EFFECTS OF LORANTHUS EUROPAEUS ETHYLACETATE AND CHLOROFORM EXTRACTS ON THE GROWTH OF RHABDOMYOSARCOMA AND RAT EMBRYO FIBROBLAST CELL LINES

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ABSTRACT

In spite of the potential usefulness of herbal drugs, numerous reports of adverse effects and fatalities have highlighted that traditional herbal medicines also need to be evaluated regarding their safety. The present study was designed to evaluate the effects of Iraqi plant, Loranthus europaeus L. (Mistletoe) ethylacetate and chloroform extracts on the growth of Rhabdomyosarcoma (RD) and Rat Embryo Fibroblast (REF) cell lines. These cell lines were incubated with different concentrations of EtAc and chloroform extracts (125, 250 and 500μg/L) and the effect on growth was estimated using crystal violet cytotoxicity assay method; the extent of growth was expressed as percent inhibition and compared with that produced by the vehicle. The results showed that 125 μg/L EtAc extract induced significantly the growth of RD and REF cells, while 250 and 500 μg/L produced pronounced cytotoxic effects; meanwhile, all concentrations of the chloroform extract produced a concentration-dependent decrease of growth in both cell lines. The cytotoxic effect of both extracts is not significantly different in both cases. In conclusion, low concentrations of Loranthus europaeus EtAc produced proliferative activity in RD and REF cells, while higher concentrations showed accelerated cytotoxic effect; meanwhile, the chloroform extract showed consistent cytotoxic activity in concentration dependent pattern.

Keywords: Loranthus europaeus, Cytotoxicity, Rhabdomyosarcoma, Rat embryo fibroblasts

INTRODUCTION

Herbal products have been traditionally used as therapeutic agents and dietary supplements in both Eastern and Western cultures. The use of medicinal plants has substantially increased in the last decades and a World Health Organization survey indicated that 70-80% of the world population still relies on herbal-based traditional medicine for their primary healthcare. Notwithstanding the potential usefulness of herbal drugs, numerous reports of adverse effects and fatalities have highlighted that traditional herbal medicines also need to be evaluated regarding their safety. It is of note that some bioactive compounds present in plants have been reported to interfere with drug kinetics and to produce adverse effects related or unrelated to their pharmacological actions, such as allergic reactions, mutagenic and carcinogenic effects, and several other toxic effects. Loranthus species, as semi-parasitic plants, are known to produce a variety of bioactive compounds, i.e., sesquiterpene lactones from Loranthus parasiticus for the treatment of schizophrenia, (+)-catechin, 3,4-dimethoxycinnamyl alcohol and 3,4,5-trimethoxycinnamyl alcohol from the Loranthus globosus with antimicrobial and antifungal properties. Many other chemical compounds such as triterpenoids from Loranthus g维nkinii, and Loranthus falcatus, flavonoids from the leaves of Loranthus kaoi and Loranthus europaeus, a cytotoxin from Loranthus parasiticus, and phenolics from Loranthus longiflorus have been reported so far. Different types of mistletoes are commonly used in the traditional medicines of various countries of the world for the management, control and/or treatment of a plethora of human disorders. Such ailments include epilepsy, asthma, bronchitis, warts, excessive or irregular menstruation, microbial infections, cancer, diabetes mellitus and hypertension. Although the literature abounds with information on the chemical constituents and pharmacological properties of some mistletoes, such information on Loranthus europaeus, commonly grown in Iraq is lacking. The present study was designed to evaluate the cytotoxic effects of Iraqi plant Loranthus europaeus L. (Mistletoe) ethylacetate (EtAc) and chloroform extracts in Rhabdomyosarcoma (RD) and Rat Embryo Fibroblast (REF) cell lines.

MATERIALS AND METHODS

The plant (Loranthus europaeus) was obtained from the local Iraqi market and authenticated by Dr Ali Al-Mosawy, Department of Biology, College of Science, University of Baghdad. The fruits of the plant (100g) were air-dried at room temperature, crashed to form gymmy powder, extracted and fractionated into two fractions according to the solvent used for extraction (chloroform and ethyl acetate fractions) using hot extraction method (60-80°C) in Soxhlet apparatus. The resulted powder was dried over night at room temperature to be extracted with the other solvents according to the required fraction. Stock solutions are prepared by dissolving 1 g of each fraction in 10 ml of solvent and chemically examined for the presence of alkaloids and flavonoids in each fraction.

Cell Line Preparation for Cytotoxicity Study

The cell lines which were used in this study were supplied by tissue culture unit/ Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR), AL-Mustansiriya University, Rat embryonic fibroblast (REF) and rhabdomyosarcoma cell line (RD). After trypsinization, 200μl of cell suspension seeds was dispensed into each well (1X104 cells/well); after shaking, the plates were incubated for 24 hrs at 37°C for cell attachment. Cell cultures in micro-titration plate (96 wells) were exposed to the plant extracts at different concentrations (125, 250 and 500μg/ml) during the log phase of growth and the effect determined after recovery time.[183]. After exposure to the plant extracts (test) and the vehicles (control), the medium was decanted off and cells in the well were gently washed two times with 0.1 ml sterile PBS. Crystal violet stain (200μl) was added to each well and the plates were incubated for 20 minutes at 37°C. After incubation, excess dye was removed by washing the wells three times with PBS. The optical density of each well was read by using a micro-ELISA reader (Organon-Teknika, Austria) at 492nm. The percent of growth inhibition was calculated according to the following equation:

\[ IR\% = \frac{A - B}{A} \times 100 \]

IR: inhibition rate, A: the optical density of control, B: the optical density of test.

Statistical analysis

Data are expressed as means±SD; unless otherwise indicated, statistical analyses were performed using unpaired t-test. If the overall F value was found statistically significant (P<0.05), further comparisons among groups were made according to post hoc Tukey's test. All statistical analyses were performed using SPSS GraphPad InStat 3 (GraphPad Software Inc., La Jolla, CA, USA) software.

RESULTS

Fig. 1 showed that the 125 μg/L EtAc extract of the plant induces growth of RD cells, while concentrations of 250 and 500μg/L
showed effective cytotoxic effect on this cell line; however, no significant differences reported between the later two concentrations regarding their cytotoxic activity. In Fig. 2, the chloroform extract fraction slightly inhibited the growth of RD cells at concentration of 125 μg/L, this effect increased significantly (P<0.05) with increasing the concentration to 250 and 500 μg/L respectively; no significant differences reported regarding the cytotoxic effects of the two later concentrations. Concerning the effect of the EtAc extract of the plant on Rat Embryo Fibroblast (REF) cell line, 125 μg/L also induces the growth of REF effectively; meanwhile, increasing the concentration to 250 and 500 μg/L produces an opposite effect, where a decrease in the growth of REF cells was reported and no significant differences between the effects of the two later concentrations were reported (Fig. 3). In Fig. 4, 125 μg/L of the chloroform extract showed weak cytotoxic effect on REF cells; this effect increased significantly with increasing the concentration to 250 and 500 μg/L (20% and 40%, P<0.05) respectively. Comparison between the cytotoxic effects of both types of extracts (EtAc and Chloroform) at the highest concentration (500 μg/L) used in both cell lines reveal no significant differences between the effects on the two types of cell lines (RD and REF) (Fig. 5).

Fig. 1: Effects of different concentrations (125, 250 and 500 μg/L) of Loranthus europaeus EtAc extract on the growth of rhabdomyosarcoma (RD) cell line. Values with non-identical letters (a,b) are significantly different.

Fig. 2: Effects of different concentrations (125, 250 and 500 μg/L) of Loranthus europaeus chloroform extract on the growth of rhabdomyosarcoma (RD) cell line. Values with non-identical letters (a,b) are significantly different.

Fig. 3: Effects of different concentrations (125, 250 and 500 μg/L) of Loranthus europaeus EtAc extract on the growth of Rat Embryo Fibroblasts (REF) cell line. Values with non-identical letters (a,b) are significantly different.
DISCUSSION

Previous chemical and pharmacological studies on some species of the Loranthaceae family have indicated the presence of several chemical compounds, including flavonoids and tyramine alkaloids, lectins and viscotoxins, arginine and polysaccharides. According to the phytochemical evaluation of the extracts, flavonoids are found to be the major constituents within all isolated fractions. Flavonoids produced many biological activities when administered both in pure form or within extracts; these include immune-regulation, anti-inflammatory, anti-oxidant, and antibacterial effects; they also played an important role in modulating cell proliferation. In the present study, only low concentrations (125μg/L) of the EtAc extract produced proliferative effect in both RD and REF cell lines, while increasing the concentration results in exaggerated cell death. Although many reports showed that flavonoids arrest cell division and decrease mitotic activity through interference with specific stages of cell cycle in time- and concentration-dependent pattern, this may not be the case when extracts that contain many other constituents (other than flavonoids) are used, and possibility of counter-effects was highly suggested in the present study.

The reported difference in the effects of increasing concentrations can be explained on the bases that in the lower concentration the flavonoid content may be not quite enough to decrease mitotic index, while such effect was counter-regulated when the concentration of the extract was increased, which may lead to the predominance of the activity of other components including the alkaloids with formation of variable adducts with DNA, proteins and other macromolecules and consequently affecting cell divisions by affecting the time of S and/or G2 phases. Similarly, moderate cytotoxic activity of Loranthus globosus EtAc extract was reported only with low doses of this extract in brine shrimp lethality assay model. In tune with the findings of the present study, some investigators have reported that the anti-proliferative and anti-metastatic effects of some Loranthaceae family extracts on melanoma xenografts were only achieved with limited dose range, which was attributed mostly to the immunomodulatory effects reported by the investigators. This finding supports the reported proliferative effects of low concentration of Loranthus EtAc extract on both cell lines. Moreover, the chloroform extract demonstrates concentration dependent cytotoxic effects on both cell lines (Figures 2 and 4); it has been reported previously that 200mg/kg of the chloroform extract of Loranthus europaeus significantly increases mitotic index in mice bone marrow cells, and phytochemical evaluation of this fraction revealed that it contains mainly alkaloids and flavonoids, and the reported effect on the genetic materials may be attributed to its alkaloid content. Mistletoe extracts are widely used in complementary cancer therapy. The composition of mistletoe extracts is very complex and some components even show additional or opposing biological effects on tumor cells. Although Mistletoe preparations are widely used among cancer patients in Europe, and a retrospective study of 700 lymphoma patients who received mistletoe extracts suggested this therapy to be beneficial and revealed no hint for tumor promoting effects, the reported diversity in the type of activity of different concentrations of Loranthus europaeus EtAc and chloroform extracts sounds the alarm to be
careful in using such types of extracts in traditional medicine, especially for treatment of inflammations and tumors.

CONCLUSION

Low concentrations of Loranthus europaeus EtAc extract produced proliferative activity in RD and REF cells, while higher concentrations showed accelerated cytotoxic effects; meanwhile, the chloroform extract of this plant showed consistent cytotoxic activity in concentration dependent pattern.

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