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Evaluation the effect of Glutamine on the viability of cultured digestive gland cells for fresh water snail *bellamyabengalensis in vitro.*

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Abstract

To evaluate the effect of amino acid (Glutamine) on the viability in vitro cultured digestive gland cell s of fresh water snail bellamyabengalensis and define the optimal concentration of glutamine for digestive cells cultured in vitro .the fresh water snails bellamyabengalensis were collected from Shamyiah river-Dewanyah province, the snail were dissection out to obtain the digestive gland cells, which cultured in (DMEM medium) medium supplemented with different concentration of Glutamine (1mM, 2mM). the digestive cells cultured in (DMEM) supplemented with glutamine showed higher viability rate compare with basal media (non-supplemented with glutamine) and control, while the medium supplemented with (2mM) glutamine had more effect on the viability of digestive gland cells than the medium supplemented with (1mM) glutamine. Glutamine have positive effect to maintain the viability of digestive gland cell for bellamyabengalensisin vitro cultured.

Keywords: Glutamine, DMEM medium

Introduction

Amino acids considers the most important material to form protein, that is why, all the cells need at least twelve essential amino-acids: arginine, cystine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, tyrosine and valine, which are L-amino acids. Beside, glutamine is another substrate has vital role in the cellular process (Haroun et al., 2001). L-glutamine is an amino acid used by naturally all invertebrates' cells grown *in vitro* culture, It is consider as energy source, fused into protein, and used in nucleic acid metabolism (Sherif et al., 2002).

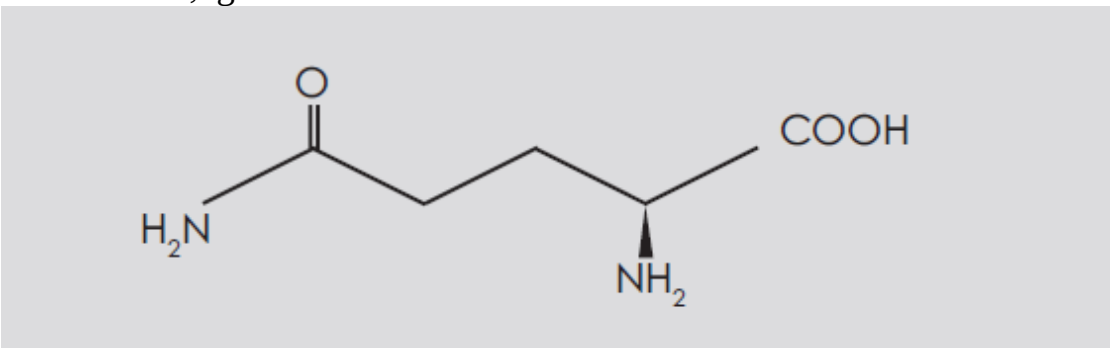

Figure (1): L-Glutamine(Corning, 2012).

L-glutamine is important demand for proliferation and maintain the viability for many sorts of cells in cells and tissue culture *in vitro* techniques (Radha&Geetha, 2012).

The concentration of glutamine ranged from(0.2 mM-1.0mM) is essential for media composition to get optimal proliferation and maintain the viability of cells; that is mean the concentration of L-glutamine is higher than the concentrations for most other amino acids that cells need (Tannock et al., 1986).

Glutamine is also substitute material to glucose for pyruvate metabolism and production of high level from energy (Zhanqiu&Xiong, 2010).

L-glutamine also considers the most dominant amino acid in the blood, approximately counting for 30-35 percent of the amino acid nitrogen in the plasma, Because glutamine contains two ammonia groups, one come from its precursor, glutamate, and the other from free ammonia in the plasma (Philip, 2001). one of the most important effects of glutamine is as nitrogen source, which maintain the normal levels of ammonia, Therefore glutamine act as a buffer which eliminate excess ammonia, the ability of glutamine to maintain the normal level of nitrogen by import and export nitrogen, makes glutamine the major canal for nitrogen transfer between different tissues in the body (Yang, 2011).

For all reasons mentioned above we designed this study to

1-Investigate the role of glutamine on the viability of digestive gland cells cultured for bellamyabengalensis in vitro.

2-Identify the optimal level of glutamine which have stronger effect to maintain the the viability of digestive gland cells cultured for bellamyabengalensis in vitro.

Material and methods.

Table1: List of equipment and chemicals that used in experiment.

Equipment |& chemical	Country
Laminar co_2 flow	Japan
Cooling centrifuge	Japan
Light microscope	Japan
Sensitive balance	Sartorius
Trypan blue	Sigma USA
Glutamine powder	Sigma USA
DMEM medium	Sigma USA
Incubator	Japan

Table2: Composition of Dulbecco's modified eagles medium (DMEM)

component	g/l
Calcium chloride	0.2
Ferric nitrate	0.0001
Magnesium sulfate	0.9767
Potassium chloride	0.4
Sodium chloride	4.4

Sodium phosphate monobasic	0.109
L-Arginine	0.084
L-Cystine	0.0626
L-Glutamine	0.584
Glycine	0.03
L-Histidine	0.042
L-Isoleucine	0.105
L-Leucine	0.105
L-Lycine	0.146
L-Methionine	0.03
L-Phenylalanine	0.066
L-Serine	0.042
L-Threonine	0.095
L-Tryptophan	0.016
L-Tyrosine	0.10379
L-Valine	0.094
Choline chloride	0.004
Folic acid	0.004
Myo-inositol	0.0072
Niacinamide	0.004
D-Pantothenic acid	0.004
pyridoxal	0.004
Riboflavin	0.0004
Thiamine	0.004
D-Glucose	4.5
Hepes	5.958
Phenol red	0.0159

Sigma (2007).

Sample collections

Fresh water snails of the species *Bellamyabengalensis*(average size of 2.5cm) were collected at Shamyiah river in Dewanyah province in April 2013 by simple hand picking method, freshly collected specimens were carried out to the laboratory of college of Science– Qadisyah university and placed in aquarium and half of fresh water in the aquarium was renewed every two days, the animals was fed with lettice and introduced to analysis, Then the specimens dissected out, and the digestive gland of *B. bengalesis* have been taken out carefully and gently and minced into small pieces to prepare its to cell culture studies. All procedures were carried out under sterile conditions in a laminar flow hood exclusively used for cell-culture purposes (Kamble & Gaikwad, 2012).

The digestive gland cells culturing using culture media.

The digestive gland explant from the Bellamyabengalensis were dissected out into small cellular fragments and placed in petri dish containing 2ml fresh water ,the petri dishes were stirred during this period in order to make easier the From

fragments disassociation.

the petri dish the suspension was taken out and put in a 15ml Falcon tube. The suspension was centrifuged at 15°C, 150xg, for 4 minutes, the supernatant was discarded and the pellet of cells resuspended in 2mL of fresh water.

Using the same parameters the pellet of cells were resuspended in 2mL of cell culture media, The cell suspension was transferred in a multi-wells and left at 15°C (Buchanan et al,2001;cristaino, 2009). Cells viability was checked every three days using trypan blue dye(Sigma,2007) ,the culture media was replaced two times per week (remove gently the old culture media close to the surface to avoid to remove many cells) (Buchanan et al, 2001; Cristaino, 2009).

Prepare glutamine supplement

To prepare 1mM from Glutamine (Glu), we weighed 0.01g from glutamine powder and solved it in 100ml distilled water, then we take out 2ml and added to the 50 ml culture media to obtain culture media have 1mM glutamine. we followed the same procedure to prepare 2mM glutamine by weigh 0.02g from glutamine powder and solved it 100 distilled water ,then take out 2ml and added to the 50ml culture media, to obtain culture media have 2mM glutamine (Haroun et al.,2001).

Results

Our study was designed to identify the effect of Glutamine in tow different concentrations as supplement to DMEM media on the viability of digestive gland cells for fresh water snail bellamyabengalensis.

Our result demonstrated that ,there was slight significant differences at the first day between the viability of cells in DMEM media supplemented with 1mM,2mM glutamine and DMEM media, when the viability was(94,92,90,3)% in DMEM media supplemented with 2mM, DMEM media supplemented with 1mM, DMEM media and control respectively.

At the third and sixth days there was significant differences between the viability of cells in DMEM media supplemented with 1mM,2mM glutamine and DMEM media, when the viability was (88,87,82,0)% at the third day and (81,78,73 ,0)% at the sixth day in DMEM media supplemented with 2mM, DMEM media supplemented with 1mM, DMEM media and control respectively.

While there was clear significant differences at the ninth and twelfth days, when the viability was (43,35,22,0)% at the ninth day and (27,0,0,0)% at the twelfth day in DMEM media supplemented with 2mM, DMEM media supplemented with 1mM, DMEM media and control respectively.

Also there was strong significant differences at the fifteenth day, When the cells still alive in DMEM media supplemented with 2mM by viability (21%), while all the cells was dead in DMEM media supplemented with 1mM, DMEM media and control by viability was (0%) (table3, figure1).

Table3:The viability of digestive gland cells using DMEM medium and DMEM medium supplemented with different concentration of Glu.

Media	1 day	3 day	6 day	9 day	12 day	15 day
Control	3	0	0	0	0	0
DME	90	82	73	52	22	0
DMEM+1mM Glu	92	87	78	64	35	0
DMEM+2mM Glu	94	88	81	70	43	27

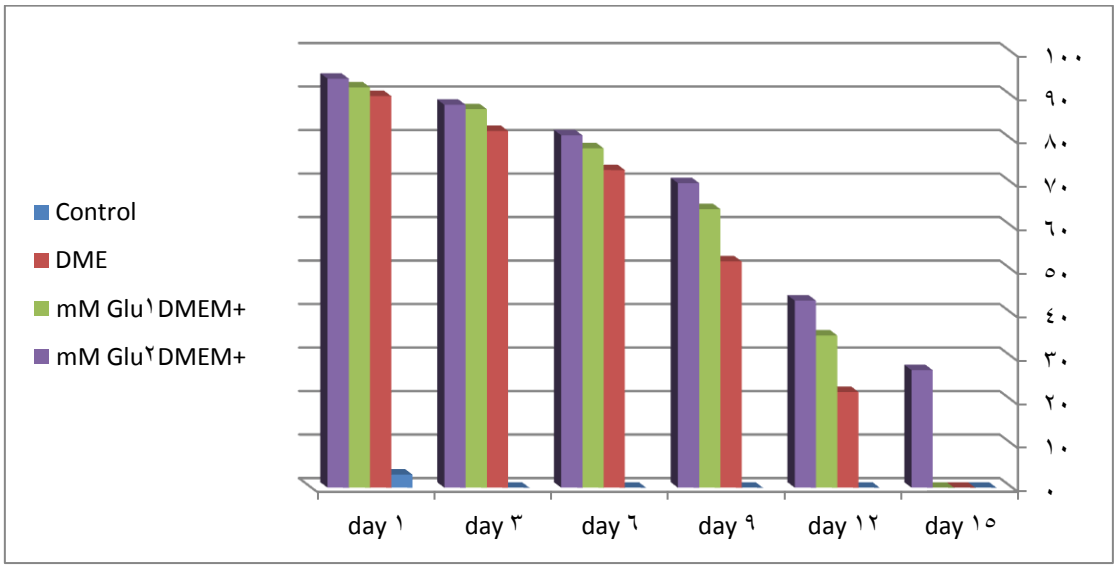

Figure1:Comparsion between the viability of digestive gland cells using DMEM medium and DMEM medium supplemented with different concentration of Glu.

Discussion

Amino Acids are the essential components in the procedure of protein synthesis. In this field there was a lot of studies have verified that amino acids can directly or indirectly control the physiological actions of the Organisms (Mathews et al.,2014). That is why we designed our study to investigate the effect of culture medium supplemented with different concentration L-glutamine on viability of digestive gland cells for *Bellamyabengalensis in vitro*. We have found out that the medium supplemented with L-glutamine in different amount (1mM, 2mM) had positive effect on viability of digestive gland cells.

Our results agree with Liu (1993) and Benson
(2000), they have been found that when amino acids especially L-glutamine added or applied to basal medium would be play a vital role on tissue culture techniques of certain species. This study also correlated with previous studies reported that when the amino acids have been added to basal medium of numerous species as a nitrogen supply in *in vitro* cultures to improve somatic *in vitro*-cultured tissues, these studies proved that amides were an acceptable nitrogen source for increased growth rate of shoots after 12 weeks *in vitro* of *Prosopis alba* (Green *et al*. 1990).

For this reason, it can be stated that in the present study Glutamine supports the growth of cells that have high energy demands and synthesize large amounts of proteins and nucleic acids, It is represent alternative energy source for rapidly dividing cells and cells that use glucose inefficiently it is can be agree with(Mathews et al.,2014).

Also our results based on previous studies that reported that cells require nitrogen atoms to build molecules such as nucleotides, amino acids, amino-sugars and vitamins(Xing et al.,2005)

Also amino acid provide the primary reservoirs of nitrogen for the synthesis of proteins, nucleic acids and other nitrogenous compounds this statement agree with Schröder conclusions (2005), he and his collogues pointed out the amides had induce effects like stimulation of cell wall formation, elongation of cells and increased cell division.

In this work, basal medium supplemented with (2 mM) of L-glutamine have more influence on the cells viability than the basal medium or basal medium supplemented with (1mM) of L-glutamine and maintained the viability of cells over double weeks, because the Glutamine contains one atom of nitrogen as amide and another atom of nitrogen as amine and it transports and delivers nitrogen freely to cells (Haroun, 2010). The present study demonstrated that the basal medium supplemented with (2Mm) of L-glutamine prolonged the cells a live for 12 days, in this point we thought that when glucose levels are low and energy demands are high, cells can metabolize amino acids for energy, Glutamine is one of the most readily available amino acids for use as an energy source and it is a major source of energy for many rapidly dividing cell types *in vitro* this reason support strongly by Groot *et al*. (2003).

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تقييم تأثير الحامض الأميني الكلوتامين على حيوية الخلايا الهضمية المزروعة لقوقع المياه العذبة بللامياينكالينسس خارج الجسم الحي

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الخلاصة

تم جمع القوقع من نهر الشاميه –محافظه الديوانيه ومن ثم تشريح القوقع للحصول على الخلايا الجسم الحي الهضمية للحصول على الخلايا الهضمية التي زرعت في الوسط الزرعي (DMEM) والوسط لتقييم تأثير الحامض الأميني (الكلوتامين) على حيوية الخلايا الهضمية المزروعة خارج الجسم الحي لقوقع المياه العذبة بلاميابنكالينسس وتحديد التركيز الأمثل للكلوتامين للخلايا الهضمية المزروعة خارج الزرعي(DMEM) مدعم بتراكيز مختلفة من الحامض الأميني (1 مل مولاري و2 مل مولاري)، الخلايا الهضمية المزروعة في الوسط الزرعي (DMEM) المدعم بالكلوتامين أظهرت معدل عالي من الحيوية مقارنه بالوسط الزرعي (DMEM) الغير مدعم و مجموعه السيطره، بينما الوسط الزرعي (DMEM) المدعم بـ(2 مل مولاري) من الكلوتامين كان لها تأثير أيجابي أكثر على حيوية الخلايا الهضمية المزروعة مقارنه بالوسط الزرعي (DMEM) المدعم بـ(1 مل مولاري) من الكلوتامين. الكلوتامين كان له تأثير إيجابي في أدامه حيوية الخلايا الهضمية المزروعة لقوقع المياه العذبة بلاميابنكالينسس خارج الجسم الحي.