New culture medium from Lentils (Lens exculenta) seeds for growth Leishmania parasite and some bacteria and fungi

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Abstract: The Lentils (Lens exculenta) were used in research of a new culture medium aimed at the growth of leishmania parasite in vitro. The medium was composed of two phases. The Lens exculenta was used in preparation (with or without added misshapen blood). In the liquid phase, dextrolite solution was used as an oral perfusion solution, instead of lock solution. This study showed increased in numbers of promastigote in new culture media and this The study showed an increase in the growth of the shape in the center of the plant, and that this increase and the number of parasites was a significant increase compared to the center of NNN-media. The average number of parasites on the eighth day of growth, which represents the peak growth in these groups (2425 and 2650) ×10⁴ cell / ml, respectively. The growth of the parasite continued but at a lower rate and good viability until the twentieth day the number of parasites reached to (500 and 466.6) ×10⁴ cell / ml, respectively. This study showed that the percentage of the parasite viability was good and increased from the second day to the highest on the eighth day (96 % and 95%) respectively. Blood added to the new medium receives good growth but lasts only 14 days with no subculture.

Key word: Lentils agar, Lens exculenta, leishmania.

Introduction

Several studies dealing with the study of antigen structure, biochemical properties in addition to susceptibility to the occurrence of various types of leishmania that reason leishmaniasis in the Middle East and tropical states (Mediterranean). These experiments require obtaining large numbers for both phases in the stage of the promastigote and amastigote. Using different culture media [1,2]. A variety of culture media have been used to cultivate leishmaniasis, and can be distributed into three central classes: liquid, biphasic, and semisolid. Whereas biphasic and semisolid media necessity blood, an important factor in multiplying parasites while most serum liquid media requires a fetus or leukocyte[3].The liquid RPMI 1640,Novy-MacNeal-NicolleMedium, Evans’ modified Tobie's medium (EMTM) and extract Peptone yeast of medium (PY) are among the important media in encouraging parasite growth in large numbers of parasites, but the defects of these media are that they are very expensive and some of them need to be prepared in fresh rabbit blood, for
example RPMI, P -Y The parasite's growth rate is lower compared to other media. [4] Cow milk, buffalo, and goats were used in the manufacture of new media for isolating and developing the parasite *L. donovani* and were more effective in isolating and growth the parasite for a long time. [5] Lentils are a plant that belongs to the leguminous family, whose scientific name is *Lens culinatus*. It is cultivated in Egypt, the Levant, southern Europe, the United States, Iran and Turkey and its origins are in the Levant region [6]. Its seeds are brownish-red, gray or black. Its diameter is never more than 13 mm. Its seeds are used in preparing foods. Its green leaves also use feed for dairy cows, and it is also used to fertilize the poor ground with nitrogen and organic matter by turning it in the soil when it is in flowering phase. The lentils contain folate at 120%, iron 50% and protein 9%, vitamin 42% B, which plays a role in freeing energy from carbohydrates. [7]

**Material and method**

**Preparation of medium**

The medium consists of two phases: the solid phase and the liquid phase

1- **Solid phase:**

It consists of the subsequent materials: Lentils 18 gm, dextrose 5gm, agar agar 10gm, blood 100ml, antibiotic (gentamicin) 1.5 ml, Distilled water 500ml.

The red lentils were ground using an electric mill mixed with distilled water, and a heated (Burner flame) can be used for the determination of facilitating and quickening the thawing, after it cooled and filtered through a medical gauze and completed the volume to a liter using distilled water, then added 20 gm of agar-agar to the medium and then sterilized with autoclave at temperature of 121 °C, 1.5 pressure for 15 minutes. Another medium was prepared in the same way but without adding blood to it. Then sterilized the ingredients with the feed with a temperature of 121 °C for 15 minutes, put the medium in a water bath with a temperature of 50 - 55 °C for the purpose of cooling it then added the antibiotic, and the medium was distributed at a rate 5ml of it in sterile glass bottles with a tight lid and left till the medium hardened, and protected at a temperature of 37 °C for 24 hours to ensure that it is free from contamination, then put in a refrigerator at 4 °C until use.

2- **Liquid phase:**

Oral rehydration salts BP was used in the preparation of the liquid phase of the medium, and it consists of the following components: Sodium chloride 2.6 gm, Potassium chloride 1.5 gm, Sodium citrate 2.9 gm, Anhydrous glucose 13.5 gm. Dissolve all contents in (1 liter) of distilled water and sterilize the medium at 121 °C for 20 minutes and press 1.5 atmosphere. Add the antibiotic and place it in sterile bottles and place it in the refrigerator at 4 °C until use. Figure (1).

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Figure 1: (A) The new lentil medium is packed in sterile vials before implantation. (B): NNNmedium.
Leishmaniasis isolate

*L. donovani* parasite and promastigote developer were obtained from the University of Al-Nahrain / Biotechnology isolated from a one and a half year old child implanted on the NNN-medium medium.

**Growing parasite in the original medium:**
To test the effectiveness of the original medium, the parasite was cultured at a rate of (0.2ml containing (1 x 10^3 cells / ml)) as follows:
- The parasite was grown in 10 vials containing the new medium of lentils (with added blood) and added 4 ml of oral perfusion solution instead of the Lock solution.
- Plant the parasite in 10 vials containing NNN-medium and add 4ml of Deadlock solution.

The numbers of the parasite were calculated using the hemocytometer on the inoculate after (2,4,6,8,10,12,14, 16,18,20) days. To measure the viability of parasite cells cultivated in the three media after (2,4,6,8,10, 12,14, 16,18,20) days, using Trypane blue stain allowing to the Hudson then Hay method [8], and examined under the (40x) For optical microscopy.

2- Preparing a new culture medium from lentil seeds to grow bacteria and fungi

**Isolates used in the study**
In this study, isolates of *Fusarium* sp. and isolates from *Staphylococcus aureus*, *Escherichia coli*, *Serratia* sp, which were obtained from the laboratories of the Department of biology, College of Science / University of Al-Mustansiriyah and preserved isolates of fungi on Sabourauds dextrose agar, while bacteria isolates were preserved on Nutrient agar. Until used.

**Preparation the lentils seeds medium.**
Lentil seeds were obtained from local markets, then were ground with an electric mill. 50 gm of ground lentils were weighed and a liter of distilled water was added to it and then boiled for 30 minutes after it cooled and filtered through a medical gauze and completed the volume to a liter using distilled water, then added 20 gm of agar-agar to the medium and sterilized the medium with a temperature of 121 °C for 15 minutes ,cold the middle to 45 °-55 C°, then an chloramphenicol was added to prevent bacterial contamination for the media over which the fungi will be grown. Pour the medium into sterile dishes [9].

**Test the ability of microorganisms to grow on the medium of the lentils**
Inoculate the medium of the lentils of the lentil seeds agar containing the antibacterial with *Fusarium* sp isolates and at that time nurtured (incubated) at a temperature of 28 C ° for a period of 48-72 hours, while inoculate the medium of the lentils seeds agar with isolates of Bacteria *Staphylococcus aureus*, *Escherichia coli*, *Serratia* sp and incubated at 37 ° C for 24 hours.

**Statistical analysis:**
The results were analyzed statistically using the SPSS Version 16 statistical analysis program using ANOVA analysis and Chi-square and by extracting the least significant difference by L.S.D. (10)

**Results and discussion**

The red lentils available in local markets are used at cheap prices in preparing a new culture medium for the growth of the parasite, *Leishmania*. The medium consists of the first solid particles, which consists of lentils and for the purpose of increasing the efficiency of the medium, dextrose and blood were added to it. In the beginning, the medium was created without the addition of dextrose and blood. The results are good, but the parasite did not continue growing for more than 14 days without a work (subculture), but when sugar and blood were added, the parasite stayed for more than 25 days, with good vitality and normal shape, and without replanting work. As for the second part, it was liquid and we replaced it with Lock's solution with the available oral irrigation solution available, In a Pharmacies is used to treat diarrhea in children. Instead of collecting salts and weighing them and then
preparing the solution, this will save time, effort and money. For the purpose of knowing the efficacy of this medium, it has been compared with the medium used globally, which is considered a cheap and efficient medium is the NNN-media medium, numbers have been calculated Parasite after (2,4,6,8,10,12,14,16,18,20) days and the results were as shown in Table (1) an increase in the number of parasites in both mediums until reaching the highest increase on the eighth day and the increase was in The parasite numbers for the new lentil medium are more than the NNN- medium, as it reached (2425,2650) x 10^4 cell / ml, respectively. Then the parasite numbers started decreasing. P until it became time after 20 days (466.6 and 500) x10^4 cells / ml, respectively, and there were significant differences between the two medium.

The cell viability of the parasite was also measured for the same days using the Trypane blue stain. The cell viability was close to both mediums until the tenth day, where the cell viability ratio was (96%) for the Lentil medium and (95%) for the NNN-media medium, and then the cell viability began to decrease with the time until the cell viability ratio reached (15%, 30%) respectively after 20 days, and this is due to the depletion of the nutrients needed by the parasite Table (2). In this study, we found the possibility of using lentils to manufacture the new medium with a high efficiency of parasite growth and that the blood was mutilated, and the sugar will enhance its continuity Increase the numbers of parasites and retention With its cell viability and for more than 20 days and without the need to renew the farm while preserving the natural parasite shape. Through the results obtained, we note that the center of lentils is more efficient and less polluted than the NNN-media medium, and this is due to several reasons, including that the use of blood transfusions led to this. The breakdown of blood cells has become more easy to use by the parasite, as blood has been placed fresh that encourages infection.

Table (1): The influence of the media on the amount of Leishmania during days 1 x 10^4 (cells / ml)

| LSD | days | media |
|-----|------|-------|
|     | 20   | 18    | 16    | 14    | 12    | 10    | 8     | 6     | 4     | 2     |
|     |      | 137.2 | 466.6 ±8.3 | 550 ±14.4 | 825 ±14.4 | 1075±14.4 | 1658.3 ±22.1 | 2200 ±14.4 | 2425 ±14.4 | 2050 ±14.4 | 600±14.4 | 358.3 ±8.3 | NNN media |
|     | 128.6 * | 500 ±14.4 | 675 ±14.4 | 1150±14.4 | 1183 ±21 | 1883.3±8.3 | 2400 ±14.4 | 2650 ±14.4 | 2350 ±14.4 | 666.6 ±8.3 | 400 ±14.4 | Lentils media |
| -- | 46.27 NS | 56.67 * | 56.67 * | 33.84 NS | 65.44 * | 56.6 * | 56.6 * | 46.2 NS | 46.2 NS | LSD |

NS: non significant( *P<0.05)

Table (2): Ratio of parasitic of cell viability in different Media

| Chi square | days | media |
|------------|------|-------|
|            | 20   | 18    | 16    | 14    | 12    | 10    | 8     | 6     | 4     | 2     |
| 13.26      | 15   | 25    | 40    | 54    | 62    | 80    | 95    | 94    | 93    | 90    | NNN media |
A new culture medium of lentil seeds for cultivating bacteria and fungi

The results of *Fusarium* sp on the medium of the lentil seeds showed their growth on the medium without any change in the shapes of cells and colonies as shown in Figure (2). The results also showed the growth of bacteria *Staphylococcus aureus*, *Escherichia coli*, *Serratia* sp and the characteristics of their typical colonies on the medium of lentil seeds and as It is shown in figures (3,4,5).

|     | 10.75 | 30  | 45  | 52  | 64  | 70  | 84  | 96  | 94  | 93  | 90  | Chi square |
|-----|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------------|
| --  | 5.4   | 8.25| 5.26| 4.81| 4.39| 1.06| 0.08| 0.00| 0.00| 0.00| 0.00| NS         |
| **  | 2 *   | *   | *   | *   | *   | NS  | NS  | NS  | NS  | NS  | NS  | P<0.05     |
| NS: non significant | *P<0.05 | **P<0.01 |

**Figure(2):** *Fusarium* sp. On lentil seeds agar incubated by 28°C for 4days.

**Figure(3):** *Escherichia coli* on lentil seeds agar incubated at 37°C for 24 h.
Numerous studies have established that the ingestion of lentil is enormously connected to the decrease in the frequency of illnesses such as diabetes, obesity, cardiovascular diseases and cancers due to its bioactive mixtures. Lentil seeds are an excellent vegetable foundation of carbohydrates, protein, iron, numerous minerals (zinc, manganese, copper, selenium, molybdenum, and boron) besides vitamins (niacin, thiamine, riboflavin, pyridoxine, folate, pantothenic acid, α, β and γ-Lentils are famous to be a good foundation of prebiotics [11,12]. Additionally, lentils are relatively little in fat and sodium, but then high in potassium contented. Furthermore, lentils must an average amount of vitamin K [13,14]. Therefor, we recycled to prepared original medium (lentils seed agar) for growing some parasites, bacteria and fungi. This is the first study conducted in Iraq and the world, which used lentils seed to prepare medium that use for growth of some fungi and bacteria and parasites. Many studies have been performed to formulate a culture medium that composed from many compound, while lentils seed agar by a few components. Preparation of a new, medium low cost, long term cultural medium consisting of inexpensive, available ingredients, and the method of
preparation is simple, easy and fast, and does not require the addition of other substances such as serum. Moreover, preserving the parasite in its shape and vitality, this indicates the availability of the materials necessary for growth. Finally, The method of preparation in which the transformation is important, as the blood was denatured with heat, and this reduces the possibility of infection with a virus that may be present in the blood.

Conclusion

Lentils (Lens exculenta) seeds were used in preparation of a new culture medium for the growth of some microorganisms (parasite, bacteria and fungi) in vitro. The results showed the ability of microorganisms tested (Leishmania, Fusarium sp, Staphylococcus aureus, Escherichia coli, Serratia sp.) to grow on the new medium Lentils (Lens exculenta) seeds. This is the first study conducted in Iraq and the world, which used Lentils to prepare medium that use for growth of some parasite, fungi and bacteria.

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