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# Simulated Microgravity Altered Hematological Indices Lipid Profile and Creatinine Kinase-Mb in *Rattus Norvegicus*
## Impact of Microgravity Induction in Rats

**Alaba Olumide Ojo*, Lawrence Adedayo, Olatunbosun Onaseso, Olufemi Oluranti, Fidelis Ejeheri, Emmanuel Timothy**

Bowen University, Iwo, Nigeria

*Corresponding Author: alaolumide_2@yahoo.com

# Simulated Microgravity Altered Hematological Indices Lipid Profile and Creatinine Kinase-Mb in *Rattus Norvegicus*

## Impact of Microgravity Induction in Rats

**Summary:** Simulated microgravity was induced in rats to mimic positive gravity in space for two weeks. Significant alterations in hematological indices were observed. Without alteration in lipid profile and creatinine kinase-MB

**Abstract:** Several studies related to simulated microgravity via various means have been carried out. However, the effect that positive microgravity exerts on plasma lipid and heart are poorly understood. This study was carried out to determine the influence of simulated positive microgravity via hind limb suspension on hematological indices, lipid profile and the heart using creatinine kinase as a marker.

Twenty-four male wistar rats were randomly divided into four groups of six rat each. Group 1 -Normal control, Group 2-Frutose control group, Group 3-Suspended animal without fructose diet and Group4-Suspended animals given fructose diet. After two weeks the animals were euthanized with chloroform, blood samples were collected via cardiac puncture and analyzed.

A significant increase in total white cell counts was observed, though, the amount of lymphocytes in group3 (60.833±11.523) was significantly lower than the control (group 1) (91.317±1.246). Also there were significant reduction in PCV and hemoglobin concentration of group3 compared to the control (group 1). There was no significant alteration in plasma lipid profile and creatinine kinase-MB.

Thus, positive microgravity simulation significantly altered hematological indices, but insignificantly affected plasma lipid profile and creatinine kinase-MB.

Key words: Hematology, Lipid, Creatinine-Kinase, Microgravity, Hindlimb.

## INTRODUCTION

The physiological systems of humans have with time gotten adapted to the earth's gravitational field, but outside the capacitance of this field the physiological systems tend to encounter numerous challenges. These challenges are experienced by astronauts that travel to space and the changes in the physiological systems include changes in cardiovascular system, lipid profile, immunological imbalances, stress markers and many other sensitive systems and parameters within the human body, although the study of most of this effects is poorly understood (Ashaolu and Ajao, 2015).

A research study carried out by Lampe and others showed that exposure to microgravity causes changes in body fluid distribution which increases hematocrit and thus causes a significant change within hem rheological values (which play a significant role in tissue perfusion) and hematological factors (Lampe *et al*.,1992).

Long time inversion of bats shows that simulated microgravity is associated with elevation of cortisol level which a marker for stress, reduced pack cell volume, reduced hemoglobin concentration and red cell count ( due to reduced erythropoietin production) as reported by Ashaolu and Ajao ,where they observed the presence of anemia, aggravated arterogenic cardiovascular risk and increased stress level as associated to bat prolonged inversion. (Ashaolu and Ajao, 2015).

Although studies of the effect of space environment should be carried out in space but due lack of funds and resources, researchers over the years have indulged in trying to mimic an environment that can help demonstrate these effects. This study will be using NASA accepted hind limb suspension of rodents by tail to simulate microgravity. The hindlimb suspension (HLS) of rodents by the tail is a well-established approach to create a ground-based model of microgravity and musculoskeletal disuse that mimics many of the physiological changes associated with space flight, as well as with prolonged bed rest (Morey-Holton *et al*., 2005; Carpenter*et al*., 2010).

The consumption of fructose has increased tremendously over the last five decades, which is to a large extent due to the development of high fructose corn syrup (HFCS), a commercial sugar additive that contains high amounts of free fructose. HFCS is often added to processed food and beverages partly because it is a powerful sweetener but even more so because the production is cheap. Although fructose in combination with fiber, vitamins and minerals, as present in fruits, is a healthy source of energy, isolated fructose, in processed food products has been associated with several health disorders such as insulin resistance and hypertension (Lowette*et al*., 2015).

The dyslipidemia observed in high fructose-fed rats included elevated triglycerides (TG), free fatty acids (FFA) and lipoprotein abnormalities (Dai and McNeill, 1995) These alterations are secondary to the development of insulin resistance (Reaven, 1989). Many studies have reported that fructose administration can have profound effects on plasma and tissue lipids levels. Michaelis *and colleagues* described an increase in total liver lipids in rats when glucose was iso-calorically substituted by either sucrose or fructose (Michaelis *et al*., 1975). This effect was attributed to the induction of various lip genic enzymes in the liver by fructose (Thorburn *et al*., 1989). The aims of this study were to estimate the effects of simulated microgravity on the heart using creatinine kinase-MB as a marker, to determine the hematological changes that accompany simulated microgravity and to determine the changes in plasma lipid profile in male *Rattus. norvegicus*.

## MATERIALS AND METHODS

Twenty four male Wistar rats (*Rattus norvegicus*), weighing between 150-200g were used in this study. The wistar rats were obtained from the animal house holdings of LAUTECH Teaching Hospital Osogbo, Osun state Nigeria. The rats were acclimatized in wooden cages at the animal house Department of Physiology, Bowen University, Iwo, Osun, State Nigeria for a week before piercing their tails. They were fed with food and water *ad*

*libitum*. The rat were divided into four groups of six rats per group viz; control group (Group 1); were not fructose loaded and not suspended , Group 2; were fructose loaded but not suspended (fructose control), Group 3;were suspended but not fructose loaded and Group 4; were fructose loaded and suspended

**PIERCING:** The tails of the suspended group were pierced close to the body about 1.5cm away from the body, the rats were anesthetized and a stainless 21g needle was inserted through the tail at level of an intervertebral disc. A cable was inserted through the needle, wrapped around to the dorsal side, it was secured and stabilized with gauze bandage and plaster and the metal ring was inserted through the intervertebral space. Then the rats were left for one week in the cages for recovery from the wound and healing **before hind limb unloading**

**HINDLIMB UNLOADING**

The methods described by Morey and Globus (2002) were used. A metal ring was attached to their tail and hung on a S-shaped hook attached to a shuttle which is also attached to the end of the bolt. The height of the animal's hind limb was adjusted to prevent any contact with the floor of the cage, which gives a tilt of 30° head downwards. The fore limb of rats maintained contact with the floor of the cage allowing the animal full access to the cage environment and ability to move 360° within the cage with access to water and feed. The rats were weighed before piercing was performed, just before hind limb unloading and after the experimental duration of two weeks of hind limb unloading.

**FIG .1 WISTAR RATS UNDERGOING SIMULATED MICROGRAVITY VIA HINDLIMB UNLOADING**

## COLLECTION OF BLOOD SAMPLE

The rats were euthanized using chloroform, after which a section through the abdominal and thoracic regions was opened in order to collect blood from the heart (Ojo and Anigbogu, 2016). Then a 5ml syringe was inserted through the apex of the heart from where blood was collected into both lithium heparinized bottles and EDTA bottles. The lithium heparinized bottles were centrifuged for 10 minutes at 3000rpm in order to separate plasma from the blood for lipid assay and creatinine kinase-MB assay while blood collected with EDTA bottle was used to analyse hematological parameters.

## HAEMATOLOGICAL ANALYSIS

An auto-digital hematological machine SFRI model-blood counter H-18 light. Blood sample collected was gently mixed. The clear blood sample was placed near the machine which operates via suction and the result were displayed within 2-3 minutes and printed via the printer connected to the machine.

Reagents used include; Haemaclair H-18; it is used to remove blockages, Diluclair H-18; it is used to wash the machine after the work, Buffer; it is the original diluent, it makes the machine to work faster, it is connected to the machine from the beginning of operation.

## PLASMA LIPID ANALYSIS

**T**hese were determined spectrophotometrically, using enzymatic colorimetric assay kits (Randox, RX MONZA) as follows: triglyceride (TG): plasma triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of Tietz (Tietz, 1990). High density lipoprotein cholesterol (HDL-cholesterol): plasma level of HDL-cholesterol was measured by the method of Wacnic and Alber (Wacnic and Alber,1978) . Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000rpm. The supernatant represented the HDL-cholesterol fraction. The cholesterol concentration in the HDL fraction which

remained in the supernatant, was determined. The value of HDL-cholesterol was expressed in unit of mg/dL.

HDL-cholesterol concentration= $A_{Sample}/A_{Standard}$×897mg/dL. **Very low density lipoprotein cholesterol (VLDL-cholesterol):** The serum level of VLDL-cholesterol was measured according to protocol of Friedewald (Friedewald *et al*., 1972).

**DETERMINATION OF CREATINE KINASE ACTIVITY**

The method of Duncan and colleagues (Duncan *et al*., 1995), as described by Saha and others (Saha *et al*., 1999) was adopted. 2.5ml of substrate (0.4M glycine +0.03M creatine phosphate + 0.062M K2CO3); pH adjusted to 8.9 with NaOH and 0.1ml of sample were mixed and incubated at 30$^{o}$C for 2 minutes. Absorbance was read 0, 1, 2 and 3 minutes at340nm against the blank.

**STATISTICAL ANALYSIS**

The results were tabulated as Mean ± SEM (standard error of mean), and were analysed using ANOVA (analysis of variance) with multiple comparism with SPSS 20, and the results were considered significant at $p<0.05$.

## RESULTS

### EFFECT ON HAEMATOLOGICAL PARAMETERS

**TABLE 1: This table shows effect of induced -ve gravity coupled with or without fructose load on haematological parameters and indices.**

| Parameters/Groups | GROUP 1 (Normal control) | GROUP 2 (Fructose control) | GROUP 3 (suspended) | GROUP 4 (Fructose loaded and suspended) |
|---|---|---|---|---|
| **WB C (/mm³)** | 3.2467±0.56277 | 4.0167±0.23010 | 7.3867±1.35254*[a] | 8.0367±0.73149*[a] |
| **RBC(mm³)** | 7.9650±0.18332 | 7.3817±0.38232 | 7.8700±0.38261 | 7.9967±1.27814 |
| **LYM (%)** | 91.3167±1.24564 | 87.4167±2.76024 | 60.8333±11.52281*[a] | 80.7167±3.61122 |
| **MONO (%)** | 4.3833±.38159 | 5.3000±1.47015 | 6.1167±0.96139 | 7.9667±1.36056 |
| **GRAN (%)** | 3.5333±0.83533 | 8.8500±2.61110 | 20.3500±7.52594 | 11.3167±3.27744 |
| **HGB (g/dl)** | 14.8333±0.30732 | 19.8500±5.69829 | 12.5117±0.94017*[a] | 14.4000±0.51381 |
| **PCV (%)** | 49.1833±0.63004 | 44.4167±1.39078 | 44.3833±1.92396* | 42.6167±2.67051* |
| **PLT(mm³)** | 590.0000±30.75603 | 601.0000±57.15126 | 667.8333±121.24065 | 763.0000±91.73513 |
| **MCV(fL)** | 61.4833±5.29764 | 60.6333±1.82399 | 56.5167±2.16790 | 58.4167±1.24457 |
| **MCHC (g/dl)** | 29.9667±0.67363 | 32.2167±0.52658 | 28.2667±0.31798[a] | 31.2167±1.97951 |
| **MCH (pg)** | 17.9667±0.37742 | 19.6000±1.15989 | 15.9833±1.18530[a] | 20.2167±2.54223 |

The results of the analysis of the hematological parameters: White blood cells count (WBC), Red blood cells count (RBC), lymphocyte count (LYM), monocyte count (MONO), granulocyte count (GRAN), hemoglobin concentration (HGB), packed cell volume (PCV), platelet count (PLT), mean cell volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were shown in Table 1.

The amount of total White blood cells in groups 3and 4 increased significantly ($p<0.05$) when compared with the control group (group 1) and fructose control (group 2). There was no significant change ($p>0.05$) in of red blood cell count when compared. The amount of lymphocytes appeared to be lower in both group 3 and group 4 significantly and insignificantly respectively when compared to that of the control group (group 1) and group 2 (fructose control).

The amount of granulocyte in both group 3 and 4 appeared to be higher than that of control group (group 1) and fructose control (group 2), but it was insignificant ($p>0.05$). The hemoglobin concentration in group 3 was significantly ($p>0.05$) higher than that of control group (group 1). Packed cell volume in group 3 and 4 were significantly lower ($p<0.05$) compared to that of the control group (group 1), and fructose control group (group 2).

Platelet count in both group 3 and 4 were insigficantly higher than that of control group (group 1) and fructose control (group 2) ($p>0.05$). Mean cell volume in both group 3 and 4 appeared to be lower than that of control group

**LIPID PROFILE**

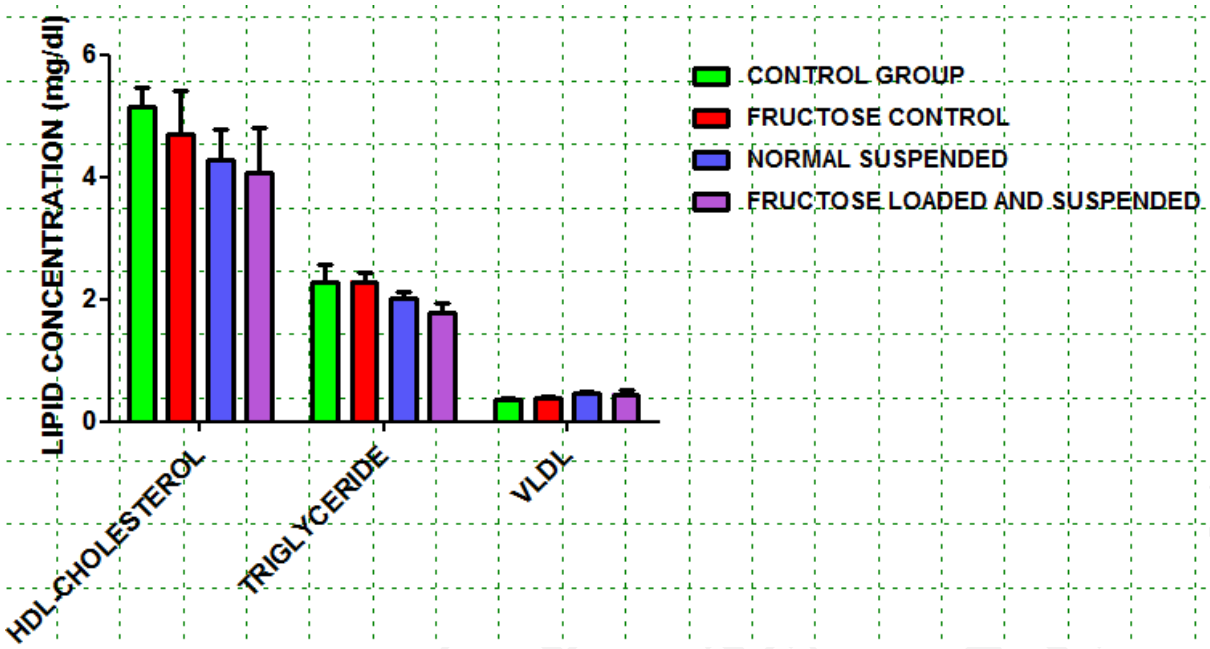


**FIGURE 2: BAR CHART SHOWING LIPID PROFILE RESULT**

Values are presented as Mean± SEM, *significant at P<0.05 when compared with group 1 and a significant at P<0.05 when compared with group 2, n=6

Figure 1 showed that the cholesterol-HDL level of group 3 and 4 were lower than that of the control group (group 1) but not significantly (p>0.05), they also appeared to be insignificantly lower when compared to that of the fructose control (group 2).

The triglyceride level of both group 3 and 4 appeared lower when compared to that of the control group (group 1) and the fructose control (group 2) but the difference in both comparison appeared insignificant.

Very low density lipoprotein (VLDL) appeared to be higher in group 3 and 4 when compared to that of the control group (group 1) but the difference was insignificant (p>0.05). The VLDL of group 3 and 4 also appeared to be higher when compared to the fructose control (group 2) but the difference was also insignificant (p>0.05).

**CREATININE KINASE-MB**

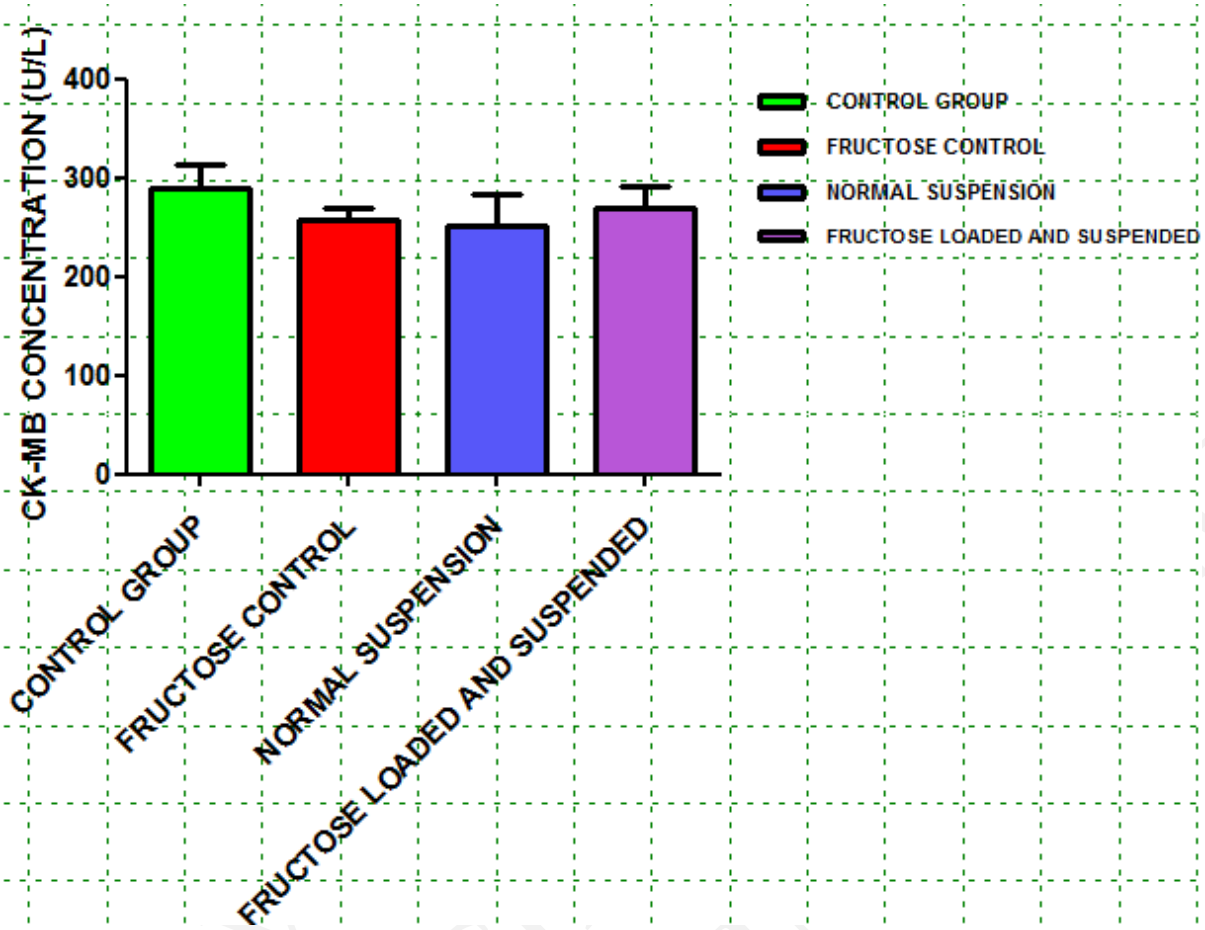


**FIGURE 2: BAR CHART SHOWING CREATININE KINASE-MB RESULT**

The creatinine kinase-MB (CK-MB) level of both group 3 and 4 appeared to be lower than that of the control group (group 1) but the difference is insignificant ($p>0.05$). The CK-MB levels of group 3 appeared to be lower than that of the fructose control (group 2), but the CK-MB levels of group 4 appeared to be higher than that of the fructose control (group 2). However both comparison were insignificant ($p>0.05$).

## DISCUSSION AND CONCLUSION

The result of this study revealed that induced positive gravity via hind limb suspension coupled with and without fructose had significant effects on the following hematological parameters; leucocytes, lymphocytes, hemoglobin, packed cell volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Moreover there was no significant difference in the effect of induced positive gravity via hind limb suspension coupled with and without fructose on lipid profile and creatinine kinase-MB when compared with the control group in this study.

Many laboratories around the world have used the rat hind limb suspension model to simulate weightlessness and to study various aspects of musculoskeletal loading and cardiovascular dysfunction. Hind limb suspension has played a significant role in space exploration because they provide data critical for the design of spaceflight experiments. Hind limb suspension model that simulate spaceflight are important for many reasons. Unlike spaceflight studies, experiments on Earth can be scheduled without concern for crew time, and modifications can be made as necessary during the experiment with little impact on cost. Furthermore, manipulations can be performed without the extensive precautions required for spaceflight experiments and the experimental duration can be varied with multiple time points measured within a single experiment (Morey and Globus, 2002). The hemodynamic changes in the tail suspension rats are similar to those found in astronauts during space flight (Convertino and Hoffler, 1992).

Investigation of hematological parameters represents a useful process in the diagnosis of many diseases as well as investigation of the extent of damage of blood tissue (Onyeyili *et al*., 1991). This is relevant since blood constituent change in relation to the physiological conditions of the animals. Hematological studies are important because blood is the major transport system of the body, and evaluation of the hematological profile usually furnishes vital information on the body's response to injury of all forms (Ojo *et al*., 2015).

Hematological constituents reflect the physiological responsiveness of the animal to its internal and external environments (Esonu *et al*., 2001).

Excess fructose consumption has been hypothesized to be a cause of insulin resistance, obesity (Elliot *et al*., 2002) , elevated LDL cholesterol and triglycerides, leading to metabolic syndrome (Basciano *et al*., 2005).

According to this study, there was significant increase ($p<0.05$) in the white blood cell count of the test groups with significant decrease ($p>0.05$) in amount of lymphocyte in group 3 and insignificant decrease in that of group 4 when compared with the control groups. The overall increase in leukocytes and the decrease in lymphocyte in group 3 is consistent with the findings by Patterson and Rayman, (2011). As of now, in spite of these changes, no illness has been attributed to these white blood cell changes. It is unknown whether or not a prolong suspension will cause further decrease in numbers as well as further dysfunction. Should this occur, the body's immune system would be compromised, making animal very susceptible to infectious disease, and possibly incapacitated by even minor illness that would otherwise easily be fended off by a normally functioning immunological system

The hemoglobin concentration of group 3 reduced significantly ($p<0.05$) and insignificantly ($p>0.05$) in group 4 when compared with that of the control groups. This decrease may impair the transport of respiratory gases in and out of body cells and thus affect the life span of this cells. Cells appear to respond to changes in the environment (Klaus et al., 1997) and to

have evolved structures that interact directly with the outside environment to sense the environmental loads placed upon them

Packed cell volume in the test groups reduced significantly ($p<0.05$) when compared to that of the control groups, this decrease is consistent with the findings. The significant decrease in packed cell volume is indicative of anaemic conditions (Ojo et al., 2015).

The amount of red blood cells counts in group 3 showed an insignificant decrease ($p>0.05$) when compared to that of the normal control group while that of group 4 was insignificantly higher ($p>0.05$). On the other hand the red blood cells of the test groups appeared to be insignificantly higher ($p>0.05$). This increase is inconsistent with the findings of other authors, which may be due to duration of experiment(Patterson and Rayman, 2011; and Ashaolu and Ajao, 2015).

The mean corpuscular hemoglobin in group 3 decreased significantly ($p<0.05$) and insignificantly ($p>0.05$) when compared to that of normal control and fructose control respectively. While that of group 4 increased insignificantly when compared with that of the control groups.

The mean corpuscular hemoglobin concentration in group 3 showed a significant decrease ($p<0.05$) when compared to that of the fructose control and insignificantly lower ($p>0.05$) when compared to that of normal control group. The mean corpuscular hemoglobin concentration of group 4 appeared to be insignificantly higher ($p>0.05$) to that of the normal control and same with that of the fructose control.

The mean cell volume of the test groups showed insignificant decrease ($p>0.05$) when compared to that of the control groups.

The amount of platelet in the test groups showed insignificant increase ($p>0.05$) when compared to that of the control groups.

The HDL-Cholesterol levels in the test groups showed insignificant decrease ($p>0.05$) when compared to that of the control groups. This is not consistent with the findings of Ashaolu and Ajao (Ashaolu and Ajao, 2015), and may be due to the duration of experiment and animal model used, because they examined on prolonged study on bats. Group 4 has the lowest level which may be due to the fructose loading they were exposed to.

The triglyceride levels in test groups showed insignificant decrease ($p>0.05$) when compared to that of the control groups, with that of group being the lowest. Although

fructose causes elevated level of triglyceride as reported by Dai and McNeill (Dai and McNeill, 1995).

The very low density lipoprotein levels in the test groups showed insignificant increase ($p>0.05$) when compared to that of the control groups, which is not consistent with previous findings (Ashaolu and Ajao, 2015).

The creatinine kinase-MB levels in the test groups showed insignificant decrease ($p>0.05$) when compared to that of the normal control groups, while when compared with the fructose control, group 4 and 3 appeared insignificantly ($p>0.05$) higher and lower respectively. The insignificant changes in the cardiac marker may be as a result of the duration of experiment which is consistent to the findings of other researchers (Zhi-Bin *et al.,* 2001; Dunlap *et al.,* 1996).

## CONCLUSION

The results of this work showed that simulated microgravity via hind limb suspension altered hematological parameters, without affecting plasma lipid profile and creatinine kinase-MB

## RECOMMEDENTATION

Further study is recommended to ascertain the findings in this study.

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