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*Full Length Research Paper*

# Exploring the effects of stress on white blood cell function in female wistar rats

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**The effect of induced physical and oxidative stress on the white blood cells (WBC) of female Wistar rats was investigated. 40 matured female Wistar rats were divided into four groups (A.B.C.D) ten per group. Group A, control, Group B, mono enucleation, Group C, bilateral enucleation and Group D, alcohol treated. The total and differential white cell counts were evaluated over the six weeks period of the induced stress. The results revealed no difference in the average total and differential white cell counts of the mono and bilaterally enucleated groups. While the alcohol treated group showed increase especially in the total WBC, neutrophils and eosinophils compared to the control group (P-value <0.05).**

**Keywords:** Induced stress, Female Wistar rats, White blood cells (WBC)

## INTRODUCTION

*Stress* has effect on almost all animals. It is the sum of all non specific biological phenomena elicited by adverse external influences. (Martin, 2000).

"Stress" according to the Oxford Advanced Learners Dictionary of current English is any mental and/or physical state which can have an adverse effect on one's health. It is also defined as any situation that upsets homeostasis and threatens ones emotional or physical well being. Causes of stress include injury, intense exercise, extreme temperatures, alcohol, cigarette smoke and malnutrition. (Hornby, 2000)

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### Oxidative stress

Oxygen is the primary oxidant in metabolic reactions designed to obtain energy from the oxidation of a variety of organic molecules. Oxidative stress results from the metabolic reactions that use oxygen, and it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems in intact cells. This definition of oxidative stress implies that cells have intact pro-oxidant/anti-oxidant systems that continuously generate and detoxify oxidants during normal metabolism. When additional oxidative events occur, the pro-oxidant systems outbalance the anti-oxidant, potentially producing oxidative damage to lipids, proteins, carbohydrates, and nucleic acids, ultimately leading to

cell death in severe oxidative stress. Mild, chronic oxidative stress may alter the anti-oxidant systems by inducing or repressing proteins that participate in these systems, and by depleting cellular stores of anti-oxidant materials such as glutathione and vitamin E. A disturbance in pro-oxidant/anti-oxidant systems results from a myriad of different oxidative challenges, including radiation, alcohol ingestion, metabolism of environmental pollutants and administered drugs (these are xenobiotics, i.e., foreign materials), and immune system response to disease or infection.(Sies,1985)

Whatever the cause the body reacts to stress in a fairly consistent way called the “stress response” or “the general adaptation syndrome”. The response generally involves elevated levels of epinephrine and glucocorticoids especially cortisol: some physiologists now define stress as any situation that raises the cortisol level. A pioneering researcher on stress physiology, Canadian biochemist Hans Selye, showed in 1936 that the general adaptation syndrome typically occurs in three stages which he called;

- The alarm reaction
- The stage of resistance
- The stage of exhaustion

**The alarm reaction**

The initial response to stress is an alarm reaction mediated mainly by norepinephrine from the sympathetic nervous system and epinephrine from the adrenal gland. Epinephrine prepares the body to take action such as to fight or to escape. It raises the blood pressure and heart rate, increases circulation to the skeletal muscles and reduces blood flow to organs that can be assigned to lower priority. It stimulates glycogenolysis and gluconeogenesis, thus raising blood glucose level. In order to ensure that there is enough glucose supplied to the brain. Epinephrine reduces the secretion of insulin and the uptake of glucose by the other organs and muscles. This is because the other organs and the muscles can utilize other sources of energy but the nervous tissue needs only glucose. Aldosterone and angiotensin levels also rise during the alarm reaction. Angiotensin helps to raise the blood pressure and Aldosterone helps to promote sodium and water conservation which helps to offset possible losses by sweating and bleeding.(Sies,1985)

**The stage of resistance**

After a few hours, the glycogen reserves get exhausted and yet the nervous keeps demanding. If the stressful situation is not resolved before the glycogen reserves are gone, the body enters into the stage of resistance in which its priority is to provide alternative sources of fuels for metabolism. This stage is dominated by cortisol. The hypothalamus secretes corticotrophin releasing hormone and the pituitary gland responds by secreting adrenocorticotropic hormone and this in turn stimulates the adrenal cortex to secrete cortisol and other glucocorticoids. Cortisol promotes the breakdown of fat and protein in glycerol, fatty acids and amino acids. The liver converts these to glucose to act as a source for the nervous tissue. Like epinephrine, cortisol inhibits the uptake by most organs. It also inhibits protein synthesis, leaving the free amino acids available for gluconeogenesis. The immune system, which depends heavily on the synthesis of antibodies and other proteins, is depressed by long term cortisol exposure. Antibody levels drop, the number of circulating leukocytes declines and lymphoid tissues atrophy.

Wounds heal poorly and a person in chronic stress becomes more susceptible to infections. Cortisol stimulates gonadal secretion of sex hormones such as estrogens, testosterone and luteinizing hormone causing disturbances to fertility and sexual function.

**The stage of exhaustion.**

The body’s fat reserves can carry it through months of stress, but when is depleted, stress overwhelms the body’s homeostasis. The stage of exhaustion sets in often marked by rapid decline and death. With its fat stores gone, the body now relies primarily on protein breakdown to meet its energy needs thus; there is a progressive wasting away of the muscles and weakening of the body. After prolonged stimulation, the adrenal cortex may stop producing glucocorticoids, making it all the more difficult to maintain glucose homeostasis. Aldosterone sometimes promotes so much water retention that it creates a state of hypertension and while it conserves sodium, it hastens the elimination of potassium and hydrogen ions. This creates a state of hypokalemia (potassium deficiency in the blood) and alkalosis (excessively high blood pH), resulting in nervous and muscular system dysfunctions. Death frequently results from heart failure, kidney failure or overwhelming infection.(Sies,1985)

**MATERIALS AND METHODS**

The experimental period of the research covered six weeks after the initial period of one month used for acclimatization in the animals` house.

A total of 40 matured Wistar rats were used. These were grouped and put into four cages of ten each and allowed to acclimatize for a month in their new environment before the commencement of the experiment. The four groups were labeled thus: Group 1-controlGroup 2-mononueleation Group 3-bilateral

enucleation Group 4-alcohol.

At the end of the one month acclimatization, all the rats were weighed and recorded.

The mono and bilateral enucleation groups were individually anaesthetized with Ketamine hydrochloride at the dose of 30mg/kg body weight and given intraperitoneally and allowed to take effect.

### Dosage of Ketamine administered

30mg /kg body weight

### Dosage of Alcohol Administered

2g/kg body weight

The orbit was exposed by reflecting the eye lids and using sharp dissecting scissors, and going posteriorly the optic nerve was sectioned and the globe removed. Bleeding points from the ophthalmic vessels were controlled by the application of firm pressure with sterile gauge. Care was taken during this procedure not to hold the rats by the neck to avoid strangulation.

They were then returned to their respective cages and having unhindered access to both tap water and continuously available feeds. All the rats were weighed twice weekly throughout the period of the experiment and average of each week recorded. The alcohol group was also weighed twice a week (Wednesdays and Fridays) and on each occasion alcohol was administered using oropharyngeal tube at the dose of 2gm/kg body weight and the calculated amount in milliliter (ml) given. Blood samples were taken twice weekly counting of the total and differential white cells` count.

The blood samples were analyzed using Coulter Counter for total white blood cell count (WBC) and WBC differential counts (including Neutrophils (N), Lymphocytes (L), Monocytes (M), Eosinophils (E) and Basophils (B).

At the end of the sixth week, the rats were sacrificed by gassing in a chloroform chamber, dissected and the reproductive organs (ovaries, Fallopian tubes and uteri) harvested. The harvested ovaries and uteri were weighed and the Fallopian tubes measured and put in labeled storage containers with Bouin`s fluid in them.

The means, standard deviations and differences between the various measured parameters were calculated using appropriate Statistical packages to see whether there is any significance or not. ANOVA was used with p value <0.05.

## RESULTS

**Table 1.** White blood count (WBC) in percentage

| WBC | | Groups | | | |
|---|---|---|---|---|---|
| | | Control | Mononucleated | Bilteral Enucleated | Alcohol Treated* |
| Total | | 12.63 | 12.74 | 12.87 | 14.2 |
| Diff | N | 18 | 20.4 | 20.6 | 21 |
| | E | 3.8 | 4.2 | 4.4 | 5 |
| | L | 73.6 | 74.1 | 75 | 74 |
| | M | 4 | 1.3 | NIL | NIL |
| | B | 0.6 | NIL | NIL | NIL |

WBC= white blood cells
N= neutrophils
E= eosinophils
L= lymphocytes
B= basophils
*Note higher values of neutrophils and eosinophils in alcohol treated group (at P-value 0.93 there is significant difference between the groups because P-value > 0.05)

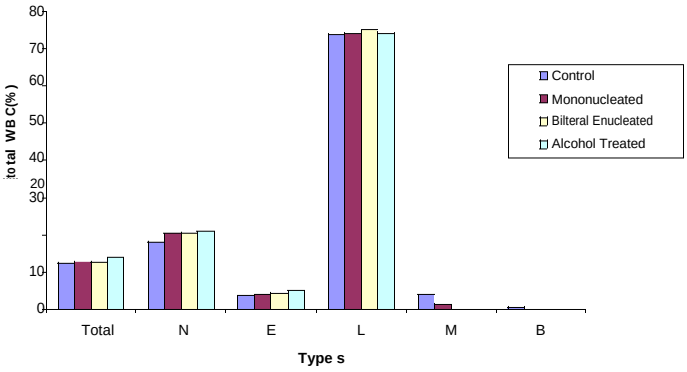


**Figure 1.** Comparison between the control and the experimental groups showing total and differential WBC.

*Note higher values of neutrophils and eosinophils in alcohol treated group (at P-value 0.93 there is significant difference between the groups because P-value > 0.05)

## DISCUSSION

Similarly, the neutrophils and eosinophils percentages rose in the alcohol treated group because of the continued effect of alcohol administration with statistically significant difference between the groups (Figure 5 and Table 3). All these have manifested in the high number of immature follicles in the alcohol treated group (Figure 4 and Table 2). The immature follicles are higher in the alcohol treated group as compared to the enucleated groups.

## CONCLUSIONS

The nature of the insulting induced stress seems to be the paramount factor in the causation of hematological and chemical changes.

Oxidative stress induced by alcohol consumption, caused some haematological effects in the female Wistar rat but the physical stress of enucleation did not cause any visible effect.

These results are also consistent with corresponding increase in the percentages of neutrophils and eosinophils especially in the alcohol treated group

## RECOMMENDATION

It is therefore recommended that further studies involving other forms of stress be carried out for longer periods of time so that more results concerning the effects of stress can be obtained.

Further hormonal and chemical studies in future will be necessary especially for comparative studies between animals` species and sexual differences within same species. Combination of various forms of stress can also be applied in future studies.

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