# EFFECT OF DIFFERENT LEVELS OF CONFINEMENT STRESS AT DIFFERENT TIME INTERVAL TO THE IMMUNE SYSTEM OF WHITE MICE (*Mus musculus L.*)

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***Abstract-*** To demonstrate how different levels of confinement stress at different time interval can affect the immune system of white mice, differential white blood cell (WBC) count was performed considering with and without application of confinement stress to male and female mice using different chamber diameters (1.0 inch, 1.5 inches, and 2.0 inches) at different time intervals (1 hour, 3 hours, 5 hours and at full confinement). Control group (without confinement) and experimental group (with confinement) were considered. Thirty mice (15 males, 15 females) were used with blood sample collected. Prepared blood smear was stained with wright-giemsa and examined under light microscopy. Differential WBC count was performed to determine the number/percentage of each type of WBC (lymphocyte, monocyte, neutrophil, basophil and eosinophil).

Results showed a distinct direct proportionality between lymphocyte count and the various levels of confinement stress demonstrated by using confinement chambers in different diameters. Generally, lymphocytes decreased in number as the diameter of the confinement chambers decreased. This means that lymphocyte count decreased as confinement stress is increased. Ironically, as the diameter of the chambers decreased, the neutrophil count increased. Hence, as lymphocyte count decreases, the neutrophil count increases both in male and female mice. Significant difference on lymphocyte and neutrophil count was shown between the experimental and control mice after 3 hours, 5 hours and in full confinement. Further, significant differences were observed between lymphocyte and neutrophil count in both male and female mice confined to the various experimental chambers.

***Index Terms***- confinement stress, leukocyte, neutrophil, white blood cell

## I. INTRODUCTION

Physiological studies have shown that stress from any source can influence the endocrine, hemopoietic and immune systems. Stress considered as a physiological response of the body to hostile environment affect not only man but also animals. When the trigger is repetitive, prolonged or unanticipated, it becomes pathological.

WBC can provide a reliable method in physiological research to study vertebrate responses since they are altered by stress and can be directly related to stress hormone levels (Davis et al, 2008).

The absolute number of each type of WBC, often more informative than its proportion can be calculated if the differential and the total number of leukocytes per volume unit are known.

In mice, the WBC count ranges from 2 -10 x $10^9$ per liter: 5-20 % (0.5 – 3.0 x $10^9$/L) neutrophils, 60-90 % (2-8 x $10^9$/L) lymphocytes, monocytes and eosinophils are minor cell types (0.05 to 0.10 x $10^9$/L). Generally, there are more monocytes than eosinophils in the peripheral blood. Basophils are very rarely observed in the peripheral blood of mice. Some authors have questioned the presence of basophils in mouse blood, however, microscopic and ultrastructural characteristics of murine basophils have been shown. (Duorak et al., 1982; Duorak, 2000; Hedrich et al., 2004).

Based from a one-hour preliminary evaluation on the effect of different levels of confinement stress to the immune system of white mice, it was observed that the mice nibbled some parts of the plastic chamber which is an indication of stress symptom (Wallace, 1976). Results showed differences on the number of the different types of leukocytes in male mice. There was a decreasing trend on the number of lymphocytes as the diameter of the chambers decreased in size (1.0 in, 1.25 in, 1.5 in. 2.0 in) after one hour confinement. Ironically, the number of neutrophils increased as the diameter of chambers also decreased after one hour of confinement. The rest of the other types of WBC like monocytes, basophils and eosinophils did not signify any increasing or decreasing trend. Significant differences were obtained in the lymphocyte and neutrophil count specifically in 1.25 in confinement chamber compared to the control cage (Domingo and Saguid, 2010).

According to Ursula et al (2007), the percentage of activated neutrophils began to fall and reached baseline levels within 8 h. It should be recalled that the mice have been subjected to only one hour confinement after collection of blood sample. Hence, this research evaluated the effect of different levels of confinement stress at different time intervals to the immune system of white mice.

## II. METHODOLOGY

The research followed Complete Randomized Design (CRD) with three replicates for both studies. To demonstrate how different levels of confinement stress at different time interval can affect the immune system of white mice, differential white blood cell (WBC) count was performed considering with and without application of confinement stress to male and female mice using different chamber diameter ( 1.0 inch, 1.5 inches, and 2.0 inches ) at different time intervals (1 hour, 3 hours, 5 hours and at full confinement). Control group (without confinement) and experimental group (with confinement) were considered. Thirty mice (15 males, 15 females) were used and with blood sample collected. Prepared blood smear were stained with wright-giemsa and examined under light microscopy. Differential WBC count was performed to determine the number/percentage of each type of WBC - lymphocyte, monocyte, neutrophil, basophil and eosinophil (Brehe and Way, 2008).

Mean differential percentages were obtained in the various types of WBC (lymphocytes, monocytes, neutrophils, eosinophils, basophils) per one hundred of WBC. Analysis of Variance, Least Significant Differences (LSD) and T-test were performed to calculate differences among the treatments, between the control and experimental mice.

## III. RESULTS AND DISCUSSIONS

Theoretically, when the body of an organism is under stress, high levels of stress regulating hormones and neurotransmitters like cortisol, norepinephrine, epinephrine, dopamine and serotonin are released that eventually leads to malfunctioning in the endocrine system, nervous system, metabolic system and immune system.

Table 1 and Table 2 show the mean differential WBC count in male and female white mice subjected to different levels of confinement stress in 1 hour.

**Table 1. Differential WBC count of male mice subjected to different levels of confinement chambers after a period of one hour.**

| Confinement chambers (in) | WBC/ Leukocytes | | | | |
|---|---|---|---|---|---|
| | Lymphocyte | Monocyte | Neutrophil | Eosinophil | Basophil |
| **1.0** | 65.33 | 9.3 | 10 | 8 | 7.3 |
| **1.5** | 65.33 | 21 | 5.3 | 4.3 | 4 |
| **2.0** | 58 | 19.3 | 7 | 8 | 7.6 |
| **Control** | 76 | 8.3 | 6.6 | 5 | 4 |
| Legend: | ns | ns | ns | ns | ns |

ns = not significant

**Table 2. Differential WBC count of female mice subjected to different levels of confinement chambers after a period of one hour.**

| Confinement chambers (in) | WBC/ Leukocytes | | | | |
|---|---|---|---|---|---|
| | Lymphocyte | Monocyte | Neutrophil | Eosinophil | Basophil |
| **1.0** | 66 | 8.3[a] | 15 | 5.6 | 5 |
| **1.5** | 67.6 | 5.6[b] | 16 | 5 | 5.6 |
| **2.0** | 59.6 | 12.3[a] | 15 | 4 | 9 |
| **control** | 73.3 | 4.6[b] | 7 | 3.6 | 11.3 |
| Legend: | ns | * | ns | ns | ns |

ns = not significant
* = significant at .05

It is shown in the 2 tables that the differential WBC count of the male and female white mice subjected to different levels of confinement stress (in various diameters of confinement chambers: 1.0 in., 1.5 in., 2.0 in) are comparable with each other except in the number of monocytes in female mice.

Despite the decreasing count of lymphocytes from the blood of male (1.0 in: 65.33, 1.5 in: 65.33, 2.0 in: 58) and female (1.0 in: 66, 1.5 in: 67.6, 2.0 in: 59.6) white mice collected on the experimental chambers as compared to the control (male: 76, female: 73.3), no significant differences were found. The increasing trend of neutrophils from both male (1.0 in: 10, 1.5 in: 5.3, 2.0 in: 7) and female (1.0 in: 15, 1.5 in: 16, 2.0 in: 15) white mice as compared to the control white mice (male: 6.6, female: 7) did not also indicate significant differences.

However, significant differences were found in the monocyte count collected in the female mice subjected to the various confinement chambers. Monocyte count at 1.0 in. and 2.0 in. confinement chambers significantly differed with the monocyte count from the female white mice that stayed in control chamber for one hour. This result could be associated to the reason that monocytes are known to be activated by estrogen and progesterone in the female white mice (Li et al, 1993 as cited by Willis C. et al 2003).

Table 3 and Table 4 show the differences on the number of the different types of leukocyte cells in male and female white mice subjected to different levels of confinement stress for a period of three hours.

**Table 3. Differential WBC/Leukocyte count in male white mice subjected to different levels of confinement chambers after a period of 3 hours.**

| Confinement chambers (in) | WBC/ Leukocytes | | | | |
|---|---|---|---|---|---|
| | Lymphocyte | Monocyte | Neutrophil | Eosinophil | Basophil |
| **1.0** | **34.6[a]** | **8** | **50[a]** | **4** | **10** |
| **1.5** | **23.3[a]** | **20.3** | **48.3[a]** | **5.3** | **2.6** |
| **2.0** | **36.3[a]** | **19.6** | **36[a]** | **4.3** | **3.6** |
| **control** | **76[b]** | **8.3** | **6.6[b]** | **5** | **4** |
| Legend: | ** | ns | ** | ns | ns |

ns = not significant
** = significant at .01

**Table 4. Differential WBC/Leukocyte count in female white mice subjected to different levels of confinement chambers after a period of 3 hours.**

| Confinement chambers (in) | WBC/ Leukocytes | | | | |
|---|---|---|---|---|---|
| | Lymphocyte | Monocyte | Neutrophil | Eosinophil | Basophil |
| **1.0** | 28[a] | 7.6 | 57.6[a] | 3.3 | 2.3[a] |
| **1.5** | 39.3[a] | 14.6 | 43.3[a] | 3.3 | 2.6[a] |
| **2.0** | 37[a] | 11.3 | 43.3[a] | 5.3 | 3[a] |
| **control** | 73.3[b] | 4.6 | 7[b] | 3.6 | 11.3[b] |
| Legend: | ** | ns | ** | ns | ** |

ns = not significant
** = significant at .01

Data on both tables show that there is a decreasing trend on the number of lymphocytes as the diameter of the chambers decreased in size after three hours of confinement. Ironically, neutrophils increased as the diameter of chambers also decreased after three hours of confinement

However, remarkable differences occurred in the number of basophils in the case of the female white mice subjected to the experimental chambers for a period of three hours as compared to the basophil count in the control mice.

This result could be due to the immediate response of the organism's system when wounded to produce thrombin which cause other responses through signal transduction making estrogen-responsive cells to function and show mitogenic responses causing increase in basophil count. (Henrikson, 1992). Besides variation in leukocyte counts is an immediate and transitory response of mice exposed in stressful stimuli ( Jensen and Rasmussen, 1969).

Table 5 and Table 6 show the differences on the number of the different types of leukocyte cells in male and female white mice subjected to different levels of confinement stress for a period of five hours.

Similar results were obtained in Table 5 and Table 6 in which significant differences occurred among the lymphocyte, neutrophil and basophil numbers of female and male mice subjected to all the confinement chambers for a period of five hours as compared to the female white mice in the control chambers.

On the case of experimental mice at full confinement, it can be observed that from the increasing and decreasing trend of lymphocyte and neutrophil numbers obtained from the three and five hour period of confinement, no existing trend of the values appeared. However, statistical analysis revealed significant differences among the lymphocyte, neutrophil and basophil numbers of female and male mice subjected to all the confinement chambers in full confinement as compared to the female mice in control chambers.

**Table 5. Differential WBC/Leukocyte count in male white mice subjected to different levels of confinement chambers after a period of 5 hours.**

| Confinement chambers (in) | WBC/ Leukocytes | | | | |
|---|---|---|---|---|---|
| | Lymphocyte | Monocyte | Neutrophil | Eosinophil | Basophil |
| **1.0** | 40.6[a] | 5 | 49.3[a] | 3.3 | 2[a] |
| **1.5** | 36.3[a] | 5 | 53.3[a] | 3 | 2.3[a] |
| **2.0** | 42.6[a] | 5.3 | 45.6[a] | 4 | 2.6[a] |
| **control** | 76[b] | 8.3 | 6.6[b] | 5 | 4[b] |
| Legend: | ** | ns | ** | ns | ** |

ns = not significant
** = significant at .01

**Table 6. Differential WBC/Leukocyte count in female white mice subjected to different levels of confinement chambers after a period of 5 hours.**

| Confinement chambers (in) | WBC/ Leukocytes | | | | |
|---|---|---|---|---|---|
| | Lymphocyte | Monocyte | Neutrophil | Eosinophil | Basophil |
| **1.0** | 31.6[a] | 7.3 | 54.6[a] | 3 | 3.3[a] |
| **1.5** | 28.6[a] | 10.6 | 53.3[a] | 5 | 2.3[a] |
| **2.0** | 28.6[a] | 15.3 | 49[a] | 4.6 | 2.3[a] |
| **control** | 73.3[b] | 4.6 | 7[b] | 3.6 | 11.3[b] |
| | ** | ns | ** | ns | ** |

Legend:
ns = not significant
** = significant at .01

Data shows in Fig. 1 that high percent mortality for both male and female mice was obtained on the 6th day of full confinement while lowest percent mortality for male mice was obtained after 4th and 8th day of full confinement.

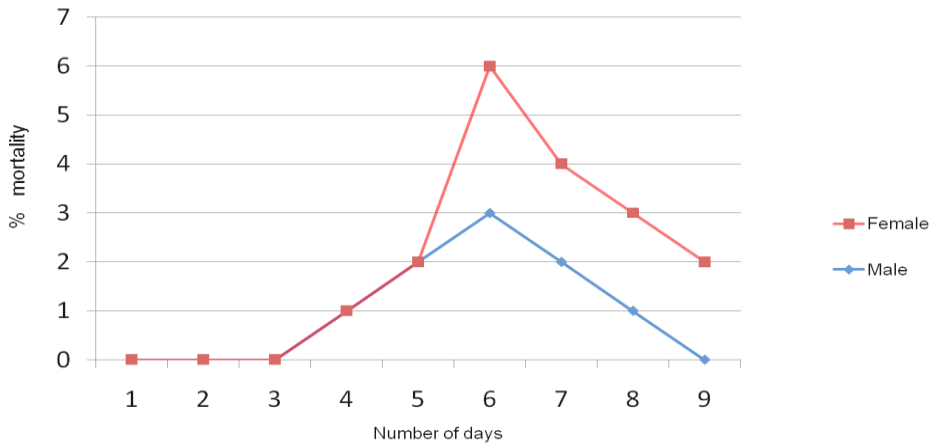


**Figure 1. Shows the percent mortality of female and male mice subjected to different levels of confinement chambers after full confinement.**

This means that male and female mice have different ways of coping to stress resulting to the varied number of tolerable days of being confined to the chambers.

Taylor et al. (2000) first proposed the idea of a unique female stress response which they termed "tend-and-befriend." The tend-and-befriend response is characterized as an oxytocin mediated stress response cascade. Estrogen has been found to increase the effects of oxytocin already in excess in females as compared with males. Testosterone and vasopressin, the counterparts of estrogen and oxytocin, present during the male stress response, "fight-or-flight," have been found to exhibit the opposite effects of oxytocin. Girdler et al.'s (1997) study showed that testosterone levels increased in males in response to stress which showed a positive correlation to levels of hostility.

Hence, despite the difference in mechanism, comparable results were controlled on the basis of hormonal secretions for both male and female mice as to the differential WBC count is concerned.

Table 7 shows the differential WBC/Leukocyte count of male white mice from different levels of confinement stress at different time intervals. Data shows that on 1.0 in. confinement chamber, it was observed that lymphocyte number of male mice decreased as neutrophil count increased from I hour to full confinement. Significant differences on the lymphocyte and neutrophil count were noted after one hour of confinement of mice as compared to the lymphocyte and neutrophil count after 3 hours, 5 hours and full confinement. This means that male mice had significantly been under stress after 3 hours of confinement to full confinement.

**Table 7. Differential WBC count in the various time intervals where male and female mice were subjected to different levels of confinement chambers**

| Male | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment | | | | | | | | | | | | | | | | | | | |
| | 1.0 | | | | | 1.5 | | | | | 2.0 | | | | | control | | | | |
| Time Interval | lymp | mono | neu | eos | baso | lymp | mono | neu | eos | baso | lymp | mono | neu | eos | baso | lymp | mono | neu | eos | baso |
| one hour | 66ª | 8.33 | 15ª | 6.67 | 5 | 67.67ª | 5.67 | 16ª | 5a | 5.67ª | 59.67ª | 12.33 | 15a | 4 | 9ª | 73.33 | 4.67 | 7 | 3.67 | 11.33 |
| three hours | 28ᵇ | 7.67 | 57.67ᵇ | 3.33 | 2.33 | 39.33ᵇ | 14.67 | 43.33ᵇ | 3.33ª | 2.67ᵇ | 37ᵇ | 11.33 | 43.33b | 5.3 | 3ᵇ | 73.33 | 4.67 | 7 | 3.67 | 11.33 |
| five hours | 31.67ᵇ | 7.33 | 54.67ᵇ | 3 | 3.33 | 28.67ᵇ | 10.67 | 53.3b | 5ª | 2.33ᵇ | 28.67ᵇ | 15.33 | 49b | 4.67 | 2.33ᵇ | 73.33 | 4.67 | 7 | 3.67 | 11.33 |
| full confinement | 28.3ᵇ | 7.67 | 55ᵇ | 6 | 3 | 29.33ᵇ | 10.33 | 49.3b | 8.67ᵇ | 2.33ᵇ | 45.67ª | 8 | 36b | 3.3 | 3ᵇ | 73.33 | 4.67 | 7 | 3.67 | 11.33 |
| | ** | ns | ** | ns | ns | ** | ns | ** | * | ** | ** | ns | ** | ns | * | ns | ns | ns | ns | ns |
| | | | | | | | | | | | | | | | | | | | | |
| Female | | | | | | | | | | | | | | | | | | | | |
| | Treatment | | | | | | | | | | | | | | | | | | | |
| | 1.0 | | | | | 1.5 | | | | | 2.0 | | | | | control | | | | |
| Time Interval | lymp | mono | neu | eos | baso | lymp | mono | neu | eos | baso | lymp | mono | neu | eos | baso | lymp | mono | neu | eos | baso |
| one hour | 65.33ª | 9.33 | 10ª | 8 | 7.33ª | 65.3ª | 21ª | 5.3ª | 4.3 | 4 | 58 | 19.3 | 7ª | 8 | 7.67 | 76 | 8.3 | 6.67 | 5 | 4 |
| three hours | 34.67ᵇ | 8 | 50ᵇ | 4 | 3.33ᵇ | 23.33ᵇ | 20.33ª | 48.33ᵇ | 5.3 | 2.67 | 36.3 | 19.67 | 36ᵇ | 4.33 | 3.67 | 76 | 8.3 | 6.67 | 3 | 3.33 |
| five hours | 40.67ᵇ | 5 | 49.33ᵇ | 3.33 | 2ᵇ | 36.33ᵇ | 5ᵇ | 53.33ᵇ | 3 | 2.33 | 42.67 | 5.33 | 45.67ᵇ | 4 | 2.67 | 76 | 8.3 | 6.67 | 5 | 4 |
| full confinement | 47.33ᵇ | 7.67 | 35ᶜ | 7 | 3ᵇ | 26.67ᵇ | 10.33ª | 52.33ᵇ | 8 | 2.67 | 33 | 8 | 47.33ᵇ | 8.33 | 3.33 | 76 | 8.3 | 6.67 | 5 | 4 |
| | ** | ns | ** | ns | ** | ** | * | ** | ns | ns | ns | ns | ** | ns | ns | ns | ns | ns | ns | ns |
| | | | | | | | | | | | | | | | | | | | | |
| Male vs Female (sig diff) | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

However, eosinophil count after one hour, 3 hours and 5 hours time interval significantly differed on the eosinophil count of those mice subjected in full confinement. At 2.0 in diameter, data revealed that lymphocyte count after 1 hour of confinement and full confinement significantly differed on the lymphocyte count of mice after 3 hours and 5 hours of confinement. On the other hand, neutrophil count and basophil count similarly differed after I hour of confinement as compared to the neutrophil and basophil count after 3 hours, 5 hours and full confinement.

The variability of the leukocyte count could be due to the reason that the effect of the stress is not continuous, the "fight and flight" reaction might have resulted to a mechanism of tiredness to make the mice fight back stress. Hence, making them rest for quite sometime and as their system get to be associated of their condition then the effect of stress on the differential WBC count is triggered.

Table 8 shows that on 1.0 in. confinement chamber, lymphocyte and basophil number of female mice decreased as neutrophil count increased after 1 hour of confinement to 3 hours and 5 hours of confinement.

Significant differences on the lymphocyte, basophil and neutrophil count were obtained after 1 hour of confinement of mice as compared to the lymphocyte and neutrophil count after 3 hours, 5 hours and full confinement. At 1.5 in confinement chamber, lymphocyte and monocyte number decreased as neutrophil count increased when the period of confinement increased from one hour to full confinement. Significant differences on the lymphocyte, monocyte and neutrophil count were noted after 1 hour of confinement of mice as compared to the lymphocyte and neutrophil count after 3 hours, 5 hours and full confinement. This means that female mice had significantly been under stressed after 3 hours of confinement, 5 hours of confinement and full confinement. At 2.0 in diameter, data revealed that neutrophil count similarly differed after I hour of confinement as compared to the neutrophil count after 3 hours, 5 hours and full confinement.

**Table 8.WBC count differences of male and female mice subjected to different levels of confinement chambers after 1 hour, 3 hours, 5 hours and full confinement**

| MALE | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | One Hour | | | | Three Hours | | | | Five Hours | | | | Final Confinement | | | |
| | 1 | 1.5 | 2 | Control | 1 | 1.5 | 2 | Control | 1 | 1.5 | 2 | Control | 1 | 1.5 | 2 | Control |
| Lymp | 65.33[b] | 65.33[b] | 58[a] | 76[a] | 34.67[a] | 23.33[a] | 36.33[a] | 76[a] | 40.67[a] | 36.33[a] | 42.67[a] | 76[a] | 47.33[a] | 26.67[a] | 33[a] | 76[a] |
| Mono | 9.33[b] | 21[b] | 19.33[b] | 8.33[b] | 8[b] | 20.33[a] | 19.67[a] | 8.33[b] | 5[b] | 5[b] | 5.33[b] | 8.33[b] | 7.67[b] | 10.33[b] | 8[b] | 8.33[b] |
| Neo | 10[b] | 5.33[b] | 7[b] | 6.67[b] | 50[c] | 48.33[b] | 36[a] | 6.67[b] | 49.33[a] | 53.33[c] | 45.67[a] | 6.67[b] | 35[c] | 52.33[c] | 47.33[c] | 6.67[b] |
| Eos | 8[b] | 4.33[b] | 8[b] | 5[b] | 4[b] | 5.33[c] | 4.33[b] | 5[b] | 3.33[b] | 3[b] | 4[b] | 5[b] | 7[b] | 8[b] | 8.33[b] | 5[b] |
| Baso | 7.33[b] | 4[b] | 7.67[b] | 4[b] | 3.33[b] | 2.67[c] | 3.67[b] | 4[b] | 2[b] | 2.33[b] | 2.67[b] | 4[b] | 3[b] | 2.67[b] | 3.33[b] | 4[b] |
| | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| FEMALE | | | | | | | | | | | | | | | | |
| | One Hour | | | | Three Hours | | | | Five Hours | | | | Final Confinement | | | |
| | 1 | 1.5 | 2 | Control | 1 | 1.5 | 2 | Control | 1 | 1.5 | 2 | Control | 1 | 1.5 | 2 | Control |
| Lymp | 66[a] | 67.67[a] | 59.67[a] | 73.33[a] | 28[a] | 39.33[a] | 37[a] | 73.33[a] | 31.67[a] | 28.67[a] | 28.67[a] | 73.33[a] | 28.33[a] | 29.33[a] | 45.67[a] | 73.33[a] |
| Mono | 8.33[b] | 5.67[b] | 12.33[b] | 4.67[b] | 7.67[b] | 14.67[b] | 11.33[b] | 4.67[b] | 7.33[b] | 10.67[b] | 15.33[b] | 4.67[b] | 7.67[b] | 10.33[b] | 8[b] | 4.67[b] |
| Neo | 15[c] | 16[c] | 15[b] | 7[b] | 57.67[c] | 43.33[a] | 43.33[a] | 7[b] | 54.67[c] | 53.33[c] | 49[c] | 7[b] | 55[c] | 49.33[c] | 36[a] | 7[b] |
| Eos | 5.67[b] | 5[b] | 4[b] | 3.67[b] | 3.33[b] | 3.33[b] | 5.33[b] | 3.67[b] | 3[b] | 5[b] | 4.67[b] | 3.67[b] | 6[b] | 8.67[b] | 7.33[b] | 3.67[b] |
| Baso | 5[b] | 5.67[b] | 9[b] | 11.33[c] | 2.33[b] | 2.67[b] | 3[b] | 11.33[c] | 3.33[b] | 2.33[d] | 2.33[d] | 11.33[c] | 3[b] | 2.33[b] | 3[b] | 11.33[c] |
| | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| | | | | | | | | | | | | | | | | |
| Male vs Female | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

No significant differences were revealed on the WBC count of the control male and female mice confined to the different levels of confinement chambers after 1 hour, 3 hours, 5 hours and full confinement.

Considering the differences of the leukocytes in the various periods wherein the female and male mice were confined in different levels of confinement chambers, Table 8 shows significant differences in all the leukocyte counts obtained after a period of 1 hour, 3 hours, 5 hours and after full confinement. However, no significant differences were attained between male and female mice WBC count.

## IV. CONCLUSION AND RECOMMENDATION

Generally, lymphocytes decreased in number as the diameter of the confinement chambers decreased. This means that lymphocytes decreased as confinement stress increased. Ironically, as the diameter of the chambers decreased, the number of neutrophils increased. Hence, as lymphocytes decreased, the neutrophil count increased in both male and female mice. Significant difference on lymphocyte and neutrophil count was shown between the experimental mice and to the mice in the control cages. However, no significant difference on the lymphocyte and neutrophil count of male and female mice existed among the treatment chambers after being confined at 3 hours and 5 hours. The effect of the different levels of confinement stress between male and female mice was comparable. Such findings, confirmed previous related physiological findings on the effect of various stressors on the WBC of mice.

Further studies should be performed considering higher levels of confinement stress at longer period of time intervals. More replicates of mice should be utilized in order to add samples for confirmatory purposes. Initial WBC count should be considered and measured to compare WBC count after the application of any form of stress. Modern techniques in differential WBC count should be performed to minimize errors in counting and characterization.

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