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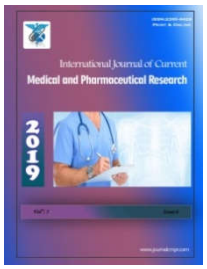

Research Article

EFFECT OF DIFFERENT *SOPHORA FLAVESCENS* EXTRACTS ON PROLIFERATION OF HEPG2 AND BT474 CELL LINES

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ABSTRACT

This study aimed to estimate the changes of proliferation of HepG2 and BT474 cell lines under the treatment with *Sophora flavescens* extracts by Ethyl acetate and Chloroform methods. The cell proliferation was evaluated by WST-1 assay. The HepG2 and BT474 cell lines were treated with Ethyl acetate extract and Chloroform extract at a concentration of 3.125, 6.25, 12.5, 25, 50, and 100 µg/ml. The results showed that the proliferation of HepG2 cell was reduced by treatment of 25 µg/ml Ethyl acetate extract. The reduced proliferation was observed in HepG2 by treatment with 25 µg/ml Chloroform extract. The Chloroform extract also induced the decrease of proliferation in BT474 cell line while there was no statistically significant difference in BT474 cell proliferation between groups by the treatment of Ethyl acetate extract. The Chloroform extract and Ethyl acetate extracts gave IC50 values of 47.08 and 45.92 µg/ml against the Hep G2 cell line. This result suggested that Chloroform extract and Ethyl acetate extracts of *Sophora flavescens* were efficient to inhibition of HepG2 proliferation.

INTRODUCTION

*Sophora flavescens*is a medicinal plant that has received much attention recently. *Sophora flavescens* contains a number of pharmacological activity compounds such as alkaloids, flavonoids, glycosides which exhibit the ability of anti-oxidant and anti-cancer activities [1-2]. The alkaloid compounds in the roots of *Sophora flavescens*are mainly matrin and oxymatrin [3]. In addition, the present of isoprenyl and lavandulyl groups of flavonoids was determined in *Sophora flavescens* by NMR [4]. Xiao-Chi Ma and colleagues used the RP-LC method to identify nine flavonoids from 26 *Sophora flavescens*[5]. Most recent studies have mainly assessed the biological effects of alkaloid compounds isolated in the roots of *Sophora flavescens* such as anti-inflammatory, anti-microbial activity [6]. Jingyu Gou evaluated the antioxidant and microbial resistance of compounds isolated from Sophora flavescens [7]. Sophora flavescens SFNP nano-form has been shown to have the anti-inflammatory effect [2]. In addition, the in vitro anti-inflammatory activity of *Sophora flavescens* grown in Russia was presented in the study of Kalinkevich and colleagues [8].

The research on the activity of *Sophora flavescens* is limited, especially the evaluation of the anti-cancer activities from *Sophora flavescens* extracts. In this study, we assessed the effect of *Sophora flavescens* extracts on the proliferation of BT474 breast cancer cells and HepG2 cell line.

MATERIALS AND METHODS

Cell culture

HepG2 and BT474 cells were thawed and cultured in DMEM-F12, supplemented with 10% FBS and 1% Penicillin/Streptomycin (all reagents were purchased from Sigma-Aldrich, St. Louis, MO, USA). All cells were maintained at 37°C, 5% CO2.

Cell treatment

Each cell line was seeded to 96-well plate with the densities 1 x 10^4 cells/well for HepG2 and BT474. The extractions of *Sophora flavescens* in 4 different solvents were used including Ethyl acetate, Cloroform. Each extraction was prepared at 10mg/ml for initial concentration, and was diluted into different concentrations: 3.125, 6.25, 12.5, 25, 50, and 100µg/ml.

Cell proliferation assay

After 48 hours with extract treatment, cell viability was assessed using the WST-1 kit (Sigma, USA). 10 µl of WST-1 was added to each of the well containing the treated cells with

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extracts. Cells were incubated with WST-1 for 3.5 hours, measured at 450 nm by Gloxmax (Promega, USA).

Statistical analysis

The experiments were triplicated. The data were analyzed for statistical significance by one-way ANOVA where P<0.05 was considered as statistically significant.

RESULTS

Effect of Sophora flavescens extracts on proliferation of HepG2

In this study, Ethyl acetate and Chloroform was employed for collection of *Sophora flavescens* extract. The effect of Ethyl acetate extracts on proliferation of HepG2 was assessed by WST-1 assay. The results showed that there was no statistically different significance of O.D. values between groups 3.125, 6.25, and 12.5µg/ml Ethyl acetate extract treatment by indicating 1.54 ± 0.05, 1.51 ± 0.04, and 1.49 ± 0.03, respectively (Figure 1).

The O.D. value was reduced from group 25 µg/ml of Ethyl acetate extract (1.33 ± 0.04). This value was strongly decreased in groups 50 and 100 µg/ml Ethyl acetate extract treatment, by demonstrating 0.65 ± 0.01 and 0.22 ± 0.01, respectively.

The effect of Chloroform extracts on proliferation of HepG2 was also estimated by WST-1 assay. The results showed that there was no statistically different significance of O.D. values between groups 3.125, 6.25 , and 12.5 µg/ml Chloroform extract treatment by indicating 1.67 ± 0.01, 1.72 ± 0.01, and 1.76 ± 0.03, respectively (Figure 2). The O.D. value was reduced from group 25 µg/ml of Chloroform extract (1.09 ± 0.05). This value was strongly decreased in groups 50 and 100 µg/ml Ethyl acetate extract treatment, by demonstrating 0.71 ± 0.04 and 0.21 ± 0.002, respectively.

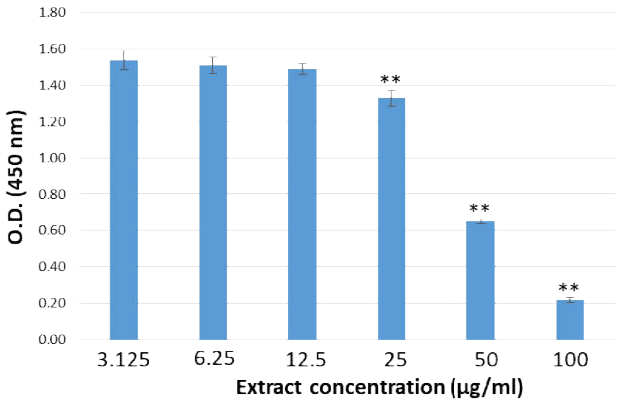

Figure 1 Effect of Ethyl acetate extract on HepG2 proliferation. **p < 0.01.

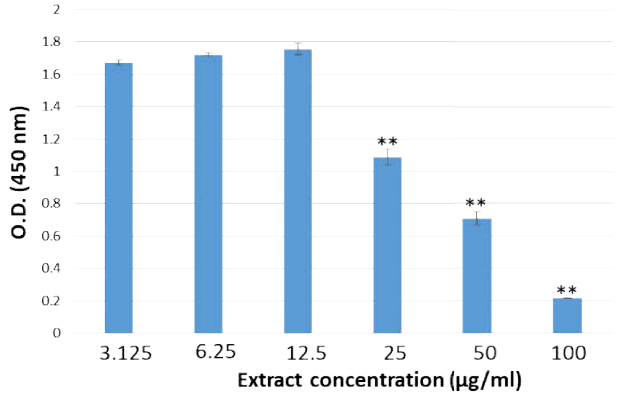

Figure 2 Effect of Chloroform extract on proliferation of HepG2 cell line. **p < 0.01.

Effect of Sophora flavescens extracts on proliferation of BT474

In this study, we also evaluated the effect *Sophora flavescens* extracts on proliferation of BT474 breast cancer cell line by WST-1 assay. The results showed that there was no statistically different significance of O.D. values between all groups which were treated with Ethyl acetate extracts (Figure 3).

However, the O.D. values was reduced from groups which were treated with Chloroform extract (Figure 4). There was no statistically significant difference of O.D. values between groups of 3.125 and 6.25 µg/ml Chloroform extract treatment, by indicating 0.70 ± 0.02 and 0.68 ± 0.001, respectively. The O.D. value were reduced from group of 12.5 µg/ml Chloroform extract treatment (0.58 ± 0.01). This value was also reduced in groups of 25 and 50 µg/ml Chloroform extract treatment, comparing to group of 12.5 µg/ml Chloroform extract treatment, however there no statistically different significance of O.D. values in these groups. The O.D. value was dramatically reduced in group of 100 µg/ml Chloroform extract treatment (0.35 ± 0.01).

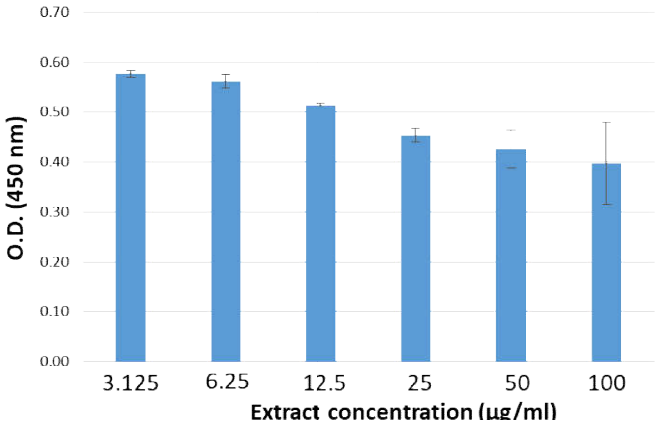

Figure 3 Effect of Ethyl acetate extract on proliferation of BT474 cell line. **p < 0.01.

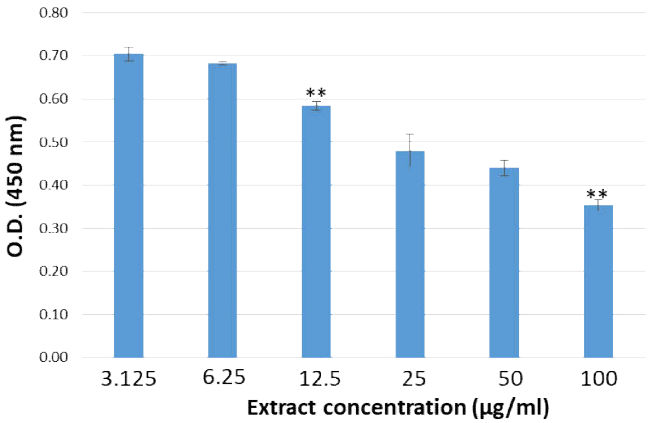

Figure 4 Effect of Cloroform extract on proliferation of BT474 cell line. **p < 0.01.

DISCUSSION

Sophora flavescens contain matrine which inhibit the invasiveness and metastasis of the human malignant melanoma cell line A375 and cervical cancer HeLa cells, as well as induce differentiation of leukemia K-562 cells [9-11]. In addition, matrine-induced autophagy in rat C6 glioma cells has been observed by electron microscopy [12]. Moreover, the

antitumor efficacies were confirmed in mice models of H22, S180 and Lewis lung tumors as well as nude mice models of human H460 and Eca-109 xenografted tumors [13].

The proliferation of mammalian cells are evaluated by the level of activity of the enzymes in the cell [14]. The cleavage process was carried out by these enzymes to convert WST-1 to formazan, which is a change in the absorbance values in the culture medium. The changes in the enzyme in the cell lead to the changes of absorbance values. In this study, the O.D. values in culture medium of HepG2 were reduced in both Chloroform extract and Ethyl acetate extracts, suggesting that *Sophora flavescens* extract is capable of reducing the possibility of proliferation of HepG2 cancer cells by inhibiting intracellular enzymes. The Chloroform extract and Ethyl acetate extracts gave IC50 values of 47.08 and 45.92 µg/ml against the HepG2 cell line. This revealed that Chloroform extract and Ethyl acetate extracts of *Sophora flavescens* were efficient in inhibition of HepG2 proliferation.

We also estimated the effect *Sophora flavescens* extracts on proliferation of BT474 breast cancer cell line. The results revealed that the Ethyl acetate extracts of *Sophora flavescens* is inefficient in inhibition of BT474 cell line proliferation. Otherwise, the Chloroform extract of *Sophora flavescens* showed an efficient inhibition of BT474 cell line by demonstrating IC50 value of 91.74 µg/ml. The IC50 value of Chloroform extract against BT474 cell line is higher than HepG2 cell line, suggesting that the *Sophora flavescens* extracts should be used for inhibition of HepG2 cell line than BT474 cell line.

CONCLUSION

In this study, we found that the HepG2 proliferation was efficiently inhibited by both *Sophora flavescens* Chloroform and Ethyl acetate extracts. The reduce of BT474 cell proliferation was demonstrated by treatment with Chloroform extracts of *Sophora flavescens*, while no inhibition was observed in treatment with Ethyl acetate extracts.

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