

Hepatoprotective and Cytotoxic Activities of Abietic Acid from *Isodon wightii* (Bentham) H. Hara

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ABSTRACT

Isodon (*Lamiaceae*) is a known source of bioactive terpenoids. Diterpenoids isolated from *Isodon wightii* (Bentham) H. Hara showed antibacterial, antiacetylcholinesterase, antioxidant, anticancer, and anticarcinogenic activities, etc., Hepatoprotective activity of ABA against lipopolysaccharide (LPS) induced liver injury in BALB/c mice was studied. Cytotoxic activity of ABA was tested against cervical cancer cells (HeLa) using MTT assay followed by propidium iodide (PI) staining to identify apoptosis. Histopathological analysis revealed that 1.5 µg/mL LPS induced liver damage was attenuated by ABA in a dose dependent manner. ABA showed cytotoxicity with IC₅₀ value of 176.28 ± 0.02 µg/mL and PI staining of treated cells showed apoptosis. This study proves that ABA would be a promising natural compound for herbal drug preparation.

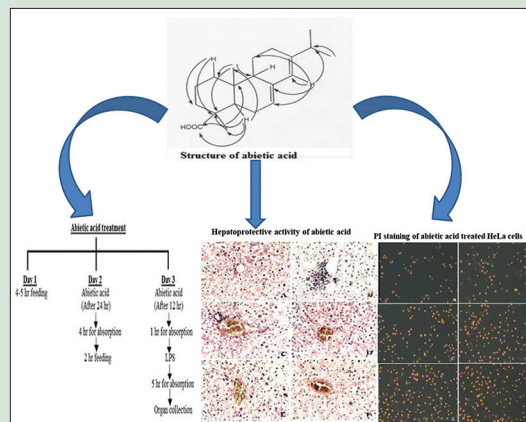
Key words: Abietic acid, apoptosis, BALB/c mice, HeLa, lipopolysaccharide

SUMMARY

- In the present study, abietic acid isolated from *I. wightii* had potent hepatoprotective effect on LPS induced liver damage in BALB/c mice. Abietic acid also showed cytotoxic activity on HeLa cells followed by apoptosis induction confirmed by PI staining.

Abbreviation Used:

ABA: Abietic acid; LPS: Lipopolysaccharide; PBS: Phosphate buffer saline; PI: Propidium iodide; NMR: Nuclear magnetic resonance; COSY: Correlation spectroscopy; HSQC: Heteronuclear single quantum correlation; HMBC: Heteronuclear multi - bond correlation; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide



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INTRODUCTION

Abietanes are known diterpenoids mostly isolated from terrestrial plants and it possess aromatic c-ring and different degree of oxygenation at several groups. The genus *Isodon* (*Plectranthus*) belongs to *Lamiaceae* reported to have a number of abietane diterpenoids with a wide range of biological activities.^[1-4] In our previous work, we reported free radical scavenging, antibacterial and antiacetylcholinesterase activities of abietane diterpenoid, abietic acid (ABA) from *Isodon wightii*.^[5] Therefore, the present study was aimed to test hepatoprotective and cytotoxic potentials of ABA.

MATERIALS AND METHODS

Plant material

The leaves of *I. wightii* (Bentham) H. Hara, a perennial herb, were collected from Coonoor, Tamil Nadu, India, during January 2014. An authentication sample was identified by Botanical Survey of India (BSI) and voucher specimen (BSI/SC/5/23/06-07-Tech. 881) has been deposited in the herbarium of BSI, Southern Circle, Coimbatore, Tamil Nadu, India.

Extraction and isolation

The dried and powdered leaves (715 g) were extracted with petroleum ether in Soxhlet apparatus at room temperature to yield crude extract (15 g). After solvent evaporation in vacuo at 45°C, the extract was

subjected to silica gel column chromatography (60–120 mesh size) and eluted with petroleum ether (100%). Further, the fractions were collected, combined, and monitored by thin layer chromatography coated silica gel G-60. Yellowish amorphous powder (82 mg) was obtained at 70th h. Based on the spectral studies (IR, ¹H NMR, ¹³C NMR, COSY, HSQC, and HMBC), the compound structure was predicted.^[5]

Animals and experimental design

The animal care and handling were done according to the regulations of Council Directive CPCSEA no: 659/02/2 about Good Laboratory Practice on animal experimentation. Adult BALB/c mice were purchased from KMCH Hospital, maintained in micro isolators with autoclaved bedding and cages, fed with autoclaved food pellets and deionized water.

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The animals were maintained under standard conditions of humidity, temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$), and light (12 h light/dark). Sterile procedures were followed to prevent unintentional introduction of microbes. Adult BALB/c mice weighing 30 g were used and randomly assigned into three groups, normal group ($n = 5$), lipopolysaccharide (LPS) treated ($n = 5$), and experimental group ($n = 5$) (compound + LPS). Mice were placed on restricted, once a day diet and given different concentrations of ABA (25, 50, 75, and 100 $\mu\text{g}/30$ g body weight) and LPS (1.5 $\mu\text{g}/30$ g body weight). The schedule for feeding and compound treatment was illustrated in Figure 1.^[6] Animals were sacrificed after 4–5 h LPS treatment. Livers were excised from animals and fixed in 10% formalin for histopathological analysis.

Cell line and culture

The human cervical carcinoma (HeLa) cell line was purchased from NCCS, Pune, India cultured in a 25 cm^2 cell culture flask containing

Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 $\mu\text{g}/\text{mL}$). The cell lines were incubated in humidified incubator at 37°C with 5% CO_2 .

MTT assay

The cell line culture in the 25 cm^2 flask was harvested using trypsin and the cell number was counted using a hemocytometer. 1×10^4 cells/100 μL medium was added in each well of 96 well plate and incubated for 24 h. Then, the cells were treated with various concentrations of ABA, dissolved in medium, and further incubated for 48 h. About 20 μL of

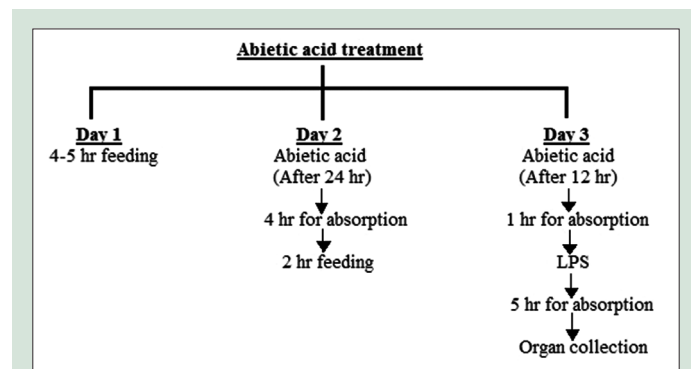


Figure 1: Schematic representation of feeding and abietic acid treatment

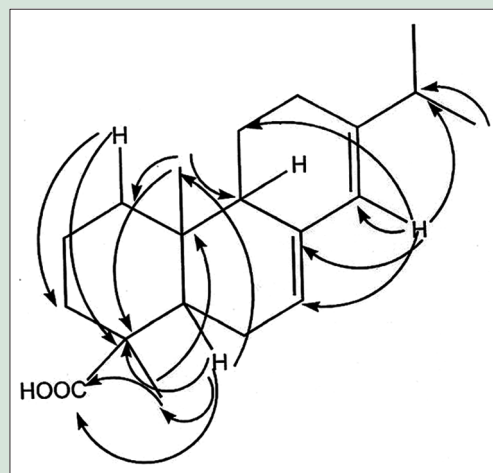


Figure 2: HMBC correlations of abietic acid

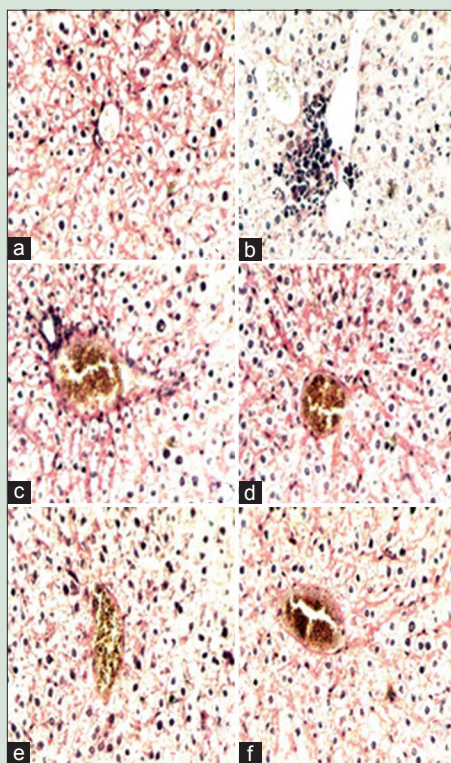


Figure 3: Hepatoprotective effect of abietic acid. (a) Normal liver, (b) lipopolysaccharide treated, (c-f) 25, 50, 75, and 100 $\mu\text{g}/\text{mL}$ of abietic acid treated lipopolysaccharide induced animal

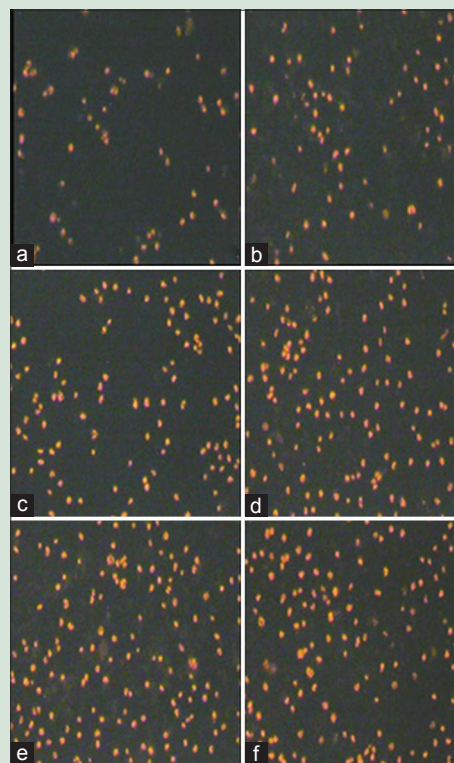


Figure 4: Apoptosis induction of abietic acid on HeLa cells was observed using propidium iodide staining. (a) Control. (b-f) cells treated with abietic acid of 25, 50, 75, 100, and 125 $\mu\text{g}/\text{mL}$, respectively

MTT (5 mg/mL) in phosphate-buffered saline (PBS) was added to each well and the plate was incubated at 37° C for 4 h. The medium was removed and 100 µL of dimethyl sulfoxide was added to each well. After 10 min of incubation at 37°C, the plate was read at 570 nm using a microplate reader.^[7] % of cell viability = $([A_B - A_A]/A_B) \times 100$, where A_B , absorption of blank sample, A_A , absorption of test sample.

Propidium iodide staining

Apoptosis of HeLa cells was assessed using uptake of fluorescent dye propidium iodide (PI).^[8] HeLa cells (1×10^4 cells/well) were seeded in a 24 well plate and grown until confluent (80%). The cells were treated with ABA at various concentrations (25–125 µg/mL) for 24 h. The cells were washed with ice cold PBS and fixed with 70% ethanol for 30 min. After fixation, the plates were rinsed again with ice cold PBS followed by staining with 200 µL of PI (500 µM) for 1 h, further the plates were washed twice with ice cold PBS, and the nuclear staining of the apoptotic cells were observed under fluorescence microscope.

Statistical analysis

Data obtained from cytotoxicity assay were expressed as mean ($n = 3$) \pm standard error. IC₅₀ values were calculated by linear regression analysis.

RESULTS

In this study, we reported hepatoprotective and cytotoxic activities of ABA [Figure 2] isolated from *I. wightii*. Hepatocytes were normal with prominent nuclei arranged around central vein on untreated liver tissue [Figure 3a]. LPS treated liver tissue had lymphocytic infiltration near the central vein and severe necrosis was seen [Figure 3b]. Histopathological data revealed that bioactive ABA reduced the rate of liver damage in LPS treated BALB/c mice. Hematoxylin and eosin stained the red blood cells in the central vein and no hemorrhage and dilation of blood vessel on ABA treated tissue. Liver tissue with congested blood vessel and no inflammation was observed on 25, 50, 75, and 100 µg/mL concentrations of ABA treated liver [Figure 3c-f], but high level of liver protection was observed with 100 µg/mL. ABA had a considerable cytotoxicity toward HeLa cells and its inhibitory concentration 50% was calculated as 176.28 ± 0.02 µg/mL. A significant increase in the number of PI positive cells was observed based on increasing concentrations of ABA. At 125 µg/mL, ABA induced more apoptosis in HeLa cells with characteristics of membrane blebbing, chromatin condensation, and nuclear fragmentation [Figure 4].

DISCUSSION

Medicinal plants have been focused to discover new anticancer drugs and many researchers have paid attention toward the plant based drugs because of its natural origin and less toxicity toward noncancerous cells.^[9] LPS participate in nuclear factor-κB and mitogen activated protein kinase pathways, which lead to the excessive accumulation of nitric oxide (NO).^[10,11] The oxidative stress caused by NO is one of the important factors for the occurrence of hepatic injury.^[12] In this study, we observed strong hepatoprotective activity of ABA against LPS induced liver damage. It is assumed that observed anti inflammatory activity of ABA might be due to the presence of hydroxyl groups present in its structure.^[13] Plant based antioxidant compounds may have a hepatoprotective activity.^[14,15] Our previous report shows antioxidant activity of ABA and it may be the reason for observed hepatoprotective action.^[5] ABA also showed significant cytotoxic and apoptotic activities in HeLa cells. Methyl abietate, a derivative of ABA, showed strong cytotoxic activity against HeLa cells.^[13] Abietane diterpenoids graciliflorin D and uncinatone isolated from *Isodon lophanthoides* var. *graciliflorus* and

Clerodendrum bungei reported to have cytotoxic activity on human carcinoma cells including B16, HGC-27, and HEK-293.^[16,17] Our study establishes hepatoprotective, cytotoxic, and apoptotic potential of ABA isolated from *I. wightii*. Overall, it is suggested that ABA is a promising natural product which could be considered for plant based medicine preparations.

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Conflicts of interest

There are no conflicts of interest.

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