

Effect of *Tinospora Cordifolia* Extract on Glucose Metabolism in Cancer Cells

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Abstract—Cancer cells in order to support their rapid proliferation alter their metabolism. Among the metabolic changes exhibited by tumor cells, is an increase in glucose metabolism and glucose dependence. Glucose metabolism rate is very high in cancer cells and it plays an important role in hydroperoxide detoxification and its inhibition has been shown to increase the production of prooxidants and cytotoxicity in cancer cells. The inhibition of the Akt pathway which is responsible for cell growth and survival, inhibits glucose consumption and induces parameters indicative of oxidative stress such as glutathione disulfide (%GSSG) and thioredoxin reductase (TR) activity in certain cancer cells. It has been hypothesized that targeting glucose metabolism may provide a selective mechanism by which to kill cancer cells. Hence after the treatment with a drug, when the levels of glucose consumption are decreased in cancer cells, it probably indicates the increase in oxidative stress resulting in cell death and that the drug is effective against that particular cancer. We have already shown that the hydroalcoholic extract of an annual herb *Tinospora cordifolia* induced cell death and generated oxidative stress in HeLa cervical cancer cell line in our previous studies. In our present study, we have performed the test for glucose consumption to support our study further.

Keywords: *Tinospora cordifolia*, HeLa cells, Glucose consumption, Altered Metabolism, Oxidative stress.

1. INTRODUCTION

Carcinogenesis is a complex, process that requires the elimination of various processes such as antiproliferative responses, programmed cell death-inducing mechanisms, and senescence. This occurs mostly through mutations in oncogenes and tumor suppressor genes [1, 2]. The tumor microenvironment plays a crucial role in the transition from precancerous lesions to carcinogenesis by exerting an adaptive pressure that selects cells for their clonal expansion [3–5]. When we talk about the metabolism of cancer cells, it is known that cancer cell mitochondria have extensive metabolic reprogramming allowing them to rely on glycolysis for production of adenosine 5'- triphosphate (ATP), despite high oxygen tension [6-8]. Cancer cells essentially shun the oxidative phosphorylation, the main energy production process in normal cells, and manage to thrive on upregulation of glucose import and metabolism [9]. Moreover, cancer cells have increased levels of gluconeogenesis, reduced pyruvate

oxidation and increased lactic acid production [10]. These metabolic differences between normal and cancer cells may be the primary cause of cancer, as proposed by Warburg, but the more likely explanation is that altered metabolism is an adaptation to support malignancy [8,11]. Aerobic glycolysis allows a cancer cell to generate ATP in varying oxygen conditions, which is advantageous for survival and proliferation [6]. Also, increase in Glucose uptake by cells eventually causes detoxification of intracellular hydroperoxides [12-16]. It has also been reported that, increasing glucose concentrations up to 10–20 mM in tissue culture media has been shown to render cells resistant to H₂O₂ -induced cytotoxicity [15]. These generally conserved features of cancer cells present an attractive target for cancer therapy, specifically those designed to generate Oxidative stress and in turn stimulate mitochondrial-mediated apoptosis. In our present Study, we have performed glucose consumption assay with varying dose concentrations at different time intervals to support our previous studies which indicated cell death and oxidative stress generation in HeLa cervical cancer cellline after treatment with Hydroalcoholic extract of the plant *Tinospora cordifolia*. [17-18].

2. MATERIALS AND METHODS

2.1 Plant Extract

Stem and leaves of *Tinospora* were collected and authenticated by Botany Department, Gujarat University (Certificate No. GU/Bot/2013 Dated. 25/10/2013). These parts of the plant were washed and shade dried under ambient temperature. After dried, the plants were sliced and ground using a steel blender. 50 g defatted (by hexane extraction) plant material was capsulated in filter paper and kept in the thimble, 500 ml solvent (water : methanol) (70:30) was added into the flask and continuous extraction was carried out in the soxhlet apparatus for 72 hours at 60-70°C till the colour in the siphon became colourless. The extract was dried at 60°C under fumehood till all the solvent got evaporated. 2-5% yield was obtained. The yield collected after drying was weighed and stored at 4°C until further use in sterile containers, as the hydroalcoholic extract.

The plant extracts for dosing were prepared with the concentrations 1 mg/ml in the Culture media with 5% DMSO and used further with various dilutions.

2.2 Cancer Cell Culture:

For cancer cell culture, HeLa cervix cell line ATCC®CCL-2™ was obtained from National Centre for Cell Science (NCCS), Pune. Cells were cultured in Minimum essential medium (MEM) with 10% fetal bovine serum and antibiotic antimycotic solution. All these supplements were procured from Hi-Media Laboratories. Cell cultures were maintained in a CO₂ incubator at 37°C. All the assays were carried out in the growth phase of the cells. For all the assays a definite number of cells i.e. 2x10⁶/ml were used.

2.3 Glucose Consumption:

Glucose consumption was measured by modified method of Nayak and Herman (1997) [19]. 2 X 10⁶ cells were seeded and were treated with various concentrations (50 µg/ml, 25 µg/ml, 12.5 µg/ml) of the extracts and glucose levels in the growth medium were measured for samples with an ACCU-CHEK® ACTIVE glucometer. Meters were used in accordance with the manufacturer's instructions, substituting culture media or glucose standards in MEM, pH 7.4, for blood. Glucose levels for zero hour and subsequently for 24, 48 and 72 hours were calculated in each well as mg/dl. Present day glucose level was subtracted from level of previous day and the amount of glucose consumed was known. Percentage consumption of glucose was measured using the formula:

$$\% \text{ Consumption} = \frac{\text{Amount of glucose consumed}}{\text{Total amount of glucose at previous day}} \times 100$$

Three independent assays were done and the values are depicted as mean percentage of glucose consumed by the cells.

2.4 Statistical analysis:

The Assay was performed in triplicate and the results were expressed as Mean ± Standard Error (S.E.M). The data was the statistically analysed by Students't' test. In all the parameters, resultant effect of treated cells was compared with untreated control cells.

3. RESULTS AND DISCUSSION:

Consumption of glucose by HeLa cells was monitored by glucometer. It was observed that consumption of glucose increased in the untreated control cells with the increase in time interval and showed 15.85 ± 0.02 %, 16.45 ± 0.08% and 16.85 ± 0.11 % consumption at 24, 48 and 72 hours (Table 1. and Fig. 1.). There was gradual decrease in the glucose intake by cells with increase in time and concentration after the treatment with *Tinospora* extract.

3.1 Tables and Figures:

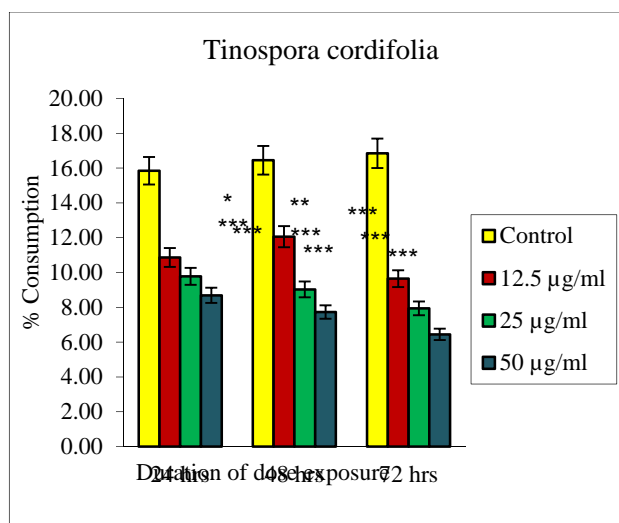
Table 1: Showing the percentage of glucose intake by HeLa cells after treatment with *Tinospora cordifolia* extract compared with untreated control cells.

Duration of dose exposure	24 hrs	48 hrs	72 hrs
Control	15.85 ± 0.02	16.45 ± 0.08	16.85 ± 0.11
12.5 µg/ml	10.86 ± 1.36*	12.06 ± 0.73**	9.65 ± 0.79***
25.0 µg/ml	9.78 ± 0.82***	9.03 ± 0.92***	7.94 ± 0.38***
50.0 µg/ml	8.69 ± 1.13***	7.73 ± 0.90***	6.45 ± 0.98***

Values are mean ± S.E.M for three individual experiments

Values are % of glucose consumed.

*= p <0.01, **= p <0.005, ***= p <0.001



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Fig. 1: Graph showing the percentage of glucose consumed by HeLa cells after treatment with *Tinospora cordifolia* extract compared with untreated control cells.

Cancer cells show increased metabolic activity, as they require high levels of energy, nucleotides, lipids, and amino acids to maintain a high rate of cell growth and proliferation. In the presence high-energy demand, a shift in cell metabolism is needed to enhance oxidative phosphorylation and to promote glycolysis. This shift could assure the survival of cancer cells, as well as their propagation [20]. Glycolysis can produce ATP at a higher rate, but at a lower yield, than oxidative phosphorylation can; this may selectively advantage cancer cells when competing for energy resources [21].

Given the ability of these pathways to help provide the metabolic fuels required for proliferation, it is perhaps not surprising that cancerous cells, which can proliferate indefinitely, often demonstrate aberrant activation of PI3K/Akt and Myc and often utilize aerobic glycolysis to meet their metabolic demands. Otto Warburg first noted nearly a century ago that cancerous cells demonstrate markedly increased rates of glucose uptake, glycolysis, and lactate production, even in the presence of normal oxygen levels [7], a phenomenon now known as ‘the Warburg effect.’ While Warburg suggested that cancer cells utilize aerobic glycolysis because damaged oxidative machinery forces a compensatory use of glycolytic metabolism [22], it is now clear that both tumor hypoxia and the very oncogenes that drive cancer cell proliferation can induce these metabolic changes [23]. Since glucose metabolism appears to play an important role in hydroperoxide detoxification then therapeutic interventions designed to inhibit glucose metabolism would be expected to increase prooxidant production and cytotoxicity in cancer cells. Furthermore, if increased Akt pathway signaling is correlated with increased rates of glucose metabolism observed in cancer cells versus normal cells, then the inhibition of Akt pathway signaling would be expected to inhibit glycolysis and increase hydroperoxide production which would preferentially kill tumor cells versus normal cells via oxidative stress. Simons and co-workers have studied the effect of LY294002 a Quercetin derivative, Perifosine and Erlotinibon known Akt inhibitors on glucose consumption in FaDu and Cal-27 human head and neck squamous cell carcinoma cells in a similar fashion and the results showed that there was decrease in consumption rate along with ROS generation after treatment. [24].

Tinospora cordifolia extract in our previous studies showed selective *in vitro* cytotoxicity, and antiproliferative activity against HeLa cancer cell line and the cause of cell death might be apoptotic induction [17]. Also, there is oxidative stress generation in HeLa cells after treatment with the *Tinospora* extract [18].

The results in our present study demonstrated that there is loss in glucose uptake after treatment of the HeLa cells with the extracts. The untreated cells continued to consume large amounts of glucose and the consumption increased with increase in time interval. It is evident that after the treatment, there is alteration in glycolytic metabolism. Hence by this study it is established that, the possible apoptosis induction by the extracts may have decreased glycolysis within the HeLa cervical cancer cells or might have driven them back into an oxidative metabolic program.

4. CONCLUSION

Further studies of the extracts on Akt pathway studies and the active component studies are still required but from our studies we can possibly state that *Tinospora cordifolia* can be considered as a therapeutic agent for various carcinomas.

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