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In vitro study of Phytic acid effect on p53 protein in cancer line

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الخلاصة:

إن دراسة التاثير السمي لمادة الفايتك اسيد على خطوط الخلايا السرطانية أظهر تاثيرا يعتمد على تركيز المادة بالمقارنة مع مجموعة السيطرة وكذلك اظهر تاثير في تحفيز انتاج بروتين P 53 باستخدام طريقة تحديد كمية البروتين Eliza.

إن انتاج البروتين قل في خط Vero بدون تاثير معنوي (34 بيكوغرام\ مل± بانحراف معياري 4) مقارنةً مع مجموعة السيطرة (64 بيكوغرام\مل± وانحراف معياري 4)، بينما اظهر خط Radomyosarcoma (232بيكوغرام\مل± بانحراف معياري 32) تاثيراً معنوياً ملحوظاً مقارنةً مع مجموعة السيطرة (686 بيكوغرام \مل بانحراف معياري 86) وكذلك أزداد في خط Glioblastoma– Multiforme cell (118 بيكوغرام\مل± بانحراف معياري 18) مقارنتا مع مجموعة السيطرة (38 بيكوغرام \مل± بانحراف معياري 8)، بينما لم تظهر اي فروق معنوية في خط Ahmed Mohamed Nahi 3 (40 بيكوغرام \مل± بانحراف معياري 10) مقارنةً مع السيطرة (44 بيكو غرام \مل± بانحراف معياري 4) مما يبين أن هنالك تاثير مختلف للفايتك اسيد على انتاج بروتين p53 في خطوط الخلايا السرطانية وان له اكثر من ميكانيكية لقتل الخلايا السرطانية.

Abstract:

Studying Phytic acid cytotoxic effect on cancer cell line showed that it is a concentration dependant effect on cancer cell line in compare with control, also have effect on the production of p53 protein using protein detection method using Eliza.

The concentration of p3 protein is decreased in Vero with no significant effect (34 pg/ml ± SD 4) compare with control (64pg/ml ±SD 4) $p > 0.05$. Radomyosarcoma cell line (232 pg/ml± SD 32) shows a significant increase in compare with control (686 pg/ml ± SD 86) $p > 0.05$. The production of p53 increase in Glioblastoma- Multiforme cell (118 pg/ml ± SD 18) in compare with control (38 pg/ml ± SD 8), while the p53 quantity was not affected in the Ahmed Mohamed Nahi 3 (40 pg/ml± SD 10) compare with control (44 pg/ml ± SD 4) $p>0.05$.which show a different inhibition mechanisms of action on cancer cell line.

Introduction:

Inositol hexaphosphate or phytic acid IP6 has been an important dietary component its present in legumes non refined cereal derivatives corn .It is a simple ringed carbohydrate with six phosphate groups attached to each carbon. In a pH range of 0.5–9.0, it adopts the sterically stable 1ax/5eq (one phosphate at carbon position 2 in the axial position and five phosphates in the equatorial position) and sterically hindered 5ax/1eq conformation over pH 9.5 [1; 2].

IP6 is contained in substantial amounts in cereals and legumes (0.4–6.4%), primarily existing as a form of salt with monovalent and divalent cations, e.g. Ca^{2+}, Mg^{2+} and K^{1+} [3].

Studies on human and rodent cancer cell lines showed that InsP6 reduces cellular proliferation rate and DNA synthesis with the enhancement of differentiation of malignant cells to a more mature phenotype, sometimes resulting in reversion to normal [4]. IP6 has been found to cause G1 cell cycle arrest in mammary cancer cell lines MCF-7 and MDA-MB 231, and in HT-29, a human colon cancer cell line [5]. *p53* and *p21waf1* mRNA increase was performed after treatment with different concentration and time of IP6 ,Real-time-QPCR based on TaqMan methodology was applied to analyze quantitatively the transcript levels of these genes.[6]

The p53 gene is the most commonly mutated gene in human cancer and more than 500 gene mutations have been described. These mutations are found in various types of malignancies; however, all mutants are not necessarily equivalent in terms of biological activity [7]. The p53 protein is highly conserved and expressed in normal tissues. Wild- type p53 is shown to be a sequence- specific transcription factor, which is directly interacting with various cellular and viral proteins. Intact p53 function is essential for the maintenance of the non-tumorogenic phenotype of cells. Thus, p53 plays a vital role in suppressing the development of cancer. The p53 tumor suppressor protein is important in the cellular response to DNA damage and other genomic aberrations. In response to DNA damage, p53 is phosphorylated at multiple sites by several protein kinases [8].

Material and Methods:

This study was carried out in the Iraqi Center of Cancer and Medical Genetic Researches (ICCMGR).

Phytic acid preparation:

A 0.05 g of about 99% purity of phytic acid powder (Sigma–Aldrich/ Germany) dissolved in 5 ml to make a stock solution concentration 10000 µg/ml, sterile by 0.22µm filter. A serial binary dilutions were (10000, 5000, 2500 1250 µg/ml) under sterile condition were prepared [9].

Cell Line and Culture:

This *in vitro* method was used to investigate the effect of phytic acid on cancerous cell lines:

- Rhabdomyosarcoma (RD) Cell Line is a human cell line, it was derived from a biopsy specimen obtained from a pelvic rhabdomyosarcoma of a 7-year-old Caucasian girl [10], passage number 46 was used.
- Glioblastoma-Multiforme primary culture is human tumor culture has derived from a human cerebral glioblastoma multiforme (GBM) obtained from a 72-year-old Iraqi male who underwent surgery for intracranial tumor. Passage 38 of Glioblastoma Multiforme cell culture was used in this study and the cells were grown on RPMI-1640 with 10% FCS. The culture of these cells was at Iraqi center for cancer and medical genetic researches (ICCMGR), [11] passage 79 was used.
- Ahmed-Mohammed-Nahi-2003 (AMN3) Cell Line is a murine mammary adenocarcinoma cell line was derived from first *in vivo* passage for a spontaneous mammary adenocarcinoma of female BALB/c mice [12]. Passages 182 of AMN3 cell line was used throughout this study and cells was maintained using Roswell Park Memorial Institute media (RPMI-1640) with 5% FCS.
- Vero Cell Line was initiated from the kidney of a normal adult African green monkey in 1962. In this study, passages 88-89 were used and the cells were maintained in minimum Essential Media with 5% FCS [13].

Cell line Preparation and Cytoxicity assay:

The growth medium was decanted off. 2 - 3 ml of trypsin- versene was added to the cell sheet and the flask shacked gently. After approximately 30 seconds most of the tyrosine- versene was poured off and the cells incubated at 37̊C until they had detached from the flask. Cells were further dispensed by pipetting in growth medium [14]. Then, 200 µl of cells in growth medium were added to each well of sterile 96- well microtitration plate. The plates were sealed with a self adhesive film, lid placed on and incubated at 37̊C.

When the cells are in exponential growth, i.e. after Lag phase, the medium was removed and serial dilutions of aqueous and methanol extracts in serum free media (SFM) (10000, 5000, 2500, 1250 or 0.0 µg/ml) were added to the wells. Three replicates were used for each concentration of each extracts. Afterwards, the plates were re- incubated at 37 ̊ C. for the selected exposure times (24, 48 or 72 hrs.).

Supernatants were removed from the wells of the microtitration plate at the end of each exposure period while maintaining sterile conditions. 100 µ l of MTT (3- (4, 5- Dimethlthiazol- 2- yl – 2,5- diphenyltetrazolium bromide, atetrazole)

solution (1mg\ml) and 50 µ l of SFM in (2: 1 v/ v) was added to each of the wells in the microtitration plate, and then covered incubated for 4 hrs. at 37 ̊ C. At the end of this incubation period, 200 µ l of Dimethyl Sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The Optical density was determined at 550 nm. using ELISA reader.

P53 detection and quantitative determination:

After cytotoxicity assay, 5000 and 0.0 µg/ml concentrations used in cytotoxicity assay that gave highest effect percentage on RD, Vero, AMGM and AMN3 cell lines after 24 were used P53 in detection and quantitative determination compared with control.

For the detection of p53 protein, we used a p53 ELISA Kit (US Biological Co., p 1001- 25A). This p53 ELISA Kit used for quantitative determination of wild- type and mutant p53 in human, mouse and rat samples. It uses a monoclonal antibody to p53 immobilized on a microtiter plate to bind p53 in the standards or samples. Purified p53 standards are provided in the kit. Apolyclonal antibody to p53 labeled with the enzyme Horseradish peroxidase is added to the standards and samples. This labeled antibody binds to the p53 captured on the plate. After short incubation, the excess sample and labeled antibody are washed out and substrate is added. The substrate reacts with the HRP- labeled antibody bound to the p53 captured on the plate. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450 nm.

Statistical: analysis*:*

Experiments data were analyzed using statistical Last Significant Design (LSD).

Results and Discussion:

Cytotoxic study to the effect of phytic acid on RD , Vero, AMGM and AMN3 cell lines show a significant effect at the concentration 10000 and 5000 µg/ml during 24 h of exposure.

According to these results there is no difference in the effect of phytic acid on the cell lines which mean the effect is not cancer cell line kind dependant. There is the same effect of the concentration 10000, 5000 µg/µl during 24h. as in table-1.

These results are associated with the study of Abdullah 2008 [9]. Therefore the less concentration give the best result 5000µg/µl were used to study the p53 effect.

Cell line	Concentration µg/ml	24 hours	48 hours	72 hours
Vero	10000	80%	87%	90%
	5000	75%	80%	85%
	2500	60%	50%	60%
	1250	60%	50%	60%
RD	10000	55%	57%	60%
	5000	50%	50%	55%
	2500	37%	0%	0%
	1250	6%	0%	0%
AMGM	10000	90%	90%	95%
	5000	85%	80%	90%
	2500	60%	60%	70%
	1250	60%	60%	70%
AMN3	10000	40%	40%	40%
	5000	40%	40%	35%
	2500	30%	20%	0%
	1250	20%	0%	0%

Table-1: The inhibition concentration of phytic acid on cancer cell line.

P53 detection kit results:

The result of the study in showing in the following fig-1:

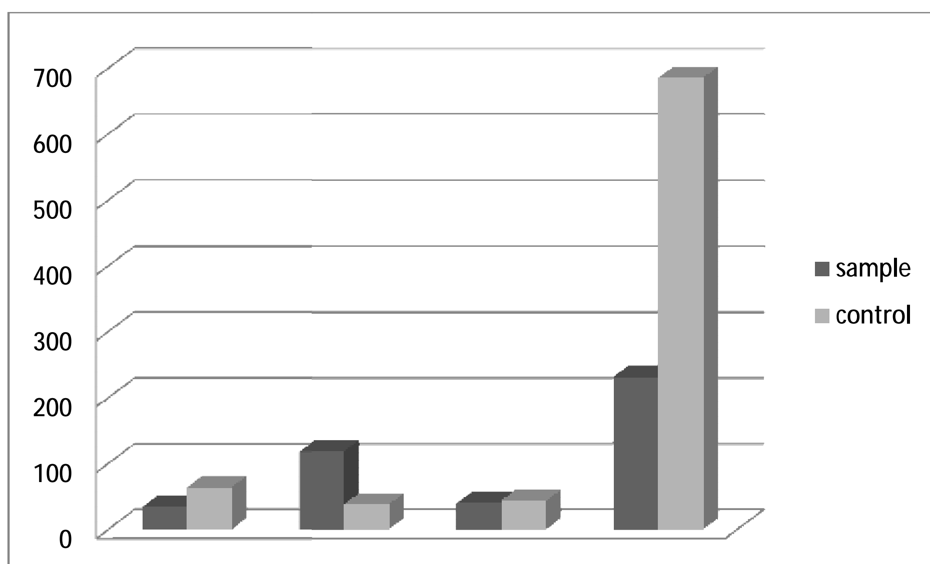

Figure 1: The effect of phytic acid on p53 protein concentration to Vero, AMGM, AMN3 and RD cell lines.

Cell lines	Means of test ±SD	Means of control ± SD
Vero	102 ±SD 4	192 ±SD 4
RD	696±SD 32	2058 ±SD 86
AMGM	354 ±SD 18	114 ±SD8
AMN3	120 ±SD10	132±SD 4

Table-2: The statistical analysis of the p53 concentration in cancer cell line.
$p>0.05$

The concentration of p3 protein is decreased in Vero with no significant effect (34 pg/ml ± SD 4) compare with control (64pg/ml ±SD 4) $p > 0.05$. RD cell line (232 pg/ml± SD 32) shows a significant increase in compare with control (686 pg/ml ± SD 86) p 0.05. the production of p53 increase in the AMGM cell (118 pg/ml ± SD 18) in compare with control (38 pg/ml ± SD 8), while the p53 quantity was not affected in the AMN3 (40 pg/ml± SD 10) compare with control (44 pg/ml ± SD 4) $p>0.05$.

InsP6 stimulated *p53* and *p21 waf1* expression at the mRNA level, with the highest increase in *p21waf1* mRNA occurring at 24 h, i.e., following the highest increase in p53 mRNA observed at 12 h. Based on these studies it may be concluded that the ability of IP6 to arrest the cell cycle may be mediated by the transcriptional up-regulation of the p53-responsive *p21waf1* genethat explain the result of AMGM increase of p53 protein after exposure to phytic acid [6].

In case of AMN3 cell line p53 protein concentration was not affected , there is other mechanism may lead to cell death rather than p53 activation like the ability of ip6 to involvement in signal transduction pathways can affect cell cycle regulation, growth, and differentiation of malignant cells [15; 16]. Derivatives of phosphatidylinositol transmit cellular signals in response to extracellular stimuli, and enzymes responsible for the phosphorylation and hydrolysis of these signaling lipids play an important role in a broad range of biological effects.

The antioxidant role of IP6 is known and widely accepted; this function of IP6 occurs by chelation of Fe^3 and suppression of ·OH formation [17].Therefore, IP6 can reduce carcinogenesis mediated by active oxygen species and cell injury via its antioxidative function. This activity seems to be closely related to its unique structure [18].

In our study we used p53 protein detection kit for normal and mutant p53 type depending on the study of Shamsuddin [19, 20, 21] and his Studies of the expression of tumor suppressor gene demonstrate that IP6 up- regulation of wild type p53 and down-regulation of the mutant form that mean the Vero an RD cell line have an increase in mutant form of p53 gene production which it reduced after the exposure to IP6 like in HepG2 cell line .this investigate that AMGM had the

wild type P53 gene production gene increased after treatment with IP6. Where AMN 3 mammary gland adenoma carcinoma in mice was dead by IP6 other mechanism but not depend on p53 mechanism.

Conclusion:

We can conclude from this study that:

1 - Phytic acid can cause inhibition concentration on different cancer cell line.

2 - Phytic acid has different mechanism of action on cancer cell line.

3 - Phytic acid has different effect on cancer cell line for the induction of p53 production.

References:

1 - Grases, F., Simonet, B.M., Vucenik,I; Prieto, R. (2001).A and excretion of orally administered inositol hexaphosphate (IP6 or phytate)in human. Bio factor .ISO press 15: Pp. 53- 61.

2 - Barrientos, L.G. & Murthy, P.P.N. (1996). Conformational studies of myoinositol phosphates. Carbohydrate Research, Vol.296: Pp. 39–54

3 - Harland, B.F. & Oberleas, D. (1987). Phytate in foods World Review of Nutrition and Diet, Vol. 52: Pp. 235–259

4 - Shamsuddin, A.M.; Yang, G. and Vucenik I (1996). Novel anticancer function of IP6: growth inhibition and differentiation of human mammary cancer cell lines *in vitro*. *Anticancer Res* Vol. 16: 3287-3292.

5 - El-Sherbiny, Y.; Cox, M.C.; Ismail, Z.A; Shamsuddin, A.M. and Vucenik, I (2001). G0/G1 arrest and S phase inhibition of human cancer cell lines by inositol hexaphosphate (IP6). *Anticancer Res* Vol. 21: Pp. 2393-2404.

6 - Węglarz1, L.; Molin, I.; Orche, A; Parfiniewicz1, B. and Dzierżewicz, Z. (2006). Quantitative analysis of the level of *p53* and *p21WAF1* mRNA in human colon cancer HT-29 cells treated with inositol hexaphosphate .Vol. 53 (2): Pp. 349–356.

7 - Milner, J. (1997). Structures and functions of the tumor suppressor p53. Path. Biol. (Paris). Vol. 45, 797-803. 8

8 - Prives, C. (1999). The p53 pathway. *Journal of Pathology* . Vol. 178: Pp 122-126.

9 - Abdullah, R. H.; Mamdoh, A. and Hassan, A. (2008). Cytotoxic effect of phytic acid on cancer cell line. Iraqi journal for cancer.Vol. (2). Pp 18–21 .

10 - Mcallister, R.M.; Melnyk, J.; Finklestein, J.Z.; Adams, E.C. and Gardner M.B. (1969). Cultivation *In Vitro* of cells derived from human Rhabdomyosarcoma. *Cancer,* Vol.24: Pp.520-526.

11 - Al-shamary ,A.A.M. (2009). Newcastle disease Virus therapy for transplanted tumors in combination with chemotherapy. PhD thesis . the *College of veterinary medicine University of Baghdad.*
12 - Al-shamary, A.A.M. (2003). The study of the Newcastle disease virus effect in the treatment of transplanted tumors in mice. MSc thesis . *College of veterinary medicine University of Baghdad.*
13 - American public health association. (1992). Compendium of methods for the microbiological examination of food. 2rd ed. *American public health association, Washington DC* Pp.43
14 - Freshney, R.I. (2005). Culture of animal cells. Amanual for basic technique. (5th ed.) xcWilley- liss. A Juhn wiley and sons. *Inc. Pup*. New York
15- Shamsuddin, A. M. (2002) Anti-cancer function of phytic acid. Int. J. Food Sci. Technol. Vol (37). Pp. 769–782.
16 - Shamsuddin, A. M. (1995). Inositol phosphates have novel anticancer function. J. Nutr. Vol. (125). Pp. 725S–732S.
17 - Graf, E. and Eaton, J. W. (1990) Antioxidant functions of phytic acid. Free Radi. Biol. Med. Vol. (8). Pp.61–69.
18 - Nelson, R. L.; Yoo, S. J.; Tanure, J. C.; Andrianopoulos, G. and Misumi, A. (1989) .The effect of iron on experimental colorectal carcinogenesis. Anticancer Res Vol. (9). Pp. 1477–1482.
19 - Shamsuddin-A-M. (1999). Metabolism and cellular functions of IP6: a review. Vol. (9): (5A) Pp. 3733-3736.
20 - Vucenik, I.; Tantivejkul, K.; Zhang, Z.S.; Cole, K.E.; Saied,I. and Shamsuddin, A. M. (1998). IP6 in treatment of liver cancer. IP6 inhibits growth and reverses transformed phenotype in HepG2 human liver cancer cell line.Anticancer research. Vol. (18). Pp. 4083-4090.
21 - Huang, C.; Ma, W.; Y.; Hecht, S. S. and Dong, Z. (1997). Inositol hexaphosphate inhibits cell transformation and activator protein 1 activation by targeting phosphatidylinositol-39 kinase. Cancer Res. Vol. (57).Pp. 2873–2878.