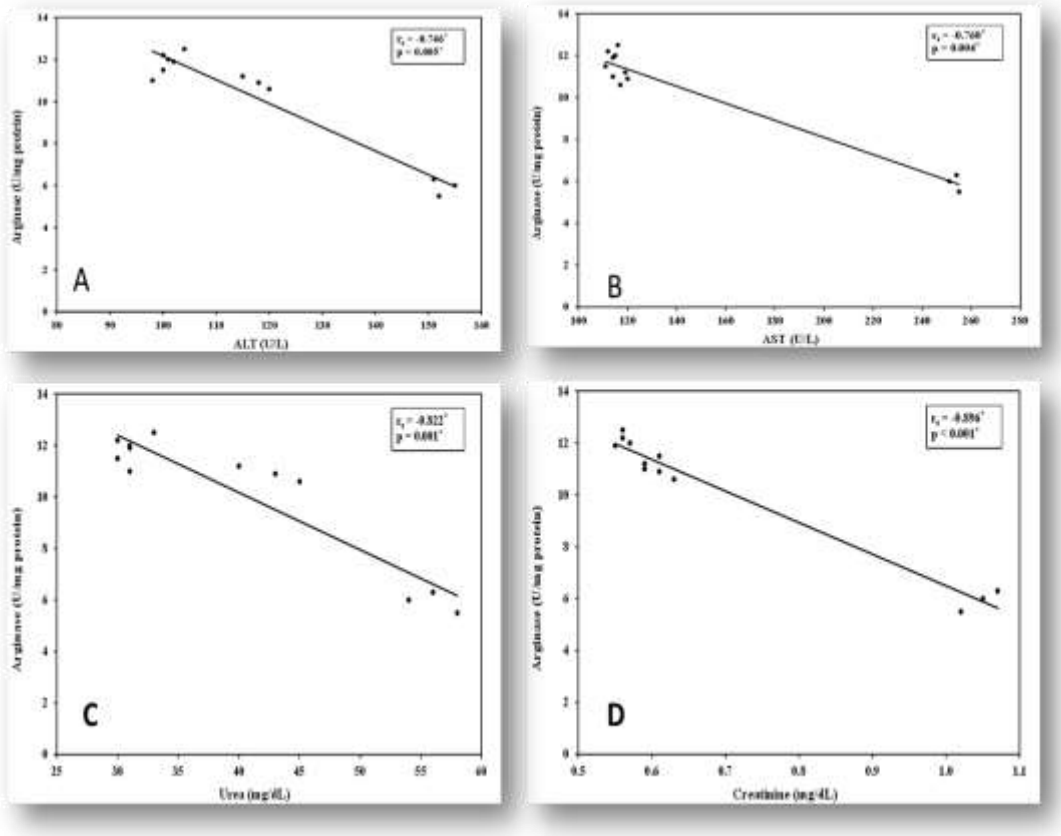

Fig.2 Correlation between the level of liver arginase-1 activity with liver and kidney function tests. (A), **Correlation between Arginase-1 and ALT (n = 12)** (B), **Correlation between Arginase-1 and AST (n = 12),** (C), **Correlation between Arginase-1 and urea (mg/dL) (n = 12)** (D), **Correlation between Arginase-1 and creatinine (n = 12).**