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In vitro Hepatoprotective activity of *Anaphyllum wightii* Schott.–An endemic plant of Southern Western Ghats

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Abstract
Anaphyllum wightii Schott. is an ethnomedicinal plant belonging to the family Araceae. This plant is endemic to the Southern Western Ghats. It is a herbaceous plant with a rhizomatous stem and pinnately compound leaves. The plant has two variations in leaves, either broad leaves or narrow leaves. The inflorescence is a spadix with a characteristically twisted spathe. The rhizome of this plant is reported to be edible, and it has importance in tribal medicine also. Tribal people use the rhizome to treat diseases like eczema, scabies, etc., and also as an antidote to snake venom. The rhizome has various pharmacological properties like anti-diabetic, anti-inflammatory, antioxidant, hepatoprotective, and anthelmintic activities. The present study was intended to compare *in vitro* hepatoprotective effects of acetone and methanolic extracts of rhizome and the methanolic extract of leaves against Hydrogen peroxide-induced hepatotoxicity in Chang liver cells. Here the acetone extract of rhizome and the methanolic leaf extracts showed significant hepatoprotective activity, whereas the methanolic extract of rhizome did not exhibit any significant hepatoprotective effect.

Keywords: *Anaphyllum wightii*, hepatoprotective, hydrogen peroxide, chang liver cells

Introduction
The liver, the largest gland in the human body, performs a wide range of vital functions, including metabolic activities. It plays a crucial role in the detoxification of our body either by the elimination of various toxic substances or by the conversion of toxic compounds into a non-toxic form. The liver also produces new molecules (proteins, cytokines, etc.) that are essential for physiological and immunological functions [1]. Liver diseases are a major health problem worldwide, especially in developing countries where hundreds of millions are affected [2]. Hepatotoxicity refers to the injury to the liver that may lead to the impaired function of the liver. Generally, the liver has the ability of regeneration and the capacity to repair any underlying damage. Hepatotoxicity occurs when liver regeneration capabilities are exhausted, and cell damage ensues [1]. There are various factors that cause liver injury and are called hepatotoxicants. Hepatotoxicants usually cause liver damage by overproduction of reactive oxygen species (ROS) and subsequent oxidative stress, lipid peroxidation, etc.

All aerobic organisms usually produce and degrade ROS, which exerts certain beneficial effects such as cytotoxicity against bacteria and other pathogens during the maintenance of normal cell function. However, when ROS are present in excess and cannot be balanced by the normal antioxidant mechanisms of the organism, the state is called 'oxidative stress.' Oxidative stress is often associated with DNA damage, protein oxidation, carbonylation, lipid peroxidation, mitochondrial dysfunction, calcium homeostasis, actin reorganization, NAD depletion, impairment of energy metabolism, and glutathione depletion in various cell types [3, 5]. Reactive oxygen species (ROS) are considered to be involved in the liver damage, which is induced under a variety of conditions, including alcohol abuse, fibrosis/cirrhosis, hepatocellular carcinoma, ischemia/reperfusion liver injury, paracetamol overdose, and viral hepatitis [6]. The antioxidant defense system of the human body, including intracellular antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione, helps to fight against highly toxic ROS [5, 4].

Research on medicinal plants and their active constituents are gaining more attention in modern drug development due to the lack of side effects, less expense, and effectiveness. The plant *Anaphyllum wightii* Schott. is a perennial herb belonging to the family Araceae. It is commonly called as 'Wight's Twisted Arum,' and in Kerala, it is locally known as 'Keerikkizhangu.' This plant is an endemic and threatened species of the Southern Western Ghats region of India [7, 8] and is ethno medicinally significant. *A. wightii* has a rhizomatous stem and pinnately compound leaves. It has two major variations in leaves, either broad or narrow. The inflorescence is spadix with a twisted spathe. The rhizome of the plant is used by the tribal communities of Kerala, such as Kani, Kadars, Madhuvars, etc. for its edible nature and medicinal value [9]. The rhizome is traditionally used in treating skin diseases like eczema and scabies and also used against snakebite [10]. Reports suggest that the rhizome possesses various pharmacological properties such as antibacterial, anti-inflammatory, antioxidant, hepatoprotective, and antidiabetic activities [11]. However, the medicinally active constituents of *A. wightii* have not yet been discovered. The present study was aimed to evaluate the *in vitro* hepatoprotective action of rhizome and the two leaf varieties (broad and narrow leaves) of *A. wightii* against hydrogen peroxide-induced hepatotoxicity on Chang liver cells.

Materials and Methods
Plant material
Both broad and narrow-leaved varieties of *A. wightii* were collected from Kallar, Kerala, identified by authenticated

literature, and the plants were established in the garden of Dept. of Botany, University of Kerala. The leaves of both varieties and the rhizome of only the broad-leaved variety were selected for the *in vitro* hepatoprotective assays.

Soxhlet Extraction

About 13 gm of the powdered samples (rhizome and two leaf varieties) were subjected to serial soxhlet extraction for about 6-8 hours using 130 ml each of the five different solvents such as petroleum ether, chloroform, acetone, methanol, and distilled water, respectively. Then methanolic extracts of the two leaf varieties and the methanolic, as well as acetone extracts of the rhizome, were selected for the *in vitro* hepatoprotective assay since the acetone extract of rhizome was more active than the methanolic rhizome extract.

Evaluation of *in vitro* hepatoprotective activity

The hepatoprotective potential of the sample extracts was evaluated *in vitro* using Chang liver cells [12, 13]. H_2O_2 was used as the hepatotoxicant at an IC_{50} concentration of 73 µM. Confluent Chang liver cells were cultured in growth media (DMEM + 10% FBS) at a density of 5 × 104 cells/well in a 96-well tissue culture plate and incubated overnight. Post-incubation, the cells were treated with varying concentrations of extracts (25, 50, & 100 µg/ml) and incubated for 24 hours; thereafter, H_2O_2 (73 µM) was added and allowed for further 24-hour incubation. Post-incubation, the treated cells were washed with PBS and incubated with MTT (containing growth medium. Finally, the medium was removed, and the formazan crystals were dissolved using DMSO. The optical density was measured at 570 nm. Untreated cells were kept as control, and percentage cell viability in treated cells was calculated.

Statistical analysis

The values are expressed as mean ± SE. A value of $P < 0.05$ was considered significant.

Results and Discussion

The liver is the major organ that plays a crucial role in eliminating toxic substances from the body. Hence improper functioning of the liver may result in severe consequences due to the accumulation of toxic components. Hepatotoxicity may occur due to various reasons such as overproduction of ROS, decreased antioxidant levels, and lipid peroxidation. Oxidative stress produced by ROS is one of the major determining factors of cellular injuries in a variety of aberrant clinical conditions, including hepato-toxicity[14]. Hydrogen peroxide (H_2O_2) is one of the ROS molecules that can induce oxidative stress and trigger apoptosis in various cell types [15].

The present study was aimed to assess the *in vitro* protective effects of rhizome and leaf extracts of *Anaphyllum wightii* against H_2O_2- induced hepatotoxicity in Chang liver cells. The cytotoxic dose of H_2O_2 for Chang liver cells was determined by MTT assay, and the IC_{50} concentration of 73 µM was used to induce liver toxicity (Table 1).

Table 1: Determination of IC_{50} Concentration of H_2O_2

Concentration of H_2O_2 (µM)	% Cell viability
6.25	90.42 ± 0.56
12.50	81.91 ± 0.92
25	73.40 ± 0.42
50	52.12 ± 0.29
100	29.78 ± 0.26
IC_{50} value	73.33

The hepatoprotective action shown by the acetone and methanolic extracts of the rhizome is represented in Table 2 (Fig. 1 & 2). The pre-treatment of the cell line with acetone extract at a concentration of 100 µg/ml could improve the percentage of cell viability to 64.85 ± 0.21%, which was comparatively higher than that shown by the methanolic extract (51.38 ± 0.61%). Hence it can be said that among the two solvent extracts of the rhizome, acetone extract showed comparatively higher hepatoprotective ability against H_2O_2-induced cytotoxicity.

In the case of the leaf methanolic extracts, both the broad and narrow leaves showed higher activity than the rhizome extracts (Fig. 3 & 4). The narrow-leaf extract showed slightly higher hepatoprotective ability (68.80 ± 0.40%) compared to that of the broad leaves (65.81 ± 0.39%), as shown in Table 3.

Table 2: The hepatoprotective activity showed by acetone and methanolic extracts of rhizome

Treatment	% Cell viability	
	Acetone	Methanol
Untreated	100 ± 0.00	100 ± 0.00
H2O2 (73 µM) + 25 µg/ml extract	53.25 ± 0.44	50.42 ± 0.48
H2O2 (73 µM) + 50 µg/ml extract	57.05 ± 1.08	51.06 ± 0.85
H2O2 (73 µM) + 100 µg/ml extract	64.85 ± 0.21	51.38 ± 0.61

Table 3: The hepatoprotective activity showed by the methanolic extracts of broad and narrow leaf varieties

Treatment	% Cell viability	
	Broad	Narrow
Untreated	100 ± 0.00	100 ± 0.00
H2O2 (73 µM) + 25 µg/ml extract	52.24 ± 0.68	56.19 ± 0.37
H2O2 (73 µM) + 50 µg/ml extract	56.30 ± 0.28	61.11 ± 0.98
H2O2 (73 µM) + 100 µg/ml extract	65.81 ± 0.39	68.80 ± 0.40

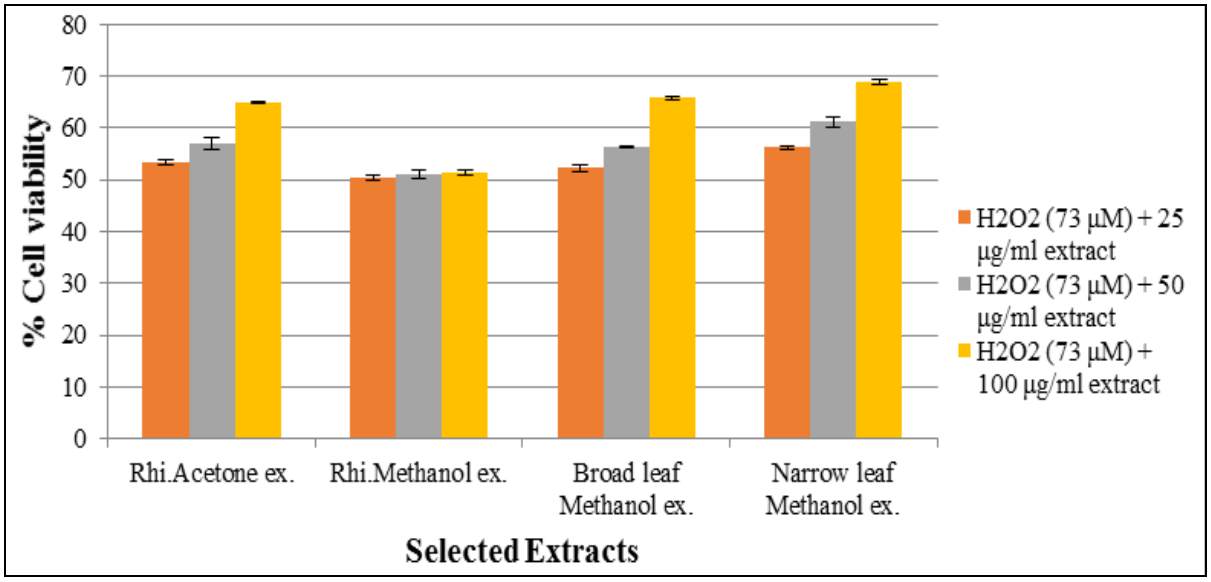

Fig 1: Hepatoprotective activity of Rhizome and Leaf extracts

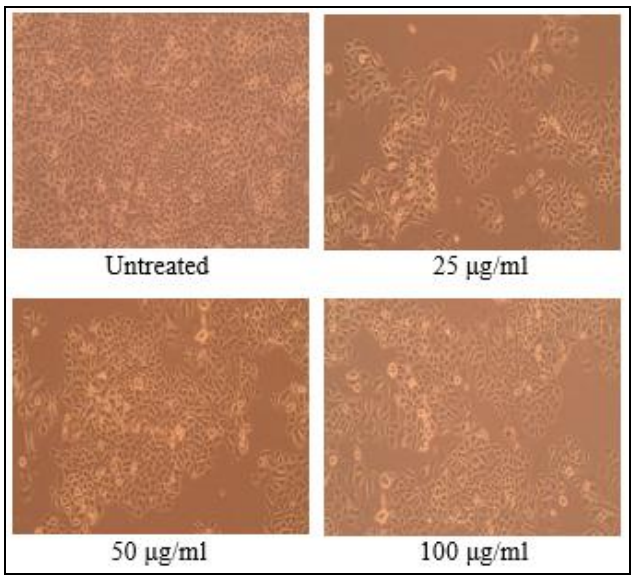

Fig 2: Hepatoprotective activity of Acetone extract of Rhizome

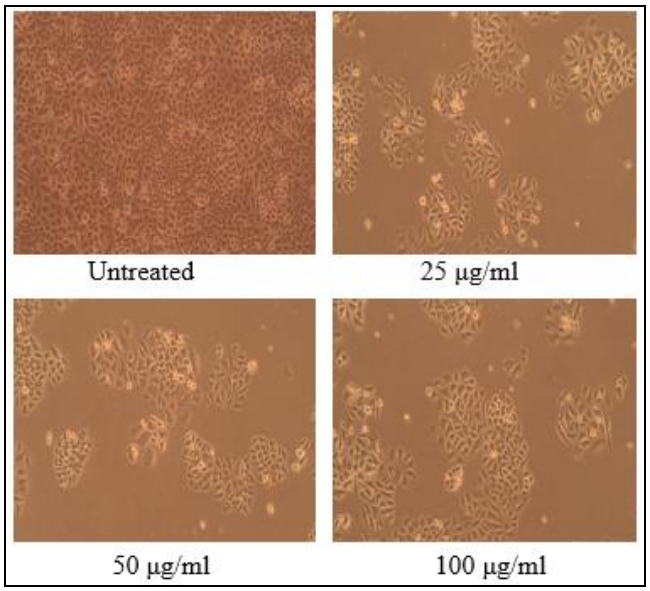

Fig 3: Hepatoprotective activity of Methanolic extract of rhizome

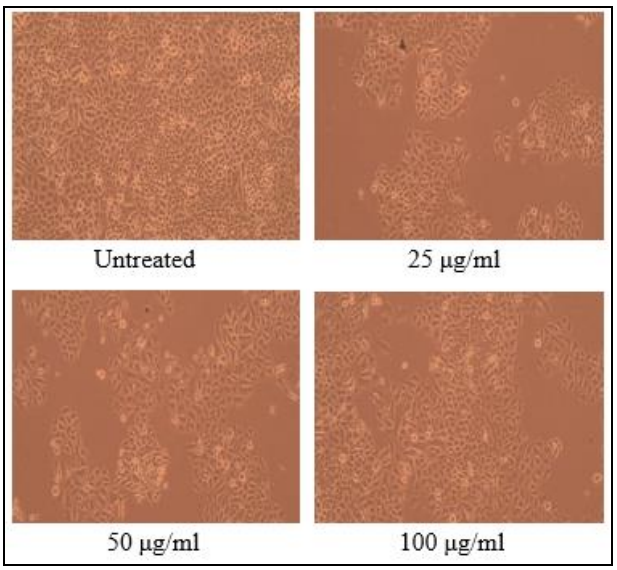

Fig 4: Hepatoprotective activity of Methanolic extract of Broad leaves

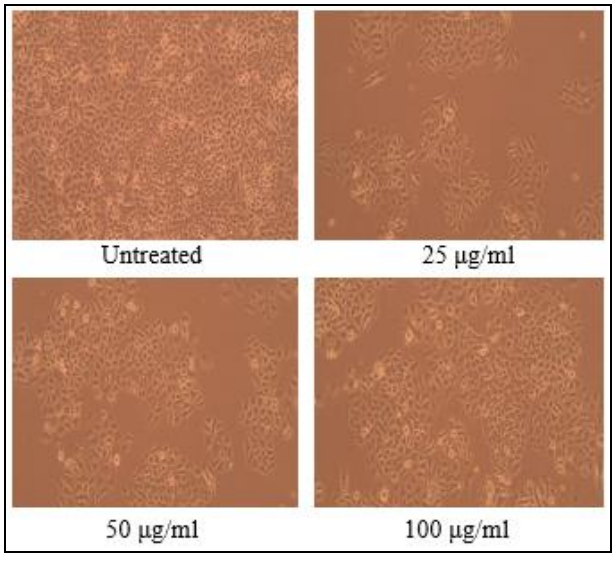

Fig 5: Hepatoprotective activity of Methanolic extract of Narrow leaves

From the present study, it is revealed that the rhizome and leaf extracts of *A. wightii* can inhibit the cytotoxicity induced by H_2O_2 in chang liver cell lines in a concentration-dependent manner. Reports suggest that the activity of endogenous antioxidant enzymes, including catalase (CAT) and superoxide dismutase (SOD), can protect cells from ROS- induced oxidative damage [4, 5]. SOD converts ROS into H_2O_2, and the enzyme catalase then converts the H_2O_2 into molecular oxygen and H_2O [16]. Also, previous studies indicated that the enzyme CAT is the most important enzyme involved in the detoxification of H_2O_2 and the protection of hepatocytes from oxidative stress [17]. Hence, it is possible that the reason behind the hepatoprotective action of the rhizome and leaf extracts might be its ability to increase the levels of endogenous antioxidant enzymes. However, further studies are needed to understand the underlying mechanism of the hepatoprotective action of these extracts and to identify the responsible bioactive compounds.

Conclusion

The *in vitro* hepatoprotective activity of the rhizome and leaf extracts of *A. wightii* against H_2O_2- induced hepatotoxicity in the chang liver cell line was evaluated in the present study. The results of the study revealed that the extracts of both rhizome, as well as the two leaf varieties, inhibited the H_2O_2- induced cytotoxicity in a concentration-dependent manner. These extracts may contain certain bioactive compounds possessing hepatoprotective action, and these compounds, in turn, may provide potential lead molecules for the development of drugs against various liver diseases in the future. However, further studies are needed to isolate and characterize the active principles that are responsible for this hepatoprotective property.

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