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SHORT COMMUNICATION

Evaluating the Hepatoprotective Activity of *Orilaithamarai Karpam* Using *In Vitro* Method

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ABSTRACT
Traditional Siddha medicine offers a wide range of formulations for maintaining liver health and treating hepatic disorders, with more than a hundred herbs recognized for their hepatoprotective properties. In the present study, the liver-protecting potential and possible toxicity of Orilaithamarai Karpam were examined using an in vitro MTT assay in $HEPG_2$ cell cultures. Exposure to CCl_4 resulted in markedly reduced cell viability (44.44%), while higher concentrations of Orilaithamarai (Nervilia aragoana) and the reference drug silymarin (100 µg/ml) improved viability to 86.40% and 88.39%, respectively. Severe cellular injury and death were prominent in the CCl_4 group, with less pronounced effects at lower NA concentrations. The results suggest that NA has both preventive and therapeutic potential against hepatotoxicity. Further investigations are necessary to identify its bioactive compounds and clarify the mechanisms responsible for its activity.
Keywords: *Siddha medicine, Kayakarpam, Hepatoprotective, Orilaithamarai Karpam.*

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INTRODUCTION

Siddha system is an ancient medical tradition that emerged in South India and is considered to be one of the India's earliest systems of medicine [1]. In Siddha medicine, the term *Kaya* refers to the human body, while *Karpam* denotes resilience or firmness comparable to stone. It is also believed to cleanse the inner self by maintaining the body in a balanced state [2]. *Kayakarpam* focuses on promoting longevity, reducing disease burden, and enhancing overall lifespan [3]. Liver is a vital organ that plays a key role in the detoxification and metabolic transformation of toxic substances. During detoxification, the hepatocytes generate reactive oxygen species (ROS), which can trigger oxidative stress, structural alterations, and cell death, leading to hepatotoxicity or liver injury. Natural products derived from medicinal plants offer promising and safer approaches for the treatment of hepatotoxicity [4]. Liver diseases are identified as the second major cause of death, amongst all digestive diseases [5]. Each year, liver diseases are responsible for nearly 2 million deaths worldwide, contributing to about 4% of all global deaths [6]. In the Siddha system, the liver is regarded as a vital organ with key roles in metabolism. Several indigenous formulations, comprising herbs, minerals, and polyherbal combinations, are traditionally used for the management of liver disorders. Liver is considered the seat of Pitta. When *pitham*- secretion of bile distributed through the influence of other humors, it results in different types of liver diseases. The imbalance in *pitham* affects the first two physical constituents, *Udal Thathukal* namely *saram* -nutritive juice and *Kuruthi*- blood. This leading to tissue depletion and obstruction in the normal flow of *pithuneer*, bile. Such aggravation of pitta is viewed as a major cause of liver diseases [7]. The present study aims to assess the hepatoprotective potential of *Orilaithamarai Karpam* using in- vitro MTT assay.

MATERIAL AND METHODS

Collection of Drug:

The whole plant was collected from Thirissur, Kerala, India.

Identification and Authentication

The plant *Orilaithamarai* was identified and authenticated by a medicinal botanist at the National Institute of Siddha, Chennai – 47.

Ingredients of *Orilaithamarai karpam*:

Orilaithamarai – *Nervilia aragoana* Gaudich [8] – The whole plant.

The whole plant of *Orilaithamarai* was dried in dark room temperature for 7 days. Then, it powdered and collected in an airtight container.

Indication:

Siruneer kulirndhu kan erichchal, kamalai, *kadiya pitham.*

Adjuvant: Ghee

Duration: 40 days

Reference: *Bogar Karpam* 300 [9], Pg.no-24

In Vitro Hepatoprotective Activity

Preparation of $HEPG_2$ cell suspension

$HEPG_2$ cell subcultures maintained in Dulbecco's Modified Eagle's Medium (DMEM) were subjected to trypsinization after removal of the spent medium. Following disaggregation, 25 mL of DMEM supplemented with 10% fetal calf serum (FCS) was added. The cells were gently pipetted to achieve uniform suspension and proper homogenization.

Seeding of cells

The homogenized cell suspension (~10000 cells) was added to each well of a 96- well culture plate along with the different concentrations of sample NA EtOH extract (25, 50, and 100 µg/mL) and single concentration (100 µg/mL) of standard silymarin incubated at 37°C in a humidified CO_2 incubator with 5% CO_2 for 12 h and then treated with 40 mM CCl_4 for 1.5 h. The cells were incubated with DMEM in DMSO (0.05% v/v) for 12 h and then treated with DMEM with 40 mM CCl_4 for 1.5 h served as a control treatment [10].

Cell viability assay

Cell viability was assessed using the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. In metabolically active cells, mitochondrial enzymes such as succinate dehydrogenase and reductase convert MTT into insoluble purple formazan crystals. The quantity of formazan formed reflects the number of viable cells, with reduced production indicating increased cytotoxicity. Following 1.5 h of CCl_4 exposure, the medium was discarded, and cells were treated with MTT (0.5 mg/mL) for 2 h. The resulting formazan crystals were solubilized using 200 µL of DMSO per well, and absorbance was recorded at 570 nm (Readwell Touch, Mumbai, India). Cell viability was calculated as the percentage ratio of absorbance values of treated samples to untreated controls [11].

RESULTS AND DISCUSSION

The in-vitro hepatoprotective activities observed in $HEPG_2$ cell lines by measuring cell viability upon damage with CCl_4 were significantly protected by the NA EtOH extract and the different concentrations of the test samples NA EtOH extract are shown in Table 1. In the $HEPG_2$ cell line, low cell viability effects were observed in the CCl_4 treated sample (44.44%), while the higher concentrations of NA EtOH extract and standard drug silymarin at 100 µg/ml showed higher cell viability as 86.40 % and 88.39%, respectively (Figure 1). The cell damage and massive cell death was occurred in CCl_4 treatment followed by test samples lower concentrations are shown in Figure 2.

Table 1: In-vitro hepatoprotective effect of NA EtOH extract against $HEPG_2$ cell line induced with CCl_4

Concentrations (µg/ml)	Cell Viability (%)
Control	100.00
CCl_4	44.44
NA 25.0	67.79
NA 50.0	75.69
NA 100.0	86.40
Silymarin	88.39

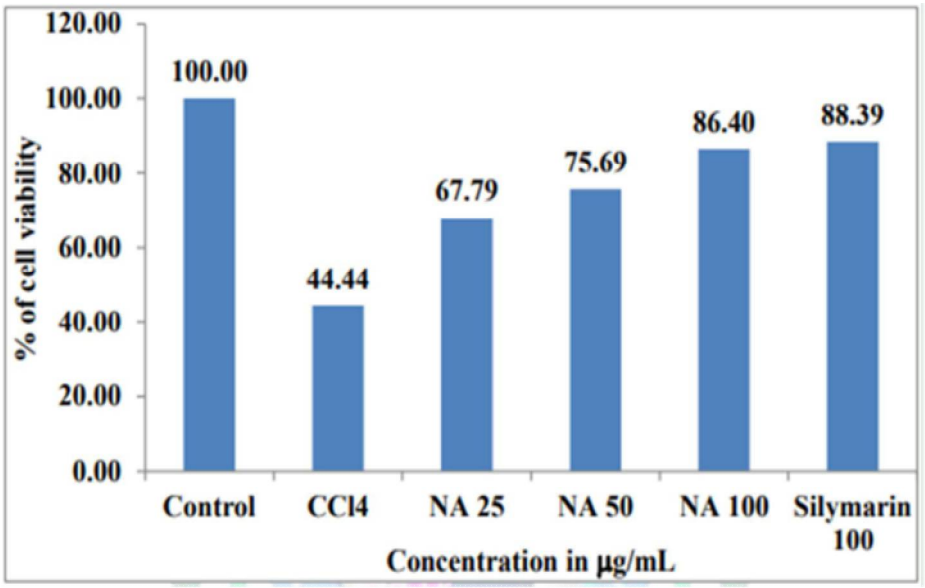

Figure 1: In vitro hepatoprotective effect of NA EtOH extract in $HEPG_2$ cell line induced with CCl_4

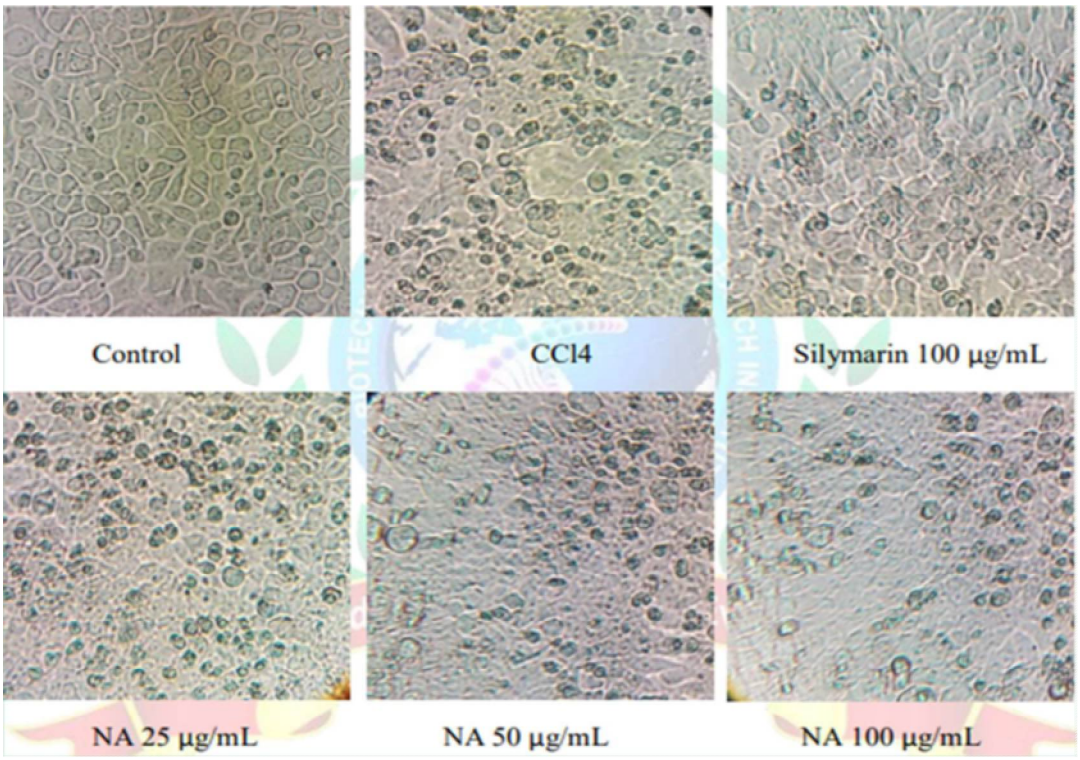

Figure 2: Hepatoprotective effect of NA EtOH extract and silymarin in $HEPG_2$ cell line induced with CCl_4

CONCLUSION

The *Orilaithamarai Karpam* has the potential to function both as a preventive and therapeutic agent against liver toxicity. However, further research is required to identify its active compounds and elucidate their mechanisms of action. Thus, it is concluded that *Orilaithamarai Karpam* has significant Hepatoprotective activity.

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CONFLICT OF INTEREST
The author(s) affirm that there are no conflicts of interest related to the research, authorship, or publication of this article.

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