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ANTI-PROLIFERATIVE EFFECT OF ASIATIC ACID ON HEP-G2 CELL LINE

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ABSTRACT

Asiatic acid (AA) is a pentacyclic triterpene in the leaf of the plant Centella asiatica (CA) is known to inhibit proliferation and induce apoptosis in several tumor cell lines. Plants are playing a significant role in human life as food, shelter and stability of the ecosystem. Most importantly to humans, it is currently estimated that 50% of all drugs in clinical use has been derived from natural products and at least 25% of all prescription drugs contain ingredients extracted from plants. In the present study, the antiproliferative activity of various concentrations (10, 20, 30, 40, 50, 60 µg/ml) of AA, a active principle of CA, on human Hep G2 liver cell lines (untreated and treated) was determined by the MTT assay based on the detection of mitochondrial dehydrogenase activity in living cells. The study reveals that the AA effectively inhibits the growth of cancer cells in concentration dependent manner and at a high of 85 % at the concentration of 50µg/ml.

Keywords: Asiatic acid, Hep G2, MTT assay, *Centella asiatica*, Antiproliferative, Mitochondrial dehydrogenase.

1. INTRODUCTION

There is an increasing interest in researches for production of biologically active compounds from natural sources. Bioactive compounds are remarkable due to prevention and/or treatment of diseases such as diabetes, cancer, liver and heart disease. Historically, the developments of novel drug were primarily through the extraction of biological active compounds from plants which were identified through medicinal use or a variety of bioactivity screening program. Researchers have examining medicinal plant through from the ancient use. In spite of the recent domination of the synthetic chemistry as a method to discover and produce drugs, the potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is still enormous [1]; [2]; [3]. Several active compounds have been discovered from plants and used directly as patented drugs like taxol, artemisinin and maprouneacin [4]; [5]; [6]. Due to

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the multitargeting effect, inexpensive and safety of plant-based products compared to synthetic agents, there is a need for more and more searching and discovering of new drugs from plants.

Centella asiatica (CA), Urban (syn. Hydrocotyle asiaticaL.) belongs to the Apiaceae family (umbelliferae). CA has immunomodulatory, antidepressive, antimicrobial, antiviral, anticonvulsant and analgesic effects [7]; [8]. CA powder and its extracts have the ability to improve venous insufficiency [8]. Some studies have investigated CA scientifically and was found to possess a number of notable pharmacological effects including anti-tumour, antimicrobial [9] antinociceptive, anti-inflammatory [10] antioxidant [11] anxiolytic [12] antipsoriatic [13] wound healing [14] antihyperglycemic [15] hepatoprotective [16] and anti-gastric ulcer [17].

Asiatic acid (AA), a pentacyclic triterpene derivative from CA has been shown to display neuroprotective properties both *in vitro* and *in vivo* [18]. In cellular systems, AA was reported to offer protection against β-amyloid-induced cell death in the neuroblastoma B103 cell line. It also reduced H_2O_2-related cell death and decreased intracellular free radical concentration. Furthermore, AA derivatives were effective at rescuing primary rat cortical cells from glutamate-induced toxicity through activation of the cellular oxidative defense pathway [19].

AA was recently reported to have antistress, hypolipidemic [20] antioxidative, anti-inflammatory and hepatoprotective activity [21] AA shares the protective effect against hepatic inflammatory injury through inhibition of 5-lipoxygenase pathway [22]. AA has been shown to promote fibroblast proliferation and collagen synthesis and to stimulate extracellular matrix accumulation in a rat wound model. In addition, AA like other triterpenes has been reported to possess other biological effects, including hepatoprotection and protective effects against b-amyloid-induced and glutamate-induced neurotoxicity. Apoptosis-inducing activity of AA mediated through increased intracellular Ca^{2+}, resulting in enhanced p53 expression in HepG2 cell [23]. Lipopolysaccharide (LPS) and D-galactosamine (D-GalN)-induced mitochondrial injury in mice could be blocked by the pretreatment of AA [24]; [25]; [26]; [27]. AA induced apoptosis and cell cycle arrest through activation of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways [26].

Phytochemicals derived from plants have been used as important source of several clinically useful anti-cancer agents such as vinblastine, vincristine, camptothecin derivatives, topotecan, irinotecan, etoposide and paclitaxel [28]. The search for anti-cancer agents from plants started in 1950s with the discovery of the vinca alkaloids, vinblastine and vincristine. As a result, the United States National Cancer Institute (NCI) initiated an extensive plant collection programme in 1960 that focused mainly in temperate regions, to expand the search of plants with anticancer properties [28]. This has led to the discovery of many novel compounds showing a range of cytotoxic activities. To date, new plant derived clinical anticancer agents have not yet reached the stage of general use but a number of anticancer agents are in a preclinical development but may take several years before they can be fully applied in medical treatment [28]. In vitro bioassays have resulted in the discovery of some novel therapeutic agents and are continually revealing

compounds from plants used in traditional medicines, which help to explain their traditional usage. They may also be valuable in providing evidence on the modes of action of plant compounds, which have shown activity in clinical or *in vivo* studies [29].

As per literature survey, there is no earlier report revealing the antiproliferative activity of AA. Hence, this attempt was made to evaluate the antiproliferative activity of AA in liver cell line (Hep-G2).

2. MATERIALS AND METHODS

AA was purchased from Sigma-Aldrich Co, USA and dissolved in physiological saline with DMSO as suspension.

2.1. Liver Cell Line (Hep-G2)

Monolayer cultures of human Chang liver cells were obtained from the National Centre for Cell Line (NCCS), Pune, India.

2.2. Anticancer Activity (MTT Assay)

The proliferation activity of cell populations-untreated and treated with AA was determined by the MTT assay based on the detection of mitochondrial dehydrogenase activity in living cells [30]. MTT is a yellow tetrazolium salt, metabolized by NAD-dependent dehydrogenase (in active mitochondria) to form a dark blue formazan product. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide), Dimethyl sulfoxide (DMSO) were obtained from Sigma-aldrich Co. Yellow color MTT is converted to the blue formazan product by metabolically active mitochondria, and the absorbance is directly proportional to the number of viable cells. MTT solution (0.5 g/L) was added to each culture well 24h after the treatment of AA at various concentrations 10, 20, 30, 40, 50µg/ml, and they were allowed to develop color for additional 4 h incubation. An equal volume of DMSO was added to stop the reaction and to solubilize the blue crystals. Samples were transferred into culture plates and the absorbance was measured at 560 nm with a reference wavelength of 690 nm after 30 seconds of shaking in a microplate reader. The toxicity percentage was calculated using the following formula:

$$\text{Toxicity Percentage} = 1 - \frac{(\text{OD extract} - \text{OD medium control}) \times 100}{(\text{OD cell control} - \text{OD medium control})}.$$

2.3. Statistical Analysis

All the values were expressed as means ± SD of six determinants. The data were statistically evaluated by ANOVA and followed by Duncan Multiple Range test (DMRT). Significance was set at $p < 0.05$.

3. RESULTS

The table 1 reveals the antiproliferative activity of AA. It inhibited the growth of cancer cells in the concentration dependent manner and at a high of 85 % at the concentration of 60µg/ml.

4. DISCUSSION

There has been a 22% increase in cancer incidence and mortality, with over 10 million new cases and over 6 million deaths Worldwide in the year 2000 and cases could further increased by in the year 2020 [31]. Liver cancer is rapidly rising and is strongly related to lifestyle; with 90% of the cases arising in people who are taking alcohol and nicotine [32]. The use of medicinal plant extracts for cancer therapy is rapidly evolving as they are affordable, with limited or no side effects. The active components present in such extracts have been shown to efficiently inhibit the process of multi-stage carcinogenesis in a synergistic manner. The identification and characterization of components with potential anti-cancer activity derived from herbal or medicinal plant extracts has been gaining attention. Earlier reports revealed that the antioxidant activity prevents development of cancers [33]. So in this context, as AA is a strong antioxidant, we examined the antiproliferative ability of AA using human liver cell lines. In the present study, AA inhibited the growth of cancer cells in the concentration dependent manner and at a high of 85 % at the concentration of 50µg/ml.

For thousands of years, natural products have been applied to treat and prevent the human cancers [34]. Importantly, nearly one hundred plant-derived compounds are currently undergoing clinical trials [35]. Meanwhile, the molecular mechanisms and targets of some compounds are still unclear. AA, a plant-derived triterpenoid compound, has anti-tumor effect in some human cancer cell lines [36]; [37]. AA was reported to inhibit cell growth and promote cell apoptosis through mitochondrial death cascade in colon cancer cells [38]. AA could also induce apoptosis in human melanoma cells by generation of reactive oxygen species (ROS) [39]; [40]. Down-regulation of the expression and secretion of vascular endothelial growth factor (VEGF) by AA could inhibit the angiogenesis of endothelial cells [41]. Moreover, AA could inhibit liver fibrosis by blocking transforming growth factor (TGF)-beta/Smad signaling in vivo and in vitro [40].

The anticancer activity AA was evaluated using MTT assay method. According to [13] CA aqueous extract suppressed the proliferation of keratinocytes cell line (SVK- 14). Coldren, et al. [42] found the anti-proliferation effect of CA aqueous extract on human dermal fibroblasts. Chen, et al. [43] reported that AA Promotes p21WAF1/CIP1 Protein Stability through Attenuation of NDR1/2 Dependent Phosphorylation of p21WAF1/ CIP1 in HepG2 Human Hepatoma Cells.

The obtained result in the present study clearly indicates that AA exhibits anticancer activity, in a dose dependant manner. 60µg/ml of AA showed more potent cytotoxic effect (84.43 %). The concentration study reveals that AA effectively inhibits the growth of cancer cells (83.65 %) even at 50µg/ml which is significant as 60µg/ml. It has been reported that antioxidant from

plants and natural food stuffs having anticancerous activity and thus great beneficial to the human life [44]. AA is also reported to have antioxidant properties due to the presence of four –OH groups in its structure. Hence, the antiproliferative activity of AA might be due to its potent antioxidant nature.

This study documents that the aciatic acid, a pentacyclic triterpene derivative from *Centella asiatica* has antiproliferative effect against HEP –G2 cell lines which reveals its anticancer property. Multiple mechanisms may interplay in its anticancer efficacy and further research on the mechanism of action of aciatic acid is to be explored.

Table-1. Anti-Proliferative effect of Asiatic acid on Hep-G2 cancer cell line by MTT assay

Concentration of TA (µg/ml)	% of cell viability	% of cell cytotoxity
10	64.00 ± 3.10^{a}	36.00 ± 1.30^{a}
20	45.57 ± 3.00^{b}	55.43 ± 1.71^{b}
30	31.03 ± 2.86^{c}	68.97 ± 3.03^{c}
40	34.72 ± 2.51^{d}	65.28 ± 3.85^{d}
50	16.35 ± 1.82^{e}	83.65 ± 4.10^{e}
60	15.57 ± 1.79^{e}	84.43 ± 3.98^{e}

Value are mean ± SD of six determinant; values sharing common alphabetic having no significant at $p<0.05$.

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5.2. Conflict of Interest

The authors declare that they have no Conflict of interest.

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