Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

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

INTESAR TARIK NUMAN

Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq Email: intesartaee@yahoo.com

Received: 09 July 2012, Revised and Accepted: 22 Aug 2012

ABSTRACT

In spite of the potential usefulness of herbal drugs, numerous reports of adverse effects Aguand 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[1]. 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[2]. 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[3] and (+)-catechin, 3,4-dimethoxycinnamyl alcohol and 3,4,5-trimethoxycinnamylalcohol from the *Loranthus globosus* with antimicrobial and antifungal properties[4]. Many other chemical components such as triterpenoids from *Loranthus grewinkii*[5], and *Loranthus falcatus*[6], flavonoids from the leaves of *Loranthus kaoi*[7] and *Loranthus europaeus*[8], a cytotoxin from Loranthus parasiticus[9], and phenolics from *Loranthus longiflorus*[10] 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[11], microbial infections[12], cancer[13], diabetes mellitus and hypertension[14,15]. Although the literature abounds with information on the chemical constituents and pharmacological properties of some mistletoes[16,17], 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-Mossawy, Department of Biology, College of Science, University of Baghdad. The fruits of the plant (100g) were air-dried at room temperature, crashed to form gummy 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[18].

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 ($1X10^4$ 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 concentration (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[19]. The percent of growth inhibition was calculated according to the following equation[20]:

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

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

Statistical analysis

Data are expressed as mean±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 500µg/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, 125µg/L also induces the growth of REF effectively; meanwhile, increasing the concentration to 250 and 500µg/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, 125µg/L of the chloroform extract showed weak cytotoxic effect on REF cells; this effect increased significantly with increasing the concentration to 250 and 500µg/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 (500µg/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).

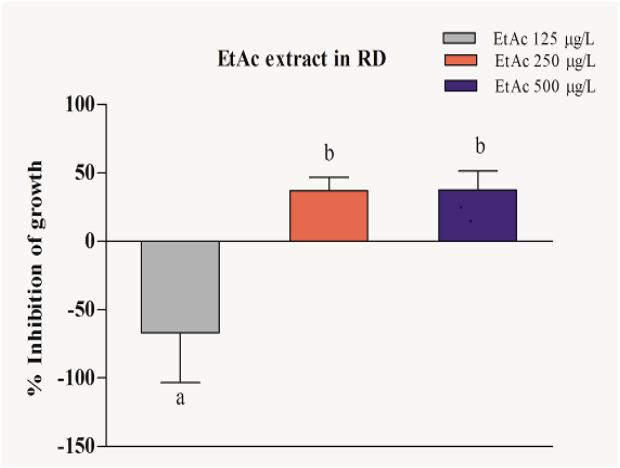

Fig. 1: Effects of different concentrations (125, 250 and 500µg/L) of *Loranthus europaeus* EtAc extract on the growth of rhabdomyosarcoma (RD) cell line. Values with non-identical letters (a,b) are significantly different.

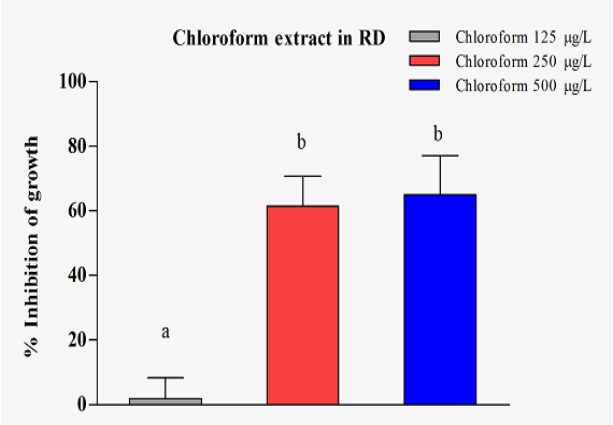

Fig. 2: Effects of different concentrations (125, 250 and 500µg/L) of *Loranthus europaeus* chloroform extract on the growth of rhabdomyosarcoma (RD) cell line. Values with non-identical letters (a,b) are significantly different.

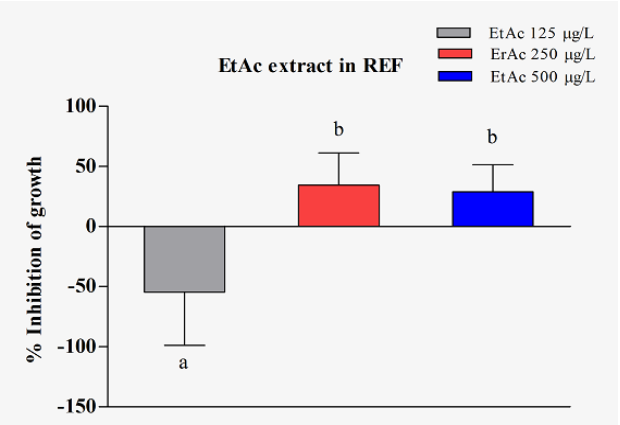

Fig. 3: Effects of different concentrations (125, 250 and 500µg/L) of *Loranthus europaeus* EtAc extract on the growth of Rat Embryo Fibroblasts (REF) cell line. Values with non-identical letters (a,b) are significantly different.

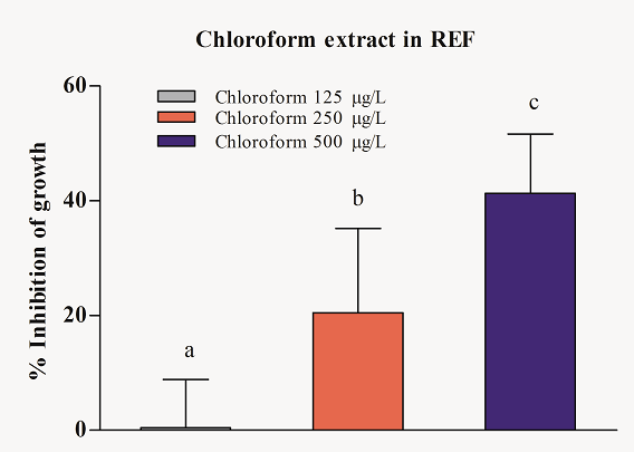

Fig. 4: Effects of different concentrations (125, 250 and 500µg/L) of *Loranthus europaeus* chloroform extract on the growth of Rat Embryo Fibroblasts (REF) cell line. Values with non-identical letters (a,b,c) are significantly different.

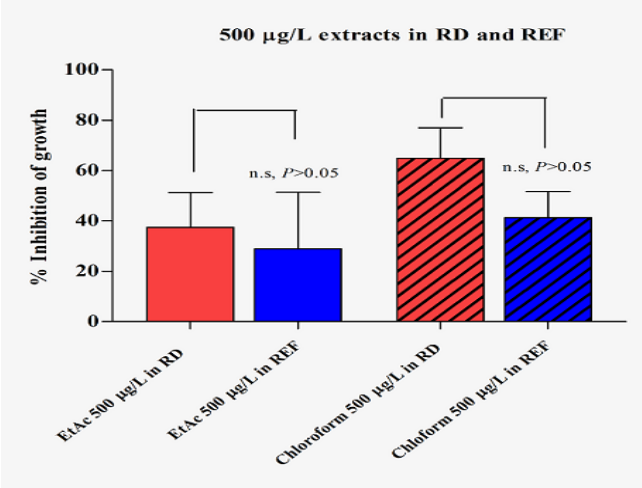

Fig. 5: Comparison between the proliferative effects of EtAc and Chloroform extracts (500µg/L) of Loranthus europaeus on Rhabdomyasarcoma (RD) and Rat Embryo Fibroblast (REF) cell lines; n.s= non-significantly different (*P*>0.05).

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[21], lectins and viscotoxins[22], arginine and polysaccharides[23]. 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-inflammation, anti-oxidant, and antibacterial effects; they also played an important role in modulating cell proliferation[24]. 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[25,26], this may be not 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[27]. Similarly, moderate cytotoxic activity of *Loranthus globosus* EtAc extract was reported only with low doses of this extract in brine shrimp lethality assay model[28]. 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 reportedby the investigators[29]. This finding supports the reported proliferative effects of low concentration of *L.europaeus* 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 *L. europaeus* significantly increases mitotic index in mice bone marrow cells, and phytochemical evaluation of this fraction revealed that it contains mainly alkaloids and flavonoids[30], and the reported effect on the genetic materials may be attributed to its alkaloid content[31]. 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[32]. Although Mistletoe preparations are widely used among cancer patients in Europe[33], 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[34], the reported diversity in the type of activity of different concentrations of *L. 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.

ACKNOWLEDGEMENT

The author thanks University of Baghdad for supporting the project and the national institute of cancer for technical assistance.

REFERENCES

1. Chan K. Some aspects of toxic contaminants in herbal medicines. Chemosphere 2003; 52:1361-1371.
2. Ernst E. Harmless herbs? A review of the recent literature. Am J Med 1998; 104:170-178.
3. Okuda T, Yoshida T, Chen XM, Xie JX, Fukushima M. Corianin from *Coriaria japonica* A. Gray and sesquiterpene lactones from *Loranthus parasiticus* Merr. used for treatment of schizophrenia. Chem Pharm Bull 1987; 35:182-187.
4. Sadik G, Islam R, Rahman MM, Khondkar P, Rashid MA, Sarker SD. Antimicrobial and cytotoxic constituents of *Loranthus globosus*. Fitoterapia 2003; 74:308-311.
5. Rahman A, Khan MA, Khan NH. Loranthol. New pentacyclic triterpenoid from *Loranthus grewinkii*. Phytochemistry 1973; 12:3004-3006.
6. Anjaneyulu ASR, Row LR, Reddy DS. Chemical constituents of *Loranthus falcatus* Linn. Curr Sci 1977; 46:850-851.
7. Lin JH, Lin YT. Flavonoids from the leaves of *Loranthus kaoi* (chao) kiu. Yaowu Shipin Fenxi 1999; 7:185-190.
8. Harvala E, Exner J, Becker H. Flavonoids of Loranthus europaeus. J Natural Products 1984; 47:1054-1055.
9. Zhou H, Zeng Z, Liu R, Chi Z. Purification and characterization of a cytotoxin from *Loranthus Parasiticus* Merr. Sichuan Daxue Xuebao 1993; 30:102-106.
10. Indrani N, Rao VS, Balasubramanian K, Reddy KK, Vijayaramayya T. Studies on *Loranthus longiflorus* Desr. Tannins. Leather Science (Madras) 1980; 27:438-439.
11. Amabeoku GJ, Leng MJ, Syce JA. Antimicrobial and anticonvulsant activities of *Viscum capense.* J Ethnopharm 1998; 61:237-241.
12. Deeni YY, Sadiq NM. Antimicrobial properties and phytochemical constituents of the leaves of African mistletoe (*Tapinanthus dodoneifolius* (DC) Danser (Loranthaceae): an ethnomedicinal plant of Hausaland, Northern Nigeria. J Ethnopharm 2002; 83:235-240.
13. Zee-Cheng RKY. Anticancer research on Loranthaceae plants. Drugs Future 1997; 22:519-530.
14. Obatomi DK, Aina VO, Temple VJ. Effects of African mistletoe on blood pressure in spontaneously hypertensive rats. Int J Pharmacog 1996; 34:124-127.
15. Osadebe PO, Okide GB, Akabogu IC. Study on anti-diabetic activity of crude methanolic extracts of *Loranthus micranthus* (Linn) sourced from five different host trees. J Ethnopharm 2004; 95:133-138.
16. Luczkiewicz M, Cisowski W, Kaiser P, Ochocka R, Piotrowski A. Comparative analysis of phenolic acids in mistletoe plants from various hosts. Acta Pol Pharm 2001; 58:373-379.
17. Rodriguez-Cruz ME, Perez-Ordaz L, Serrato-Barajas BE, Juarez-Oropeza MA, Mascher D, Paredes-Carbajal MC. Endothelium-dependent effects of the ethanolic extract of the mistletoe *Psittacanthus calyculantus* on the vasomotor responses of rat aortic rings. J Ethnopharm 2003; 86:213-218.
18. Singh N, Juyal V, Gupta AK, Gahlot M. Preliminary phytochemical investigation of extract of root of *Bergenia lgulata.* J Pharmacy Res 2009; 2(9):1444-1447.
19. Menard RH, Stripp B, Gillette JR. Decreased testosterone formation in several species and changes in hepatic drug metabolism. Endocrinol 1974; 94:1628-1636.
20. Menard RH, Bartter FC, Gillette JR. Spironolactone and cytochrome P450, impairment of steroid 21-hydroxylation in the adrenal cortex. Arch Biochem Biophys 1976; 173:395-402.
21. Fernandez T, Wagner ML, Varela BG, Ricco RA, Hajos SE, Gurni AA, *et al*. Study of an argentine mistletoe: the hemiparasite *Ligaria cuneifolia* (Loranthaceae). J Ethnopharmacol 1998; 62:25-34.
22. Park JH, Hyun CK, Shin HK. Cytotoxic effects of the components in heat-treated mistletoe (*Viscum album*). Cancer Lett 1999; 139:207-213.
23. Sinha A, Taylor WH, Khan IH, McDaniel ST, Essko JD. Glycoside primers of *Psittacanthus cucullaris*. J Nat Prod 1999; 62:1036-1038.
24. Lee T, Chen CL, Shieh ZH. Study on antioxidant activity of *Echinacea purpurea* L. extracts and its impact on cell viability. Afr J Biotechnol 2009; 8:5097-5105.
25. Ruska G, Gutzeit HO, Muller JL. Structurally related flavonoids with anti-oxidative properties differentially affect cell cycle progression and apoptosis of human acute leukemia. Nutr Research 2005; 25(2):143-155.
26. Kobayashi T, Nakata T, Kuzumaki T. Effect of flavonoids on cell cycle progression in prostate cancer cells. Cancer Lett 2002; 176(1):17-23.
27. Yvonne CM, Hebels GA, Marcel HM, Ralph WH, *et al*. Binary PAH-mixtures cause additive or antagonistic effects on gene expression but synergistic effects on DNA adduct formation. Carcinogenesis 2007; 28(12):2632-2640.
28. Islam R, Khurshid Alam AHM, Hossain MA, Mosaddik MA, Sadik G. Biological screening of Bangladeshi mango mistletoe bark extracts. Fitoterapia 2004; 75:405-408.
29. Thies A, Dautel P, Meyer A, Pfuller U, Schumacher U. Low-dose mistletoe lectin-I reduces melanoma growth and spread in a SCID mouse xenograft model. Br J Cancer 2008; 98:106-112.
30. Hassan AF, Numan IT, Al-Sammarrae KW, Hussain SA. Hematopoietic toxicity of *Loranthus europaeus* chloroform extract: In vitro study. Int J Comprehen Pharmacy 2011; 2(7):1-4.
31. Ji L, Chen Y, Wang Z. Intracellular glutathione plays important roles in pyrrolizidine alkaloid clivorine-induced toxicity on L-02 hepatocytes. Toxicol Mech Methods 2008; 18(8):661-664.
32. Melzer J, Iten F, Hostanska K, Saller R. Efficacy and safety of mistletoe preparations (*Viscum album*) for patients with cancer diseases. Forsch Komplementmed 2009; 16:217-226.
33. van der Weg F, Streuli RA. Use of alternative medicine by patients with cancer in a rural area of Switzerland. Swiss Medical Weekly 2003; 133(1516):233-240.
34. Stumpf C, Rosenberger A, Rieger S, Troger W, Schietzel M. Mistletoe extracts in the therapy of malignant, hematological and lymphatic diseases–a monocentric, retrospective analysis over 16 years. Forschende Komplementarmedizin und Klassische Naturheilkunde 2000; 7(3):139-146.