Plant Archives Volume 20 No. 1, 2020 pp. 2871-2874 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DETERMINATION THE EFFECT OF DIFFERENT CALCIUM CA^{2+} LEVELS ON HEPATOCYTES DIVISION IN WHITE RATS *RATTUS NORVEGICUS*

Ali A. Ghazzay

College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq.

Abstract

In this study we tried to determine possible roles of Ca^{2+} on hepatocytes division**.** Calcium have been demonstrated to be vital mediators. The period of cell cycle showed too effected by calcium. In the this paper we tried to report the relation between calcium concentration and the cellular proliferation ratio for hepatocytes of white rats we divided the experimental animals into four groups each group contains five experimental animals at the same age and weight, the results of this study showed that the cellular proliferation ratio increased with the calcium concentration increasing, where the ratio of hepatic division progressed to get 35.33 ± 0.33^{C} at concentration 20mg/ml, 55.33 ± 0.33^{B} at concentration 40mg/ml and 70.33 ± 0.33^{A} at concentration from Ca^{2+}.

Key words: *Ca^{2+}. Proliferation*, hepatocytes, white rats.

Introduction

Calcium have important role,involved in cellular division processes, In dividing cells the phase of cellular division (mitosis) is marked at anaphase by significant changes in cytosolic calcium concentration, which are much more strongly expressed in embryos than in somatic cells (Barritt *et al.*, 2010). In hepatocytes of white rats, it appears natural to consider that the release of calcium from internal stores, like the endoplasmic reticulum (ER), is an important step in the cascade of mitotic events (Zhang *et al.*, 2008) the releasing of Ca^{2+} from internal stores because of increasing the inositol (Aromataris *et al.*, 2008; Chaube *et al.*, 2009; Cheng *et al.*, 2005) triphosphate (IP3) (Palee *et al.*, 2016) the introduction of Ca^{2+} to the experimental animals facilities and cause spindle dissolution and period of anaphase is beginning (Aromataris *et al.*, 2008).

It is well reported that intracellular Ca^{2+} is significant for tissue homeostasis through regulation of cell cycle and apoptosis, In other words intracellular calcium Ca^{2+} has been reported to regulate cell proliferation at all phases of the cell cycle, from gene expression to mitosis (Sato *et al.*, 2009) resent studies demonstrated that the liver regeneration and homeostasis influenced by extracellular calcium concentration (Cheng *et al.*, 2005) Also, the alteration and modifications of intracellular calcium pathway could alter liver regeneration in the rat have been reported (Lewis, 2007; Palee *et al.*, 2016).

It has been documented that the activation of transcription factors like CREB, NF-κ B, or NF-AT and for immediate early genes like c-fos or c-jun determined by Ca^{2+} signals was determined (Sato *et al.*, 2009) It is also reported that the Ca^{2+} have influence on activation of the RAS pathway (Litjens *et al.*, 2004).

Subsequently the nuclear calcium signals have crucial effect on gene transcription, by tow pathways either from the diffusion of cytosolic calcium to the nucleus, or from an $InsP_3$ -mediated calcium release in the nucleus (Suresh *et al.*, 2015) previous studies reported that hepatocyte calcium signals Ca^{2+} have been shown and regulation have been shown and regulation liver cell division. (Rychkov *et al.*, 2005). Other papers demonstrated the significant impact of mitochondrial calcium concentration in the regulation of apoptotic processes and documented that mitochondrial calcium overload can be one of the pathways leading to the swelling of mitochondria and to the rupture of the outer membrane, in turn releasing pro-apoptotic molecules in the cytosol (Soboloff *et al.*, 2012).

The purpose of the present work is aimed at discussing

****Author for correspondence :*** E-mail: Ali.Ghazay@qu.edu.iq

Table 1: Compare to control group.

Concentration	Division ratio (mean ± SE)
Negative control	5±0.57^D
Ca^{2+} (20mg/ml)	35.33±0.33^C
Ca^{2+} (40mg/ml)	55.33±0.33^B
Ca^{2+} (60mg/ml)	70.33±0.33^A
LSD	1.602

o LSD : least significant difference.

the relation and physiological impact between the increasing of calcium concentration and the proliferation and the viability of hepatocytes in white rats it attempts to provide a possibility quantitating the effects of different external of calcium concentrations upon hepatocytes cellular division,

Our results indicate that the interactions between Ca^{2+} and cell cycle progression and positive physiological impact of Ca^{2+} on hepatocytes proliferation.

Materials and Methods

Forty adult male waster rats weighting 200±10 g were used in this study. They were fed with standard diet pellets and allowed food and water for an acclimation period of two weeks. The animals were maintained in temperature (25±1°C). Humidity was kept at 50% with adequate ventilation.. The rats were randomly divided into 4 groups each consisting of ten animals. Group I received normal saline, group II received 20 mg/ml Ca^{2+} for one week, group III received 40mg/ml Ca^{2+} for one week and group IV received 60mg\ml for one week. Animals were sacrificed on day 7, livers were removed and then tissue preparation was performed then the viability of hepatocytes detected by trypan blue dye (Wu. *et al.*, 2014,Zha0 et.al,2015).

Results and Discussion

Investigation of the possible role of Ca^{2+} on the proliferation and induced the division of hepatocytes Previous reports using microinjected Ca^{2+} suggest that this messenger diffuses through gap junctions in hepatocyte doublets. However little information is available on the ability of Ca^{2+} to effect on the division of hepatocytes study (Soboloff *et al.*, 2012)

In the present study, we first aimed to further investigate if Ca^{2+} can stimulated the hepatocyte division and the ratio of proliferative cells effected by different concentration of Ca^{2+}. To investigate this possibility we used three different concentration of Ca^{2+} (20 mg/ml, 40 mg/ml and 60 mg/ml) respectively as well as control group.

We have shown that focal stimulation of Ca^{2+} on the proliferation and regulation division of hepatocytes in different concentration, the present study data showed that the ratio of proliferation hepatocytes induced by introduced 20mg/ml of Ca^{2+} to experimental animals and increased to 35.33±0.33 with significant differences ($P<0.05$) compare with control group 5±0.57 (Table 1, Fig. 1).

Our results also reported that the proliferation induced by physiological effect of Ca^{2+} on the regulation of hepatocytes division,there was significant differences ($P<0.05$) when the group III received 40mg/ml Ca^{2+} for one week and the ratio of proliferation increased to 55.33±0.33 compare to control group 5±0.57 (Table 1, Fig. 1).

The present study data demonstrated there was significant differences ($P<0.05$) at the proliferation ratio of hepatocytes when group IV received 60mg\ml for one week and the ratio of proliferation increased to 70.33±0.33 compare to 35.33±0.33 in group II that received 20 mg/ml Ca^{2+} for one week and control group 5±0.57 that received normal saline.

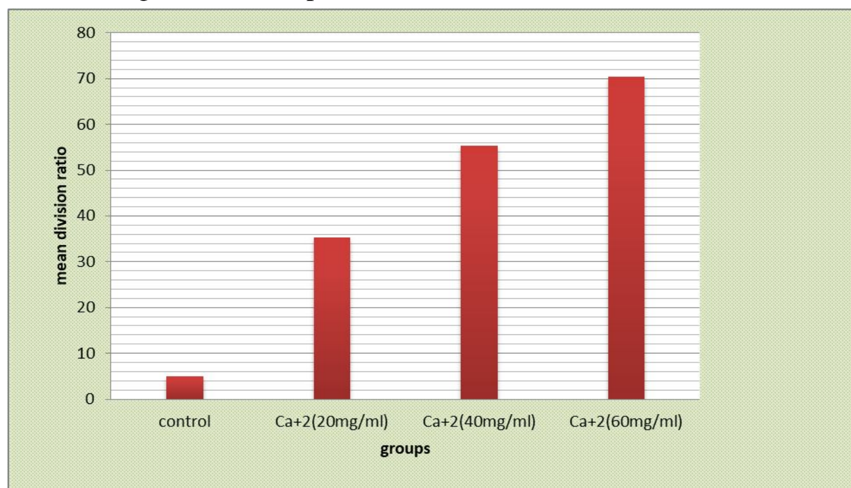

Fig. 1: The relation between ratio of hepatocytes proliferation and Ca^{2+} concentration.

The present paper thought that Ca^{2+} have crucial physiological impact,our results agree with previous studies that reported that Ca^{2+} important molecules involved in cell division acts as a second messenger In addition, it is involved in providing the energy needed by the cell. Its role in nerve conduction is also known (Uguz *et al.*, 2016).

The rise of Ca^{2+} concentration lead to increasing proliferation ratio and regulation of hepatocytes growth and division when we applied (20, 40, 60)mg/ml respectively these data seem to be

compatible with other studies that reported Ca^{2+} binds to and activates several hormones and molecules. Moreover, it is reported that it has a stimulating effect in the division of leukocytes. When the amount of Ca^{2+} in the blood rises to the level of 15 mg/ml it serves as stimulus for the division of leukocytes (Bai *et al.*, 2002)

Also there was a lot of papers documented that Calcium is reported to take key role in the end of G1 phase, transition to S phase, entry into M phase and the completion of this phase in the cell cycle when calcium introduced to the experimental animal in different concentrations (Lewis, 2007; Oh. *et al.*, 2009).

It was found in the study performed on hepatocytes that the cell proliferation was increasing as a result of the fact Ca^{2+} molecules released from the endoplasmic reticulum into the cytoplasm inside the cell, the level of Ca^{2+} required for passing to G2 / M phase after S phase. these findings proofed in previous studies that indicated there was no significant change in G2 / M phase due to insufficient Ca^{2+} amount (Palee *et al.,* 2016; Roderick and Cook, 2008).

Our findings indicated that the high level from calcium concentration have key role in increasing the proliferation and viability of hepatocytes based on other data the reported sufficient amounts of Ca^{2+}, which is needed for the formation of cyclin D-CDK4 (cyclin-dependent kinase 4) required for cell division (Cheng *et al.,* 2005; Rosado *et al.,* 2015)

pp34 is the protein product of a yeast gene whose counterpart has been found to regulate cell cycle transitions,calcium at concentration 15 mg/ml that lead ultimately to DNA synthesis and cell cycle progression cause rapid pp34 phosphorylation. Treatments with sufficient extracellular calcium and increased it to (20mg/ml) stimulate DNA synthesis and cell cycle onset because of phosphorylation pp34 (Rychkov *et al.,* 2005; Wu *et al.,* 2014).

Mitosis promoting factor (MPF) is a cytoplasmic activity found during mitosis or meiosis in frog and starfish oocytes the formation of active MPF are regulated by kinase and phosphatase activity and effected by calcium amount (Suresh *et al.,* 2015) these approaches may be state the most important reasons that involved with increasing of proliferation for rats hepatocytes when sufficient amount of calcium introduced to experimental animals. Other paper reported that extracellular Ca^{2+} from 1.0 mM to 0.1 mM led to a gradual decrease in the rate of proliferation these results disagree with our results (Roderick and Cook, 2008).

Extracellular Ca^{2+} is required at multiple distinct points in the cell cycle in mammalian cells. When proliferating mouse or human fibroblasts were placed into media containing low Ca^{2+} content to normal levels enabled cells to undergo DNA synthesis within hours Cells were most sensitive to the depletion of extracellular Ca^{2+} at two points during the cell cycle, in early G_1 and near the G_1/S boundary (Palee *et al.,* 2016).

The consequences of intracellular Ca^{2+} pool depletion included inhibition of DNA synthesis, protein synthesis and nuclear transport Furthermore, depletion of intracellular Ca^{2+} stores at any point during G_1 to S resulted in an accumulation of cells in a G_0-like state even when cells have partially replicated DNA, Therefore, normal cells require both extracellular and intracellular Ca^{2+} for proliferation, with cells being the most sensitive to Ca^{2+} depletion during G_1 (Lewis, 2007; Sato *et al.,* 2009).

References

Abed, S.A. and M.A. Salim (2019). The first record of Asian Pied starling gracupica contra Linnaeus, 1758 (Aves, Sturnidae) in Iraq. *Eco. Env. & Cons*., **25(1):** 106-110.

Abed, Salwan Ali (2017). Occurrence of Anatidae in Sawa Lake: A Ramsar Wetland Site in Southern Iraq. *Journal of Advanced Zoology. J. Adv. Zool*., **38(1):** 43-51.

Aromataris, E.C., J. Castro , G.Y. Rychkov, G.J. Barritt (2008). Store-operated Ca^{2+} channels and stromal interaction molecule 1 (STIM1) are targets for the actions of bile acids on liver cells. *Biochim. Biophys. Acta.,* **1783:** 874-885.

Bai, X.C., F. Deng, A.L. Liu, Z.P. Zou, Y. Wang and Z.Y. Ke *et al.,* (2002). Phospholipase C-gamma1 is required for cell survival in oxidative stress by protein kinase C. *Biochem. J.,* **363(Pt 2):** 395-401.

Barritt, G.J., T.L. Litjens, J. Castro, E. Aromataris and G.Y. Rychkov (2009). Store-operated Ca^{2+} channels and microdomains of Ca^{2+} in liver cells. *Clin. Exp. Pharmacol. Physiol.,* **36:** 77-83.

Chaube, S., A. Tripathi and S. Khatun *et al*., (2009). Extracellular calcium protects against verapamil-induced metaphase-II arrest and initiation of apoptosis in aged rat eggs. *Cell. Biol. Int.,* **33:** 337-343.

Cheng, S., Q. Wu and F. Yang *et al*., (2005). Influence of DL-β-hydroxybutyric acid on cell proliferation and calcium influx. *Biomacromol-ecules.,* **6:** 593-597.

Ewaid, S.H. and S.A. Abed (2017). Water quality index for Al-Gharraf river, southern Iraq. *Egypt. J. Aquatic Res*., **43(2):** 117-122. http://dx.doi.org/10.1016/j. ejar.201703001.

Ewaid, S.H., S.A. Abed and N. Al-Ansari (2020). Assessment of Main Cereal Crop Trade Impacts on Water and Land Security in Iraq. *Agronomy.,* **10:** 98.

Ewaid, S.H., S.A. Abed and N. Al-Ansari (2019). Crop Water

Requirements and Irrigation Schedules for Some Major Crops in Southern Iraq. *Water.,* **11:** 756.

Ewaid, S.H., S.A. Abed and N. Al-Ansari (2019). Water Footprint of Wheat in Iraq. *Water.,* **11:** 535.

Lewis, R.S. (2007). The molecular choreography of a store-operated calcium channel. *Nature.,* **446:** 284-287.

Litjens, T., M.L. Harland, M.L. Roberts, G.J. Barritt, G.Y. Rychkov Fast (2004). Ca^{2+} dependent inactivation of the store-operated Ca^{2+} current (I(SOC)) in liver cells: a role for calmodulin. *J. Physiol.,* **558(Pt 1):** 85-97.

Oh, Y.J., J.H. Youn, Y. Ji, S.E. Lee, K.J. Lim and J.E. Choi *et al.,* (2009). HMGB1 is phosphorylated by classical protein kinase C and is secreted by a calcium-dependent mechanism. *J. Immunol.,* **182:** 5800-5809.

Palee, S., N. Apaijai, K. Shinlapawittayatorn, S.C. Chattipakorn and N. Chattipakorn (2016). Acetylcholine attenuates hydrogen peroxide-induced intracellular calcium dyshomeostasis through both muscarinic and nicotinic receptors in cardiomyocytes. *Cell. Physiol. Biochem.,* **39:** 341-349.

Roderick, H.L. and S.J. Cook (2008). Ca^{2+} signalling checkpoints in cancer: remodelling Ca^{2+} for cancer cell proliferation and survival. *Nat. Rev. Cancer.,* **(8):** 361-375.

Rosado, J.A., R. Diez, T. Smani and I. Jardín (2015). STIM and Orai1 variants in store-operated calcium entry. *Front Pharmacol.,* **6:** 325.

Rychkov, G.Y., T. Litjens, M.L. Roberts and G.J. Barritt (2005). ATP and vasopressin activate a single type of store-operated Ca^{2+} channel, identified by patch-clamp recording, in rat hepatocytes. *Cell. Calcium.,* **37:** 183-191.

Salim, M.A. and S.A. Abed (2017). Avifauna Diversity of Bahr Al-Najaf Wetlands and the Surrounding Areas, Iraq. *Jordan Journal of Biological Sciences*., **10(3):** 167-176.

Salim, M.A. and S.A. Abed (2019). The first oriental honey buzzard *Pernis ptilorhynchus* (Temminck, 1821) in Iraq. *Eco. Env. & Cons*., **25(1):** 1926-1929.

Salwan Ali Abed and A. Salim Mudhafar (2018). Breeding observations of the Black-winged Kite *Elanus caeruleus* (Desfontaines, 1789) in Iraq, *Zoology and Ecology*, **28(1):** 21-24, DOI: 10.1080/21658005.2017.1415833.

Salwan Ali Abed *et al.*, (2019). *J. Phys. Conf. Ser*., 1294 072025.

Sato, H., T. Takeo, Q. Liu, K. Nakano, T. Osanai and S. Suga *et al.,* (2009). Hydrogen peroxide mobilizes Ca^{2+} through two distinct mechanisms in rat hepatocytes. *Acta Pharmacol. Sin.,* **30:** 78-89.

Soboloff, J., B.S. Rothberg, M. Madesh and D.L. Gill (2012). STIM proteins: dynamic calcium signal transducers. *Nat. Rev. Mol. Cell. Biol.,* **13:** 549-565.

Suresh, K., L. Servinsky, J. Reyes, S. Baksh, C. Undem and M. Caterina *et al.,* (2015). Hydrogen peroxide-induced calcium influx in lung microvascular endothelial cells involves TRPV4. *Am. J. Physiol.,* **309:** L1467.

Uguz, A.C., A. Öz, M. Nazýroglu Curcumin (2016). Inhibits apoptosis by regulating intracellular calcium release, reactive oxygen species and mitochondrial depolarization levels in SH-SY5Y neuronal cells. *J. Recept Signal Transduct Res.,* **36:** 395-401.

Wu, M.M., E.D. Covington and R.S. Lewis (2014). Single-molecule analysis of diffusion and trapping of STIM1 and Orai1 at endoplasmic reticulum–plasma membrane junctions. *Mol. Biol. Cell.,* **25:** 3672-3685.

Zhang, X., D. Wheeler, Y. Tang, L. Guo, R.A. Shapiro and T.J. Ribar *et al.,* (2008). Calcium/calmodulin-dependent protein kinase (CaMK) IV mediates nucleocytoplasmic shuttling and release of HMGB1 during lipopolysaccharide stimulation of macrophages. *J. Immunol.,* **181:** 5015-5023.

Zhao, P., X. Yan and X.H. Wang (2015). induced hepatocyte apoptosis by intracellular calcium homeostasis. *Chin. J. Cell. Biol.,* **37:** 1257-1262.