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Cytotoxicity Effect of Glucokinin Isolated from *Bauhinia variegata* Against Several Cancer Cell Lines

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Abstract: Cancer is the major health issue around the world, in spite of the development in the diagnosis and therapy methods. The discovery of new anticancer drugs from plants become the new trend in the scientific research. The phytochemicals, consider selective in their action and specially not to be harmful for normal cell like other cancer treatments. In the current study, leaves of *Bauhinia variegata* were collected from Baghdad University garden in spring of 2019 and Glucokinin was isolated and purified from the methanol extract of *B. variegata* by using column chromatography. The cytotoxicity effects of Glucokinin were investigated via MTT assay against cancer cell lines HepG2, A549 and WRL normal cell line at 24, 48 and 72h. by using different concentrations. The result showed that, the highest cytotoxicity of Glucokinin at 400 µg/ml was 46.53% (std. ±5.8) at 72h. against A549 cell line. And the half maximal inhibitory concentration (IC_{50}) of Glucokinin that inhibit the A549 cancer cell line was 114.5 µg/ml at 72h. The greatest cytotoxicity effect against HepG2 was 71.75% (std.±6.5) at 72h. The (IC_{50}) of Glucokinin that inhibit the proliferation of HepG2 cancer cell line was 51.24 µg/ml at 72h. Additionally, Glucokinin showed high IC_{50} against WRL normal cell line. In conclusion, these finding show the probability of using Glucokinin as anticancer agent.

Keywords: Medicinal plants, *Bauhinia variegata* , Glucokinin, cytotoxicity.

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Introduction

Cancer still the major health problem in the world with more than 1.8 million new cancer cases are expected to be diagnosed in 2020 (1). A large number of efforts have been made to minimize the harmful side effects of drugs during the process of cancer therapy (2).

Medicinal plants are considered as a rich source of wide variety of ingredients which can be used for the development of drug. Anticancer properties of several medicinal plants are used to find a lead compound that can block the development of cancer (3). Plants and plant derived products are a revolutionizing field as these are Simple, safer, eco-friendly, low-cost, fast, and less toxic as compared with conventional treatment methods (4).

Bauhinia variegata L which commonly known as mountain ebony, orchid-tree, poor-man's orchid, camel's foot and Napoleon's hat, belongs to the family Leguminosae (5). It was planted in garden, park and roadsides as ornamental plant in many warm temperate and subtropical regions. It was native to Southeast Asia and grows in tropical and subtropical climate (6). All parts of the plant (leaves, flower buds, flower, stem, stem bark, seeds and roots) were used in traditional medicine. It was traditionally used in the treatment of bronchitis, leprosy, tumors, astringent, tonic, anthelmintic, antidiabetic, laxative and for piles, used in the treatment of

worm infestations, diarrhea, and piles (7). Pharmacological studies showed that *B. variegate* can be used as anticancer, antioxidant, hypolipidemic, antimicrobial, anti-inflammatory, nephroprotective, hepatoprotective, antiulcer, immunomodulating, molluscicidal and wound healing effects (8). A wide range of chemical compounds isolated so far from the plant are β-sitosterol, kaempferol –3-glucoside, tannins, carbohydrates, amides, reducing sugars, vitamin C, crude protein, fibers, calcium, phosphorus, quercetin, rutin, quercitrin, apigenin, apigenin -7-O- glucoside, heptatriacontan -12, 13 diol and dotetracontan -15- en -9- ol (9,7). In addition, the presence of insulin-like molecules which known as Glucokinin was demonstrated in the leaves of *B. variegata* where a protein was found that has a partial amino acid sequence identical to that of bovine insulin (10, 11). The activity of this Glucokinin on serum glucose levels Swiss albino (CF1) diabetic mice was similar to that of commercial swine insulin used as control (12,13). Since, Glucokinin shares amino acid sequence with human insulin it suggests that it might communicate with insulin mediated signal transduction by binding to insulin receptors (14). The cytotoxicity effects of crude extract of *B. variegata* were observed by many studies (15, 16, 17, 18). While the cytotoxicity of Glucokinin not studied yet. Therefore, in this study we aimed to investigate the effect of Glucokinin on several cancer cell lines.

Materials and Methods

Plant preparation and extraction

The fresh leaves of *B. variegata* were collected from the gardens of Baghdad University. The washed leaves dried under shad, then placed in oven for couples of hours and powdered. Fifty gram from the powdered leaves of *B. variegata* were taken and placed in 350 ml from %70 methanol for one week with shaking frequently. After that, the residues were taken off by filter paper, by using the rotary evaporator the methanol was removed until dryness (19).

Partial and final purification of Glucokinin

By using column chromatography, partial separation for flavonoids is done by using open glass column (2×17) cm that filled with Sephadex LH20 (20). Two gram from plant crude extract dissolved in 3 ml from 70% methanol were added to the column. Only the positive results tested by $FeCl_3$ 1% test for polyphenols identification are collected (21). The positive tubes that were collect from the first column were concentrated then added to the second sephadex LH20 column. The positive tubes for $FeCl_3$ 1% were more identified and screened by HPLC and FTIR according to (22).

The Cell Line

The Cell lines that used in this study were HepG2 (Liver hepatocellular cancer cells) , A549 (lung cancer cell line) these cancer cell line were compared to WRL (normal human hepatic cell line) which used as control. All cell lines kindly provided from Centre for Natural Product Research and Drug Discovery, Department of Pharmacology, Faculty of Medicine, University of Malaya Kuala Lumpur. The cell lines cultured as recommended.

Cytotoxic Assay on Cell Line

A 0.2 ml of prepared cells concentration (1×10^5 cells/well), in the exponential phase, were added to the 96-well plats, then placed in CO_2 incubator at 37Ċ for not more than 24 hrs (23, 24). Serial concentrations (6.25, 12.5, 25, 50, 100,200 and 400µg/ml) were added and three replicates were used to each concentration. The exposure time were 24, 48 and 72 hrs. after that, the samples and media were removed from the plates, and washed with worm PBS. A 50- 100 µl of MTT working solution dye was added to each well and incubated at 37 Ċ for 4 hrs. At the end of last incubated period the dye was removed from the plates, then 50-100 µl of DMSO was added to each well to dissolve the MTT-formazan crystals (25). Finally the plates became ready to reading by ELISA reader at 550 nm. Following equation was used to calculate the percentage of cell viability:

Cell viability (%) = {OD sample (mean)}/{OD control (mean)}×100

Concentration that required inhibiting 50% of cell growth was used as a stricture for cytotoxicity. These data used to obtain the IC50 values by using the Graphpad prism program.

Results and Discussion

In the current study the cytotoxicity effect of the pure Glucokinin that isolated from *B. variegate* was examined for the first time against several cancer cell lines for different times. The inhibition rate (cytotoxic effects) of Glucokinin against A549 and HepG2 were illustrated in Table (1), that shown increased in time depended manner especially at 72h where shown the best inhibition rates, also it can be seen that the high concentration gave the best inhibition. These results indicate that, Glucokinin can be effective in a time and concentration depended manner. These findings were agreed with other study that suggested the *B. variegata* extract contain bioactive compounds that gave to its effect as antitumor in dose respond manner (15).

The IC_{50} of the Glucokinin against HepG2 cell line at exposure time 24h.,48h. and 72h. was shown in the Fig 1, 2 and 3 respectively. The IC_{50} decreased in time depending manner, while it was 73.57µg/ml at 24h., become 51.24µg/ml at 72h. Also the IC_{50} for Glucokinin against A549 cancer cell line at different time 24, 48 and 72h. of treatment were shown in Fig 4, 5 and 6 respectively. There is decreasing in IC_{50} depending on time of exposure, while the IC_{50} decrease from 238.6 to 179.6 to 114.5 µg/ml at 24, 48 and 72h. respectively. In addition, the IC_{50} against normal cell WRL was high comparing with IC_{50} against cancer cell lines.

As mentioned the Glucokinin shared amino acids with bovine insulin so that it suggested to share the same signal transduction and this signals transduction play a significant role in the developing cancers and it consider as important target for drug discovery. For this reason, Glucokinin might be a possible substance for new anticancer agent searching. Among approved anticancer drugs, approximately 50% are either natural products or their derivatives (26) primarily from plants, microorganisms, and seeds (27). Numerous plants have been reported to have anticancer effects (28) or to complement conventional therapeutics by targeting various hallmarks of cancer (29). Many anticancer drugs derived from plants were useful clinically include vinblastine irinotecan, topotecan and paclitaxel (30). Several biological processes and

biological interactions occur between flavonoids, phenolic compounds or polyphenols, with enzymes, proteins that make them toxic to the cell or inhibit the growth of cells (31). Several reports indicated that the cytotoxicity and anticancer properties of natural plants were due to the existence of flavonoid compound and it play a significant function in chemoprevention through showing its effects on transduction of signal and angiogenesis of cells (32,33). In 1998, Bravo reported that polyphenolic compounds, including flavonoids are important for cancer prevention (33).

Table (1): Cytotoxic effects of different concentration of purified Glucokinin on HepG2 and A549.

Conc. µg/ml	HepG2						A549					
	24		48		72		24		48		72	
	I.R.	std	I.R.	std.	I.R.	std	I.R.	std	I.R.	std.	I.R.	std
6.25	**4.32**	±0.6	**3.9**	±1.7	**3.7**	±0.8	**4.75**	±0.2	**4.21**	±1.6	**4.9**	±1.5
12.5	**4.98**	±3.0	**3.12**	±1	**3.64**	±1.4	**4.86**	±1	**3.47**	±0.7	**4.4**	±2
25	**3.2**	±0.9	**5.44**	±2.8	**24.5**	±3.5	**4.28**	±0.8	**5.67**	±1.7	**5.13**	±1.8
50	**25.23**	±5.3	**36.03**	±5.2	**37.04**	±4.4	**5.17**	±1.2	**8.29**	±2	**5.9**	±1.8
100	**37.92**	±6.1	**45.52**	±2.7	**48.33**	±1.4	**6.75**	±2.1	**22.84**	±3.2	**24.94**	±3.9
200	**49.61**	±1.3	**59.72**	±3.6	**63.14**	±5.1	**13.46**	±1.2	**22.82**	±9.7	**35.61**	±5.6
400	**58.95**	±2.7	**68.79**	±2.8	**71.75**	±6.5	**20.02**	±0.8	**36.04**	±2.3	**46.53**	±5.8

I.R.= Inhibition rate , Std= standard deviation.

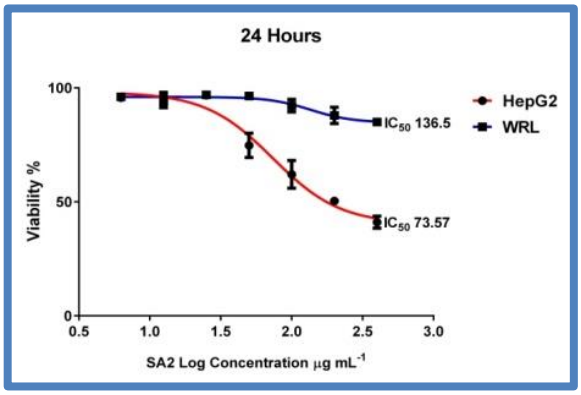

Figure (1): The IC_{50} of Glucokinin against HepG2 cell line at 24h.

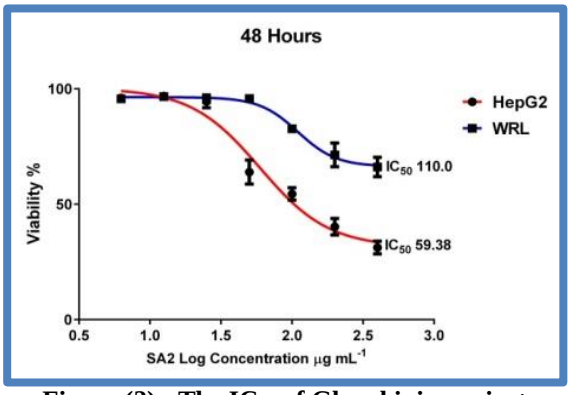

Figure (2): The IC_{50} of Glucokinin against HepG2 cell line at 48h.

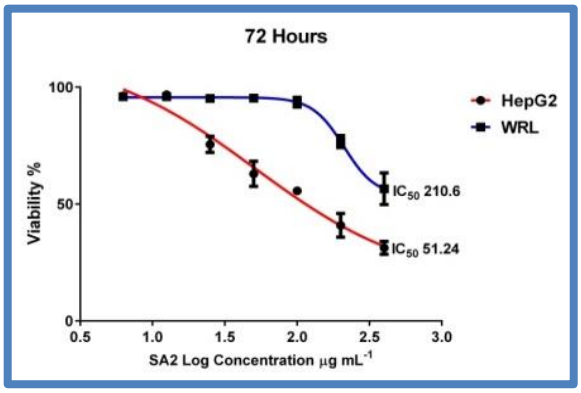

Figure (3): The IC_{50} of Glucokinin against HepG2 cell line at 72h.

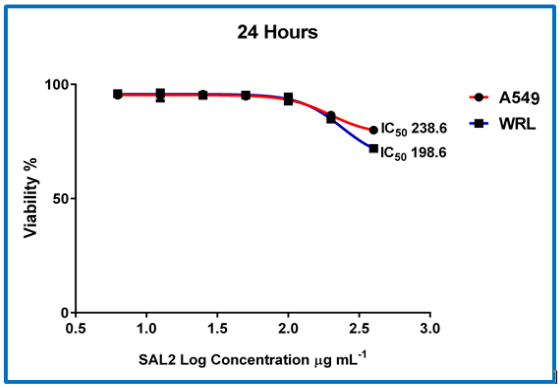

Figure (4): The IC_{50} of Glucokinin against A549 cell line at 24h.

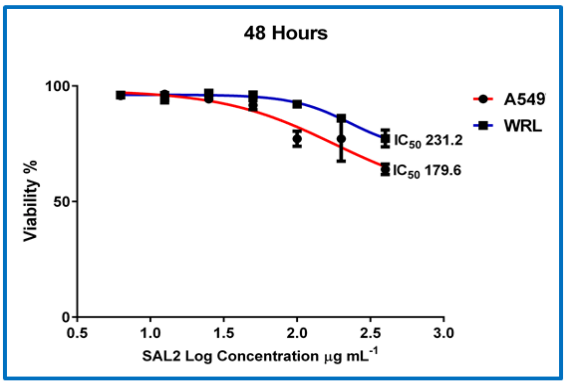

Figure (5): The IC_{50} of Glucokinin against A549 cell line at 48h.

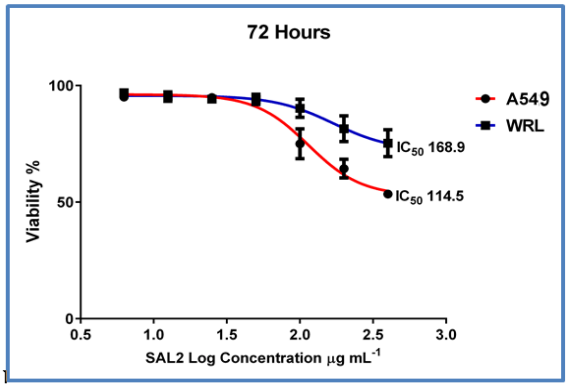

Figure (6): The IC_{50} of Glucokinin against A549 cell line at 72h.

Conclusion

In the present study the Glucokinin isolated from the methanol extract from leaves of *B. variegate* showed a potential role in the developing of a novel drug in the area of cancer treatment by suggesting mechanism including signal transduction. Forward working with Glucokinin against other cell line will continue.

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