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Effectiveness of Lime Concentration As A Modification of Turk's Reagent in Leucocyte Type Count Examination

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ABSTRACT

Background & Objective: Turk solution has a composition of glacial acetic acid, gentian violet, and distilled water. Lime (*Citrus aurantifolia S*) has a pH of 2.0 which is almost the same as acetic acid. Both components are weak acids that can lyse blood cells other than white blood cells. This study aims to determine the effectiveness of lime concentration as a modification of Turk's reagent in counting the number of leukocytes and knowing the concentration of 1%, 2%, or 3% which is more effective in counting the number of leukocytes.
Method: This study aims to provide an overview of the effectiveness of lime concentration as a modification of turk reagent composition in counting the number of leukocytes. The research method used is the Counting Room Method. This research was conducted in August 2024 at the clinical pathology laboratory of the Muhammadiyah Makassar Polytechnic. The number of samples in this study was 4 blood samples obtained from 4 correspondents with treatment, lime concentrations namely 1%, 2%, and 3%, and turk reagent as control.
Result: At 1% concentration, the average difference in leucocyte count between the control and the sample was 1,150 cells/mm^3, at 2% concentration a difference of 2,788 cells/mm^3 was obtained with the control, and at 3% concentration a difference of 3,638 cells/mm^3 was obtained.
Conclusion: It can be concluded that the concentration of lime that is effective as a modification of Turk's reagent in counting the number of leukocytes is a concentration of 1%.

Keywords: Lime; Turk's Solution; Modified; Leukocytes.

Introduction

Leukocytes have a role in the body's defense system to prevent foreign objects that cause disease from entering the body (Salman, et al., 2021). There are two ways to check white blood cells, namely manually and automatically. For manual methods turk reagent as a solution is used to check the number of leukocytes, which has a composition of glacial acetic acid, gentian violet, and distilled water (Nugraha, 2017). There is one composition of turk reagent, acetic acid, which has a pH that is almost the same as the substances contained in lime. Lime (*C. aurantifolia S*.) is one type of citrus fruit that contains citric acid with a pH of 2.0. Both components are weak acids and can lyse blood cells other than white blood cells (Kahfi, et al., 2022).

Lime (Citrus aurantifolia S.) contains limonene essential oil and 7% citric acid, bioflavonoids, proteins, enzymes, pectin, fats, and pigments (chlorophyll and carotene) (Sethpakdee 1992 in Prastiwi & Ferdianyah 2017).

There are advantages possessed by lime that glacial acetic acid does not have, which can be taken into consideration, namely easily obtained, environmentally friendly, not a chemical, not toxic, and contains citric acid which is a weak acid that can lyse cells other than leukocytes (Lestari, et al., 2018)

Based on the author's experience in one of the Puskesmas in one of the regions, that has used a Hematology Analyzer tool in routine blood tests, sometimes the tool experiences problems that can hinder the routine blood examination process, one of which is the examination of leukocytes, therefore the ATLM at the location uses a hemocytometer as an alternative, in case of emergencies, but sometimes the turk reagent expires due to a long time not using a hemocytometer tool in leukocyte examination.

This manual leukocyte examination method uses a haemocytometer to count the number of leukocytes in the Improved Neubauer counting chamber. This examination uses Turk's reagent as the dilution solution. This Turk reagent sometimes has expired or has exceeded the expiration date determined by the product, even though the Turk reagent stored is still available in large quantities. The use of Turk reagents that have passed the time limit will affect the results achieved. The composition of turk reagent contains 2% acetic acid and 1% gentian violet. When reacting with leukocytes, it will cause leukocytes to absorb the solution, which causes acetic acid to lyse cells other than leukocytes along with the coloring of their nuclei and granules by gentian violet (Nurbidayah & Maulida, 2019).

Objective

This study aims to determine the effectiveness of lime concentration as a modification of Turk's reagent in counting the number of leukocytes and knowing the concentration of 1%, 2%, or 3% which is more effective in counting the number of leukocytes.

Method

This study aims to provide an overview of the effectiveness of lime concentration as a modification of turk reagent composition in counting the number of leukocytes. The research method used is the Counting Room Method. This research was conducted in August 2024 at the clinical pathology laboratory of the Muhammadiyah Makassar polytechnic. The number of samples in this study was 4 blood samples obtained from 4 correspondents with treatment, lime concentrations namely 1%, 2% and 3% and turk reagent as control.

TABLE 1. The population of this study was lime (*C. aurantifolia S*). The samples used were lime juice concentrations of 1%, 2%, and 3%.

Concentration Variation	Volume of lime juice (µl)	Volume Gentian Violet µl)	Volume Aquades Steril (ml)
1%	200 µl	100 µl	10 ml
2%	200 µl	100 µl	10 ml
3%	200 µl	100 µl	10 ml

The technique used in this sampling uses Purposive Sampling, which is a sampling technique based on criteria. The criteria of this sample are green and fresh lime. The tools used in this study are dry cotton, alcohol swab, tourniquet, syringe, EDTA tube, tube rack, micropipette, micropipette tip (white, yellow & blue), haemocytometer (improved Neubauer counting chamber, cover glass, Thoma leukocyte pipette, suction rubber) petri dish, counter, microscope, microscope tissue, test tube, funnel, knife, and filter paper. The materials used in this study were 2% glacial acetic acid, 1% gentian violet, sterile distilled water, water, lime juice, and 3 ml venous blood. Following Research Procedure.

a. Preparation of Lime Juice

Lime juice was collected in a test tube while filtered at least twice. A concentration of 100% was obtained without the addition of any solution, after obtaining a concentration of 100%, concentrations of 1%, 2%, and 3% were made by means of 1% concentration: 100% concentration of lime as much as 2 µl and distilled water as much as 198 µl; 2% concentration: 100% concentration of lime as much as 4 µl and distilled water as much as 196 µl; 3% concentration: 100% concentration of lime as much as 6 µl and distilled water as much as 194 µl.

b. Preparation of Solution Concentration

Preparation of turk reagent is made as a standard solution by mixing acetic acid solution as much as 200 µl, gentian violet as much as 100 µl and distilled water as much as 10 ml in a ratio of 2: 1: 100.

The following is a table of concentration variations for making lime juice modification solution.

c. The venous blood collection procedure is as follows:

Prepare the tools and materials that will be used to collect blood samples. Place the tourniquet approximately 10 cm above the elbow crease. select the median cubital vein. Then palpate to determine the position of the vein. Clean the skin on the part to be punctured using 70% alcohol cotton and allow it to dry. The vein is punctured with the pinhole facing upwards, then the tourniquet is released after the blood volume is sufficient. A cotton swab is placed at the puncture site and the needle is immediately removed, pressed against the cotton for a while, and then plastered.

d. Leukocyte count examination

Sucked the blood sample right at the 0.5 mark, removed the remaining blood on the tip of the pipette, then inserted the tip of the pipette in the reagent Turk, keeping the blood from flowing down into the reagent, the pipette was held in a 45-degree tilt position and then the reagent turk was slowly sucked until mark 11. To keep the air bubbles from forming, lift the pipette from the Turk reagent, close the pipette tip using the fingertip, and then release the suction rubber and close it with the other fingertip. Then homogenised the thoma pipette for 10-15 seconds.

Place a completely clean counting chamber and cover glass on a flat table, remove all the

liquid in the capillary tube (approximately 3-4 drops), and immediately touch the pipette tip to the surface of the counting chamber at an angle of 30 degrees to the edge of the counting chamber. Due to capillarity, the counting chamber will fill up slowly. The counting chamber is then left for 2 or 3 minutes to allow the leucocytes to settle.

Results

Based on research on the effectiveness of lime concentration as a modification of turk reagent in the examination of leukocyte counts conducted at the Makassar Polytechnic Clinical Pathology Laboratory, the results can be seen in the following table :

TABLE 2. Leucocyte count results using 1%, 2%, and 3% Turk and modified lime solutions

Sample	Control (Cell/mm^3)	Concentration (Cell/mm^3)					
		1%	Difference	2%	Difference	3%	Difference
1	4.950	3.250	1.700	2.050	2.900	1.450	3.500
2	6.250	4.900	1.350	3.350	2.900	1.950	4.300
3	5.100	4.200	900	2.250	2.850	1.600	3.500
4	5.500	4.850	650	3.000	2.500	2.250	3.250
Average			1.150		2.788		3.638

Source : Data first 2024

Discussion

Based on the results of the leukocyte count research that has been carried out, namely by using lime juice (*Citrus aurantifolia S*) concentrations of 1%, 2%, and 3% modified to replace the role of 2% acetic acid in lysing leukocytes in Turk's reagent, different leukocyte counts are obtained.

In this study, the average results of the difference from the examination of different leukocytes in each concentration were obtained. At a concentration of 1% the highest difference was in sample 1 by 1,700 cells/mm3 lower than the control and the lowest difference was in sample 4 by 650 cells/mm^3 lower than the control with an average difference of 1,150 cells/mm^3, at a concentration of 2% the highest difference was in samples 1 and 2 by 2.900 cells/mm^3 lower than the control and the lowest difference was in sample 4 at 2,500 cells/mm^3 lower than the control with an average difference of 2,788 cells/mm^3, and the 3% concentration obtained the highest difference in sample 2 at 4,300 cells/mm^3 lower than the control and the lowest difference was in sample 4 at 3,250 cells/mm^3 lower than the control with an average difference of 3,638 cells/mm^3. Among these three concentrations, the 1% concentration is closer to the results of the leukocyte count examined using turk solution as a control, because it has a smaller difference than the 2% and 3% concentrations In the examination of the leukocyte count using the modified lime juice concentration of 1%, the results obtained have the least difference, or the value obtained is close to the value of the control solution.

Whereas in the examination of the leukocyte count using a modified solution of 2% concentration lime juice, the number decreased from the control. However, no erythrocyte cells were found in the field of view, making it easy to calculate the type of leukocytes. As well as in the examination of the leukocyte count using 3% concentration lime juice, the results obtained are very far from the control value, because too high a

concentration can cause blood cells to be lysed including leukocytes.

Leukocytes can be maximally stable at a concentration of 3% acetic acid. However, when it exceeds this limit, it will cause leukocytes to lyse and if it is too low it can also cause erythrocyte cells and platelets to not lyse completely. Therefore, the administration of acid will greatly affect the examination of the leukocyte count (Nurbidayah & Maulida, 2019).

The acid content in lime can also affect the dilution process because the purpose of adding this diluent solution is to dilute leukocytes while lysing erythrocytes and platelets. However, this study has a different interpretation from the research of Ika Maulidya and Nurbidayah with the title of using lemon juice (citrus limon) as an alternative reagent to Turk reagent for leukocyte counts. In the research of Ika Maulidya and Nurbidayah, the examination of leukocytes using a modified concentration of 2% had results with a difference of 1,000 cells/mm3 higher than the control. Whereas in this study, the 2% concentration had an average difference of 2,440 cells/mm^3 compared to the control, while the 1% concentration had a difference of 1,150 cells/mm^3.

Factors that may affect the difference in results from previous studies with this study are differences in the pH of lemon and lime. Lime (*C. aurantifolia S*.) is one type of citrus fruit that contains citric acid with a pH of 2.0 (Kahfi, et al., 2022). Meanwhile, lemon contains citric acid with a pH of 2.74 (Trisnawati, et al., 2019). Lime has a higher acidity level than lemon, because the lower the pH value, the more acidic it is. And the length of time the examination is delayed also affects the examination sample which is characterised by a decrease in the number of blood cells. In leucocytes, the average decrease was 0.17 103/µl (Artati, et al., 2022).

Conclusion

From the results of the examination of the leukocyte count using Turk's reagent modification, where acetic acid is replaced with lime juice at a concentration of 1%, 2%, and 3% compared to Turk's solution, it can be concluded that the concentration of lime is effective as a modification of Turk's reagent in counting the number of leukocytes. Of the 1%, 2% or 3% concentration of lime which is more effectively used as a modification of Turk's reagent is 1% concentration.

From this study, the 1% concentration is closest to the control but still has a large enough difference, so it is recommended for further researchers to conduct research with lime concentrations below 1%.

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Conflict of Interest

There is no conflict of interest in this research.

References

1. Aliviameita, A. & Puspitasari, 2019. *Buku Ajar HEMATOLOGI*. Septi Budi Sartika, M.p ed. Jawa Timur: UMSIDA Press
2. Artati, Naim, H. N., Yusril, M. & Armah, Z., 2022. Waktu Simpan Whole Blood Cell (WBC) Terhadap Jumlah Leukosit, Eritrosit Dan Trombosit. *Media Analis* Kesehatan, Volume 13, p. 2
3. Kahfi, M. S., Aryani, D. & Purnomo, F. O., 2022. Variasi Konsentrasi Air Perasan Jeruk Nipis (Citrus Aurantifolia Swingle) Sebagai Pengganti Komposisi Larutan Turk Untuk Hitung Jumlah Leukosit Di Laboratorium Rs Hasanah Graha Afiah. *Jurnal Kesehatan Tambusai,* 3(1), pp. 117-118
4. Lestari, R. K., Amalia, E., Yuwono. 2018. Efektifitas Jeruk Nipis (*citrus aurantifolia*

swingle) sebagai Zat Antiseptik Pada Cuci Tangan. JKK, Volume 5, No 2. pp:55-65.

5. Nugraha, G., 2017. *Panduan Pemeriksaan Laboratorium Hematologi Dasar*. Jakarta: a
6. Nurbidayah, M. & Maulida, I., 2019. Penggunaan Air Perasan Lemon (Citrus Limon) Sebagai Reagen Alternatif Pengganti Larutan Turk Untuk Hitung Jumlah Leukosit. *Jurnal ERGASTERI,* 6(2), p. 2
7. Prastiwi, S. S., & Ferdiansyah, F. (2017). REVIEW ARTIKEL: KANDUNGAN DAN AKTIVITAS FARMAKOLOGI JERUK. *Farmaka, 15*, 2.
8. Salman, Y., Nadia, N. & Wahidah, R., 2021. Perbedaan Hasil Jumlah Leukkosit dengan Modifikasi Air Perasan Jeruk Nipis (Citrus aurantifolia Swingle) dan Asam Cuka sebagai Pengganti Komposisi Larutan Turk. *Jurnal Kesehatan Indonesia,* Volume XII, p. 1
9. Trisnawati, I., Hersoelistyorini, W. & Nurhidajah, 2019. Tingkat Kekeruhan, Kadar Vitamin C Dan Aktivitas Antioksidan Infused Water lemon dengan Variasi Suhu dan Lama Perendaman. Jurnal Pangan Dan Gizi dan lain-lain.