Global Academic Journal of Medical Sciences

Available online at www.gajrc.com
DOI: https://doi.org/10.36348/gajms.2024.v06i05.001

OPEN ACCESS

ISSN: 2706-9036 (P)
ISSN: 2707-2533 (O)

Original Research Article

Effects of *Rubus idaeus extracts* on some Cancerous Cell Lines *in vitro* Study Using MTT Assay

Eissa Ahmed Eissa[1*], Basil Hazem Salih[1], Omar Saeb Sabbar[1], Raneen Saadi Yassin Khalaf[1]

[1]Graduated from Department of Pathology, College of Applied Science, Samarra University

***Corresponding Author**
Eissa Ahmed Eissa
Graduated from Department of Pathology, College of Applied Science, Samarra University

Article History
Received: 25.07.2024
Accepted: 02.09.2024
Published: 04.09.2024

Abstract: Our study investigated the cytotoxicity of *Rubus idaeus* alcoholic and aqueous extracts prepared from ripe blackberries were collected from the farmlands north of Baghdad, the extracts prepared at the same starting concentration, and tested on two cell line, the result show high toxic effects increase with the increase of concentration. The three extracted components from *Rubus idaeus* (purified flavonoid, polysaccharides, and carotenes) showed cytotoxic effect towards both: the primary cell culture of normal hepatic cells (HepG2 and L20B cell), and cancer hepatic cell lines (HepG2 and L20B cell) at 100µg/ml concentration for 24 hours treatment. Purified flavonoid exerted the potent effect on both cell lines among the other two extracted components. The cytotoxicity assay was held only for the purified flavonoid to investigate the mechanism by which the purified flavonoid affected living cells toward apoptosis. The most significant reduction ($p \leq 0.05$) in cell viable count was at the concentration 100µg/ml which appear to cause the induction of cell death via mitochondrial pathway for HepG2 and L20B cell line after 24 hours exposure. The purified flavonoid had no effect on the HepG-2 cell cycle. The Immunomodulation effect for the purified flavonoid and the extracted polysaccharides on normal human peripheral blood lymphocytes showed that the purified flavonoid suppress lymphocytes proliferation, while the later increased lymphocytes proliferation significantly. The immune stimulating effect of the polysaccharides caused alteration in IL-17 and level estimated by ELISA technique towards IL-2 elevation for the normal human blood lymphocytes against IL-17 level after 4 hours exposure at concentrations (250 and 500 µg/ml), while toxicity results were shown after 4 hours.

Keywords: *Rubus idaeus*, Cytotoxic activity, Mtt assay.

INTRODUCTION

Rubus idaeus contain a wide range of bioactive phytochemicals and are rich in vitamin C and total phenolics [1], including flavonoids, anthocyanins and ellagitannins, it is the flavonoids and anthocyanin pigments that give raspberries their characteristic color [2]. Many of the health benefits derived from blackberry *Rubus idaeus* consumption are due to their content of polyphenols, which are responsible for many of the biological activities of these berries, including antioxidant, anti-inflammatory and anticancer properties [3].

The anticancer property of blackberry *Rubus idaeus* has been attributed to their content of vitamin C, anthocyanins and ellagitannins. Vitamin C contributes about 20% of the total anticancer capacity, anthocyanins about 25% and the largest

contributor to antioxidant capacity is made by the ellagitannins at more than 50% [4]. Ellagitannins are an uncommon phytochemical found in only a limited number of berry species, including cloudberry and raspberry and to a limited extent in strawberries [5].

Berry bio actives, including blackberry, have many roles in cancer prevention according to [6]. Laboratory studies show berry bio actives protect against oxidative DNA damage by direct scavenging of reactive oxygen species (ROS), often considered a first line of defense against the multistage process of carcinogenesis [7]. The Fruit is rich with many active constituents of important biological activity among them; polysaccharides, Betaine, carotene, zeaxanthin, ascorbic acid, riboflavin, nicotine, thiamine, fat, protein, and about nineteen kinds of amino acids and twenty one kinds of minerals. *Rubus idaeus* polysaccharides estimated to comprise (5-8%) of the dried fruit, gave the fruit sweet taste, considered as a kind of Proteoglycan which composed from six kinds of monosaccharide, these are arabinose, glucose, galactose, mannose, rhamnose and xylose. The glycoconjugate isolated from the fruit of *Rubus idaeus* possess pronounced immune activity [8]. The color component of *lycium* fruit "zeaxanthin" is a group of caroteniods, that make up 0.03–0.5% of the dried fruit. The predominant caroteniods present as zeaxanthin dipalmitate (also called physalien or physalin). *Rubus idaeus* possess fruit is considered as one of the best food source of zeaxanthin [9]. The aim of this study is to evaluate the effect of *Rubus idaeus* on viability of two cancer cell lines: Human hepatocellular carcinoma HepG2 cell line and the mouse cell line $L_{20}B$ might get the plant fruites extracts an attention for being promise anticancer products.

METHOD

Sample Preparation: During the month of November 2023, ripe blackberries *Rubus idaeus* were collected from the farmlands of Tarmia area to the north of Baghdad. Identified and authenticated as *Rubus idaeus.* by Professor Dr. Ali Biology departments, college of science University of Baghdad.

The blackberries *Rubus idaeus* fruits were then washed, cleaned, weighed and preserved at -20 c^0 for using it in extraction.

Aqueous and alcoholic extracts were prepared from blackberry juice according to [8].

After preparation of extracts, the solvent (water or alcohol) was evaporated and the prepared dry extract was weighed and dissolved by DMSO in order to prepare the concentrations for cytotoxic study [9].

Rubus idaeus extracts stock solution was made by mixing 400µl of extracts with 10 µl Dimethylsulfoxide(DMSO) and complete the volume up to one ml using serum free medium to get the concentration of 400µl extracts/

RESULTS AND DISCUSSION

Effect of Extract on normal human lymphocytes using MTT assay:

Berry bio actives are also effective in inhibiting the formation of carcinogen-induced DNA adducts, enhancement of DNA repair and inhibition of carcinogen-induced tumorigenesis in animal models [10]. In addition, berry bio actives modulate signaling pathways involved with cellular proliferation, apoptosis, inflammation, angiogenesis and cell cycle arrest [11].

Table 1: Cytotoxic effect % of *Rubus idaeus extract*

Concentration of extract mg/ml	Cytotoxic effect %
10	50
20	122
30	132
40	203
50	243
100	265
control	**68**

Level of IL-10 in Human cell culture supernatant using ELISA kit (Simplest of ABCAM) at 450 nm.

Table 2: Level of IL-10 in HepG2 and L20B cell lines *in vitro*

Time exposure	Concentration of extract	Concentration of IL-10 pg/ml
4 Hours	10	9.18
	50	18.00
	100	20.76
	Control	14.54

Level of IL-17 in Human cell culture supernatant using ELISA kit (Simplest of ABCAM) at 450 nm.

Table 3: Level of IL-17 in HepG2 and L20B cell lines *in vitro*

Time exposure	Concentration of extract	Concentration of IL-17 pg/ml
4 Hours	10	132.500
	50	687.870
	100	784.875
	Control	598.875

Our study investigated the cytotoxicity of *Rubus idaeus* alcoholic and aqueous extracts prepared from ripe blackberries were collected from the farmlands north of Baghdad, the extracts prepared at the same starting concentration, and tested on two cell line, the result show high toxic effects increase with the increase of concentration. This due to their capacity to stimulate or inhibit protein phosphor relation that regulate cell function or by counterbalancing the effect of cellular protein tyrosine phosphatases [12]. As a result of the relatively poor prognosis and response to conventional chemoradiotherapy, there is a great need for new effective agents. Renewed attention in recent years to natural therapies has stimulated a new wave of research interest in traditional practices. Herbs have become a target for the search for new anticancer drugs. About half of the drugs used in clinical practice come from natural products [13]. Various *in vitro* studies about the mechanism of the plant cytotoxicity were differ from one cell culture to another depend on whether whole plant extract was used or any of the plant component. In fact, many nutritive and nonnutritive phytochemicals with diversified pharmacological properties. In a study, it was reported that LBP and APS possess effective immunostimulatory effects when used in vaccination programs against Foot and mouth disease virus [14, 15], Newcastle disease virus (NDV) and Infectious bursal disease virus (IBDV) [16, 17]. The appropriate concentration and antiviral action of APS on the propagation of H9N2 AIV (Avian influenza subtype H9N2belongs to the low pathogenic avian influenza virus (AIV) group; considered to be a common cause of disease epidemics) in chick embryo fibroblasts (CEF) was investigated. *Rubus idaeus* polysaccharide (APS) effectively increases the expression of IL-10, IL-17 promotes cell growth, and improve humoral immunity, and boosts both T cells and B cells [18]. *Rubus idaeus*polysaccharides (LBP) can stimulate moderate immune responses therefore could potentially be used as a substitute for oil adjuvant in veterinary vaccines. Ling and his collagous [19].

REFERENCES

1. Camille, S., Bowen-Forbes, Yanjun, Z., & Muralledharan, G. (2010). Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits. *Journal of Food Composition and Analysis, 23*, 554-560.
2. Jing, Z., Dong, Y. L., & Wang, Y. (2009). Analysis of flavonoid from leaves of cultivated Lycium barbarum. *Plant foods for Human Nutrition, 5*, 1007.
3. John, P. C. I. (2002). The Cell Cycle. 3rded. Cambridge University Press, London.
4. Cavanagh, H., Hipwell, M., Wilkinson, J. (2003). Antibacterial Activity of Berry Fruits Used for Culinary Purposes. *Journal of Medicinal Food*, *6*(1), 57 – 61.
5. Thomas, F. (1999)."The Physicians' Desk Reference PDR for Herbal Medicines". First edition, Medical Economic Company. New Jersey.
6. Zhang, X. (2007). "WHO monographs on selected medicinal plant". vol.3. The World Health Organization library, Spain.
7. Egyptian Pharmacopoeia. (1972). General Organization for Government Printing, Cairo, vol. 2,3rd.ed.
8. Bruni, A., Medici, E., Andreotti, C., Fantin, M., Muzzoli, M., & Dehesa, R. (2003). Chemical composition and biological activities of Isphingo essential oil, a traditional Ecuadorian spice from *Ocotea quixos* (Lam.) Kosterm. (Lauraceae) flower calices. *Food Chem.*, *85*, 415–421.
9. Chen, M. (1997). Pharmacopeia of the People's Republic of China, English Edition, vol.1, pp: 22. Chemical industry press, Beijing, China.
10. Chen, Z., Kwong, B. H. T., & Ha, S. C. (2008). Activation of T lymphocytes by polysaccharide-protein complex from L.barbarum. *International Immunophamacology, 8*, 1663-1671.
11. Mcallister, R. M., Melnyk, J., Finklestein, J. Z., Adems, E. C., & Gardener, M. B. (1969). Cultivation *In vitro* of cells derived from human Rhabdomyosarcoma. *Cancer, 24*, 520-526.
12. Freshney, I. R. (2001). Culture of animal cells: A manual for basic technique. Wiley-Liss publication, New York.
13. Wu, X. J., Stahl, T., Hu, Y., Kassie, F., & Mersch-Sundermann, V. (2006). The production of reactive oxygen species and the mitochondrial membrane potential are modulated during onion oil-induced cell cycle arrest and apoptosis in A549 cells. *J. Nutr.*, *136*, 608-613.
14. Khadhri, A., El Mokni, R., Mguis, K., & Ouerfelli, I. (2011). Variability of two essential oil of *Ammi*

visnaga a traditional Tunisian medicinal plant. *J of Medical plant Research, 20*, 5079-5082

15. Zani, F., Massino, G., Benvenuti, S., Bianchi, A., Albasini, A., Melegari, M., Vampa, G., Bellotti, A., & Mazza, P. (1991). Studies on the genotoxic properties of essential oils with Bacillus subtilis rec-assay and Salmonella/microsome reversion assay. *Planta Med.*, *57*, 237–241.
16. McKenna, K., Beignon, A., & Bhardwaj, N. (2005). Plasmacytoid Dendritic Cells: Linking Innate and Adaptive Immunity. *J. Virol., 79*, 17–27.
17. Metzelder, S., Wang, Y., Wollmer, E., Wanzel, M., Teichler, S., Chaturvedi, A., ... & Burchert, A. (2009). Compassionate use of sorafenib in FLT3-ITD–positive acute myeloid leukemia: sustained regression before and after allogeneic stem cell transplantation. *Blood, The Journal of the American Society of Hematology*, *113*(26), 6567-6571.
18. Miao, Y., Xiao, B., Jiang, Z., & Guo, J. (2010). Growth inhibition and cell cycle arrest of human gastric cancer cells by *Rubus idaeus* polysaccharide. *Medical Oncology, 27*, 785-790.
19. Ming, M., Guanhua, L., Zhanhai, Y., Guang, C., & Xuan, Z. (2009). Effect of the *Rubus idaeus* polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet *in vivo*. *Food Chem, 113*, 872–877.