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Histological Study and Cytotoxic Effect of *Globularia alypum* Leaves

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Abstract: The phenomenon of drug resistance poses a major problem to a successful chemotherapy. Therefore, we have to look for newer component with effective modes by incorporating them into the test bench. Our purpose was to study the localization site of resin of *Globularia alypum* (L.). Also, we investigated its antitumor activity against the Hep-2 cell and Vero cell in vitro by the observation under an inverted microscopy. Hep-2 cells and Vero cell were seeded at a density of 10^5 or 10^6 cells /mL and cultured in the presence of the various concentrations of the extract for 24 h. A study of the anatomy of *G. alypum* using double coloration has highlighted the presence of globular trichomes which can be a secretary site of resin. An *in vitro* cytotoxicity experience showed that the most of Hep-2 cell and Vero cell in monolayer with a density of 10^6 cells/well were destroyed with concentration of 26 and 13 mg/ml.

Keywords*:* cytotoxic effect, *Globularia alypum,* Hep-2 cell, Vero cell, double coloration

I. Introduction

Cancer is presently the second most important cause of mortality; nearly 100,000 persons die each year [1]. Cancer victim usually has to have a billion cancer cells in his body before he dies. But a cancer does not become obvious until there are 109 or 1010 cells – hundreds of millions of malignant cells [1] and [2]. The chemotherapy primarily affects cells in the process of division. The greatest problem of chemotherapy is that not only malignant cells, but also numerous healthy of tissues in the body contain cells and other tumorigenic stem cells demonstrate resistance to chemotherapy which makes successful treatment extremely difficult [3]. The study of medicinal plant as a source of potential novel agent for the treatment of cancer and other diseases has greatly expanded the scope of natural product drug discovery. In some African countries, there are also herbal remedies which have been in use for treatment of many diseases treated by the traditional healers. The effectiveness of these herbal remedies can be evaluated according to the pharmacological research of natural product drugs. The medicinal plants specialist has accorded special attention to *Globularia alypum* (L.). *Globularia* is a genus of about 22 species of flowering plants, native to Central and Southern Europe, Macaronesia, Northwest Africa and South-west Asia [4]. It is a wild plant belonging to *Globulariaceae* family [4]. It is traditionally used to treat as a hypoglycemic agent, laxative, somatic, purgative, intermittent fevers, gastric ulcers and it is used as a cataplasm to mature abscesses [5-8].

More recently, it has been found that the hydromethanolic extract of *G. alypum* has an antioxidant effect [9]. Several biological studies on this plant have shown it to be active against lymphocytic leukaemia P-388 and HeLa cells [10] and [11]. Es-Safi, et al. [9] and Taskova et al. [12] reported the presence of glycosidic iridoids, phenolic acids, flavonoids and a lignan diglucoside.

II. Experimental Section

II.1. Plant material

The leaves of *Gobulaira alypum* L. were harvested on the hills of Medea (North West Algeria), during the period from April to May 2009. After drying in the air, the dried leaves are grounded in a blender. The decoction was prepared by boiling 26 g of the leaf powder in 1 L of distilled water for 20 min. The extract was filtered through a cellulose filter. However, a 26 mg/ml aqueous extract was used to prepare samples from 13 to 1 mg/ml. The obtained solutions were filtered using the sterile syringe filter with a 0.22 µm.

II.2.Histological study

The histological studies were carried out on leave's thin cross sections which were stained with methyl green in combination with Congo red. Fresh hand sections were prepared from *G. alypum* by using a razor blade. The thin cross sections were thoroughly soaked with sodium hypochlorite for duration of 15 to 20 min and washed with distilled water, then the cross sections were treated with acetic acid and stained for 3-5 min with 0.1% methyl green and lignified cell walls will stain green. The cross sections were rinsed with distilled water and stained for 5 min with 2% Congo red and the pecto-cellulose cell walls will stain red. After washing with distilled water, the obtained cross sections were examined and microscopically analyzed with various ocular-objectives.

II.3.Cell culture

Human epithelial type 2 Hep-2 cells are considered to originate from a human laryngeal carcinoma and Vero cells are derived from the kidney of an African green monkey. This adherent cell line was grown in medium Grown in Dulbecco's modified by Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were kept at 37°C in a humidified atmosphere containing 5% CO_2.

II.4.In vitro cell culture studies

Hep-2 cells were seeded at a density of 10^5/100 µL of cell suspension in 180 µl culture medium and cultured in the presence of 40 µL of the concentration of extract 26 and 2.66 mg/mL into 16 wells from 96 wells microplates. Hep-2 cells and Vero cell were seeded at a density of 10^6/100 µL of cell suspension with 180 µL culture medium and cultured in the presence of 40 μL of the various concentrations of extract 26, 13 , 6.66, 2.66, 1.60 and 1 mg/ml, respectively into 16 wells from 96 wells microplates. The plate was incubated for 24 h at 37°C in in a humidified atmosphere containing 5% CO_2.

II.5.Morphological observation of cells

The 96-well micro culture plates into which the Hep-2 cell or Vero cell had been seeded were observed by inverted microscopy. Images of the changes in the conformation of the cells were captured and recorded.

III. Results and Discussion

III.1.Histological study

Microscopic observation of photonic cross freehand on the leaf of *G. alypum* showed that the leaf from the outside to the inside is formed: Epidermis forms initially as a single layer of cells which are walls are highly elongated, which have very thin walls. The internal tissues consist of a palisade parenchyma (PP) with elongated cells located below the epidermis and the cells have thin walls. However, a few other larger veins may extend from the epidermis. The veins become progressively smaller as they move from the midrib to the leaf blade (Figure 1). Epidermal cells elongate to become unicellular trichomes (Ut), or divide to form bicellular trichomes (Bt), having no basal stalk which can be secretary of resin (Figure 2).

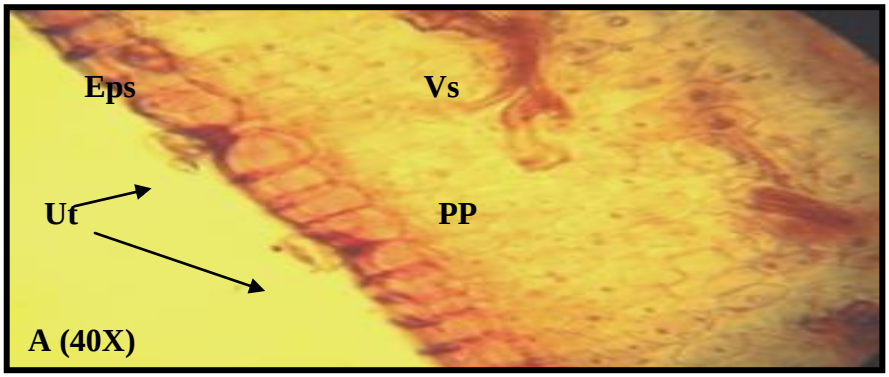

Fig 1 .Leaf anatomy of Globularia alypum , Eps : epidermis , Vs : secondair veins, PP: palisade parenchyma,Ut : unicellular trichomes.

Fig 2. Leaf anatomy of Globularia alypum, (B) Ut : unicellular trichomes, (C) Bt :bicellular trichomes.

III.2.Cytotoxicity effect of Globularia alypum extract

In the present work, we studied the effect of different concentrations obtained from *G. alypum* on cell proliferation of cell line Hep-2 at a density of 10^5 cells/well. The 96-well plates were placed under inverted microscope. A camera recorded the changes in cell morphology in the varying concentration in order to observe the effect that exhibited the high anti-proliferative activity.

A remarkable cytotoxic effect of aqueous extracts against Hep-2 cells has been observed from cell morphology, which broke up or rounded into groups in 16-well plate. Most of the cells in monolayer

were not completely destroyed in 10-well plate at a concentration of 2.66 mg/ml, compared to the control (Figure 3).

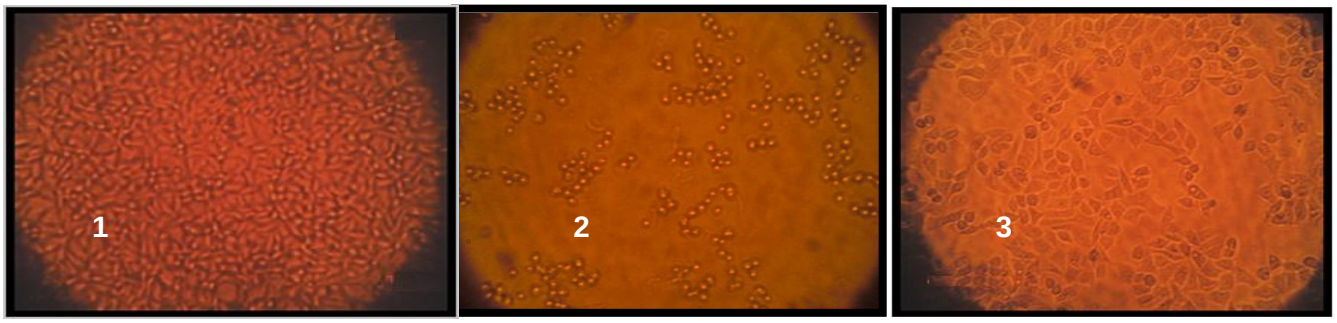

Fig 3. Effect of extract aqueous on morphology of human laryngeal carcinoma Hep-2 for, 0, 26, 2.66 mg/ml (picture 1-3, respectively) using an inverted microscope (Gr x 40).

Figure 4 shows the effect of various concentrations of extract on Hep-2 cell proliferation at a density of 10^6 cells/well. After 24 h of incubation, the aqueous extracts caused from 15-well plate of large rounded polymorphic, shrunken nuclei, which were rounded into many groups (Figure 6), while some of the cells in monolayer were not completely destroyed in 7-well plate at a concentration of 13.33 mg/ml. The tumor cell grew with concentrations of 6.66, 1.62 and 1.15 mg/ml, which numerous rounded cells were observed flouting on cell monolayer compared to the control group. We found that the high dilution had no significant effect on Hep-2 cell. Figure 5 shows the effect of different concentrations of *G. alypum* on Vero-cells proliferation at a density of 10^6 cells/well. The aqueous extract caused cytotoxic effect against Vero cell in 16-well plate, and showed a round shaped form with the nuclear condensation (Figure 6), while untreated cells did not show these apoptotic characteristics and their observation showed that the most of cells in monolayer were destroyed, which were rounded into groups in 15, 13-well plate, with concentrations of 13 and 6.66 mg/ml, respectively. At the concentration of 1.60 and 1 mg/ml, there is no production of a cytotoxic effect. It has often observed that low dilutions act well, when high dilutions *had no effect*. The results obtained in the present study indicate that the aqueous extract of *G. alypum* may be a potential source of natural anticancer substances.

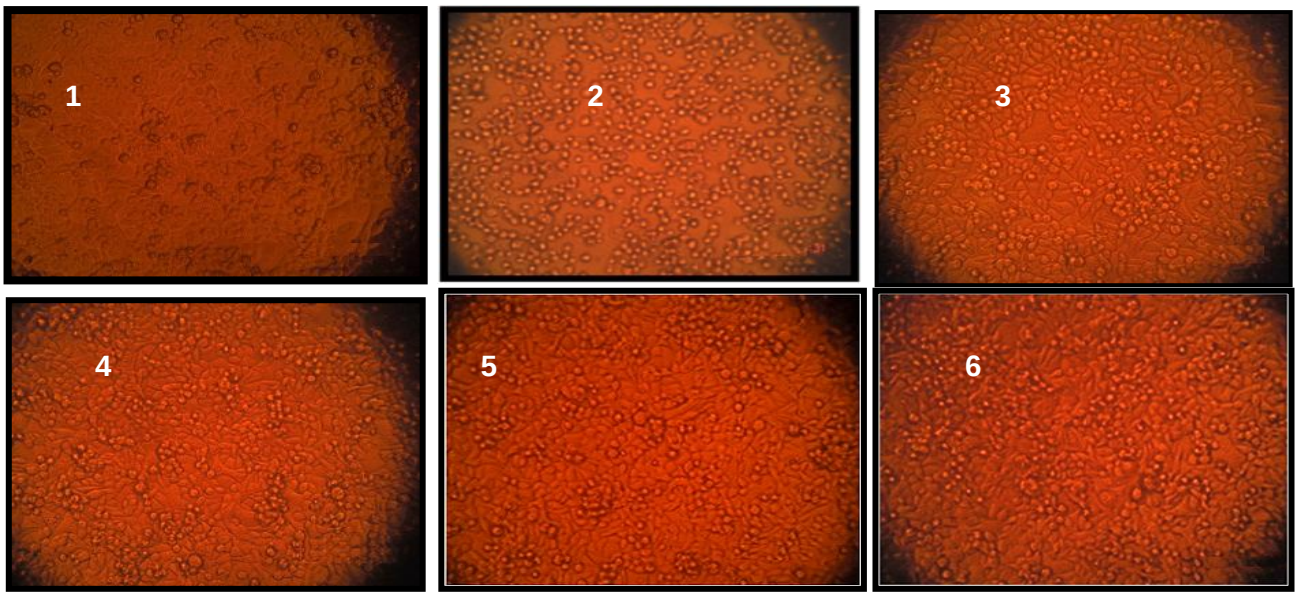

Fig 4: Effect of extract aqueous on morphology of Hep-2 for, 0, 26, 13.33, 6.66, 1.60 and 1 mg/ml (picture 1-6), respectively using an inverted microscope (Gr x 40)

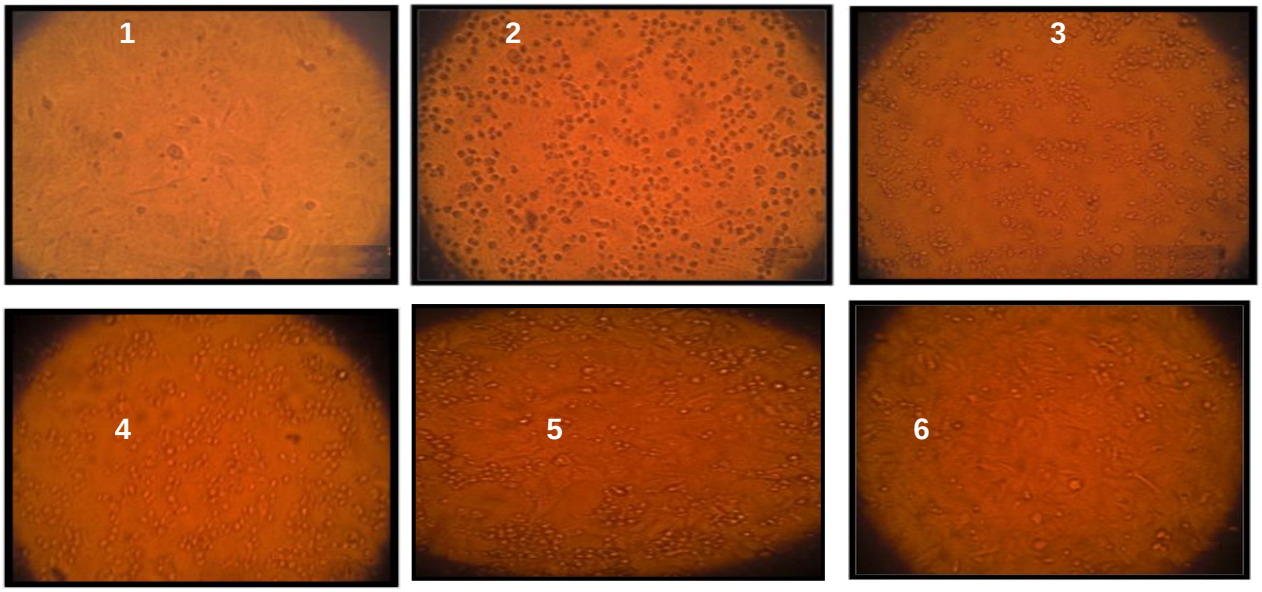

Fig 5. Effect of extract aqueous on morphology of Vero cells for 0, 26, 13.33, 6.66, 1.60 and 1 mg/ml (picture 1-6), respectively using an inverted microscope (Gr x 40).

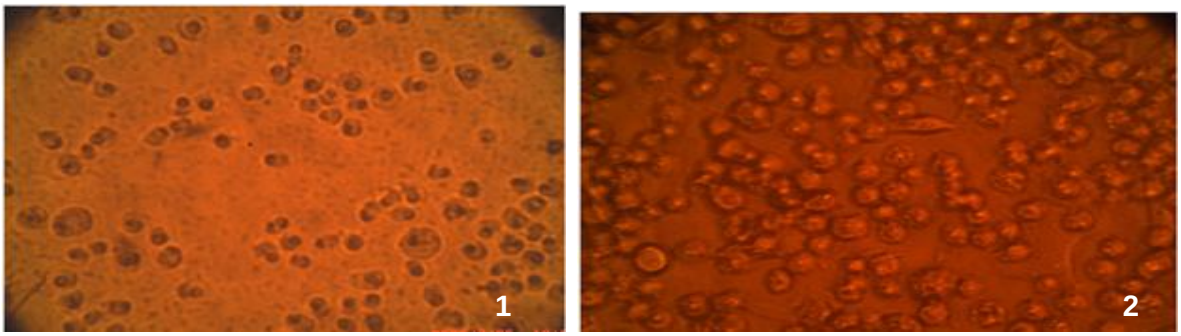

Fig 6. Effect of extract aqueous on morphology of Vero (1) and Hep-2 cell (2) using an inverted microscope (Gr x 100).

IV. Conclusion

A histological study by the technique of double staining with methyl green and Congo red has identified the glandular trichomes, which are specialized in the synthesis of resin. In the present study, a culture of Vero cell and Hep-2 cell for toxicity assay to verify the effect of the extract aqueous of *G. alypum* was used. The obtained results indicated that the effect at high concentration of 26 and 13 mg/ml was better than low concentrations on Hep-2, and the concentrations of 26, 13 and 6.66 mg/ml gave good cytotoxic effect on Vero cell.

V. References

[1] Preedy V.R; Watson, R.R. Handbook of Disease Burdens and Quality of Life. New York: Springer 2010; p. 4161.

[2] Hicks R. Understanding Cancer. Australia: Lulu. Huon media 2009; p. 55-56.

[3] Candelaria M; Gallardo-Rincón; Arce C; Cetina L; Aguilar-Ponce ;J.L Arrieta O; Serrano A; Perez-Plasencia C; Gonzalez-Fierro A; de la Cruz-Hernandez E; Revilla-Vazquez A; Chavez-Blanco A; Trejo-Becerril C; Perez-Cardenas E; Taja-Chayeb L; Camargo M.F; Robles E and Dueñas-Gonzalez A. A phase II study of epigenetic therapy with hydralazine and magnesium valproate to overcome chemotherapy resistance in refractory solid tumors ,BMC Cancer 2007; 7:A27.

[4] Watson R.R; Preedy V.R and Zibadi S. Polyphenols in Human Health and Disease. Academic Press: Elsevier 2013; p. 727.

[5] Aït Youssef M. Les plantes médicinales de Kabylie. Paris: Ibis Press 2006; p155-159.

[6] Ziyya A; Legssyer A; Mekhfi H; Dassouli A; Serhrouchni M and Benjelloun W. Phytotherapy of hypertension and diabetes in oriental Morocco. J. Ethnopharmacol 1997 ; 58(1): 45-54.

[7] Jouad H; Maghrani M and Eddouks M., 2002. Hypoglycaemic effect of Rubus fructicosis L. and Globularia alypum L. in normal and streptozotocin-induced diabetic rats. J. Ethno –pharmacol 2002; 81(3): 351–356.

[8] Leporatti ML ; Ghedira K. Comparative analysis of medicinal plants used in traditional medicine in Italy and Tunisia, Journal of Ethnobiology and Ethnomedicine 2009; 5:31.

[9] Es-Safi N.E; Khlifi S; Kerhoas L; Kollmann A; El Abbouyi A and Ducrot P.H. Antioxidant Constituents of the Aerial Parts of Globularia alypum growing in Morocco, J. Nat. Prod 2005; 68: 1293-1296.

[10] Fehri B; Tebbett I; Freiburger B; Karlix J. The immunosuppressive effects of Globularia alypum extracts, Phytoth Res 1996; 10: 539–540.

[11] Ben Mansour R; Gargouri B; Gargouri B; Elloumi N; Ben Haj Jilani I; Ghrabi-Gammar Z and Lassoued S. Investigation of antioxidant activity of alcoholic extract of Globularia alypum L. Journal of Medicinal Plants Research; 2012; 6(25):4193-4199.

[12] Taskova R.M; Gotfredsen C.H and Jensen S.R. Chemotaxonomy of Veroniceae and its allies in the Plantaginaceae. Phytochemistry 2006; 67(3): 286–301.

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