Toxicology Reports 1 (2014) 629–638

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

# Amelioration of bromobenzene hepatotoxicity by *Withania somnifera* pretreatment: Role of mitochondrial oxidative stress

Mahima Vedi, Mahaboobkhan Rasool, Evan Prince Sabina *

*VIT University, Vellore 632014 Tamil Nadu, India*

ARTICLE INFO

*Article history:*
Received 6 June 2014
Received in revised form 19 August 2014
Accepted 19 August 2014
Available online 27 August 2014

*Chemical compounds studied in this article:*
Bromobenzene (CID:7961)
Glutathione (CID: 124886)
Pyrogallol (CID: 1057)
Tricarboxylic acid (CID:643757)

*Keywords:*
Antioxidant
Lipid peroxidation
Glutathione
Bromobenzene
Oxidative stress

ABSTRACT

The present study investigated the possible protective role of *Withania somnifera* (Linn.) Dunal (Solanaceae) root powder against bromobenzene-induced oxidative damage in rat liver mitochondria. Administration of bromobenzene (10 mmol/kg body weight) to rats resulted in increased levels of liver marker enzymes, lipid peroxidation, TNF-α, IL-1β and VEGF. There was also marked depletion in the levels of mitochondrial enzymes and antioxidant activity. Pre-treatment with *W. somnifera* significantly decreased the levels of liver marker enzymes, TNF-α, IL-1β, VEGF and ameliorated histopathological manifestations in bromobenzene-treated rats. The molecular docking analysis predicted that the pro-inflammatory mediator NF-κB showed significant interaction with selected various active components of *W. somnifera* (withaferin A, withanolide D and withanolide E). This study demonstrates a good protective effect of *W. somnifera* against bromobenzene-induced oxidative stress.



## 1. Introduction

Bromobenzene ($C_6H_5Br$) is a well-known organic solvent and has profound use in the manufacture of various drugs and chemicals. It is metabolized in the liver by primary cytochrome enzymes to form various oxides of bromobenzene, of which bromobenzene-3, 4-oxide is highly reactive. Bromobenzene-3, 4-oxide is sequestered by binding with reduced glutathione (GSH) and subsequently depleting hepatic glutathione levels. This results in reduced protection against intracellular reactive oxygen species (ROS) leading to secondary events such as mitochondrial dysfunction, changes in membrane permeability and oxidative stress [1]. Mitochondria is a vital site for energy metabolism and ATP production. Hence, its malfunction leads to cellular damage and contributes to a wide range of diseases [2]. It can be suggested that mitochondrial dysfunction would be diminished by enriching the mitochondria with antioxidants, thereby reducing the oxidative stress.

Recent studies have focussed on the potential of various natural compounds against liver pathological conditions. Silymarin and *N*-acetyl-L-cysteine are being already used

*Abbreviations:* BB, Bromobenzene; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GST, glutathione-s-transferase; WS, *Withania somnifera*; ROS, reactive oxygen species.

* Corresponding author at: School of Biosciences and Technology, VIT University, Vellore, 632014 Tamil Nadu, India. Tel.: +91 9080494445; fax: +91 4162202324.
*E-mail address:* eps674@gmail.com (E.P. Sabina).

http://dx.doi.org/10.1016/j.toxrep.2014.08.009

in the clinical treatment of liver injury and they exhibit a potent hepatoprotective activity, but with certain limitations such as gastric irritation, allergies and limited efficacy [3,4]. This indicates that there is still the need of finding highly effective and reliable drugs with minimal side effects for the prevention of acute liver failure.

*Withania somnifera* (Linn.) Dunal (Solanaceae) is a well-known Ayurvedic medicine known to possess pharmacological properties such as antistress, antioxidant, immunomodulating and anti-arthritic activities [5,6]. These properties may be due to the presence of various biologically active chemical constituents such as alkaloids (isopellertierine, anferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanoloides with a glucose at carbon 27 (sitonidoside XI and X) [7,8]. The roots of *W. somnifera* are the most pharmacologically active part of the plant and are known to possess free radical scavenging and antioxidant activity [9]. Therefore, an attempt was made to evaluate the hepatoprotective effect of *W. somnifera* root powder against bromobenzene-induced acute liver necrosis which has not been reported to the best of our knowledge. Silymarin has been used as the standard reference drug in the present study. Also, the effect of selected active components of *W. somnifera* on NF-κB was analyzed using molecular docking.

## 2. Materials and methods

### 2.1. Animals

Wistar albino rats of either sex, weighing 120–150 g were obtained from animal house, VIT University, Vellore. They were fed with commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water ad libitum. The animals were maintained according to the guidelines recommended by the Committee for the Purpose of Supervision and Control of Experiments on Animals (CPSCEA), Government of India, Chennai, Tamil Nadu. Experimental procedure for the present study has been approved by the ethical committee (VIT/IAEC/VIIth/17) of VIT University, Vellore, India.

### 2.2. Drugs and chemicals

Commercially available *W. somnifera* root powder was obtained from Indian Medical Practitioners Co-operative Stores and Society (IMCOPS, Chennai, India). Silymarin, a standard hepatoprotective drug, was obtained from Micro Labs Ltd (Goa, India). All other reagents used were standard laboratory reagents of analytical grade and were purchased locally. The effective dosage of bromobenzene [10] and *W. somnifera* [11] were based on the basis of previous studies. Aqueous suspension of silymarin (100 mg/kg body weight) and *W. somnifera* (250 and 500 mg/kg body weight) were made in double distilled water for administration to rats.

## 3. Experimental procedure

Animals were allocated randomly in six groups of six animals each. In this study, all group of rats except group I and group VI received bromobenzene dosage (intragastric tube) only once. The animals were treated as shown in Table 1:

**Table 1**
Experimental animal design for the study.

| Groups | Treatment |
|---|---|
| Group I | Normal control (received single dose of 0.1 ml coconut oil through intragastric intubation once and sacrificed after 19 h) |
| Group II | Bromobenzene (10 mmol in 0.1 ml coconut oil by intragastric intubation) once and sacrificed after 19 h |
| Group III | *Withania somnifera* (250 mg/kg, orally) for 8 days and a single dose of bromobenzene (10 mmol/kg in 0.1 ml coconut oil, intragastric intubation) on the 8th day and sacrificed after 19 h |
| Group IV | *Withania somnifera* (500 mg/kg, orally) for 8 days and a single dose of bromobenzene (10 mmol/kg in 0.1 ml coconut oil, intragastric intubation) on the 8th day and sacrificed after 19 h |
| Group V | silymarin (100 mg/kg, orally) for 8 days and a single dose of bromobenzene (10 mmol/kg in 0.1 ml coconut oil intragastric intubation) on the 8th day and sacrificed after 19 h |
| Group VI | *Withania somnifera* (500 mg/kg, orally) for 8 days and sacrificed after 19 h |

All six groups were fasted for 24 h before and 19 h after the administration of silymarin/*W. somnifera*/bromobenzene/coconut oil. After the collection of blood, samples from liver tissues (approximately 0.05–0.1 g) were homogenized in phosphate buffer (pH 7.4) to give 20% w/v homogenate [12]. This homogenate was centrifuged at 3000 g and 4 °C for 10 min; the supernatant was stored at −20 °C until analysis.

### 3.1. Serum biochemical parameters, antioxidant status, plasma ceruloplasmin and total sulfhydryl group determination

The activities of aspartate aminotransferase (AST), alkaline aminotransferase (ALT), alkaline phosphatase (ALP), albumin, total bilirubin and direct bilirubin were determined according to the manufacturer's protocol using kits (Autospan diagnostics, Bangalore, India) in the serum of control and experimental rats.

Antioxidant status was determined in the plasma and liver tissue of control as well as experimental rats. Lipid peroxidation was determined by the procedure of Ohkawa et al. [13] and malondialdehyde (MDA), which forms as end product of lipid peroxidation, was measured. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-s-transferase (GST) and reduced glutathione were also evaluated [14–18]. Total protein was estimated according to the method of Lowry et al. [19] using bovine serum albumin as standard. Furthermore, plasma ceruloplasmin [20] and total sulfhydryl groups [21] were measured in plasma and liver respectively in the control and experimental rats.

### 3.2. Isolation of mitochondria

For the isolation of liver mitochondria, the method of Johnson and Lardy [22] was followed and the protein