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RESEARCH ARTICLE

Preparation a New SHE-Medium Replacement of RPMI1640-Medium using Oral Rehydration Solution

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Abstract

The study showed that the use of the new culture medium which called (SHE-medium) prepared by using oral rehydration salts, led to increase the ratio of cell viability of PMNs and lymphocytes compare with RPMI 1640-medium on 24,48,72 h. The results shows that ratio of cell viability of PMNs in SHE-medium were 89%,91% ,93% respectively, while in RPMI 1640-medium were 80%,90%,93% respectively while the ratio of cell viability of lymphocyte in SHE-medium were 88%,90% ,91% respectively, compare with RPMI 1640-medium which are 80%,85%,90% respectively. But when tested the effect of SHE-medium on THP-1 cell line and REF cells and comparison with RPMI 1640-medium, the results shows that the ratio of cell viability on SHE-medium were 84.33% and 74.33% respectively, while in RPMI 1640-medium were 86.67% and 80.67% respectively.

Keywords: *1640-medium, SHE-medium, Oral rehydration solution.*

Introduction

Media of RPMI1640 was developed by Moore and his co-workers in 1966 at the Roswell Park Memorial Institute. Hence, the acronym RPMI. RPMI 1640 Medium was originally developed to culture human leukemic cells in suspension and as a monolayer. Roswell Park Memorial Institute (RPMI) 1640 Medium has since been found suitable for a variety of mammalian cells, including HeLa, Jurkat, MCF-7, PC12, PBMC, astrocytes, and carcinomas [1-4].

It's medium is unique from other media because it contains the reducing agent glutathione and high concentrations of vitamins. RPMI 1640 medium contains biotin, vitamin B_{12}. In addition, the vitamins inositol and choline are present in very high concentrations. RPMI 1640 Medium contains no proteins, lipids, or growth factors.

Therefore, RPMI 1640 Medium requires supplementation, commonly with 10% Fetal Bovine Serum (FBS). RPMI 1640 Medium uses a sodium bicarbonate buffer system (2.0 g/L), and therefore requires a 5-10% CO_2 environment to maintain physiological pH [1]. Because RPMI 1640 is very complex, expensive, very sensitive to acidic, and it is not available in the local market, Therefore, the study aimed to finding substitutes medium for RPMI 1640, locally available, inexpensive, easy to preparation and use, highly efficient and highly stable pH therefore used oral rehydration salts (BP), which is used to treat severe diarrhea in children and is composed of very simple components and is available and can be prepared easily and without any cost material comparative to the RPMI medium and can be used for the same purposes and the same effectiveness.

The medium was named SHE-medium, SHE abbreviation was used to label the medium and refers to the initials of the names of the researchers.

Materials and Methods

Preparation Inhibitor Human Serum

The serum (O $^{+}$ blood group) was preheated before used in a water bath (56 ° C) for 30 minutes to inhibit complements inactivation. After cooling, it was filtered through Millipore filter with a diameter of 0.22 µm, added to the recorded medium [5].

Preparation of SHE-medium

The oral rehydration salts buffer phosphate was used, it contains the following ingredients. Sodium chloride 2.6 gm, potassium chloride 1.5 gm, Sodium citrate 2.9gm and anhydrous glucose 13.5gm.

about 0.025gm of the contents of the sachet was solvent in 50 ml of distilled water and sterilized with heatstroke 121 ° C for 20 minutes and compress 1.5 g , then placed in sterile bottles with added 2.5 ml of inhibitor human serum. Antibiotic Pencillin-G antagonist (10000 units) and Streptomycin 10 mg / ml were added. The pH was adjusted at pH7.1 then placed in the refrigerator at 4 ° C until use.

Preparation of RPMI-1640 Medium

The medium was prepared according to the French company Biomereiux, which dissolved 13.65 g of RPMI 1640 medium in one liter of distilled water, and 10% of the fetal calf serum was added to it from English Flow-Laboratories. Inactivated at (56) c° for 30 minutes, then 0.2 mL of Pencillin-G (10,000 units) and Streptomycin (10 mg / ml) were added, set the pH to (7.2) and sterile through the filters Millipore diameter (0.22) micrometer and then distributed in sterile tubes and freeze-keeping (-20) C° until use.

Isolation White Cells with Multiple PMNs of Peripheral Blood

The PMNs cells were isolated according to method Cech and Lehrer [6]. Then the blue Trypan dye was used to know the number of living cells and the number of cells was calculated by means of the blood sample Haemocytometer and the models were used, which gave a percentage of 95% or more.

Test the SHE-medium Effect on White Cells

The number of cells was determined to be 1 × 10^{4} cells / ml and divided into 12 groups (6 groups suspended in the new medium SHE-medium and the remaining one were suspended in the RPMI-1640 media and incubated at 37°C then after 24,48 and 72 hours the cells were calculated by using blue Trypanus dye.according to the following equation:

(Cell Viability %) = Number of living cells (non-colored) \ total number of cells

Test the SHE-medium Effect on Lymphocytes

lymphocytes were isolated according to methodAd'hiah, [8].Then Suspend cells in 1 ml of SHE-medium medium containing 5% of the inactivated human serum (56 ° C for 30 min) and then count the cells using the haemocytometer.

The cell viability was determined by mixing 10 microliters of cell suspension with 90 µl of trypan blue dye for three minutes, and then counting the percentage of cells that did not appear in color (live cells) ,determined the viability according to the previous equation was mostly 95%. The tubes were incubated in an incubator at 37 ° C (24, 48 and 72 hours). The cell viability was measured and counted after 24 hours, 48 hours, 72 hours.

Maintenance of Cell Cultures

In this experiment two types of cells were used: the line of cancer cell leukemia (THP-1 cell line), the line of cells derived from a leukemia patient, rat embryonic fibroblast (REF cells) and obtained from the Iraq biotech Cell Bank The control group consists of RPMI-1640 supported by Fetal bovine serum and units / mL (penicillin 100) and streptomycin (100 µg / mL) while the test cells were stored in the new SHE-medium and incubated at 37 ° C for 3 days, cell viability was measured by using a trypan blue [7].

Results and Discussion

In this study, oral rehydration salts BP was used to prepare a new medium (SHE-medium) for the maintaining of PMNs , Lymphocytes, THP-1 cell line and REF cells. measured when the ratio of cell viability of PMNs in SHE-medium compare with RPMI 1640-medium on 24,48,72 h, were 89%, 91%, 93% respectively, while in RPMI 1640-medium were 80%, 90%, 93% respectively(Table 1).

Table 1: Percentage of PMNs viability in SHE-medium and RPMI-1640medium

Medium	Time (hour)		
	Cell viability %		
	24hr	48hr	72hr
SHE-medium	93%	91%	89%
RPMI-1640 **Medium**	93%	90%	80%

While the ratio of cell viability of lymphocyte in SHE-medium were 88%, 90%, 91%, respectively, compared with RPMI 1640-medium which are 80%,85%,90% respectively (Table 2).

Table 2: Percentage of Lymphocytes viability in SHE-medium and RPMI-1640medium

Medium	Time (hour)		
	Cell viability%		
	24	48	72
SHE-medium	91%	90%	88%
RPMI-1640 medium	90%	85%	80%

Moreover, when tested the effect of SHE-medium on THP-1 cell line and REF cells and comparson with RPMI 1640-medium, the results shows that ratio of cell viability on SHE-medium were 84.33% and 74.33% respectively, while in RPMI 1640-medium were 86.67% and 80.67% respectively (Figures 1 and 2).

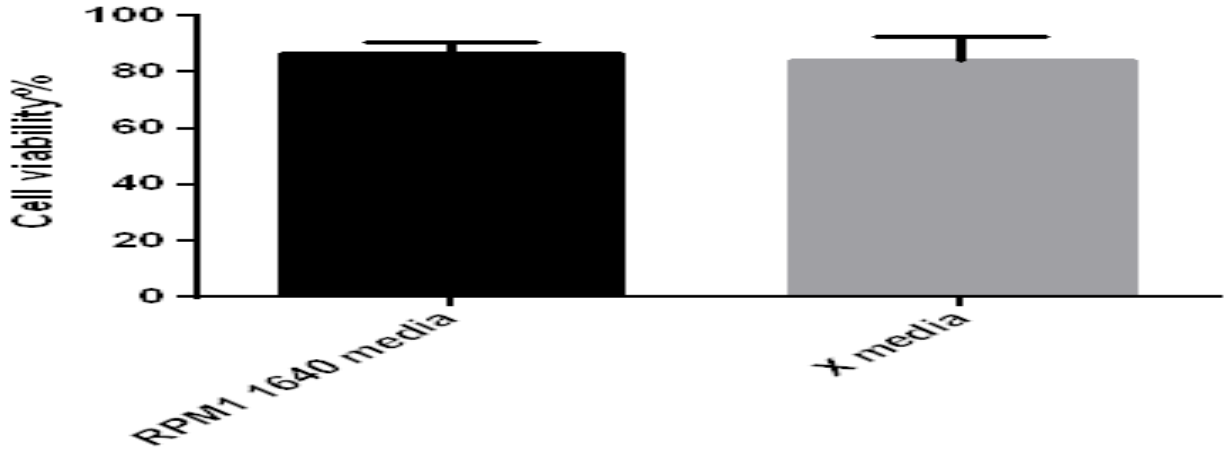

Figure 1: Percentage of THP-1 cell line cell viability in the SHE-medium (X media) and RPMI1640-medium

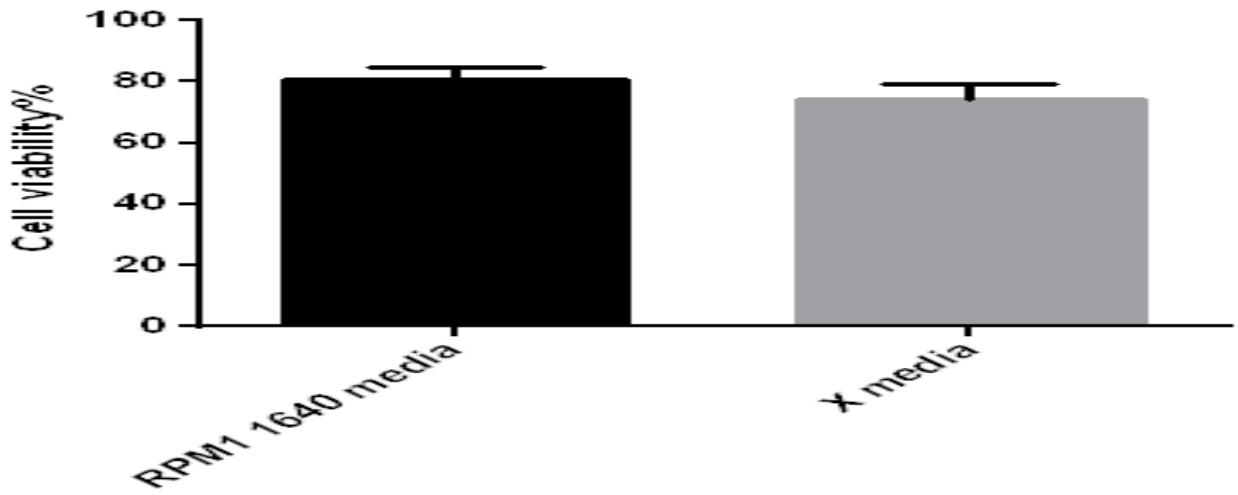

Figure 2: The percentage of REF cell viability in SHE-medium (X media) and RPMI1640-medium

It was also observed that the new medium was compensated for the preservation of cell forms in both natural cells form Fig.(4) or LUKEMA cancer cells Figure (5).

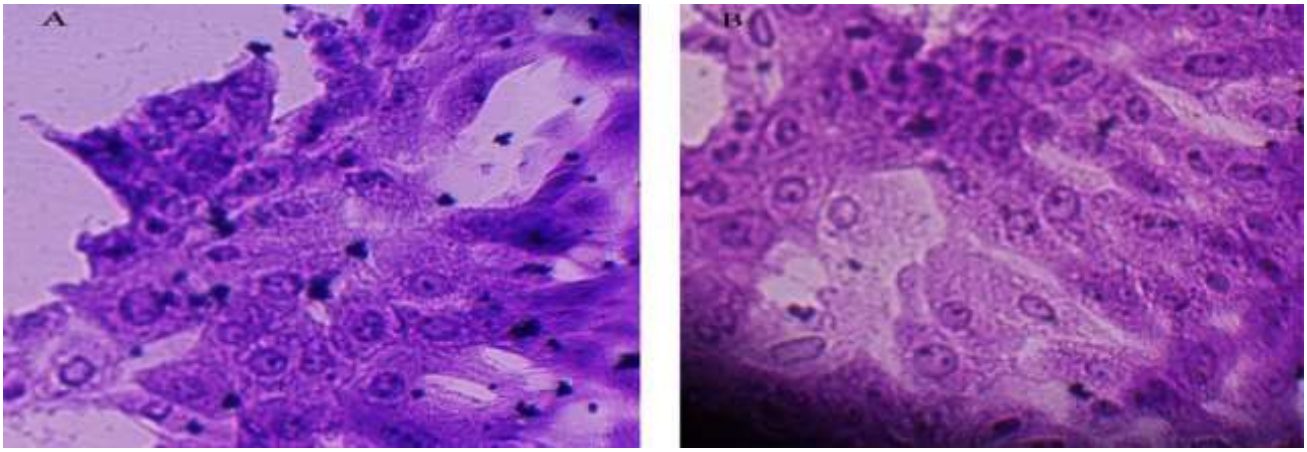

Figure 4: The form of natural cells REF cells after adding it: (A) RPMI-medium , (B):SHE-medium

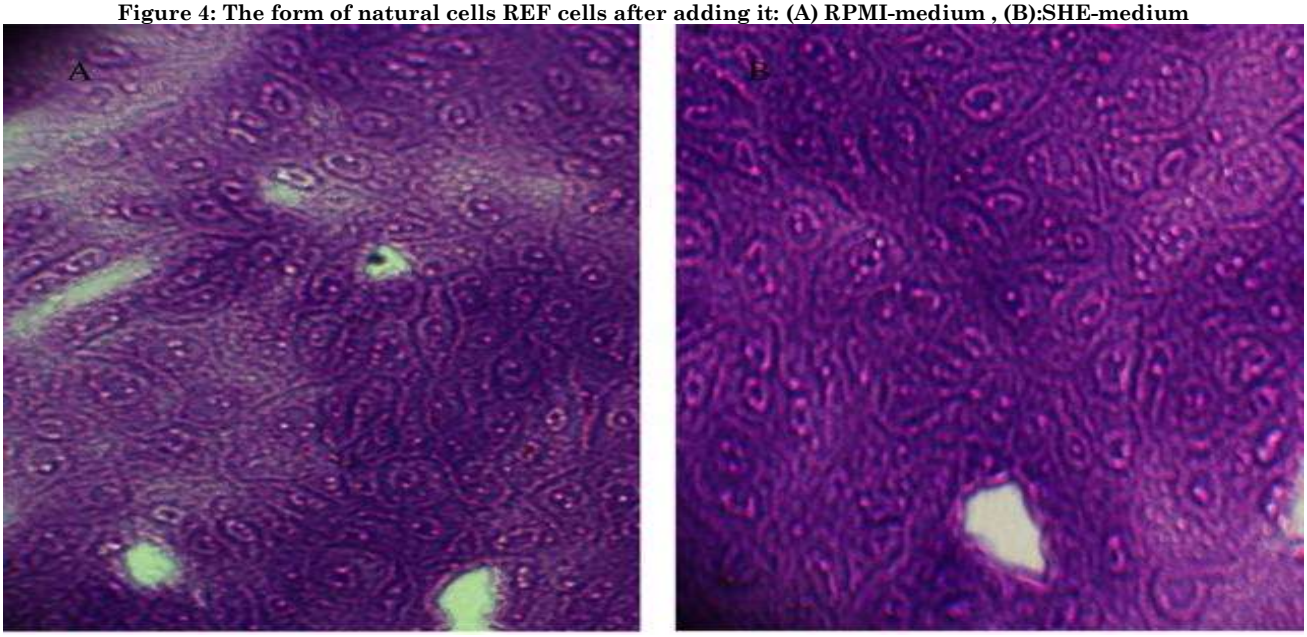

Figure 5: Shape of cells THP-1 cell line after adding, (A): medium RPMI-medium and . (B):SHE-medium

The results of this study showed the possibility of using the oral rehydration salts (BP) in the manufacture of the new medium that highly efficient for the growth of blood cells and added by the inactivated serum will enhance the continuity of cell viability and for more than three days without resorting to changing the medium between time and period compared to the RPMI-1640medium, the RPMI-1640 medium contain of L-glutaminewhich is benefit for both plasma cell differentiation and lymphocyte transformation [9].

Which is Rapid contamination and the procedures of preparation of the medium is complicated. The RPMI-1640medium sterilized by filtrating through 0.22 µm filter paper, which is more susceptible to contamination compared to the new medium (SHE-Medium) that sterilized by autoclave.

Moreover, the new medium SHE-medium is efficient in maintaining THP-1 cell line cell and natural cells REF, which supports the possibility of being used as an alternative to RPMI-1640 medium when the absence or the difficulty of preparation and (SHE- Medium) consist of NaCl and KCl which is important of electroneutral transport process for the growth of lymphocyte [10].

Conclusion

In conclusion, new low-cost, long-term implantation of cheap, accessible and affordable ingredients, the method of preparation is simple, easy and fast and does not require the addition of other materials, also the conservation of cells in their form and vitality indicates the availability of materials necessary for growth and for a period exceeding three days.

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