Effect of diosmin on P13K and AKT expression on NDEA induced hepatocellular cellular carcinoma in rats - an *in vitro* study.

VA Muralidharan, R Gayathri*, J Selvaraj, V Vishnu Priya

Department of Biochemistry, Saveetha Institute of Medical and Technical Sciences, Chennai, India

Abstract

Introduction: Diosmin is a flavone found in *Teucrium gnaphalodes* and in some citrus fruits indicated for the treatment of venous disease. Dismosin is also used to hemorrhoids and leg sores caused due to poor blood flow. Diosmin is not recommended for use in children or women during pregnancy. Diosmin is not recommended for treating the rectal mucosa, skin irritations, or wounds, and should not be used to treat dermatitis, eczema or urticaria. The oral administration of diosmin as a protective agent normalized the altered levels of AFP, LPO, antioxidant enzymes, pro and anti-apoptotic proteins as well as caspase-3 and -9 proteins. In a previous study, desmosin is a flavanone glycoside that is found in the citrus species and showed antioxidant, hepatoprotective as well as anticancer activity. Previous study on diosmin reveals that it acts as an anti-diabetic drug in type -2 diabetes mellitus Also acts as a potent anti-inflammatory drug. Pi3k gene is a master regulatory gene for cancer.

Activation of the pi3k pathway leads to the development of tumors and gives resistance to anticancer therapeutic drugs. Phosphatidylinositol 3-Kinase (PI3K)–AKT signaling pathway regulates the expression of several downstream target genes that inhibit apoptosis and promote cell proliferation. The effect of its deregulation on the clinical outcome of human tumors has recently become a subject of intense investigation. Activation of this pathway has been associated with aggressive phenotype and poor prognosis in brain tumors (glioblastoma and neuroblastoma) as well as in various carcinomas, including breast, prostate, bladder, colon, and lung carcinomas

Aim: To determine the effect of diosmin on Pi3k and Akt expression in NDEA induced hepatocellular carcinoma in experimental rats.

Materials and methods: The present experimental study was approved by the institutional animal ethics committee. Adult male Wistar albino rats were obtained and maintained in clean propylene cages in an air-conditioned animal house, fed with standard rat pelleted diet orally for 16 weeks. At the end of the treatment, animals were anesthetized and blood was collected through cardiac puncture, sera were separated and stored. Gastrocnemius muscle from control and experimental animals was immediately dissected out and used for assessing the various parameters. Hepatic carcinoma by administering (NDEA) and the changes are then assessed using real time PCR.

Results and discussion: Pi3k /AKT pathway is an intra-cellular signalling pathway important in regulating the cell cycle. Therefore, it is directly related to cellular quiescence proliferation cancer and longevity DIRK or AKT mRNA. In the current study there was a Dec 2 animals indicating the induction of hepatocellular cancer, whereas in the treatment group (group 3 treated with the diosmin) there is significant increase in the expression of PI3K or Akt which was equivalent to control oxidative stress. A similar study on cioskinagnin proves to be effective in pulmonary syndrome.

Conclusion: Animals treated with diasmin clearly exhibited an improvement in Hepatocellular cancer, thus diasmin can augment anti-cancer therapy.

Keywords: Diosmin, Akt expression, P13k signalling pathway, NDEA induced, Innovative technology, Novel method.

Accepted on November 15, 2021

Introduction

Diosmin is a flavone found in *Teucrium gnaphalodes* and in some citrus fruits indicated for the treatment of venous disease. Dismosin is also used to hemorrhoids and leg sores caused due to poor blood flow [1]. Diosmin is not recommended for use in children or women during pregnancy. Diosmin is not recommended for treating the rectal mucosa, skin irritations, or wounds, and should not be used to treat dermatitis, eczema or urticaria. The oral administration of diosmin as a protective agent normalized the altered levels of AFP, LPO, antioxidant enzymes, pro- and anti-apoptotic proteins as well as caspase-3 and -9 proteins [2]. In a previous study, desmosin is a flavanone glycoside that is found in the *Citrus* species and showed antioxidant, hepatoprotective as well as anticancer activity [3]. N-Nitrosodimethylamine is a volatile, combustible, yellow, oily liquid nitrosamine with a faint characteristic odor that decomposes when exposed to light and emits toxic fumes of nitrogen oxides when heated to decomposition [4]. Hepatocellular Carcinoma (HCC) is the most common type of primary liver cancer. Hepatocellular carcinoma occurs most often in people with chronic liver diseases, such as cirrhosis caused by hepatitis B or hepatitis C infection [5].

In the previous studies, the results showed that CA reduced the activities of **Alanine Aminotransferase** (ALT) and **Aspartate Aminotransferase** (AST) in liver and serum. CA also reduced the levels of Interleukin-6 (IL-6), IL-1β, Tumor Necrosis Factor-α (TNF-α), **methane** dicarboxylic **aldehyde** (MDA), and stored the activity of Superoxygen Dehydrogenases (SOD) in serum. CA could obviously attenuate the hepatic pathological alteration. Furthermore, CA effectively inhibits the **phosphorylations** of Phosphatidylinositol 3 Kinase (PI3K), **protein kinase B** (Akt), **Mammalian Target of Rapamycin** (mTOR) [6]. Evidence has emerged indicating the hepatoprotective effect of purslane on CCl4-induced liver damage and acetaminophen-induced liver injury through the anti-inflammatory and antioxidative properties [7].

Pi3k and akt expression was checked on other materials and carcinomas but in this study the expression was determined with the help of dismosin in hepatocellular carcinoma [8]. Our team has extensive knowledge and research experience that has translate into high quality publications [9-28]. The main aim of our study was to determine the effect of diosmin on pi3k and akt expression in N-Nitroso Diethylamine (NDEA) induced hepato cellular carcinoma in rats.

Materials and Methods

Chemicals

All chemicals and reagents used in this study were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA. PCB was procured from Sigma Chemical Company St. Louis, MO, USA; Total RNA Isolation Reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from Genet Bio, South Korea purchased from Promega, USA. Interleukin-1β and β-actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India.

Animals

The present experimental study was approved by the institutional animal ethics committee (IAEC no.: BRULAC/SDCH/SIMATS/IAEC/12.2019/048). Adult male Wistar albino rats, weighing 180–200g, were obtained and maintained in clean propylene cages at the Biomedical Research Unit and Laboratory Animal Centre (BRULAC), Saveetha dental college and hospitals, Saveetha university, India in an air-conditioned animal house, fed with standard rat pelleted diet (Lipton India Ltd., Mumbai, India), and clean drinking water was made available ad libitum. Rats were divided into 3 groups, each consisting of 6 animals.

Experimental Design

Group 1: Control (Vehicle control, rats were intraperitoneally (i.p.) administered with the vehicle (corn oil) for 30 days.

Group2: Rats hepatocellular induced carcinogenic rats 0.01%N-Nitrosodiethylamine (NDEA) given orally for 16 weeks it was dissolved in corn oil at a dose of 2mg/kg body weight (b.wt) intraperitoneally daily at 10:00 am for 30 days.

Group 3: Cancer bearing rats (diosmin treated rat of 200mg per Kg/body weight per day at 10 am through oral route for 28 days)

At the end of treatment, animals were anesthetized with sodium thiopental (5 mg/kg, i.p), and 20 ml of normal saline was perfused through the left ventricle, to clear blood from the liver, and other organs. Gastrocnemius muscle was dissected out and used for the assay of various parameters.

Assessment of Akt expression mRNA

A 10% homogenate of the tissue was prepared in 0.1M tris-hydrochloric acid buffer at a pH of 7.4 .This was centrifuged and the clear supernatant was used for further analyses.

Isolation of total RNA

Total RNA was isolated from control and experimental samples using TRIR (Total RNA Isolation Reagent) kit. Briefly, 100 mg fresh tissue was homogenized with 1 ml TRIR and the homogenate was transferred immediately to a microfuge tube and kept at -80°C for 60 min to permit the complete dissociation of nucleoprotein complexes. Then, 0.2 ml of chloroform was added, vortexed for 1 min and placed on ice at 4°C for 5 min. The homogenates were centrifuged at 12,000 xg for 15 min at 4°C. The aqueous phase was carefully transferred to a fresh microfuge tube and an equal volume of isopropanol was added, vortexed for 15 sec and placed on ice at 4°C for 10 min. The samples were centrifuged at 12,000 xg for 10 min at 4°C. The supernatant was discarded and RNA pellet was washed with 1 ml of 75% ethanol by vortexing and subsequent centrifugation for 5min at 7,500 xg (4°C). The supernatant was removed and RNA pellets were mixed with 50 µl of autoclaved Milli-Q water and dissolved by heating in a water bath for 10 min at 60°C.

Quantification of RNA

Diluted RNA sample was quantified spectrophotometrically by measuring the Absorbance (A) at 260/280 nm. 40 µg of RNA in 1 ml gives one absorbance at 260 nm. Therefore, the concentration of RNA in the given sample can be determined by multiplying its A260 by 40 and dilution factor. The purity of RNA preparation can be calculated using the ratio between its absorbance at 260 and 280 nm. A ratio of absorbance at 260/280 nm> 1.8 is generally considered as good quality RNA. The purity of RNA obtained was 1.8.

Reverse Transcriptase – Polymerase Chain Reaction (RT – PCR)

RT-PCR is an approach for converting and amplifying a single stranded RNA template to yield abundant double stranded DNA products. 1. First strand reaction: complementary DNA (cDNA) is made from the mRNA template using oligo dT,

dNTPs and reverse transcriptase. 2. Second strand reaction: After the reverse transcriptase reaction is complete, standard PCR (called the "second strand reaction") is initiated. Principle RT-PCR is a method used to amplify cDNA copies of RNA. It is the enzymatic conversion of mRNA into a single cDNA template. A specific oligo deoxynucleotide primer hybridized to the mRNA and is then extended by an RNA dependent DNA polymerase to create a cDNA copy. First strand DNA synthesis The RT kit was purchased from Eurogentec (Seraing, Belgium). Reagents 1. 10X RT buffer: One vial containing 1.4 ml of 10X RT buffer. 2. EuroScript reverse transcriptase: One tube containing 75 µl of Moloney Murine leukemia virus reverse transcriptase (3750 U at 50 U/µl).

Quantitative real time PCR principle

The purpose of a PCR (Polymerase Chain Reaction) is to make a huge number of copies of a gene. There are three major steps in a PCR, which are as follows: Denaturation at 94°C for 3 min: During the denaturation at 94°C for 2-5 min, the double strand melts open to single stranded DNA, all enzymatic reactions stop. Annealing at 54°C- 65°C for 30 sec: Ionic bonds are constantly formed and broken between primer and the single stranded template to ensure the extension process. Extension at 72°C for 30 sec: Primers that are in positions with no exact match get loose again (because of the higher temperature) and don't give an extension of the fragment. The bases (complementary to the template) are coupled to the primer on the 3' side (the polymerase adds dNTP from 5' to 3', reading the template from 3' to 5' side; bases are added complementary to the template). Because both strands are copied during PCR, there is an exponential increase of the number of copies of the gene.

Reagents

1. 2X Reaction buffer: The PCR master mix kit was purchased from Takara

Bio Inc., Japan. Contains TaKaRa Ex Taq HS (a hot start PCR enzyme) dNTP

Mixture, Mg2+, Tli RNase H (a heat-resistant RNase H that minimizes PCR

inhibition by residual mRNA), and SYBR Green I.

2. Forward primer (10 µM)

3. Reverse primer (10 µM)

4. cDNA- Template

5. Autoclaved milli Q water

6. Primers: The following gene specific oligonucleotide primers were used.

Procedure

Procedure real time PCR was carried out on CFX 96 real time system (Bio-Rad). The reaction mix (10 µl) was prepared by adding 5 µl of 2X reaction buffer, 0.1 µl of sense and antisense primer, 1 µl of cDNA and 3.8 µl of sterile water. The thermal cycler protocol was as follows: Initial denaturation at 95°C for 3 min, followed by 40 cycles of PCR, denaturation at 95°C for 10 sec, annealing at 60°C for 20 sec and extension at 72°C for 20 sec. All reactions were performed in triplicate along with No Template Control (NTC).

Melt curve analysis was performed using the thermal cycling programmed at 50-95°C for each sample to determine the presence of multiple amplicons, non-specific products and contaminants. The results were analysed using CFX 96 Real Time system software (Bio-Rad). As an invariant control, the present study used rat β-actin.

Results

Figure 1 represents the Akt expression on mRNA assay in which x axis represents the fold chain over control and y axis represents the study groups in which green colour represents the control , red represents the NDEA induced rats and blue represents the diosmin treated rats.

It is evident that fold change over diosmin treated is greater than NDEA induced Akt expression on mRNA proving that it has significant effect on Akt gene on mRNA.

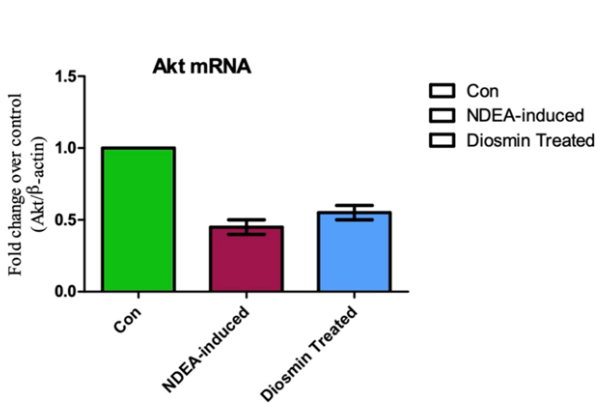

Figure 1. *Represents the Akt expression on mRNA.*

Figure 2 represents the P13k expression on mRNA assay in which x axis represents the fold chain over control and y axis represents the study groups in which green colour represents the control, red represents the NDEA induced rats and blue represents the diosmin treated rats.

It is evident from the graph that the fold change over control on p13k gene on mRNA expression of diosmin treated is as equal as the control and greater than the NDEA induced thus proving that the drug is more effective on the p13k expression.

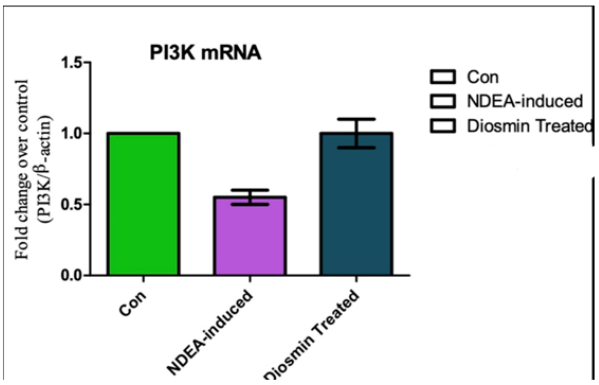

Figure 2. *Represents the Pi3k mRNA expression.*

Discussion

Pi3k/AKT pathway is an intracellular signaling pathway important in regulating the cell cycle. It is directly related to cellular quiescence, proliferation, cancer and longevity. In the current study there was decreased expression of pi3k/ AKT mRNA in group 2 animals, indicating the induction of hepatocellular cancer, whereas in the treatment group (group 3 has been treated with Diosmin) there is significant increase in the expression of pi3k/ AKT mRNA which was equivalent to control [29]. Hesperidin is a flavanone glycoside that is found in the *Citrus* species and showed antioxidant, hepato protective as well as anticancer activity. This study investigated the effect of hesperidin on the PI3K/Akt pathway as a possible mechanism for its protective effect against Diethylnitrosamine (DEN)-induced Hepatocellular Carcinoma (HCC) [30]. Uncontrolled tumour cell proliferation plays the most crucial role in hepato-cellular carcinoma growth. This study investigated whether diosmin can inhibit cell proliferation in human hepato-cellular carcinoma HA22T cells. We found a significant inhibitory effect by diosmin on HA22T cell viability as revealed by MTT assay [31]. PCNA is a useful marker for proliferative activity, which functions as a cofactor of DNA polymerase and as an important marker for evaluating the proliferation of several cancers, including hepato-cellular carcinoma. In normal cells, c-Myc is induced upon growth factor stimulation and constitutively high in transformed cells. The c-Myc overexpression is estimated to occur in 70% of human tumours. Our results showed that diosmin significantly inhib- ited PCNA, c-Fos, and c-Myc in HA22T cell levels. There is increasing evidence supporting pi3k as an attractive target in cancer therapy. Our results revealed that diosmin treatment resulted in a dose- dependent increase in p53 level. It was found that pi3k might directly facilitate cytochrome c release [32]. MDM2 is an onco-protein that is over-expressed in a range of human cancers. Under non-stressed conditions, the p53 level is tightly controlled by MDM2 through a well-established auto-regulatory feedback loop [33]. It induces MDM2 gene expression, which in turn leads to pi3k inactivation and degradation [34]. MDM2 is reported to be one of the main factors causing pi3k degradation *via* the proteasome-related pathway [35]. Accordingly, the western blot assay showed that diosmin inhibited MDM2 expression in a dose-dependent manner. It is also found that pi3k, pp53, p21 and p27 are significantly upregulated in the presence of diosmin [36]. According to previous studies, to show that asparanin A causes G2 / M arrest in HepG2 cells, the the authors showed that cyclin A and Cdk1 were downregulated to varying degrees after treatment with asparagine. Exposure to asparanin A also resulted in increased levels of the pCdk1 protein. In a recent study conducted, HKH40A was used to treat Hep3B cells and was found to increase p-Cd25C and p-Cdk1 expression in a dose-dependent manner. Treatment of HA22T hepatocarcinoma cells with different doses of diosmin also had similar results, leading to cell cycle arrest in the G2 / M phase.

One of the previous studies indicated that P13k is a key regulatory enzyme useful in the regulation of the particular enzyme known as the puncture of rubella which affects the cerebellum and corpus callousness of the brain, also the spinal cord leading to the formation of nagri bodies and further leads to anglers reaction key enzyme is the key enzyme in the cell regulatory network. It is widely distributed in the animal and plant kingdoms and appears to be essential in the regulation of a number of physiological processes *via* dephosphorylation of a cohort of specific target proteins. Inhibition of P13k by OA promotes the cell cycle progression at the start/restriction point [37]. However, the inhibition of PP2A activity at later stages causes mitotic defects. Therefore, specific inhibition of P13k can cause significant cytotoxic effects. It has also been previously reported that cantharidin inhibits the activity of a purified catalytic subunit of Pi3k rat pheochromocytoma cells. The norcantharidin analogues that inhibit Pi3k correlate well with their observed anti-cancer activity against a panel of five cancer cell lines: A2780 (human ovarian carcinoma), G401 (human kidney carcinoma), HT29 (human colorectal carcinoma), H460 (human lung carcinoma) and L1210 (murine leukemia) [38]. A recent study showed that sorafenib plus bortezomib significantly suppressed P13k hepatocellular carcinoma cell xeno-graft tumour growth, down-regulated p-Akt expression, and up- regulated PP2A activity [39]. It was also found that OA reversed diosmin-mediated down-regulation of p-PI3K, p-Akt and pMDM2, suggesting that PP2A targeting might be a possible way to influence the critical phase [40]. It was also found that diosmin has no cytotoxic effects on rat primary hepatocytes. Further experiments demonstrated that diosmin dramatically suppressed HA22T cell proliferation and tumour growth in the nude mice model. Diosmin showed the strongest effect at a concentration of 30 mg/kg. Based on these findings, diosmin up-regulated PP2A, leading to decreased p-PI3K, p-Akt, and p-MDM2, and further upregulated cell cycle checkpoint proteins such as the increased expression of p53, p21, p27, p-Cdc25C and p-Cdk1; and decreased expression of c-Fos, c-Myc, cyclin A, cyclin D, cyclin E, Cdc25C, Cdk1, and PCNA [41]. Many studies showed that the administration of hesperidin significantly decreased the elevation in liver function enzymes, serum AFP level, and oxidative stress markers. Diosmin, being a flavonoid rich in citrus fruits, acts as an anti-cancer agent. Previous studies on Diosmin against Hepatopulmonary syndrome also prove the same. Effect of diosmin on pi3k and akt expression in N-Nitroso Diethylamine (NDEA) induced

hepato cellular carcinoma in rats, an *in-vitro* study. In conclusion, the hepatoprotective activity of diosmin is mediated *via* its antioxidation and downregulation of the PI3K/Akt pathway.

Conclusion

From the study it is evident that the pivotally increased inhibition of the PI3K and akt pathway induced by the administration of with diosmin indicates that this drug is a promising candidate for further clinical trials and clinical investigation to enhance cancer therapy management.

Conflict of Interest

All the authors declare no conflict of interest in the study.

Acknowledgement

We thank Saveetha dental college for supporting us to conduct the study.

Source of Funding

The present study was supported by the following agencies: Saveetha dental college, Saveetha institute of medical and technical science, Saveetha university, Prompt paper products private LTD.

References

1. Ren X, Luo W. Exploration of pro-apoptotic effect of thymoquinone on oral squamous cell carcinoma cells through PI3K/Akt signaling pathway. Cell Mol Biol. 2019;65(1):61.
2. Tokuzen N, Nakashiro KI. Abstract 3468: Antitumor effect by inhibition of PI3K/AKT/mTOR and RAS/MAPK pathways in human oral squamous cell carcinoma cells. Mol Cell Biol/Genet. 2018;78(13).
3. Fransson SM, Abel F, Eriksson H, et al. Abstract 2237: Analysis of the PI3K/Akt signaling pathway in neuroblastoma shows stage dependent expression of PI3K isoforms. Moland Cell Biol. 2012;72(8).
4. Kumar RS. The Antioxidant defense system induced by methanol extract of Careya arborea in N-Nitroso-diethylamine (NDEA) induced hepatocarcinogenesis. J Complement Integrat Med. 2008;5(1).
5. Zeng Y, Lian S, Li D, et al. Anti-hepatocarcinoma effect of cordycepin against NDEA-induced hepatocellular carcinomas via the PI3K/Akt/mTOR and Nrf2/HO-1/NF-κB pathway in mice. Biomed Pharmaco. 2017;95:1868–1875.
6. Issinger OG, Guerra B. Phytochemicals in cancer and their effect on the PI3K/AKT-mediated cellular signaling. Biomed Pharmaco. 2021;139:111650.
7. Matsuura B. Effect of antiandrogen treatment on hepato-cellular carcinoma. Hepatology. 1995;22(4):A196.
8. Bhatt P. Solid lipid nanoparticle of hesperidine exerts diethylnitrosamine/phenobarbital-induced hepatocellular carcinoma via alteration of PI3K/Akt pathway. Ann Hepato Biliary Pancreat Surg. 2021;25:S222–S222.
9. Wu F, Zhu J, Li G, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16). Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):3297–305.
10. Chen F, Tang Y, Sun Y, et al. 6-shogaol, a active constiuents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating NrF2 signaling in human epidermal keratinocytes (HaCaT cells). J Photochem Photobiol B. 2019;197:111518.
11. Li Z, Veeraraghavan VP, Mohan SK, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway. J Photochem Photobiol B. 2020;203:111773.
12. Babu S, Jayaraman S. An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. Biomed Pharmacother. 2020;131:110702.
13. Malaikolundhan H, Mookkan G, Krishnamoorthi G, et al. Anticarcinogenic effect of gold nanoparticles synthesized from Albizia lebbeck on HCT-116 colon cancer cell lines. Artif Cells Nanomed Biotechnol. 2020;48(1):1206–1213.
14. Han X, Jiang X, Guo L, et al. Anticarcinogenic potential of gold nanoparticles synthesized from Trichosanthes kirilowii in colon cancer cells through the induction of apoptotic pathway. Artif Cells Nanomed Biotechnol. 2019;47(1): 3577–3584.
15. Gothai S, Muniandy K, Gnanaraj C, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. Biomed Pharmacother. 2018;107:1514–1522.
16. Veeraraghavan VP, Hussain S, Balakrishna JP, et al. A comprehensive and critical review on ethnopharmacological importance of desert truffles: Terfezia claveryi, Terfezia boudieri, and Tirmania nivea. Food Rev Int. 2010;1–20.
17. Sathya S, Ragul V, Veeraraghavan VP, et al. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. Environ Nanotechnol Monit. 2020;14:100333.
18. Yang Z, Pu M, Dong X, et al. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitro and in vivo studies. Arab. J. Chem. 2020;13(5):5501–5516.
19. Rajendran P, Alzahrani AM, Rengarajan T, et al. Consumption of reused vegetable oil intensifies BRCA1 mutations. Crit Rev Food Sci Nutr. 2020;1–8.
20. Barma, M D, Muthupandiyan I, Samuel SR, et al. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021;126:105132.
21. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021;31(2):285–286.

22. Samuel SR, Kuduruthullah S, Khair AMB, et al. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent. 2021;31(3):436–441.
23. Tang Y, Rajendran P, Veeraraghavan VP, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from Scutellaria baicalensis on human osteoblast-like MG-63 cells. Mater Sci Eng C. 2021;119(3): 111656.
24. Yin Z, Yang Y, Guo T, et al. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. Environ Toxicol. 2021;36(6):1011–1020.
25. Veeraraghavan VP, Periadurai ND, Karunakaran T, et al. Green synthesis of silver nanoparticles from aqueous extract of Scutellaria barbata and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). Saudi J Biol Sci. 2021;28(7):3633–3640.
26. Mickymaray S, Alfaiz FA, Paramasivam A, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway. Saudi J Biol Sci. 2021;28(7):3641–3649.
27. Teja KV, Ramesh, S. Is a filled lateral canal – A sign of superiority? J Dent Sci. 2020;15(4):562–563.
28. Kadanakuppe S, Hiremath S. Social and behavioural factors associated with dental caries experience among adolescent school children in Bengaluru city, India. J adv med. 2016;14(1):1–10.
29. Bryant LK, Jones KJ, Hinton CV. Abstract 501: CXCR4 induced ROS accumulation through PI3K/AKT. Mol Cell Biol. 2014;74(19).
30. Owada S, Shimoda Y, Tsuchihara K, et al. Abstract 1133: Glucose deprivation-induced intracellular reactive oxygen species activates the PI3K-AKT axis. Mol Cellr Biol. 2012;78(8).
31. Okui G, Tobiume K, Rizqiawan A, et al. AKT primes snail-induced EMT concomitantly with the collective migration of squamous cell carcinoma cells. J Cell Biochem. 2013;114(9):2039–2049.
32. Zhang H, Wang Y, Cao S, et al. Expression of PI3k, akt, p-akt, bad, and p-bad in radiosensitivity induced by aresenic trioxide in human esophageal carcinoma. J Clin Oncol. 2014;32(15):e15039–e15039.
33. Saritha K, Setty OH. Protective Effect of Hemidesmus indicus on the mitochondrial function in NDEA induced hepatocellular carcinoma. Planta Medica. 2013;79.
34. Reddy GP, Kartik R, Rao CV, et al. Chemopreventive effect of Cissampelospariera againstn-nitroso diethylamine induced hepatocellular carcinoma. J Clin Exp Hepatol. 2013;3:S101.
35. Antoniv TT, Ivashkiv LB. Interleukin-10-induced gene expression and suppressive function are selectively modulated by the PI3K-Akt-GSK3 pathway. Immunol. 2011;132(4):567–577.
36. Somoza V. Hypoglycemic Effect of Hydrophobic BCAA Peptides Is Associated with Altered PI3K/Akt Protein Expression.
37. Li B. ID1-induced activation of PI3K/AKT/NFkB pathway : mechanisms and significance in esophageal cancer. University of Hong Kong. 2019.
38. Nguyen CN, Kim HE, Lee SG. Caffeoylserotonin protects human keratinocyte HaCaT cells against H2O2-induced oxidative stress and apoptosis through upregulation of HO-1 expression via activation of the PI3K/Akt/Nrf2 pathway. Phytotherapy Res. 2013;27(12):1810–1818.
39. Radisavljevic ZM, Flecha BG. TOR kinase and ran are downstream from PI3K/Akt in H2O2-induced mitosis. J Cell Biochem. 2004;91:1293–300.
40. Li F, Yao J, Hao Q, et al. miRNA-103 promotes chondrocyte apoptosis by down-regulation of Sphingosine kinase-1 and ameliorates PI3K/AKT pathway in osteoarthritis. Bioscie Reports. 2019;39(10).
41. Jang Y. Endurance exercise activates PI3K/AKT/mTOR signaling in MPTP-induced Parkinson's disease. Sports Science. 2020;38:89–94.

*Corresponding to:

Kavitha S

Department of Biochemistry

Saveetha Institute of Medical and Technical Sciences

Chennai

India

E-mail: kavithas.sdc@saveetha.com