EFFECTS OF PARACETAMOL-INDUCED LIVER DAMAGE ON SOME HEMATOLOGICAL PARAMETERS: RED BLOOD CELL (RBC) COUNT, WHITE BLOOD CELL (WBC) COUNT, AND PACKED CELL VOLUME (PCV) IN WISTAR RATS OF EITHER SEX

*Seriki A. Samuel, Adebayo O. Francis, Oshagbemi Ayomide, Uche-Orji Onyinyechi
Department of Physiology, Faculty of Medical Sciences Bingham University, Karu, Nigeria.

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ABSTRACT
Paracetamol is a widely used analgesic and antipyretic. It is classified as a mild analgesic commonly used for the relief of headaches and other minor aches and pains. While it is generally safe for use at recommended doses (1000mg per single dose and up to 3000mg per day for adults human), acute overdoses of paracetamol can cause potentially fatal kidney, brain and liver damage, and in rare individuals, a normal dose can do same. The current study investigates the effect of paracetamol-induced liver damage on some haematological parameters in wistar rats. Twenty wistar rats were randomly allocated in two groups of 10 each, and were well fed for the duration of the research work. Rats in group 1(control) were fed with just normal rat feeds. But the rats in group 2 were, in addition, given over dose (300mg) of paracetamol for two days. On the third day, they were sacrificed using chloroform. The results showed that there was a significant decrease in Red Blood Cell (RBC) count between the control group and the treated (p<0.05). There was also a significant increase in white Blood Cell (WBC) count between the control group and treated group (P<0.05). There was also a significant decrease in the Packed Cell Volume (PCV) values between the control group and the treated group (P<0.05). Paracetamol 300mg/kg administered for two days significantly reduced RBC count and PCV, and increased WBC count of the wistar rats. Serum transaminase, an important indication of severe liver damage also increased.

Corresponding author
Seriki Samuel Adinoyi
Department of Physiology,
Bingham University, Karu, Nigeria
+2348036041121
sammlampese@yahoo.com


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INTRODUCTION

Liver

The liver is a vital organ present in vertebrates and some other animals. Its main functions are to take up nutrients and to store them, and to provide the nutrients to the other organs of the body. Also, the liver takes up potentially damaging substances like bacterial products or drugs delivered by the portal blood or microorganisms that reach the circulation [1].

Haematology and Blood Parameters

The study of blood and its formation is known as haematology. Blood is a specialized body fluid that delivers necessary substances to the body’s cells such as nutrients and oxygen. It also transports waste products away from those same cells. Blood is the most important body fluid that governs vital functions of the body like respiration, circulation, excretion, osmotic balance and transport of metabolic substances. Circulation of the blood within the cardiovascular system is essential for transportation of gases, nutrients, minerals, metabolic products and hormones between different organs. Blood is composed of two components, namely; Plasma (55%) and Blood cells (45%).

Plasma is a straw coloured clear liquid part of the blood. It contains 91-92% of water and 8-9% solids [2].

The blood cells are of three main types namely;
1. Red Blood Cells (RBC) or Erythrocytes
2. White Blood Cells (WBC) or Leukocytes
3. Platelets or Thrombocytes

Red Blood Cells are the non-nucleated formed elements in the blood, and the count normally, ranges between 4.2-5.9 million/mm³ in humans. It has a shape of biconcave disc, about 7µm in diameter and 2.2µm thick. Their unique shape relates to their function of transporting oxygen. The red colour of the cells is due to the presence of haemoglobin. They are mainly formed in the hematopoietic stem cells of the bone marrow by erythropoiesis and have a life span of 120 days.

White Blood Cells, on the other hand, are the colourless and nucleated formed elements of blood. They are larger in size and lesser in number when compared with the red blood cells. WBCs are either granulocytes or agranulocytes. Generally they are;
1. Neutrophils (54-62%)
2. Eosinophils (1-3%)
3. Basophils (less than 1%)
4. Monocytes (3-9%)
5. Lymphocytes (25-33%)

The Life span of each white blood cell and functions differ. The white blood cell count ranges between 5000-10000/mm³ in human.

Platelets are the formed elements of the blood. They are small colourless, non nucleated and moderately refractive bodies. Platelet count ranges between 100000-140000/mm³ [3].

Packed cell volume (PCV) is an important parameter used to express the proportion of red blood cell in percentage and this is significant in the diagnosis and treatment of conditions such as anaemia. The normal range is 38-42% in females and 40-45% in males. Also, blood indices such as mentioned below are sometimes measured because they have diagnostic values;
1. Mean corpuscular volume
2. Mean corpuscular haemoglobin
3. Mean corpuscular haemoglobin concentration

Blood parameters are probably the more rapid and detectable variations under stress and fuel for assessing the health condition of a patient. The importance of haematological parameters in clinical biochemistry, population genetics and medical anthropology is well established. Speculations have shown that they may be used as valuable indicators of disease or stress in animals [4].

Paracetamol (Acetaminophen)

Paracetamol or acetaminophen chemically named N-acetyl-p-aminophenol is a widely used over-the-counter analgesic (pain reliever) and antipyretic (fever reducer). It is classified as a mild analgesic that is commonly used for the relief of headaches and other minor aches and pain and is a major ingredient in numerous cold and flu remedies. In combination with the opioid analgesics, paracetamol can be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients [5]. The onset of analgesia is approximately 11 minutes after oral administration of paracetamol [6] and its half-life is 1-4 hours. Though paracetamol is used to treat inflammatory pain, it is not generally classified as an NSAID (Non-Steroidal Anti-Inflammatory Drugs) because it exhibits only weak anti-inflammatory activity.

While it is generally safe for use at recommended doses (1000mg per single dose and up to 3000mg per day for adults human), acute overdoses of paracetamol can cause potentially fatal kidney, brain and liver damage, and in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. In the western world, paracetamol toxicity accounts for most causes of acute liver failure and for most drug overdoses [7, 8].

It is the active metabolite of phenacetin, once popular as an anagelsic and antipyretic in its own right, but unlike phenacetin and its combinations, paracetamol is not considered carcinogenic at therapeutic doses [9]. The words acetaminophen and paracetamol both come from a chemical name for the compound: para-APAP, for acetyl-para-minophenol [8].

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Paracetamol-induced liver damage

Acetaminophen is safe and well tolerated when taken in the usual therapeutic dose. However, overdose of paracetamol is fairly common and often associated with hepatic and renal damage in both humans and experimental animals. In large dosages, acetaminophen produces acute liver and kidney necrosis in most mammalian species. Paracetamol-induced liver necrosis has been studied extensively, but the extra-hepatic manifestations of paracetamol toxicity are currently not well described. Acute renal failure is not uncommon and occurs in approximately 1-2% of patients with acetaminophen overdose. Most instances of paracetamol toxicity resulted from large, single overdose [10]. The toxicity of acetaminophen is closely related to its metabolism in both the liver and extra hepatic tissues. Acetaminophen is mainly metabolized by sulfation and glucuronidation. A small proportion is metabolized through cytochromes CYP2E1 and to a lesser extent CYP1A2 and CYP3A4, which produce a reactive metabolite, N-acetyl-p-benzoquione imine, NAPQI [11]. Liver paracetamol toxicity is associated with depletion of glutathione (GSH) followed by covalent binding of reactive paracetamol metabolites to tissue proteins [12] though a cause and effect relationship between covalent binding and toxicity has not yet been clearly established, the severity of paracetamol-induced hepatic damage is correlated with the extent of GSH depletion and covalent binding of radiolabel derived from APAP [13].

Paracetamol toxicity occurs in 3 phases;
Phase I - begins within hours of overdose
Phase II- begins within 24hours-72 hours of overdose
Phase III- begins within 3-5 days of overdose

The current study is to determine the effects paracetamol-induced liver damage on RBC count, WBC count and PCV in wistar rats of either sex. With understanding is expected to guide in prescription and use of paracetamol to avoid the damaging effect of overdose.

MATERIAL/METHODOLOGY

Materials

Chemicals and Reagents; Distilled water, paracetamol tablets, White cell diluting fluid, Red cell diluting fluid, Chloroform, Corn oil, Alanine Amino transferase diagnostic kit, Aspartate Amino transferase diagnostic kit

Equipments; Micro-hematocrit centrifuge, Weighing balance, Syringe and needle, EDTA bottles, Plain bottles, Neubaur counting chamber, Beaker, Red cell pipette, White cell pipette. Measuring cylinder, Microscope, Dissecting set, Dissecting board

Animals; 20 Wistar rats (2-4 weeks old) weighing 130 to 200

Animal Grouping: The animals were randomly allocated in two groups (10 per group). The rats were fed with water and rat feed for the whole duration of the research work.
Group 1 - Normal control given rat feed only
Group2- Also fed with rat feeds like those of Group 1, but given over dose (300mg) of acetaminophen, intra peritoneal administration for two days, and on the third day they were sacrificed using chloroform.

Bingham University Ethical Committee on the handling of experimental animals was consulted, and approval was gotten for all the entire procedure.

BLOOD COLLECTION

Blood samples were collected into EDTA bottles and serum bottles through the jugular vein

DETERMINATION OF SERUM TRANSAMINASES

Using Randox kit –

Transferases are made up of a group of enzymes which catalyse the interconversion of amino acids and alpha (α) keto acids (α-ketoglutteric acid) by transfer of amino groups. They are called amino transferases ( AST and ALT) or amino transaminases (GOT and GPT).

Principle of ALT:

Glutamic- pyruvic transferases are measured by monitoring the concentration of pyruvate hydrozone formed with 2,4-dintrophenylhydrazine [14].

ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate to form L-glutamate and pyruvate. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. Concomitantly, NADH is oxidized to NAD⁺, as illustrated below;

\[
\text{ALT} \quad \text{L-Alanine} + \alpha \text{–Ketoglutarate} \rightarrow \text{L-Glutamate} + \text{Pyruvate} \\
\text{LDH} \quad \text{Pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{Lactate} + \text{NAD}^+ 
\]

The rate of change of the absorbance difference between 340 nm and 405 nm is due to the conversion of NADH to NAD⁺ and is directly proportional to the amount of ALT present in the sample.
Principle for AST: Glutamic–oxaloacetic transaminase is measured by monitoring the concentration of oxaloacetate hydrozone formed with 2,4-Dinitrophenyl hydrazine [15].

AST catalyzes the reaction of L-aspartate and α-ketoglutarate into oxaloacetate and L-glutamate. Oxaloacetate is converted to malate and NADH is oxidized to NAD⁺ by the catalyst MDH.

\[
\text{AST} \quad \text{L-aspartate} + \alpha\text{-ketoglutarate} \rightarrow \text{Oxaloacetate} + \text{L-glutamate}
\]

\[
\text{MDH} \quad \text{Oxaloacetate} + \text{NADH} \rightarrow \text{Malate} + \text{NAD}^+ 
\]

The rate of absorbance change at 340 nm/405 nm caused by the conversion of NADH to NAD⁺ is directly proportional to the amount of AST present in the sample.

Method for AST and ALT: Two test tubes labelled blank and sample were used for each of the test, 0.1ml of serum sample was added to the sample tube, 0.5ml of solution 1 was added to the blank. Mix and incubate for exactly 30 mins at 37°C. 0.5ml of solution 2 was added to blank and sample tube. Mix and allowed to stand for exactly 20mins at 25°C. 5ml of sodium Hydroxide was added to the test and sample tube. The tubes were mixed and absorbance was read at 540nm against the blank. The activity for AST and ALT were obtained from a standard table.

DETERMINATION OF HAEMATOLOGICAL PARAMETERS

Determination of red blood cell count

Red blood cells were determined by visual counting method, using an improved Neubaur counting chamber [16]. Blood sample from the EDTA bottle was drawn into the red cell pipette up to the 0.5mark. The end of the pipette was wiped with a cotton wool. The red blood cell diluting fluid was drawn from a beaker into the pipette up to the 101 mark. The end of the pipette was wiped again. Both ends of the pipette were closed and the pipette was vigorously shaken to ensure the mixing of the fluid. This was done for about two minutes, then left to stand for about 5 minutes.

The counting chamber was cleansed thoroughly, placed on a horizontal flat surface and the cover slip was carefully put into position with firm pressure. The suspension was again mixed for about three minutes and two drops were discarded. Holding the chamber at the angle 45°C with the tip of the pipette slightly touching the edge of the cover slip, the chamber was filled with the fluids, carefully avoiding the spilling of the fluid into the channels.

The chamber was placed at the microscopic stage. Using the×40mm objective lens, the cells on the counting chamber were viewed and counted. The cells counted were those located at the top 16 box squares on the left and right side corners, lower left and right side corners and middle.

Calculations for red blood cell count;

Let N represent the number of cells counted in four large squares

Area of each small square = 1/400mm²
Depth of chamber = 1/10mm
Volume of fluid over each small square = 1/400mm² × 1/10mm

\[=1/4000mm^3\]

If N cells are counted in 80/4000mm³ of diluted blood then, 1mm³ of diluted blood contains \[1÷80/4000\]
\[= N\times4000/80\]

Dilution factor =200

1mm³ undiluted blood contains; \[N\times4000/80\times200 \text{cells/mm}^3\]
\[=N\times10000 \text{cells/mm}^3\] or \[N\times10000 \times10^6 \text{L of blood}\]

Determination of white blood cell count

Red blood cells were determined by visual counting method, using an improved Neubaur haemocytometer counting chamber.

Blood sample from the EDTA bottle was drawn into the white cell pipette up to the 0.5mark. The end of the pipette was wiped with a cotton wool. The white blood cell diluting fluid was drawn from a beaker into the pipette up to the 11 mark. The end of the pipette was wiped again. Both ends of the pipette were closed and the pipette was vigorously shaken to ensure the mixing of the fluid. This was done for about two minutes, then left to stand for about five minutes.

The counting chamber was cleansed thoroughly, placed on a horizontal flat surface and the cover slip was carefully put into position with firm pressure. The suspension was again mixed for about three minutes and two drops were discarded. Holding the chamber at the angle 45°C with the tip of the pipette slightly touching the edge of the cover slip, the chamber was filled with the fluids, carefully avoiding the spilling of the fluid into the channels.

The chamber was placed at the microscopic stage. Using the×40mm objective lens, the cells on the counting chamber were viewed and counted. The cells counted were those located at the top 16 box squares on the top left and right side corners, lower left and right side corners and middle.
Calculations for white blood cell count: 
Let N represent the number of cells counted in four large squares 
Area of each square= 1mm$^2$
Depth of chamber= 1/10mm
Volume of fluid over each square = 1mm$^2 \times 1/10$mm
If N cells are counted in 4/10mm$^3$ of diluted blood then, 1mm$^3$ of diluted blood contains $N \times 10/4$ cells
Dilution factor = 20
1mm$^3$ of undiluted blood contains: $N \times 10/4 \times 20$ cells/mm$^3$ = $N \times 50$ cells/mm$^3$ or $N \times 50 \times 10^6$L of blood

Determination of Packed cell volume
Packed cell volume was determined by micro haematocrit technique [17].

Principle:
A volume of anti-coagulated blood is placed in a glass tube which is centrifuged so the blood will be separate into three layers: Red cells, Buffy coat (WBC and platelets) and plasma. Ideally there should be complete separation of the three layers. Haematocrit is the ratio of the height of red cells column to that of the whole tube.

The method applied here was the micro tube method using capillary tubes and this was as a result of its advantage of short time of centrifugation and better packing of the red cells.
1. The blood sample to be used as fresh as possible and well mixed
2. Using the capillary action, allow blood to enter the tube stopping at 10-15mm from one end. Wiping the outside of the tube.
3. Seal the dry end by pushing into plasticine two or three times
4. If heat sealing is used the dry end of the tube is rotated in a fine Bunsen Burner flame
5. The tube is placed into one of the centrifuge plate slots, with the sealed end against the rubber gasket of the centrifuge plate
6. A record of sample number against centrifuge plate number is kept
7. Centrifuge is done for five minutes
8. PCV is read in the micro haematocrit reader
9. The haematocrit reader is expressed in percentage.

STATISTICAL ANALYSIS
Significant differences between experimental groups were estimated using unpaired Student’s t-test. The difference between the groups means were tested using the least significant differences (LSD) p-values <0.05 were considered statistically significant and results are expresses as the mean ±S.E except if otherwise stated.

RESULTS

Table- Mean ± SEM of control group and treated group (given 300mg/kg of paracetamol) for two days.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>300mg/kg of paracetamol</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (× 10$^6$/µL)</td>
<td>3.74±0.29</td>
<td>2.67±0.23*</td>
</tr>
<tr>
<td>WBC (×10$^3$/µL)</td>
<td>1.76±0.13</td>
<td>2.37±0.24*</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.55±3.05</td>
<td>23.35±2.93*</td>
</tr>
<tr>
<td>AST (IU)</td>
<td>31.48±5.38</td>
<td>67.72±7.19*</td>
</tr>
<tr>
<td>ALT (IU)</td>
<td>31.48±5.38</td>
<td>20.76±1.82*</td>
</tr>
</tbody>
</table>

Key: *p < 0.05.

When all the groups were compared, there was significant difference between control group, and the group given 300mg/kg of paracetamol which is the treated group. The values were represented using mean ±SEM and the P value was indicated
Effect of Paracetamol on RBC

The RBC values decreased from 3.74±0.29 for the control group to 2.67±0.23 for the treated group. There was a significant difference between the control group and the treated group as indicated by the decrease in the RBC values (p<0.05) (see table).

Effect of Paracetamol on WBC

The WBC values increased from 1.76±0.13 for the control group to 2.37±0.24 for the treated group. There was a significant difference between the control group and treated group as indicated by the increase in the WBC values (P< 0.05) (see table).

Effect of Paracetamol on PCV

The PCV values decreased from 33.55±3.05 for the control group to 23.35±2.93 for the treated group. There was a significant difference between the control group and the treated group as indicated by the decrease in the PCV values (P<0.05) (see table).

Paracetamol toxicity (effect on ALT and AST)

The ALT and AST values increased from 8.67±1.24 for the control group to 20.76±1.82 for the treated group and 31.48±5.38 for the control group to 67.72±7.19 for the treated group respectively. There were significant differences between the control groups and treated groups as indicated by the increase in both the ALT and AST values (P<0.05) (see table).

DISCUSSION

Results show that 300mg of paracetamol induced liver damage as indicated by the increased levels in the serum transaminases; ALT and AST (p<0.05) (see table). The increases in serum enzyme activities are roughly proportional to the extent of tissue damage. The damage caused significant changes in the red blood cell count, white blood cell count and packed cell volume of rats in the treated group which was given paracetamol overdose. [16].

It is evident that treatment of rats with paracetamol overdose caused significant (p<0.05) decrease in RBC count which could indicate that there were destruction of matured RBC and reduction in the rate of erythropoiesis. It also suggests that paracetamol has the potential to inhibit erythropoietin release from the kidneys. Paracetamol also caused significant decrease in PCV value (P<0.05) which could indicate the induction of anaemia. The significant increase (P<0.05) in total white blood cell count could be a result of the body defence mechanism trying to protect the body from being vulnerable to infections following the liver damage.

CONCLUSION

Paracetamol overdose in wistar rats causes liver damage, with extent of damage depending on the dose. This damage brings about alterations in the red blood cell count, white blood cell count and packed cell volume. Therefore, it is advised that caution be taken with the rampant use of paracetamol. And also, no form of abuse of the drug should be encouraged because of the fatal effect its overdose has on the liver cell that goes further to affect the blood cells. Only qualified medical practitioners are advised to prescribe paracetamol in the recommended dose to avoid these damaging effects.

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REFERENCES