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Effects of *Citrus sinensis*(Orange) and *Lycopersicon esculentum* Miller (Tomato) Juices On the Hematological Parameters of *Rattus albus*(Albino Rat)

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ABSTRACT

This research determined the effects of *Citrus sinensis* and *Lycopersicon esculentum* Miller juices on the hematological parameters of *Rattus albus*. To achieve its objectives, the study utilized quantitative-experimental method. For this purpose, nine, two-weeks-old male albino rat weighing 100-150g were used. Rats were kept under room temperature in a 12-hour alternate light and dark cycle and provided with free access to water and foods. Rats were divided into three equal groups namely the control group and experimental groups administered with 2ml of orange and 2ml of tomato juice respectively for 6 days for three continuous weeks. Blood were collected through saphenous vein sampling technique starting at the end of acclimatization and every 7th day of the week. The obtained blood samples were used for determination of hematocrit, red blood cell (RBC) count, hemoglobin, platelet count, white blood cell (WBC) and differential count including neutrophil, lymphocyte, monocytes, eosinophil and basophil and indices such as the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The results revealed that administration of orange juice obtained a significant differences (p-value <0.05) in the neutrophil and lymphocytes compared to their baseline results. After two weeks of administration, only the neutrophil count showed a significant difference, whereas WBC count, hematocrit concentration, eosinophil, and lymphocytes obtained a significant difference throughout three weeks' time. Furthermore, significant differences (p-value <0.05) were observed on the values of monocyte after one week and two weeks of the administration of tomato extracts, whereas WBC count, monocyte, lymphocytes and eosinophil count after three weeks of administration. Therefore, it is then concluded that bioactive substances present in orange and tomato extract like lycopene and vitamin C induces the proliferation of white blood cells in the blood circulation. Hence, it was proven that orange and tomato have a protective effect which may serve as an alternative treatment options in patients with leukopenia.

Keywords
Citrus sinensis; lycopersicon esculentum miller*;* hematological parameters; *rattusalbus*.

1. INTRODUCTION

Food is considered as a basic need for human. Nevertheless, consuming unhealthy food instead of a balance diet degrades the quality of life. In that context, vegetables and fruits contain essential nutrients such as vitamins, minerals, folate, dietary fiber, plant sterols,

carotenoids and various phytochemicals that are needed for human daily diet [1]. These nutrients help to reduce mortality and prevent chronic diseases, including various cancers, cardiovascular diseases, and even mental illnesses [2-4]. This is due to the different phytochemicals that are present on those fruits and vegetables which function as antioxidants, phytoestrogens, and anti-inflammatory agents [5-6].Currently, limited studies had been conducted to determine the extent of the effects of these phytochemicals in the blood parameters of human.

Previous study of Mallick and Khan [7] studied the effects of *Citrus sinensis*on blood coagulation and hematological parameters of white rabbits. It was found outthat in the red blood cell count at all three doses (2ml/kg, 5ml/kg and 8ml/kg), a significant increase was observed while on hemoglobin level determination, a significant increase only manifested at moderate and high dose of *Citrus sinensis*. On the other hand, other blood parameters showed a non- significant difference at any dose of *Citrus sinensis*after 60 days of administration.

Based on the information above, the researchers attempted to validate the study of Mallick and Khan [7] about the effects of *Citrus sinensis*on the hematological parameters of white rabbits. In the present study, the researches included tomatoes for it is one of the popular fruit or vegetable produced worldwide which contains the most powerful antioxidants such as lycopene and vitamin C which are the same components found with orange [8]. Primarily, this study would determine the effects of orange and tomato juice administration to the hematological parameters of albino rat. Eventually, it will provide awareness to the people on their blood cell parameters about the possible effects of high consumption of these fruits.

2. METHODOLOGY

2.1 Research Design

In this study, quantitative-experimental control group design was utilized. This research design allowed the researchers to carry out experimental studies to determine the effects of orange and tomato juice on the hematological parameters. It was evaluated by examining the complete blood count (CBC) result of albino rat before and after the administration of fruit juices through oral route administration.

2.2 Subject of Study

The researchers used convenience sampling technique. The study was carried out on through experimentation with nine male albino rats. These rats were divided into three groups, first group comprised the control group, and the second group and third group are experimental groups on which administered with orange and tomato juice respectively. Each group comprised of three albino rats that were housed in a transparent glass with wire mesh tops under room temperature and twelve (12) hours light and dark cycle with free access to water and food pellets.

2.3 Instrumentation

The researcher utilized testing technique as an instrument in collecting the data. The main tool that was utilized in data gathering was the CBC results of albino rat to determine the effects of orange and tomato juices. It was obtained by collecting whole blood samples of the subject in ethylene diamine tetraacetic acid (EDTA) microtainer tube using saphenous vein extraction technique.

The automated instrument from the laboratory that was utilized was the Sysmex XS-800i automated hematology analyzer that examines the RBC count, WBC count, platelet count, RBC indices and WBC differential count in less than a minute. These were used to obtain the results and ensure accurate analysis of hematological parameters.

2.4 Materials and Equipment

Materials used for orange and tomato juice extraction included electronic blender and the pureed juice were filtered using cheesecloth and strainer. Fruit juices were administered to the first and second group. Each contained three (3) albino rats via oral route administration using 1ml syringe. Blood sampling was done using EDTA microtainer tubes and syringe with 23 gauge needles. Whole blood samples collected in EDTA microtainer tubes for CBC were analyzed using the Sysmex XS-800i automated hematology analyzer.

2.5 Preparation of Orange and Tomato Juices

Fresh oranges and tomatoes were purchased from the Cantimbuhan Market Complex in City of Dasmariñas, Cavite. Fruits samples were prepared and washed prior to blending procedure. Fruits were blend and pureed. After which strainer and cheesecloth were placed over the plastic bowl to allow the filtration of the pureed juice. Fibrous content were eventually squeezed to release more of the juice into the plastic bowl. Juice was poured off from the bottle and was set aside in cool place prior to administration.

2.6 Experimental Animals

Nine (9) male albino rats weighing about 100-150 grams were purchased from the Dasma Albino Rat Pet Services in Dasmariñas, Cavite and were used in the study. Nine albino rats were housed in a transparent glass with wire mesh tops and wheat husk bedding. They were kept under room temperature in a 12-hour light-dark cycle and with free access to food and purified water. Albino rats were acclimatized for one week to room conditions before experimentation.

2.7 Administration of Juices to Albino Rats

Each albino rat was administered orally with 2 ml of fruit juices (orange and tomato) once a day during the afternoon for three continuous weeks. The sterile syringes were prepared containing 2 ml of fruit juices and were administered to albino rats. Fit bottle were used to restrain the rat and the tip of the syringe were inserted into the mouth.

2.8 Blood Sampling

Blood samples were collected for the determination of hematological parameters. Each albino rat undergone syringe method blood extraction technique with 23 gauge needle and 0.25ml of blood samples were collected through saphenous vein technique and immediately transferred to EDTA microtainer tube for analysis every 7th day of the week for three consecutive weeks. Aseptic technique was applied using 70% ethanol.

2.9 Determination of Hematological Parameters of Albino Rats

Blood samples were analyzed using the Sysmex XS-800i automated hematology analyzer. Each blood samples was aspirated on the sample aspirating probe of the instruments and tested for CBC with platelet count. Prints out of the results were then saved for proper documentation. The instrument is a five-part differential counter on which results generated include the RBC count, WBC count, hemoglobin determination, hematocrit level, platelet count, WBC differential count (neutrophil, lymphocyte, monocyte, eosinophil, and basophil) and blood indices (MCV, MCH, and MCHC).

3. RESULTS AND DISCUSSION

The results displayed include complete blood count results of control group rat, orange group rat, and tomato group rat. Statistical data obtained were carried out by comparing the complete blood count result of 1^{st}, 2^{nd} and 3^{rd}week administration of orange and tomato juices from the baseline and control group. Interpretations of the results were based on the normal reference range of hematological parameters depicted in Table 1 of rats according to Kumar [9]. The effects were based on the administration of 2ml/125 g body weight of the albino rats with fruit juice extracts.

Table 1. Normal reference range of blood cell parameters of albino rats

Hematological parameters	Units	Reference range
RBC count	(x 10^{12} / L)	6.76-9.75
WBC count	(x 10^{9} / L)	6-18
Hematocrit	(L/L)	0.36-0.54
Hemoglobin	(g/L)	110-192
Platelet count	(x 10^{9} / L)	500-1300
Neutrophil	(L/L)	0.10-0.30
Lymphocyte	(L/L)	0.65-0.85
Monocyte	(L/L)	0.00-0.05
Eosinophil	(L/L)	0.00-0.06
Basophil	(L/L)	0.00-0.01
MCV	(Fl)	48-70
MCH	(pg)	26-32
MCHC	(g/dL)	26.5-58.0

Table 2 presented the mean CBC results of albino rats administered with orange juice. Based on the normal reference of blood parameters of rats presented on Table 1, hematological parameters of the control group revealed a non-anemic, with normal WBC and platelet count results right before subjected to experimentation. This expected range of values were also manifested among the orange group rats with a markedly thrombocytopenic (decreased platelet count).

Throughout the three weeks of administration of orange juice extract on the albino rats, erratic change in their RBC count is noted. From the baseline result, an increased-decreased pattern of the count occurred week after week. The same pattern of RBC count result among the control group is also manifested throughout three weeks' time.

For WBC, both the control group and experimental group showed a similar pattern of changes in the results. It started with a normal baseline result which suddenly decreased after a week and steadily increased after 2 weeks up to 3 weeks after the administration. Hematocrit of the subjects from the control group, decreased from its baseline after a week, until it shoot up on the 2ndweek and even more higher on the 3rdweek. This slightly varied among the orange group in which a sharp increased result is noted throughout the entire three weeks of testing.

Hemoglobin level of both control and experimental group were within the normal range (NV= 110-192 g/L) at the start of administration. While on the control group, haemoglobin level tends to decrease on the 1stweek and peak on the third week. The experimental groups' hemoglobin on the other hand, showed a decreased and increased pattern after 1 week, 2 weeks, and 3 weeks respectively. Platelet count on the other hand, for the control group and experimental group, showed a marked high level in all the testing time at which the highest

was after 3 weeks, which was different on the experimental group, wherein all the platelet counts on baseline and after 1 week was not within the normal range (NV=500-1300 $x10^9$/L).

Differential count for both the control and orange group clearly showed a lymphocytosis. Basophil result revealed an absence of the cell throughout the testing time because in the circulation, it was just present in a range of 0-0.01 L/L.

Blood Indices (MCV, MCH and MCHC) were indirectly computed parameters taken from the values of RBC count, Hct and Hb concentration. Variations in the results were just the representation of those directly measured parameters. Based on the values presented in Table 2, the MCV result of the control group has a clear increased pattern as compared to the orange group. On the other hand, MCH and MCHC from the control and experimental groups did not show a predictable pattern throughout the 3 weeks of experimentation time.

Table 2. Mean CBC results of albino rats administered with orange juice

Parameters	*RBC* ($x10^{12}$/L)	*WBC* ($x10^9$/L)	*Hct* (L/L)	*Hb* (g/L)	*Plt* ($x10^9$/L)	*N* (L/L)	*L* (L/L)	*M* (L/L)	*E* (L/L)	*B* (L/L)	*MCV* (fL)	*MCH* (pg)	*MCHC* (g/dL)
Control Group													
Baseline	7.8	14.23	0.45	165	708.67	0.37	0.5	0.12	0.03	0	57	31.13	37.07
After 1 Week	6.65	10.63	0.41	150	602.67	0.19	0.713	0.09	0.01	0	57.57	22.6	39.33
After 2 Weeks	9.06	15.58	0.57	175.33	564.67	0.27	0.59	0.14	0	0	62.5	19.3	31.2
After 3 Weeks	7.83	17.5	0.61	176.33	894.33	0.11	0.8	0.08	0.013	0	64.63	22.73	39.3
Orange Group													
Baseline	6.66	10.3	0.4	153.33	357	0.36	0.54	0.1	0	0	60.37	23.03	38.2
After 1 Week	6.19	7.97	0.4	145	232.33	0.17	0.76	0.067	0.0033	0	60.17	23.53	39.07
After 2 Weeks	6.87	13.67	0.46	166.67	638.33	0.19	0.68	0.12	0	0	57.9	24.57	34.3
After 3 Weeks	6.57	27.1	0.48	157.67	703.33	0.11	0.78	0.05	0.02	0	63.73	23.63	37.53

Note: N=3. RBC - Red Blood Cell, WBC - White Blood Cell, Hct – Hematocrit, Hb – Hemoglobin, Plt – Platelets, N – Neutrophil, L – Lymphocyte, M – Monocyte, B – Basophil, E – Eosinophil, MCV - Mean Corpuscular Volume, MCH - Mean Corpuscular Hemoglobin, MCHC - Mean Corpuscular Hemoglobin Concentration

As tabulated in Table 3, albino rats belonging to the experimental group (tomato) has a baseline blood picture of normocytic, normochromic with WBC and platelet count within the normal range. A similar hematological parameters result was seen with the control group as well.

After one week of administration of 2mL of tomato juice, a profound decreased in the RBC, Hct, Hb and WBC level occurred, whereas platelet count was highly above the baseline result in the experimental group. This was noticeably the same with the control group wherein their RBC, WBC, Plt, Hb and Hct were all decreased. Differential count between the two groups varied with their changed pattern as compared from their baseline results, yet both showed a predominance of lymphocytes. In blood indices of experimental group, only MCV was seen as increasing result, while in the control group both the MCV and MCHC increased from their baseline results in 3 weeks' time.

On the 2nd week of experimentation, RBC, WBC, Hct and Hb continue to increase even much higher compared to its first week of administration in tomato group. The platelet count result, however, was not going into this pattern as it suddenly decreased. Comparing this trend pattern with the control group somewhat showed a similar direction of result with the tomato group. Differential count showed a comparable blood picture presentation with both the control and tomato group with declined result seen only in the lymphocytes.

On the last week after administration, tomato group results of the WBC count, Hct, Hb and Plt steadily increased as seen, as well with the control group laboratory test results. Differential count presented a consistent proliferation of lymphocytes for both the two

groups. On the other hand, blood indices (MCV, MCH, and MCHC) were all slightly above from the previous week of administration.

Table 3. Mean CBC results of albino rats administered with tomato juice

Parameters	*RBC* ($x10^{12}$/L)	*WBC* ($x10^9$/L)	*Hct* (L/L)	*Hb* (g/L)	*Plt* ($x10^9$/L)	*N* (L/L)	*L* (L/L)	*M* (L/L)	*E* (L/L)	*B* (L/L)	*MCV* (fL)	*MCH* (pg)	*MCHC* (g/dL)
Control Group													
Baseline	7.8	14.23	0.45	165	708.67	0.37	0.5	0.12	0.03	0	57	31.13	37.07
After 1 Week	6.65	10.63	0.41	150	602.67	0.19	0.713	0.09	0.01	0	57.57	22.6	39.33
After 2 Weeks	9.06	15.58	0.57	175.33	564.67	0.27	0.59	0.14	0	0	62.5	19.3	31.2
After 3 Weeks	7.83	17.5	0.61	176.33	894.33	0.11	0.8	0.08	0.013	0	64.63	22.73	39.3
Tomato Group													
Baseline	6.8	11.6	0.4	158	641.7	0.223	0.673	0.1	0	0	58.7	23.23	39.53
After 1 Week	6.53	9.1	0.38	147.33	849.3	0.187	0.73	0.08	0	0	58.8	22.6	38.47
After 2 Weeks	8.05	17.1	0.45	159	811.3	0.24	0.61	0.15	0	0	56.13	19.83	35.37
After 3 Weeks	7.45	19.8	0.51	185	855	0.14	0.78	0.01	0.01	0	61.03	23.03	39.77

Note: N=3. RBC - Red Blood Cell, WBC - White Blood Cell, Hct – Hematocrit, Hb – Hemoglobin, Plt – Platelets, N – Neutrophil, L – Lymphocyte, M – Monocyte, B – Basophil, E – Eosinophil, MCV - Mean Corpuscular Volume, MCH - Mean Corpuscular Hemoglobin, MCHC - Mean Corpuscular Hemoglobin Concentration

Investigation of the significant variation in the CBC results of both the control and treatment groups were taken using One-Way ANOVA and Tukey Post Hoc Test for parameters that are normally distributed and Kurskal-Wallis Test and Mann-Whitney U Test for parameters that were not normally distributed against the baseline results to allow judgment as to reject the null hypothesis for p-values less than 0.05 level of significance.

Table 4 summarizes the p-values obtained by control and experimental groups (orange and tomato) with the CBC parameters throughout the 3 weeks post fruit juice administration against their corresponding baseline results. Results revealed that the parameters like RBC count, Hb, platelet counts, MCV and MCHC did not have a significant difference in all the groups (control and experimental groups) throughout experimentation duration on which all their p-values were >0.05. This signified that administration of tomato and orange juice had no effects on those aforementioned parameters in reference to the result of control group. This finding is in accordance with the result of the study by Alagbe [10] with sweet orange peel administration as food supplements on the subjects. However, the present study result in the RBC count vary with the result of the study of Shaista [11] and Ganwani [12] and even in the study of Mallick and Khan [7] which stated that tomatoes and orange contain vitamin C that enhances RBC production. The variation in the result may be due to differences in the duration of testing and amount of tomato given to the subjects.

WBC count on the other hand, showed a statistically significant variation on the tomato group and orange group after the 3rd week of administration with p-values of 0.05 and 0.031 respectively. This is regarded as a valid result as the control group did not have a significant difference between its baseline values throughout the entire testing time. This indicated that the bioactive substances of the tomato and orange induced proliferation of WBC in the circulation of the rats maybe through marginal mobilization according to Levy [13]. This profound leukocytosis was not a response to infection or other factors as the control group was not statistically varied compared with its baseline result throughout the time of testing. This result proved the study of Sharma [14] and Alagbe [10]which stated that bioactive component lycopene present in the tomato and orange caused oxidative damage to the WBC which eventually induced WBC proliferation.

For hematocrit determination, continuous administration of 2 ml of fruit juices daily for a period of 2 weeks showed a non-significant p-values among the group (control and

experimental) where all the p-values were >0.05. Nevertheless, after the last week of testing time, orange group demonstrated statistically different results from its baseline (p-values of 0.028). This, however, may not be considered a meaningful variation as the control group also showed a significant result (p-values of 0.041) on that time. This probably was due to the factors not related to fruit juice bioactive substances. Considering factors included environmental factors or physiological factors as stated in the study of Wei et al. [15] although these were beyond the scope of the study as these were not confirmed on this study.

Differential counts on the other hand, showed an interesting result. For neutrophil, orange group got a p-value which was statistically significant after the 1st and 2nd week of administration but not on its 3rd week. This result was regarded as the transient effect of the orange juice administration to the neutrophil count of the rats at a span of 2 weeks duration only. Tomato group on the other hand, showed a non-significant result on this type of WBC (neutrophil) throughout the entire fruit juice administration. For the lymphocytes, again, orange juice manifested an erratic effects on this WBC as it showed a significant values after 1st week and 3rd week only but not on the 2nd week. Tomato juice on the other hand is more effective in causing effect on the monocyte throughout the testing time all its p-values 0.005, 0.000 and 0.000 respectively were all <0.05. However, it is difficult to establish if the bioactive substance in the tomato causing the effect would have a lowering or increasing effect, on which after 1 week and 3 weeks of administration had a lower result than its baseline but on the second week it had a higher result compared to the baseline. Eosinophil instead, increased on the third week of administration of the orange and tomato juices. This generated statistically significant p-values (0.034 and 0.025 respectively) for orange and tomato. This indicated that a longer duration of administration induced an eosinophil, neutrophil and lymphocyte proliferation because of their bioactive substances.

Blood indices did not show a significant p-value throughout the experimentation except on the MCH of tomato group on the 2nd week. This parameter unlike the others was only obtained through calculation from RBC count and Hb concentration. The perceived effect as the significant difference was not the direct effect of the fruit juice administration on this parameter but rather on the RBC count taken in relation with its hemoglobin concentration.

Table 4 p-values on significant difference between the CBC results among groups against their baseline result

Baseline vs. 1st week													
Parameters	RBC	WBC	Hct	Hb	Plt	N	L	M	E	B	MCV	MCH	MCHC
Control Group	0.54	0.555	0.836	0.64	0.559	0.827	0.184	0.25	0.817	-	0.376	0.658	0.706
Orange Group	0.977	0.275	0.998	0.783	0.936	0.003*	0.008*	0.662	0.317	-	0.825	0.513	0.985
Tomato Group	0.988	0.708	0.943	0.756	0.447	0.512	0.224	0.005*	1.000	-	1.000	0.943	0.979
Baseline vs. 2nd week													
Parameters	RBC	WBC	Hct	Hb	Plt	N	L	M	E	B	MCV	MCH	MCHC
Control Group	0.463	0.955	0.146	0.839	0.325	0.827	1.000	0.743	0.317	-	0.275	0.05	0.086
Orange Group	0.998	0.268	0.106	0.475	0.586	0.007*	0.071	0.845	1.000	-	0.507	0.513	0.462
Tomato Group	0.498	0.158	0.54	1.000	0.6	0.86	0.16	0.000*	1.000	-	0.84	0.070*	0.47
Baseline vs. 3rd week													
Parameters	RBC	WBC	Hct	Hb	Plt	N	L	M	E	B	MCV	MCH	MCHC
Control Group	1.000	0.623	0.041*	0.8	0.162	0.060	0.056	0.108	0.814	-	0.127	0.513	0.715
Orange Group	1.000	0.05*	0.028*	0.959	0.428	0.101	0.005*	0.313	0.034*	-	0.507	0.513	0.993
Tomato Group	0.867	0.031*	0.071	0.131	0.426	0.046*	0.014	0.000*	0.025*	-	0.873	0.998	1.000

*Note: * p-value is consistent significant at <0.05 alpha ;n=3 ;*
(-) = omitted

4. CONCLUSION

Based on the data generated on the effects of tomato and orange juice extract administration daily for a period of three weeks on the albino rats at the formulation of 2ml/ average 125 g weight, the study came up with the following conclusions:

(1) Orange extract contained bioactive substances like lycopene and vitamin C that increased the WBC count, lymphocyte and eosinophil count when administered for three consecutive weeks given on daily basis only after the third week of administration.

(2) Tomato extract was effective as well in increasing the WBC count after the third week of administration as well as with the monocyte and eosinophil.

(3) Results revealed that orange extracts administration obtained a significant difference in the neutrophil and lymphocytes after one week of administration compared to their baseline results. After two weeks of administration, only the neutrophil induced a significant variation, furthermore, throughout three weeks of administration of orange extract, WBC, hematocrit, lymphocyte, and eosinophil obtained a statistically significant variation as compared to the pre-administration time.

(4) Tomato administration induced a significant difference in the values of monocyte after one week and two weeks of treatment compared to the baseline values. However, only the MCH result showed a significant difference on the 2^{nd}week of administration. Throughout three weeks' time of experimentation, results revealed that monocyte, neutrophil, lymphocyte, eosinophil and WBC showed a significant difference (p value <0.05) compared to their baseline result.

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