Original Article

Ameliorating Effects of β-Myrcene, a Monoterpene in Many Plants, on Thioacetamide-Induced Acute Hepatic Encephalopathy in Rats

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ABSTRACT

Hepatic encephalopathy (HE) is a clinical syndrome that can result from acute and chronic liver disorders, such as hepatitis, liver failure caused by alcohol or drugs, autoimmune diseases, metabolic diseases, cirrhosis, different types of tumors, and infections. This study aimed to investigate the effects of different doses of Beta-myrcene (β-myrcene) on the improvement of HE caused by thioacetamide (TAC) in male rats. To induce liver failure and acute damage in the studied animals, TAC was administered to rats at a dose of 100 mg/kg of body weight through an intraperitoneal (IP) injection with 24-hour intervals for seven consecutive days. After the oral treatment of rats with β-myrcene at doses of 10, 25, and 50 mg/kg/day for seven days, the cerebral edema (brain water content, BWC), the serum level of liver enzymes (aspartate aminotransferase, alanine transferase, alkaline transferase, total protein, and bilirubin), ammonia, and the level of oxidant-antioxidant factors (lipid peroxidation [MDA], glutathione peroxidase [GPx], catalase [CAT], and superoxide dismutase enzymes [SOD]), were evaluated. β-myrcene dose-dependently reduced BWC in TAC-induced acute HE in rats. In TAC rats treated with β-myrcene, especially at doses of 25 and 50 mg/kg, the serum levels of these liver enzymes and ammonia were significantly moderated (P<0.001), compared to the untreated TAC rats. The analysis of the obtained results revealed that the treatment of TAC rats with β-myrcene, especially at doses of 25 and 50 mg/kg, significantly reduced the oxidative stress marker MDA (P<0.001), whereas it significantly increased the antioxidant enzymes SOD, CAT, and GPx (P<0.001). Therefore, it can be concluded that the treatment of TAC rats with β-myrcene, especially at doses of 25 and 50 mg/kg, significantly reduced the oxidative stress marker MDA, whereas it significantly increased antioxidant enzymes and subsequently improved TAC-induced acute HE in rats.

Keywords: Antioxidant, Liver, Monoterpene, Oxidative stress

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1. Introduction

Hepatic encephalopathy (HE) is a clinical syndrome that can result from acute and chronic liver disorders, such as hepatitis, liver failure caused by alcohol or drugs, autoimmune diseases, metabolic diseases, cirrhosis, different types of tumors, and infections (1). Liver malfunction causes disruption in the urea cycle and increases the level of ammonia in the plasma and brain (1). The increase in the concentration of serum ammonia and the subsequent increase in the level of ammonia in the brain cause damage to this vital organ, which is the main organ affected in hyperammonemia (2). For this reason, brain damage caused by liver failure is called HE syndrome, in which the patient has a loss of consciousness or cognitive-motor disorders, and if the person is not treated, it leads to coma and death (2). Among different types of HE, in type A, which occurs following severe necrosis and acute liver failure, serum ammonia levels rise rapidly, and neurological complications appear (3). Oxidative stress has been considered one of the main factors involved in the pathogenesis of acute and chronic liver impairments, such as poisoning with toxins, biliary obstruction, alcohol abuse, liver ischemia, and infections (4). Excessive secretion of reactive oxygen species and nitrogen species and suppressed antioxidant activity disrupt some cellular functions via lipid peroxidation (5). As a result, more attention has been given to antioxidants, or free radical scavengers, for controlling acute and chronic liver damage. Beta-myrcene/β-myrcene (7-methyl-3-methylene-1,6-octadiene) is the main monoterpene found as a principal constituent in various herbs, such as hops and cannabis (6). This compound displays various pharmacological and therapeutic properties, including antinociceptive, anti-inflammatory, anticancer, antimicrobial, sedative, and antidiabetic effects (6). Previous studies have reported that β-myrcene represents promising antioxidant activities through increasing the level of glutathione reductase (GR), glutathione peroxidase (GPx), and superoxide dismutase (SOD) enzymes (6). Since the function of antioxidants is to collect, neutralize, or eliminate free radicals inside the cell and the environment outside it (7) and that β-myrcene is a promising antioxidant agent, it is expected that this compound reduces free radicals and the oxidative damage caused in different animal models, including the acute model in rats suffering from HE. Therefore, this study aimed to investigate the effects of different doses of β-myrcene on the improvement of HE caused by TAC in male rats.

2. Materials and Methods

2.1. Chemicals

β-myrcene ($C_{10}H_{16}$, $H_2C{=}CHC(=CH_2)CH_2CH_2CH{=}C(CH_3)_2$), with a purity of 90%, and thioacetamide (TAC), with a purity of ≥99.0%, were prepared from Sigma-Aldrich (St. Louis, MO, USA). All other materials used in this study were of analytical purity.

2.2. Animals

Overall, 60 male Wistar rats weighing 180-220 g were kept in standard laboratory conditions at a temperature of 22±2°C and 60% humidity in a 12-hour light-dark cycle with food and water *ad libitum*.

2.3. Induction of Acute Liver Failure and HE

In order to induce liver failure and acute damage in the studied animals, TAC was administered to rats at a dose of 100 mg/kg of body weight through an intraperitoneal (IP) injection with 24-hour intervals for seven consecutive days (8).

2.4. Study Design

Rats were randomly assigned into six groups of 10, including 1) healthy rats receiving normal saline, 2) rats receiving TAC (100 day/kg/mg), 3) rats receiving TAC (100 mg/kg/day)+β-myrcene (10 mg/kg/day for seven days), 4) rats receiving TAC (100 mg/kg/day)+β-myrcene (25 mg/kg/day for seven days), 5) rats receiving TAC (100 mg/kg/day)+β-myrcene (50 mg/kg/day for seven days), and 6) rats receiving the highest dose of β-myrcene (50 day/kg/mg). To ensure safety, treatment with β-myrcene was orally started one hour after the IP injection of TAC. To avoid hypoglycemia in rats followed by liver damage caused by TAC, their drinking water throughout the study contained dextrose at 1% (v/w).

2.5. Sample Collection

Followed by the administration of β-myrcene, the rats were euthanized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and then blood samples were collected from the abdominal aorta and centrifuged at 6000 rpm for 10 min to collect the era.

2.6. Evaluation of Cerebral Edema

After collecting the brain specimens of rats from all tested groups, the samples were weighed and kept in an oven (Esco Scientific, South Africa) at 120°C for 24 h. After dying, the samples were again weighed. Finally, the volume of water in the brains of the tested rats was determined using the following formula (9):

$$\%\text{Brain water content (BWC)} = \frac{(\text{initial brain weight} - \text{dried brain weight})}{(\text{initial brain weight})} \times 100$$

2.7. Biochemical Study

The serum levels of liver enzymes (aspartate aminotransferase [AST], alanine transferase [ALT], alkaline transferase [ALP], total protein [TP], and bilirubin [TB]) and ammonia were evaluated using Roche diagnostic kits and the Roche Cobas C 111 Biochemistry Analyzer (Germany), according to the manufacturer's protocols.

2.8. Assessment of Oxidative Stress Factors

To determine the level of oxidative stress markers, the Lipid Peroxidation (MDA) Assay Kit (ab118970) and Nitric Oxide Assay Kit (ab272517) were used, according to the manufacturer's instructions.

2.9. Assessment of Antioxidant Enzymes

To determine the level of antioxidant enzymes, namely GPx, Catalase (CAT), and SOD, Glutathione Peroxidase Assay Kit (Colorimetric) (ab102530), Catalase Activity Assay Kit (Colorimetric/Fluorometric) (ab83464), and

Superoxide Dismutase Activity Assay Kit (Colorimetric) (ab65354) were used, respectively, according to the manufacturer's instructions.

2.10. Statistical Analysis

The collected data were entered into SPSS software (version 26.0) and were then analyzed using a one-way ANOVA. $P<0.05$ was considered statistically significant.

3. Results

3.1. Evaluation of Cerebral Edema

As shown in table 1, β-myrcene dose-dependently reduced BWC in TAC-induced acute HE in rats. A significant reduction ($P<0.001$) was observed in BWC in groups receiving β-myrcene at doses of 25 and 50 mg/kg, compared to the TAC group.

3.2. Biochemical Assessment

The results showed that in TAC rats, the levels of liver enzymes (AST, ALT, ALP, and TB) and ammonia were significantly increased ($P<0.001$). On the other hand, in TAC rats treated with β-myrcene, especially at doses of 25 and 50 mg/kg, the serum levels of these factors were significantly moderated, compared to the untreated TAC rats ($P<0.001$) (Table 2).

3.3. Assessment of Oxidant/Antioxidant Enzymes

The obtained findings revealed that in TAC rats, the level of the oxidative stress marker MDA was significantly increased ($P<0.001$), while the level of antioxidant enzymes (GPx, CAT, and SOD) was significantly reduced ($P<0.001$). The analysis of the obtained data revealed that the treatment of TAC rats with β-myrcene, especially at doses of 25 and 50 mg/kg, significantly reduced the oxidative stress marker MDA ($P<0.001$), whereas it significantly increased the antioxidant enzymes SOD, CAT, and GPx ($P<0.001$) (Figure 1).

Table. 1. Effects of various doses of β-myrcene on the brain water content (BWC) in Thioacetamide (TAC)-Induced Acute Hepatic Encephalopathy in rats. * p<0.001 significant difference compared with control group. φ p<0.001 significant difference compared with TAC group.

Groups	% of BWC
Normal saline (control)	61.3 ± 4.6
TAC (500 mg/kg)	88.3± 2.6*
TAC + β-myrcene (10 mg/kg)	84.3±3.51*
TAC + β-myrcene (25 mg/kg)	70.3±3.15 φ
TAC + β-myrcene (50 mg/kg)	63.3±3.66 φ
β-myrcene (50 mg/kg)	59.3±3.51 φ

Table. 2. Effects of various doses of β-myrcene on the serum level of liver enzymes aspartate aminotransferase (AST), alanine transferase (ALT) and alkaline transferase (ALP), total protein (TP), bilirubin (TB), and ammonia in Thioacetamide (TAC)-induced acute hepatic encephalopathy in rats. * p<0.001 significant difference compared with control group. φ p<0.001 significant difference compared with TAC group.

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TB (mg/dL)	Ammonia (µg/dL)
Normal saline (control)	161.6±23.3	74.7±8.6	217.3±16.4	0.34 ±0.16	107.66±13.3
TAC (500 mg/kg)	431.3±36.6*	266.3±21.0*	492.6±46.6*	1.86± 0.33*	519.6±36.6*
TAC + β-myrcene (10 mg/kg)	315.3±26.6*	177.4±19.6*	401.3±28.6*	0.93±0.51*	446.6±41.3*
TAC + β-myrcene (25 mg/kg)	244.3±19.6*, φ	116.3±13.3 φ	306.3±25.6*φ	0.66±0.33 φ	306.3±26.6*, φ
TAC + β-myrcene (50 mg/kg)	191.3±23.6 φ	89.6±8.3 φ	239.6±31.3 φ	0.56±0.16 φ	212.3±36.6*, φ
β-myrcene (50 mg/kg)	172.6±20.3 φ	80.6±10.3 φ	251.6±15.2 φ	0.51±0.13 φ	98.6±13.3 φ

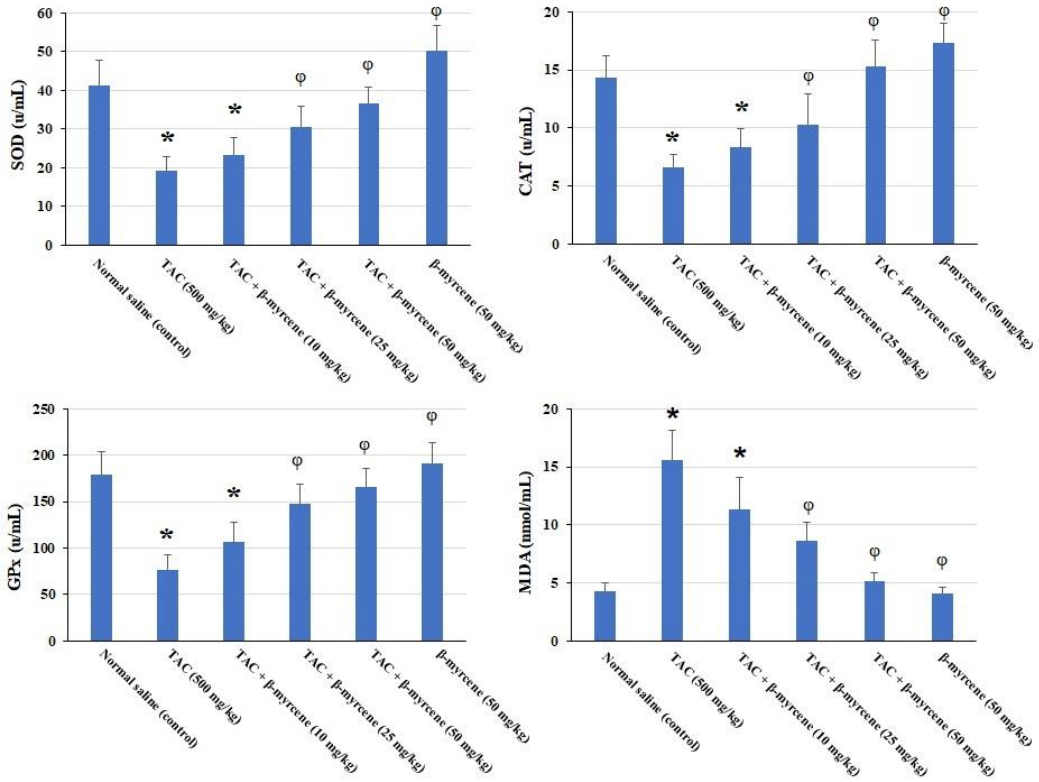

Figure 1. Effects of various doses of β-myrcene on the level of glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase enzymes (SOD), and lipid peroxidation (MDA) in Thioacetamide (TAC)-induced acute hepatic encephalopathy in rats. * $p<0.001$ significant difference compared with control group. φ $p<0.001$ significant difference compared with TAC group.

4. Discussion

It has been proven that oxidative stress is the main factor in the pathogenesis of acute and chronic liver disorders and their resulting conditions, such as HE (10). Therefore, more attention has been given to antioxidant agents, or free radical scavengers, for controlling acute and chronic liver damage (11). This study aimed to investigate the effects of different doses of β-myrcene on the improvement of HE caused by TAC in male rats. It was found that β-myrcene dose-dependently reduced BWC in TAC-induced acute HE in rats. In addition, in TAC rats treated with β-myrcene, especially at doses of 25 and 50 mg/kg, the serum levels of AST, ALT, ALP, TB, and ammonia were significantly moderated, compared to the untreated TAC rats ($P<0.001$). Consistent with these findings, Cardia et al. (2022) reported that in rats with acetaminophen-induced liver injury, pretreatment with β-myrcene at doses of 100 and 200 mg/kg for seven days improved liver damage and moderated liver cell function and the serum levels of ALT and AST. These findings indicated the potent hepatoprotective effects of β-myrcene through improving liver function (12). It was also found that the treatment of TAC rats with β-myrcene, especially at doses of 25 and 50 mg/kg, significantly reduced the oxidative stress marker MDA ($P<0.001$), whereas it significantly increased the antioxidant enzymes SOD, CAT, and GPx ($P<0.001$). It has been shown that monoterpenes have potent antioxidant effects, which can be linked to the presence of conjugated double bonds that cause chain-breaking antioxidant activity (13-15). Several studies have reported the *in vivo* antioxidant effects of β-myrcene (16-18). In a study conducted by Ciftci et al., the results showed that the treatment of female rats subjected to the environmental contaminant 2,3,7,8-tetracholorodibenzo-p-dioxin (TCDD) with β-myrcene at a dose of 200 mg/kg/day for one or two months resulted in a significant reduction in hepatic lipid peroxidation via the activation of antioxidant and radical scavenger properties (16). Another study conducted by Ciftci et al. showed that β-myrcene at a dose of 200 mg/kg/day had promising neuroprotective effects on cerebral ischemia/reperfusion injury in C57Bl/J6 rats

through increasing the level of antioxidant enzymes, such as GPx and SOD (17). Bonamin et al. (2014) showed that the oral administration of β-myrcene at a dose of 7.5 mg/kg improved ethanol-induced gastric ulcers in male rats through antioxidant effects via raising the tissue levels of GPx, GR, and total glutathione (18). Therefore, it can be concluded that the treatment of TAC rats with β-myrcene, especially at doses of 25 and 50 mg/kg, significantly reduced the oxidative stress marker MDA, whereas it significantly increased the antioxidant enzymes and subsequently improved TAC-induced acute HE in rats.

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Authors' Contribution
R. Z. designed and supervised the study and wrote the manuscript. F. S. Z. conducted the experiments, obtained the data, and reviewed and edited the manuscript. All authors agreed upon the final version to be published.

Ethics
This experimental study received permission from the Ethical Board of the College of Pharmacy, Rajiv Gandhi University of Health Sciences, Bangalore, India (No. 2022-10Q0607).

Conflict of Interest
The authors declare no conflicts of interest.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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