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## The Effect of Ginger Extract on Glycoproteins of Raji Cells

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**Abstract:** Protein glycosylation is associated with the development and progression of specific diseases, including cancers. The ginger rhizome is known to have anti-cancer and anti-fungal properties. This investigation was carried out to study the effect of ginger on glycoproteins of Raji cells. A 10% yield of ginger extract was mixed with 0.01% DMSO and added to  $6 \times 10^4$  Raji cells at different concentrations for 24, 48 and 72 h at 37°C. Their half maximal inhibitory concentration ( $IC_{50}$ ) was determined and analyzed statistically using Graphpad prism software. Cell extracts were prepared and their glycoproteins purified using lectin-affinity chromatography (Q proteome total glycoprotein and O glycoprotein kits) and SDS PAGE was carried out.  $IC_{50}$  of ginger extract on Raji cells was  $20 \mu\text{g mL}^{-1}$  at 72 h with  $<0.01$  significance. Silver staining of purified glycoproteins in Raji cells indicated the presence of O-glycans and N-glycans. N-linked mannose and N-linked sialic acids were detected with the total glycoprotein kit. O-linked galactose and O-linked sialic acids were identified with the O-glycoprotein kit. Ginger reduced the expression of O-linked sialic acid and also N-linked mannose on Raji cells but had no effect on other glycoproteins. Sialic acid is now well known as a cancer marker and investigations are on to use it as a drug-target in cancerous tissues.

**Key words:** Raji cells, ginger extract, glycoproteins, sialic acid

## INTRODUCTION

Glycoproteins can be defined as proteins attached covalently to oligosaccharides. These sugars are generally fixed on the protein after its translation and are known as post translational glycosylation. It should be noted that secreted extracellular proteins are often glycosylated. Glycoproteins are very important components in cells taking part in a number of functions from cell-cell association to signaling (Drake *et al.*, 2006). It is interesting that biomarkers for diagnosis and drug targets in cancer are glycoproteins (Ruddock and Molinari, 2006), such as CA125 in ovarian cancer (O'Brien *et al.*, 2001), breast cancer with Her2/neu (Nustad *et al.*, 1996) and PSA in prostate cancer (Cirisano and Karlan, 1996). Altering the heterogeneity or changing the entire glycan can change the structure of the protein completely. These changes on the cell surface and in body fluids have been shown to correlate with the development of cancer and other disease states and have also been reported as novel drug targets and markers for prognosis (Bubley *et al.*, 1999). In cancer, besides radiation therapy and the relatively new targeted therapies, chemotherapy is still

looked upon as one of the standard lines of treatment but it is beleaguered by resistance in some cases (Pao *et al.*, 2005). Alternative herbal treatment is the trend for therapy of many diseases and is now being considered in cancer.

Edible plants containing phytochemicals which interfere with certain stages (Surh *et al.*, 1998) of cancers. Ginger which is the common name for the rhizome of *Zingiber officinale* Roscoe (Zingiberaceae), plays an important role in treatment of many traditional and alternative medicines (Mills and Bone, 2000). The active compounds in this rhizome are gingerols, shogaols, paradols and zingerone (Jolad *et al.*, 2004) which are widely studied and shown to be responsible for its antioxidant, anti-inflammatory, antiemetic and gastroprotective activities (Aggarwal *et al.*, 2009). Ginger's chemopreventive and anti-neoplastic effects have been demonstrated by a number of preclinical investigations. Ginger has also been reported to down-regulate NF- $\kappa$ B-regulated gene products which are involved in cellular proliferation and angiogenesis (Shukla and Singh, 2007). It has a suppressive effect on IL-8, Vascular Endothelial Growth Factor (VEGF) and ovarian cancer cells (Rhode *et al.*, 2006).

The history of Raji cells date back to 40 years ago when the first continuous human cell line of hemopoietic origin was obtained from a Nigerian patient with Burkitt's Lymphoma. This lymphoma primarily involves B cells and influence their antigen presentation and T cell recognition. (Karpova *et al.*, 2005). Raji cells lines of Burkitt's lymphoma origin were soon found to harbor the Epstein-Barr virus (EBV) which resulted in discovery and isolation of this virus. Raji cells are good hosts for transfection studies on hemopoietic and other cell cancers. Raji cells are a good model for study of effect of ginger on lymphoma cells (Drexler and Minowada, 1998).

Hence, we thought that a study of the effect of ginger extract on Raji cells and their glycoproteins could provide preliminary data for the mechanism of this anti carcinogenic phytochemical. We interestingly observed that sialic acid had obviously decreased after ginger extract treatment. Sialic acid is now well known as a cancer marker and studies are on to target it in metastatic tissue for therapeutic aims.

## MATERIALS AND METHODS

**Preparation of ginger extract:** Three grams of oily ginger extract obtained from mixing 30 g of dried ground rhizome (*Zingiber officinale* Roscoe) with 300 mL methanol in a soxhlet device, after evaporation at room temperature (Wahida, 2010).

The ginger extract was mixed with 0.01% DMSO and a range of concentrations were made with RPMI. The solution was sterilized with 0.2  $\mu$ m filters.

**Cell culture:** Raji cells were cultured in RPMI 1640 with 10% fetal calf serum and 10  $\mu$ L penicillin streptomycin antibiotic. The cells were grown in 0.01% DMSO alone to rule out any effect on the cell lines (referred to as native cells) and then in different concentrations of ginger extract for determination of their  $IC_{50}$ ;  $6 \times 10^4$  cells were grown with the alcoholic extract of ginger in duplicates (referred to as treated cells) 5 to 2000  $\mu$ g  $mL^{-1}$  for 24, 48 and 72 h in 12 well plates in an incubator at 37°C with 5%  $CO_2$  and their  $IC_{50}$  determined by the method below (Sun *et al.* 2004).

**Viability of cells:** Raji cells were checked for viability by staining with trypan blue and counted in a Neobar slide at 24, 48 and 72 h and their  $IC_{50}$  determined. Cells were kept at -80°C (Altman *et al.*, 1993).

**Statistical analysis:** Standard deviation and significance was carried out by student's t test using Graph pad prism.

**Lysis of cells:** Raji cells were lysed after transferring them to -10°C with a special buffer containing protease inhibitor cocktail and then kept for 30 min on ice, vortexing every few minutes and then centrifuged at 10,000 g at 4°C and supernatant was collected. The amount of protein was determined by Lowry's method Lowry *et al.* (1951), mixed with sample buffer as described and then loaded onto the gel using Laemmli's method (Laemmli, 1970).

SDS-Polyacrylamide gel electrophoresis was carried out using 7.5% gel using the method of Laemmli (1970). The samples were prepared in reducing buffer as required using 50  $\mu$ g to 300  $\mu$ g  $mL^{-1}$  protein for different stainings like Coomassie blue for detection of proteins (Meyer and Lambert 1965), periodic acid schiff's staining (PAS) for detection of polysaccharides (Konat *et al.*, 1984) and silver staining for detection of glycoproteins (Blum *et al.*, 1987).

**Glycoprotein analysis:** was carried out using lectin affinity chromatography with Q-proteome kit for total glycoproteins and O-glycans (Hage, 1999). The cells were lysed in lysis buffer containing inhibitor cocktail and passed through the respective affinity lectin columns. The cell extract was loaded onto the lectin column and washed with loading buffer, and then eluted with the respective buffer. The flow through with the washing buffer (FT) and the eluant (Elu) which was obtained with the respective eluting buffer from all the columns were checked by SDS PAGE. Total glycoprotein kit consisting of concanavalin A (Con A) lectin affinity column for detection of N-linked mannose glycoproteins and wheat germ agglutinin (WGA) affinity column for N-linked sialic acids. The O proteome kit also consisted of two lectin affinity columns, Peanut agglutinin (PNA) for O-linked galactose units and jackfruit lectin *Jacalin: Artocarpus integrifolia* (AIL) lectin for detection of O-linked sialic acids. The samples were passed through the lectin column and then eluted with their specific buffers. The flow through and the eluants were collected after which SDS PAGE was carried out and gels were stained with silver.

## RESULTS

Growth of cells were completely dependent on dose and time so that with increase of time, at higher concentrations of ginger extract, number of viable cells showed a very obvious decrease. Figure 1 shows Raji cell viability after 24 h with  $p < 0.05$  as compared to native cells, Fig. 2 also shows the same but after 48 h with  $p < 0.01$  and Fig. 3 after 72 h with  $p < 0.01$  significance. At all three time points,  $IC_{50}$  of the cells were recorded at 20  $\mu$ g  $mL^{-1}$  of ginger extract.

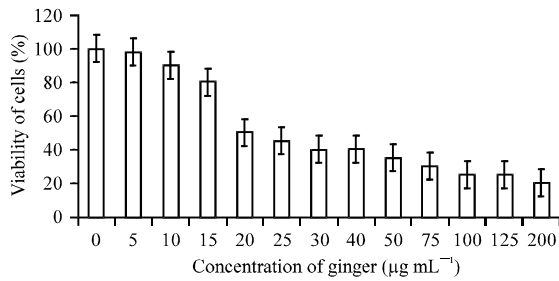


Fig. 1: Effect of different concentrations of ginger extract on Raji cells after 24 h. The bars on the histograms show standard deviations,  $p < 0.01$  by students' t test as compared to native cells.  $IC_{50}$  is  $20 \mu g mL^{-1}$

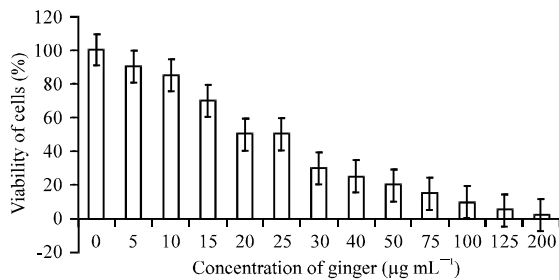


Fig. 2: Effect of different concentrations of ginger extract on Raji cells after 48 h. The bars on the histograms show standard deviations,  $p < 0.01$  by students' t test as compared to native cells,  $IC_{50}$  is  $20 \mu g mL^{-1}$

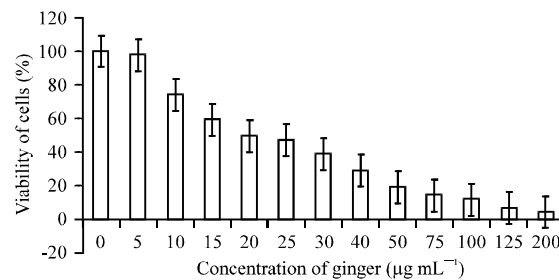


Fig. 3: Effect of different concentrations of ginger extract on Raji cells after 72 h. The bars on the histograms show standard deviations,  $p < 0.01$  by students' t test as compared to native cells.  $IC_{50}$  is  $20 \mu g mL^{-1}$

SDS PAGE of gels stained by Coomassie blue (Fig. 4) showed the presence of lighter protein bands at 55 and 65 KD after ginger treatment indicating reduction of proteins. The glycoproteins were stained by PAS (Fig. 5) and it can be seen that the two glycoproteins of 65 and 75 KD seen in native cells are missing in the treated ones.

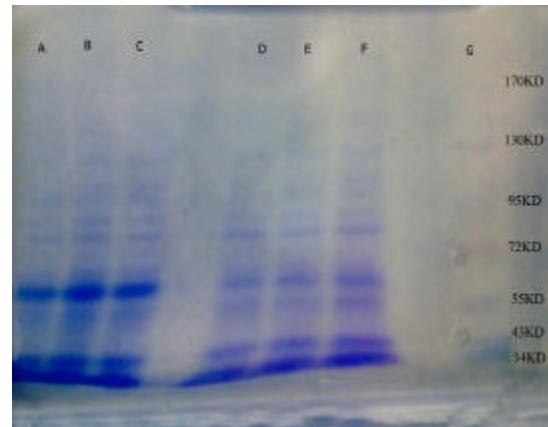


Fig. 4: Coomassie blue stained 7.5% SDS-PAGE for total proteins, Lane A-C native cell extract, lanes D-F treated cell extract, lane G contains MW markers. It should be noted that proteins of 65 and 55 KD have lessened in treated cells

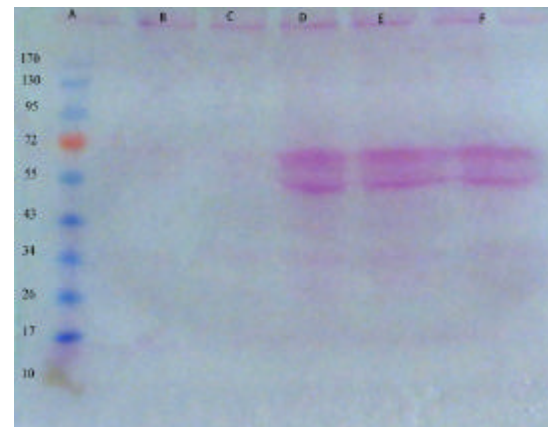


Fig. 5: PAS staining of SDS-PAGE for glycoproteins, Lane A MW markers, Lanes B and C treated cell extract and D to G native cell extract. Ginger reduces the expression of glycoproteins noticeably especially at 65 and 55 KD

Lectin affinity columns of total Q-proteome kits isolated N-linked mannose glycoproteins Fig. 6 with the Con A column and N linked sialic acids with the WGA column (Fig. 7). The three bands of 17, 26 and 35 KD indicating N linked mannose glycoproteins were missing in treated cells (Fig. 6). N-linked sialic acids were detected at 20 KD with no difference before and after ginger treatment (Fig. 7). Using O-Proteome kit for O-linked glycoproteins, the lysed cells were passed through two affinity columns, AIL for detection of O-linked sialic acids

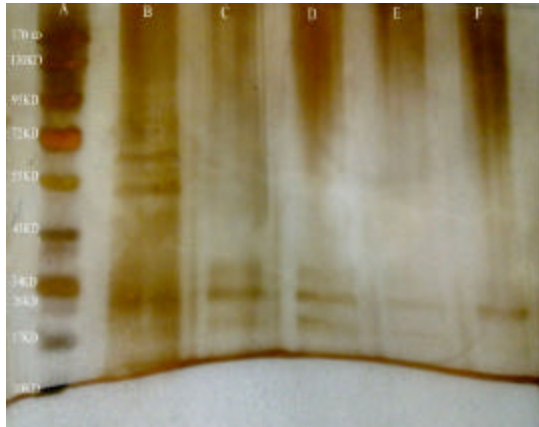


Fig. 6: Silver staining of reducing 10% SDS PAGE of purified glycoproteins obtained from Con A lectin for detection of N-linked mannose glycoproteins. Lane A contains MW markers, lane B contains FT ( treated ) and lanes C and D contain Elu (native ) shows three bands at 34, 26 and 17 KD and Lanes E and F contain Elu (treated) showing only the 26 KD band indicating the effect of ginger on N-mannose glycoproteins

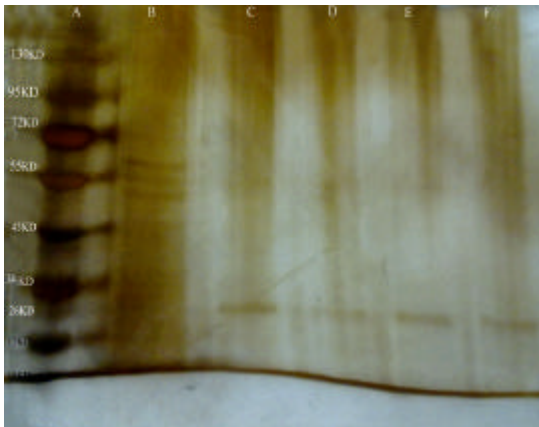


Fig. 7: Silver staining of reducing 10% SDS PAGE of purified glycoproteins obtained from WGA column for N-linked sialic acids. Lane A contains MW, lane B contains FT ( native) lanes C and D (Elu native) showing a single band at 26 KD and Lanes E and F contain Elu (treated) show the same band showing no effect of ginger on N-sialic acid glycoproteins

and PNA for O-linked galactose units. Ginger treatment seemed to have no effect on O-linked galactose units (seen at 35 KD) but, O-linked sialic acid

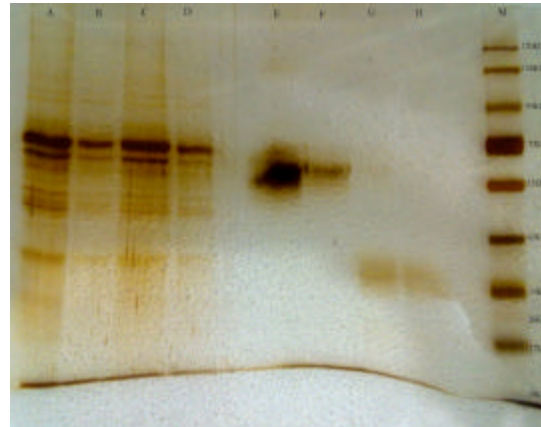


Fig. 8: Silver staining of reducing 10% SDS PAGE of purified glycoproteins obtained from AIL lectin column for detection of O-sialic acids and PNA column for O-galactose units. Lane A( FT native) from AIL, lane B ( FT treated ) from AIL, lane C (FT native) PNA and lane D (FT treated ) from PNA. Lane E ( Elu native) from AIL column, lane F ( Elu treated) from AIL, Lane G is (Elu native) from PNA and lane H (Elu treated ) from PNA and lane M contained MW, Lane F show the decrease of O- sialic acid in treated cells at 65 and 55 KD and Lane G and H show no effect of ginger on N-galactose units at 36 KD

(at 55KD and 65 KD) had noticeably reduced after ginger treatment (Fig. 8).

## DISCUSSION

Our studies have shown that proliferation of Raji cells was inhibited by ginger at  $IC_{50}$  of  $20 \mu g mL^{-1}$ . It also had a direct effect on the surface glycoproteins especially O-sialic Acid (OSA) of Raji cells. It is interesting that the two bands seen at 55 and 65 KD which were severely affected by ginger extract in PAS gel (Fig. 2) were OSA as seen in the purified glycoprotein gel (Fig. 8).

Sialic Acid, (SA) or N acetyl neuraminic acid, is a very important component of glycoprotein structure and glycolipids on the outer surface of the cells. They impart a negative charge to the cell surface due to their acidic nature and are important in cell-to-cell or cell-to matrix interactions. SA residues on the cell surface may also be involved in masking cell surface antigens and may serve as receptors for lectins, virus particles, some hormones and antibodies (Uslu *et al.*, 2003). Aberrant glycosylation is a characteristic feature of cancer cells, in particular, altered sialylation is closely associated with malignant

properties, including invasiveness and metastatic potential (Miyagi *et al.*, 2012). These glycoconjugates are released into the circulation through increased turnover, secretion, or shedding from malignant cells. Increased quantities of glycoconjugates such as Total Sialic Acid (TSA) and Lipid Bound Sialic Acid (LSA) have been detected in the serum of head and neck cancer patients (Miyagi *et al.*, 2012), infect, SA levels have been used for detection of prostate cancer in patients with non-diagnostic levels of prostate-specific antigen (Michalakakis *et al.*, 2012). It is interesting that like the other reported cancer cells, native Raji cells showed an increased expression of OSA as can be seen in Fig. 8 lane E. This could be the result of expression of the different genes of the different enzymes involved in the asialylation process. It is reported that enhanced expression of  $\alpha$ 2-6-linked sialic acids on N-glycans in the sequence Sia $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-R (Sia6LacNAc units) often correlates with human cancer progression, metastatic spread and poor prognosis. Increased Sia6LacNAc is explained by up-regulation of the *ST6GAL1* gene encoding the enzyme  $\beta$ -galactoside: $\alpha$ 2-6-sialyltransferase (ST6Gal-I) (Hedlund *et al.*, 2008; Harduin-Lepers *et al.*, 2012). Increased expression is reported in carcinomas of the colon, breast and cervix, choriocarcinomas, acute myeloid leukemia and in some brain tumors (Tian *et al.*, 2012). Role of these  $\alpha$ 2-6-Linked Sialic Acids and their modulation of carcinoma differentiation *in vivo* has also been demonstrated (Hedlund *et al.*, 2008). Latest techniques using hydrazide chemistry and mass spectrometry have also shown altered expression of sialylated glycoproteins in breast cancer (Tian *et al.*, 2012).

It is of great relevance that our results show that ginger extract has targeted O-sialic acids, (Fig. 8, lane F) at 55 and 65 KD can be seen in its poor expression in treated cells. SA and its enzymes are now regarded as potential targets for therapy. An interesting study has shown that an antibody to De-N-Acetyl sialic acid containing Polysialic Acid Identified an Intracellular Antigen SEAM 3 (SEAM 3-reactive antigen or S3RA), expressed in human melanomas induced apoptosis in four cell lines tested (Steirer and Moe, 2011). This helped a study which was made to target SA in metastatic tissues for therapeutic aims. These mechanisms could include vaccines against SA and its inhibitors and conjugates, antibodies, anti cancer drugs and anti metastatic drugs once more involving the sialic acid mechanism (Lu *et al.*, 2012). Our study shows that ginger effects the OSA in Raji cells. Raji cells being tumor cells have a large amount of O SA as compared to normal cells. The effect of gingerol in prevention and inhibition of skin cancer in mice has been proved and this is due to different compounds like vanillyl ketones, paradol and gingerol (Shukla and Singh, 2007;

Rhode *et al.*, 2006). A similar effect of gingerol in reduction of carcinogenic tumors in the digestive system of rats were observed (Yasmin Anum *et al.*, 2008) and also its inhibitory properties on human colon cancer (Zick *et al.*, 2011). In fact, mice fed with water extract of ginger exhibited an inhibitory effect on ovarian tumors (Kim *et al.*, 2005).

Ginger has been reported as having an inhibitory effect in different cancers. We have also shown it to have an inhibitory effect on Raji cells. Gingerol has been shown to prevent metastasis of lung cancer in mice and it is seen to be due to stimulus of host immune system (Yasmin Anum *et al.*, 2008). Anti-angiogenetic properties have been reported in ginger which helps to prevent tumor growth and metastasis (Kim *et al.*, 2005). Recent studies from UK have also show that ginger has a role in controlling prostate cancer (Khan *et al.*, 2008). Our studies have also shown an inhibitory effect of ginger extract on Raji cell growth with IC<sub>50</sub> at 20  $\mu$ g mL<sup>-1</sup>.

Ginger extract has an inhibitory effect on it and the amount of OSA in Raji cells is obviously lessened after treatment. As, SA reduction in sera is an indicator of response to anti-cancer treatment it is interesting to notice that OSA is targeted by ginger extract though the exact mechanism by which it is so will have to be further studied.

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