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Preparation of new culture medium for culturing *Leishmania* parasite by using Semolina and Rock candy

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ABSTRACT

This study demonstrated that the using of a new culture preparation by using Semolina and Rock candy with misshapen blood and addition of a small amount of dextrose to the liquid phase led to a better growth of *Leishmania* parasite. The new culture can able to the survival growth of parasite for 20days without need re culturing and with kept the ability of growth despite surpassed growth period logarithmic stage. The parasite remain is active and in a natural form, as opposed to the solid culture containing Lock solution (NNN media) which have lower number without survival the parasite growth more than 15 days. After 5 days the parasite number reached to (12200 cells/ml) in new culture compared with the NNN media which have the parasite number were (12184.75 cells/ml) and when measuring the ratio of cell viability in two cultures was equal reached to 96%, then begin gradually decrease in both cultures, but the cell viability ratio remained good even after 15days in the new culture was 70% and only 45%in NNN media.

KEY WORDS: *Leishmania*, NNNmedia, Promastigotes.

1. INTRODUCTION

Leishmania species are protozoa with indirect life cycle (promastigotes and amastigotes). Promastigotes are basically cultivated in nutrient media of animal origin and in liquid media to which animal serum or blood is added (Chang and Fish, 1983). A widely use medium is the Brain Heart Infusion, mostly supplemented with serum. This medium displays a hazard for defilement of the recombinant item e.g. with infections or with prion proteins mindful for ox-like spongiform encephalopathy (Robb, 1975). Many culture media used for cultivated *Leishmania* parasite like semisolid culture media like: NNN media Evans' modified Tobie's medium (EMTM), liquid RPMI 1640, and Peptone-yeast extract medium (P-Y) and TC-199 medium (Silva, 2011).

Rock candy (also called rock sugar) is a kind of sweet made out of generally expansive sugar gems. This sweet is framed by permitting a supersaturated arrangement of sugar and water to take shape onto a surface reasonable for precious stone nucleation the name comes from the medieval era, and in turn lends its name to a British candy called rock (Richardson, 2002). Semolina is the rough, sanitized wheat middling's of durum wheat utilized as a part of making pasta, breakfast oats, puddings, and couscous (Merriam, 2012). Semolina is gotten from the Italian word semola, signifying grain (OED, 2012). This study compares classic culture media to the new media

2. MATERIALS AND METHODS

Parasites: It was obtained from the department of biology science AL-Nasirya University. *L.donovani* strain was maintained in vitro by serial passage in semisolid medium NNN media.

Preparation of the culture medium: New culture medium consisted of the two phases:

Solid phase: consisted of the following ingredients: Semolina (18.5g), Dextrose 5gm, Agar 10gm, Blood 100ml, Gentamicin 1.5ml, Distilled water 500ml.

In the beginning the grinding semolina and sugar by using electric grinder so for the purpose of facilitating and accelerating the dissolve in distilled water and can be used warmly (flame Brenner) for the purpose of accelerating the solvent then add Agar, blood and antibiotics.Then sterilized components, Then 5ml was distributed among six 25-cm^2 flasks, left until the center-hardening, and incubated at a temperature 37^0C for 24 hours to make sure they are free of contamination, and then placed in the refrigerator for 4^0C till in use.

Liquid phase: Consisted of the following ingredients: Sodium chloride (Nacl) 9 gm, distilled water 1000ml and dextrose 3gm. All These ingredients dissolved in one liter of distilled water and adjusted pH to 7.4. NNN (Novy-MCNeal-Nicolle) medium prepared According to Kang and Norman (1970).

Culturing the parasite in the new culture: To test the efficiency of the new center has been growing parasite as a following: $1x10^3$ parasites were counted and cultivated in 18 vials of the new culture and added to 4 ml of sodium chloride solution instead Lock solution. $1x10^3$ parasites were counted and cultivated in 18 vials of the NNN-media and added to 4 ml of Lock solution. Enumerated the numbers of the parasite in the culture daily, and after 20, 15, 10, 7, 5 day.

Examination of culture media: One drop of sample was examined on direct microscopic examination and counted the number of parasite in 1ml of sample by use this equation (Al-Idrrise, 2008).

No. of (C) or (T) ×15.25= No. of (C) or (T) in one ml sample.

Estimation of parasite viability: To measure the viability of cells cultured parasite after 20, 15, 10, 7, 5, day by using Trypane blue stain according to method of Hudson and Hay (1980).

Statistical analysis: Data are reported as mean± standard deviation and the inter group variation performed by t-test.

3. RESULTS

In this study, preparation of new culture media for culturing of the *Leishmania* parasite and the advantage of this medium its component are available in local markets with speed and ease preparation, it is characterized by high competence in parasite development and maintain its vitality and activity for a longer period from the NNN media.

It was to get a good and increasing growth occur in both mediums with a note higher growth occur in new media, the peak of growth reach in the fifth day and the parasite numbers in new media was 12200 (cells/ml) compared with the NNN media was 12184.75 (cells/ml). Then the numbers of parasites began to gradually decrease with the passage of time, but it was noted that the parasite number of in the new media more in numbers in addition to retaining vitality and natural shape after 15 to 20 days and the numbers of parasite reached to 5917 and 1525 (cells/ml), respectively.

While decreased occur in parasite numbers in NNN media was reached 915 (cells/ml) after a 15-day and then the death of the parasite after less than 20 days with a note that there were significant differences between the two mediums at the level ($P \leq 0.05$) and ($P \leq 0.001$) as shown in table.1.

When measuring the viability rate of cell ratio it was equal in two media, amounting to 96% in the phase logarithmic parasite after 5 days, but then began a gradual decrease in the new media and remained good viability even after 15 days, amounting to 70%, while in the NNN media amounted to only 45%. After 20 days and reached the viability of the cells to 40% of the new media table.2.

Observed by the results of this study, the new media laboratory prepared is the best than the NNN media as It retained its ability to sustain the growth of the farm, although it goes beyond the period of growth logarithmic, The parasite is active and is a natural form, unlike the NNN media containing Locke solution as it was parasite tends to balled after 10 days

Numbers of parasite(cell\ml)					
Day medium	1	2	3	4	5
New media	2897.5±3.6	4575±15.8	6481.5*±4.1	9485.5*±1.6	12200±7.1
NNN media	2821.25±3.8	4803.75±11.2	6100*±7.9	9150*±7.2	12184.75±6.4

Numbers of parasite(cell\ml)				
Day medium	7	10	15	20
New media	8128.25**±2.2	6435.5**±1.58	5917**±1.4	1525±15.81
NNN media	6938.75**±6.4	3050**±7.9	915**±4.5	0

*significant ($p<0.05$) differences

**significant ($p<0.001$) differences

Table.2. The viability of *L. donovani* promastigotes in different media

medium	**Time (day)**				
	5	7	10	15	20
New media	96	90	85	70	40
NNNmedia	96	89	80	45	0

DISCUSSION

We need to prepare new media for cultivation *leishmanias* is parasite for can conduct experiments more and more study of the importance of this parasite and spread to Iraq, especially to children and causing them to death.in this study Prepared the media from simple ingredients and cheap ,method of prepare a very brief and fast way.

In this study growth in new media very best than NNN media and less contamination occurred than NNN media, it was noted that sub culture done every two days will help to get rid of the pollution gradually and maintain the vitality of the parasite, also the parasite remain more active and maintain on normal shape of parasite. The continuation of the growth and survival of the parasite active longer this indicates the presence of nutrients in the center of the longest, which provides an opportunity for the survival of the parasite longer.

Parasite usually cultivated in liquid media to which blood or animal serum is added (Chang and Fish, 1983). A commonly used medium is the Brain Heart Infusion partially supplemented with serum. This medium revelation a risk for contamination of the recombinant product e.g. with viruses or with prion proteins responsible for bovine spongiform encephalopathy (BSE; Mad Cow's disease) (Robb 1975, Yamamoto and Akama 1969).

Li moncu (2004), prepared new liquid culture medium with chemicals that can be obtained economically and commercially called P-Y culture medium then after 10 subsequent passages**,** the culture medium prepared was evaluated as being quite inexpensive, simple, and successful compared with other commercially available liquid

culture media .all previous media recurred blood, serum salts and other chemicals therefore it's become very expensive also with several parasite passage can cause changes occur in shape of parasite.

Biphasic Novy-MacNeal-Nicolle (NNN) culture medium, which has been used for a long time, is preferred because it is far cheaper than other commercial media but this media easy preparation, low cost and best than NNN media for parasite growth.

Semolina contain many important compound like fat, protein, carbohydrate, iron, vitamins and many minerals all these compound are important for parasite growth. Semolina also contains Vitamin E, which prevents harmful oxidation of cell membranes and DNA also contains iron and all necessary for the growth of these materials *leishmania.*

Rock sugar is a type of confection composed of relatively large sugar crystals. It can be used as a substitute for glucose and lactose in the manufacturing new media.

Fritsche (2007), reported that after glucose consumption, pH raised and resulted finally in morphological changes of the cells, which appeared to be very thin and partially degraded in YE medium also Hemin is an essential component for growing *Leishmania* species, but the detailed function inside the cell is currently unknown. It is important as pro synthesis group of different proteins, a source of energy and essential as an intracellular regulator for metabolic pathways involved protein synthesis and in respiration (Srivastava, 1997; Pal and Joshi-Purandar 2001).

4. CONCLUSION

We believe that new culture medium, having characteristic such as it's in expensive and its easy preparation that can be done whenever desired, can be used successfully in in vitro cultivation for various studies of *Leishmania* species.

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