

# Research Journal of Immunology

ISSN 1994-7909

**science**  
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## **Immunomodulatory Effect of Warm Water Swim-stress in Adult Female Sprague Dawley Rats**

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### **ABSTRACT**

This study investigates the immunomodulatory effect of warm water swim-stress. Twenty Sprague Dawley rats, divided into 4 groups were used. Control Group (CG) rats were not exposed to any kind of stress. Experimental Group 1 (EG1) rats were exposed to mild/short-term stress for 3 min, experimental Group 2 (EG2) rats were exposed to moderate stress for 6 min, while experimental group 3 (EG3) rats were exposed to severe/long-term stress for 10 min. All experimental rats were exposed to warm water swim-stress once daily for 20 consecutive days. CD<sub>4</sub> cell count, Total White Blood Cell (TWBC) Count and Differential White Blood Cell (DWBC) count were determined. The results of this experiment showed that there was a significant increase in the CD<sub>4</sub> cell counts and TWBC counts of EG1 and EG2 when compared with CG. However, there was a significant decrease in the CD<sub>4</sub> cell and TWBC counts of EG2 when compared with EG1. There was a significant decrease in the CD<sub>4</sub> cell and TWBC counts of EG3 when compared with CG, EG1 and EG2. There was a significant decrease in neutrophil counts of EG1 and EG2 when compared with CG. There was also a significant increase in lymphocyte counts of EG1 and EG2 when compared with CG. However, there were no significant alterations in the neutrophil and lymphocyte counts of EG3 when compared with CG. The present study showed that mild/short term and moderate warm water swim stress stimulate the immune response, while severe/chronic stress suppresses the immune system.

**Key words:** Stress, swimming, CD<sub>4</sub> cells, immunity, temperature

### **INTRODUCTION**

Stress is related to the levels of circulatory white blood cells, immunoglobulin and antibody titers. A number of studies have associated stress with immunomodulation (Weisse *et al.*, 1990; Naliboff *et al.*, 1991; Bachen *et al.*, 1992; Brosschot *et al.*, 1992; Sieber *et al.*, 1992; Zakowshi *et al.*, 1992). It is also established that communication between the central nervous system and the immune system occurs through bidirectional signals linking the nervous, endocrine and immune systems. Studies have shown varying effects of acute and chronic forms of stressors on immunological parameters (Segerstrom and Miller, 2004). Marshland *et al.* (1995) reported that acute psychological stress caused a decrease in circulating CD 19 lymphocytes and an increase in both CD8 and CD56 lymphocytes. Studies of Connor *et al.* (1997) also associated forced swim stress

test with a significant reduction in both the Total White Blood Cell (TWBC) count and lymphocytes levels. Stress has also been associated with increased phagocytic capacity (Ortega *et al.*, 1993), increased Natural Killer (NK) cell activity (Naliboff *et al.*, 1991; Herbert and Cohen, 1993; Benschop *et al.*, 1998) and alteration in cytokines production (Cheng *et al.*, 1990; Shu *et al.*, 1993; Dugue, 2000).

Stress-induced immunomodulation has been associated with sympathoadrenal activity (Bachen *et al.*, 1995; Yokoyama *et al.*, 2000). Bachen *et al.* (1995) reported that immune responses did not change following administration of a non selective adrenoceptor antagonist, labetalol, prior to exposure to stress. Their findings demonstrated that immunologic responses to stress are dependent on sympathetic nervous system. However, there has been no study that documents the effect of warm swim-stress on immunological parameters. Therefore this study determines the immunomodulatory effect of warm swim stress in rats.

## **MATERIALS AND METHODS**

**Experimental animals:** The experiment was conducted in the Animal House of the Ladoko Akintola University of Technology (LAUTECH), Ogbomosho, Oyo State, Nigeria in 2009. Twenty adult female Sprague dawley rats weighing 200-230 g were used. The rats were housed in a wire mesh cage and allowed to acclimatize for 2 weeks under a maintained room photoperiodic condition of 12:12 h light-dark cycle. The rats fed *ad libitum*.

The rats were randomly divided into 4 groups (n = 5): Control Group (CG), experimental group 1 (EG1), Experimental Group 2 (EG2) and Experimental Group 3 (EG3).

**Experimental procedure:** All experimental groups (EG 1, EG2 and EG 3) were exposed to warm water swim stress of different forms. However; CG rats were not exposed to any form of stress.

Stress was induced as described by Burgin *et al.* (1996) with some modifications. This method involves swimming of the experimental rats in warm water while varying the temperature of the water and duration of exposure. EG 1 rats were exposed to mild/short-term stress by swimming them in warm water at 35°C±1 for 3 min, once in a day. EG 2 rats were exposed to moderate stress by swimming them in warm water at 37°C±1 for 6 min, once in a day. EG 3 rats were exposed to severe/long-term stress by swimming them in warm water at 40°C±1 for 10 min, once in a day. All experimental rats were exposed to warm water swim-stress for 20 consecutive days.

**Measurements:** On the 20th day after swimming the experimental rats, all rats were sacrificed by cervical dislocation and blood samples were collected by cervical puncture. CD<sub>4</sub> cell counts, TWBC counts and Differential White Blood Cell (DWBC) counts were determined.

**Determination of CD<sub>4</sub> counts:** CD<sub>4</sub> cell count was determined using flow cytometry technique described by Bentwich (2005). A micropipette was used to pipette 20 µL of monoclonal antibodies into labelled test tubes. Twenty microliter of blood sample collected into EDTA bottle was mixed with the monoclonal antibodies in the labelled test tube. The mixture in the labelled test tube was incubated for 15 min. After incubation, 800 µL of buffer was added to the mixture in the labelled test tube. Then the CD<sub>4</sub> cell count was read using a cytoflow cell counter.

**Determination of TWBC count:** Total white blood cells count was determined using the standard laboratory technique procedure. With the use of a micropipette, 0.38 mL of Thürk solution was

measured and mixed with 0.02 mL of the blood sample in a test tube. The solution was mixed and a drop from the solution was dropped on the counting chamber. The counting chamber was then covered with a cover slip and mounted on a light microscope. White blood cells were viewed by the use of a low power eye piece. Total number of white blood cells count was estimated as: Number of white blood cells counted  $\times 100 \text{ mm}^{-3}$ .

**Determination of DWBC count:** Differential white blood cells count was determined using the standard laboratory technique procedure. A drop of the blood sample was dropped on a plain glass slide and a blood smear with a head and a tail was made. The blood smear was allowed to dry, after which the glass slide was inserted into methyl alcohol for about 30 sec. The glass slide was then stained with Wright's Stain for about 2 min. After staining, the glass slide was washed by inserting the glass slide into distilled water and removing it almost immediately. The glass slide was dried after washing and a drop of immersion oil was placed towards the tail of the smear. The glass slide was then mounted on the light microscope and white blood cells were viewed with the immersion oil objective lens (100x). The proportions of the different kinds of white blood cells were calculated as percentage.

**Statistical analysis:** Data obtained are presented as mean $\pm$ SEM. The statistical analysis was carried out using Analysis of Variance (ANOVA) for comparison between the 4 groups followed by multiple students't-tests. The level of significance was set at  $p < 0.05$ .

## RESULTS

There was a significant increase in the  $\text{CD}_4$  cell counts of EG1 and EG2 when compared with CG and EG3. EG3 showed a significant decrease in  $\text{CD}_4$  cell counts when compared with all other groups. There was also a significant decrease in the  $\text{CD}_4$  cell counts of EG2 when compared with EG1 (Fig. 1).

There was a significant increase in TWBC counts of EG1 and EG2 when compared with CG and EG3. However, there was a significant decrease in TWBC counts of EG2 when compared with EG1. There was a significant decrease in TWBC counts of EG3 when compared with all other groups (Fig. 2).

There was a significant decrease in the neutrophil counts of EG1 and EG2 when compared with CG. There was no significant difference in the neutrophil counts of EG3 rats when compared with that of CG rats. There was no significant difference in the neutrophil counts of EG2 when compared with EG1. There was no significant change in the neutrophil counts of EG3 when compared with all other groups (Table 1).

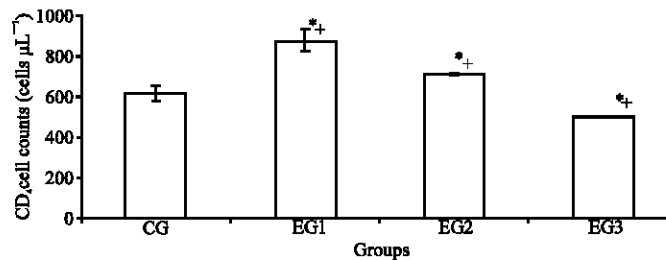


Fig. 1: Effect of warm water swim-stress on  $\text{CD}_4$  cell count. \* $p < 0.05$  vs. CG, + $p < 0.05$  vs. other groups. CG: Control, EG: Experimental group

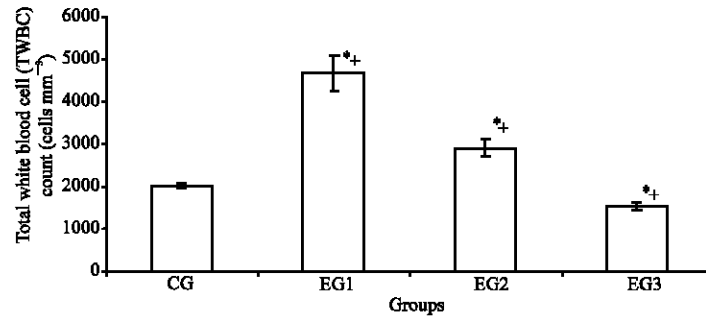


Fig. 2: Effect of warm water swim-stress on Total White Blood Cell (TWBC) count. \* $p < 0.05$  vs. CG, † $p < 0.05$  vs. other groups. CG: Control, EG: Experimental group

Table 1: Effect of warm water swim-stress on differential white blood cell counts

| Effect (%)  | CG         | EG1         | EG2          | EG3        |
|-------------|------------|-------------|--------------|------------|
| Neutrophils | 20.2±1.281 | 13.4±1.327* | 15.0±1.1612* | 14.2±2.973 |
| Eosinophils | 0.0±0.1    | 0.4±0.4     | 0.0±0.1      | 0.4±0.4    |
| Lymphocytes | 79.8±1.281 | 86.2±1.685* | 85.0±1.612*  | 85.4±3.124 |

\* $p < 0.05$  vs CG. CG: Control, EG: Experimental group

There was a significant increase in the lymphocytes counts of EG1 and EG2 when compared with CG. However, there was no significant difference in the lymphocytes counts of EG3 when compared with other groups. There was no significant decrease in the lymphocytes counts of EG2 when compared with EG1 (Table 1).

There were no significant changes in the eosinophil counts across all groups (Table 1).

## DISCUSSION

This study documents the effects of exposure to varying degree and duration of warm water swim-stress on immunological parameters in adult female Sprague Dawley rats. Previous studies of Segerstrom and Miller (2004) showed that short-term stress enhances the immune system, while long-term stress suppresses the immune system. This immunomodulation has been associated with the release of catecholamines (Naliboff *et al.*, 1991). Increase in catecholamine has been reported to suppress the natural killer cells and alter cell numbers via redistribution (Naliboff *et al.*, 1991). Changes in epinephrine levels are thought to affect lymphocyte migration from the bone marrow, extremities and the thymus (Glaser *et al.*, 1992) to other areas of the body.

The results of this study show a significant increase in the CD<sub>4</sub> and TWBC counts in rats exposed to mild/short-term warm water swim-stress when compared with rats of CG. The significant increase in the CD<sub>4</sub> and TWBC levels of rats exposed to mild/short-term warm water swim stress may be due to an increase in the release of adrenergic stress hormones which increases the synthesis and release of cytokines, consequently enhancing the immune system. This is in consonance with previous studies (Shu *et al.*, 1993; Rassnick *et al.*, 1994) that reported increase in immunological parameters in mild forms of stress.

This study also shows that as the degree and duration of stress/stressor increased as in the EG2 rats, there was a significant decrease in the CD<sub>4</sub> and TWBC counts when compared with those of EG1 exposed to mild/short-term warm water swim-stress. However, there was a significant increase

in the CD<sub>4</sub> and TWBC counts of EG2 when compared with the CG. This suggests that moderate stress also enhances the immune system but not as much as mild stress. EG3 rats that were exposed to severe/long-term stress had a significant reduction in CD<sub>4</sub> and TWBC counts when compared with that of CG, EG1 and EG2. This shows that severe/long-term stress has a suppressive effect on the immune system. This is similar to previous studies (Cheng *et al.*, 1990; Sheperd and Shek, 1998). The immunosuppression seen can be associated with increased circulatory levels of cortisol and catecholamine seen in severe stress, that is known suppress the immune system when not used up by the body.

There was a significant decrease in neutrophil counts of rats exposed to mild/short-term (EG1 rats) and moderate (EG2 rats) warm water swim-stress when compared with that of CG rats. There was also a significant increase in lymphocyte counts of rats exposed to mild/short-term and moderate warm water swim-stress when compared with that of CG rats. This may be due to rapid alteration of cell numbers via redistribution that results from increase in the release of catecholamine. This is in agreement with previous studies (Naliboff *et al.*, 1991; Glaser *et al.*, 1999) that reported that changes in epinephrine levels reflect lymphocytes migration. However, there was no significant decrease in neutrophil and lymphocyte counts following exposure to severe/long-term warm water swim-stress (EG3 rats) when compared with that of CG, suggesting that severe/long-term stress has no effect on the neutrophil and lymphocyte levels.

## CONCLUSION

In conclusion, mild/short term and moderate warm water swim stress stimulate the immune system, while severe/chronic stress suppresses the immune system. This study shows that the immunomodulatory effect of warm water swim stress is associated with CD<sub>4</sub> cell count.

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