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SUB-ACUTE EFFECT OF ASPIRIN ON SOME HAEMATOLOGICAL PARAMETERS IN WISTAR RATS

***[1]Mba, C., [2]Osita, C. and [3]Ogbo, I.**

[1]Department of Medical Laboratory Science, Faculty of Allied Health Science, Bayero University, P.M.B. 3011, Kano State, Nigeria

[2]Department of Medical Laboratory Science, Faculty of Health Science, Ebonyi State University, P.M.B. 53 Abakaliki, Ebonyi State, Nigeria.

[3]Department of Haematology and Blood Group Serology, Ebonyi State University Teaching Hospital, P.M.B 077Abakaliki,Ebonyi State, Nigeria

Corresponding Author: cmentor348@gmail.com+2348038847686

ABSTRACT

Back ground: Aspirin is one of the oldest analgesics used to relieve minor aches and pains, an antipyretic to reduce fever, an anti-inflammatory drug and still a widely used non-steroidal anti-inflammatory drug(NSAID).

Aim: This study is aimed to understand the effect of aspirin on haematological parameters of albino wistar rats (*Rattus norvegicus*). The human genome is very similar to that of rodents and due to this genome closeness it helps scientists to study them and to gain better understanding of human physiology and for new drug formulation and effects.

Methods: In this study, ten (10), 8 weeks old adult albino wistar rats with an initial average weight of 180±18.5 were grouped into 2 of five (5) animals each. The first group (control) was fed with poultry feed and water, the second group (test) was given food, water and dose of aspirin at a dose of 300mg/kg body weight twice daily for 7 days. The high dosage is necessary to maintain the plasma salicyclate levels; this is because the clearance rate of aspirin in mice is several folds higher as compared to humans. Haematological parameters such as packed cell volume, haemoglobin, total and differential white blood cell count and platelet count were measured.

Results: The aspirin treated rats showed a significant decrease in haemoglobin, packed cell volume, platelet count and a significant increase in total white blood cell count, a decrease in neutrophil as compared to control, and an increase in lymphocyte count as compared to the control.

Conclusion: The present investigation indicates that aspirin reduces platelet "by inhibiting the production of thromboxane, reduces haemoglobin level due to depressed erythropoiesis and elevated leucocyte by enhancing the rate of leucopoiesis, hence, the adverse effects of aspirin may include bleeding, thrombocytopenia, haemolytic anaemia and agranulocytosis.

Keywords: Aspirin and haematological parameters.

INTRODUCTION

Aspirin was first isolated by Felix Hoffman, a chemist with the German Company Bayer in 1897 under the direction of Arthur Eichengrun (Sneader, 2000).Aspirin is part of a group of medications called non-steroidal anti-inflammatory drugs (NSAIDs) but differ from most other NSAIDs in the mechanism of action(Sneader, 2000). Aspirin is rapidly absorbed from the stomach and small intestine primarily by passive diffusion across the gastrointestinal tract and rapidly hydrolyzed to salicylic acid by esterase in the in the GL mucosa and plasma salicylic acid is widely distributed throughout the

body, with the highest concentration in the plasma (Marcia, 2007). Aspirin is rapidly converted in the body to salicylic acid, which is responsible for most of the actions. Other actions are the result of acetylation of certain macromolecules including cyclooxygenase (COX) (Tripathi, 2008).

Haematological parameters are those parameters that are related to the blood formed element, some authors regard these haematological parameters as full blood count (Baker *et al*., 2000).

The mechanism of aspirin's analgesic, anti-inflammatory and antipyretic properties is believed to be due to the following mechanism:

- Suppression of prostaglandins and thromboxane.
- COX-l and COX-2 inhibition.
- Additional mechanism

Suppression of prostaglandins and Thromboxane

Aspirin's ability to suppress the production of prostaglandins and thromboxane is due to its irreversible inactivation of the cyclooxygenase (COX) enzyme required for prostaglandins and thromboxane synthesis(Vane, 1971). Aspirin acts as an acetylating agent where an acetyl group is covalently attached to a serine residue in the active site of the COX enzyme. This makes aspirin different from other NSAIDs, which are reversible inhibitors (Vane, 1971)

COX-1 and COX-2 Inhibition

There are at least 2 different types of cyclooxygenase (COX) namely COX-1 and COX -2. Aspirin irreversibly inhibits COX-1 and modifies the enzymatic activity of COX-2. COX-2 normally produces prostanoids, most of which are pro-inflammatory. Aspirin modified COX-2 produces lipoxins most of which are anti-inflammatory (Warner *et al*., 2002).

Aspirin has been shown to have at least three additional modes of action. It uncouples oxidative phosphorylation in cartilaginous and hepatic mitochondria by diffusing from the inner membrane space as a proton carried back into the mitochondria matrix, where it ionizes once again to release protons (Simon, 2010).

In addition, aspirin induces the formation of nitric oxide radicals in the body, which has been shown in mice to have an independent mechanism of reducing inflammation (Lisa, 2010)

The chemical properties of Acetylsalicylic acid (ASA) decompose rapidly in solutions of ammonium acetate or of the acetates, carbonates, citrates or hydroxides of the alkali metals. ASA is stable in dry air, but gradually hydrolysis in contact with moisture to acetic and salicylic acids. In solution with alkalis, the hydrolysis proceeds rapidly and the clear solutions formed may consist of acetate and salicylate (Carstensen*et al.,*1985).

Physical properties of aspirin which is an acetyl derivative of salicylic acid, is a white, crystalline, weakly acidic substance with a melting point of 1350C (1250F), boiling point of 140°C (284°F) (decomposes). The acid dissociation constant (pKa) for acetylsalicylic acid is 3.5 at 250 (Tripathi, 2008).

The pharmacokinetic data of aspirin are as follows

- Bioavailability: Rapid and completely absorbed
- Protein binding: 99.6%
- Metabolism: Renal
- Elimination of the drug is dose dependent (Tripathi, 2008).

The pharmacological actions of aspirin are as follows

1. Analgesic, antipyretic, anti-inflammatory action: the analgesic action is mainly due to obtunding of peripheral pain receptors and prevention of PG-mediated sensitization of nerve endings. Aspirin resets the hypothalamic thermostats and rapidly reduces fever by promoting heat loss but does not decrease heat production. In addition to COX inhibition, quenching of free radicals may contribute to its anti-inflammatory action.
2. Metabolic effects: cellular metabolism is increased; especially in skeletal muscles due to uncoupling of oxidative phosphorylation increases heat production.

3. Acid base and electrolyte balance: Aspirin produces significant changes in the acid-base and electrolyte composition of body fluids.

4. Blood: Aspirin even in small doses irreversibly inhibits TXA2 synthesis by platelets. Thus, it interferes with platelet aggregation and bleeding time is prolonged to twice the normal value. Long-term intake of large doses decreases synthesis of clotting factors in the liver and predisposes to bleeding but can be prevented by prophylactic vitamin k therapy (Tripathi, 2008).

Haematological parameters:

ii. White blood cell count
iii. Hemoglobin estimation
iv. Platelet count

Blood is a circulating tissue composed of fluid plasma and cells. It is composed of different kinds of cells (occasionally called corpuscles); these formed elements of the blood constitute about 45% of whole blood. The other 55% is blood plasma, a fluid that is the blood's liquid medium, appearing yellow in color. The normal pH of human arterial blood is approximately 7.40 (normal range is 7.35-7.45), a weak alkaline solution. Blood is about 7% of the human body weight, so the average adult has a **Red Blood Cells** are the most numerous cells in the blood. In adults, they are formed in the marrow of the bones that form the axial skeleton. Mature red cells are non-nucleated and are shaped like flattened, bilaterally indented spheres, a shape often referred to as ”biconcave disc” with a diameter 7.0-8.0μm and thickness of 1.7-2.4μmblood volume of about 5 liters, of which 2.7-3 liters is plasma. They are primarily involved in tissue respiration. The red cells contain the pigment hemoglobin which has the ability to combine reversibly with oxygen. In the lungs, the hemoglobin in the red cell combines with oxygen and releases it to the tissues of the body (where oxygen tension is low) during its circulation. Carbondioxide, a waste product of metabolism, is then absorbed from the tissues by the red cells and is transported to the lungs to be exhaled. The red cell normally survives in the blood stream for approximately 120 days after which time it is removed by the phagocytic cells of there reticuloendothelial system, broken down and some of its constituents reutilized for the formation of new cells.

White Blood Cellsare a heterogeneous group of nucleated cells that are responsible for the body’s defenses and are transported by the blood to the various tissues where they exert their physiologic role, e.g. phagocytosis.WBCs are present in normal blood in smaller number than the red blood cells. Their production is in the bone marrow and lymphoid tissues (lymph nodes, lymph nodules and spleen). The normal cell values ranges from 4-10 x 10^9/L in apparently healthy individual. White cell counts in infancy and childhood tends to be greater than in adults with values as high as 25 x 10^9/L at birth. The count drops over the seven days of life to about 14 x 10^9/L. The leucocytes count may be increased due to factors such as ingestion of food, physical exercise, and emotional stress. (Maton, 2007).There are five distinct cell types each with a characteristic morphologic appearance and specific physiologic role. These are:

- Polymorphonuclear leucocytes/granulocytes
  - Neutrophils
  - Eosinophils
  - Basophiles
- Mononuclear leucocytes/agranulocytes
  - Lymphocytes
  - Monocytes

(cheesbrough, 2000)

Platelets are small, non-nucleated, round/oval cells/cell fragments that stain pale blue and contain many pink granules. Their size ranges 1-4μm in diameter. They are produced in the bone marrow by fragmentation of cells called megakaryocytes which are large and multinucleated cells. This process is regulated by thrombopoietin, a hormone usually produced by the liver and kidneys and the average life span of a platelet is 7-10 days.

The physiological range for platelet is 150-400 x 10^9/L. Their primary function is preventing blood loss from hemorrhage. When blood vessels are injured, platelets rapidly adhere to the damaged vessel and with one another to form a platelet plug. During this process, the soluble blood coagulation factors are activated to produce a mesh of insoluble fibrin around the clumped platelets. This assists and strengthens the platelet plug and produces a blood clot which prevents further blood loss fibrin around the clumped platelets. This assists and strengthens the platelet plug and produces a blood clot which prevents further blood loss. Platelets release a multitude of growth factors including platelet – derived growth factor (PDGF), a potent chemotactic agent and transforming growth factor- B, which stimulates the deposition of extracellular matrix. Both of these growth factors have been shown to play a significant role in the repair and regeneration of connective tissues (Connels, 2008).Wistar rats are an outbreed strain of albino rats belonging to the species *Rattus norvegicus*. This strain was developed at the wistar institute in 1906 for use in biological and medical research, and is notably the first rat strain developed to serve as a model organism at a time when laboratories primarily used *Musmusculus* or the common house mouse. More than half of all laboratory rat strains are descended from the original colony established by physiologist Henry Donaldson, scientific administrator Milton J. green man and genetic researcher embryologist Helen Dean King (Wistar institute retrieved 2007). The Wistar rat is currently one of the most popular rat strains used for laboratory. Its long ears, wide head and having a tail length that is always less than its body length, characterize it. (Clause, 1998).The human genome is very similar to that of rodents than mouse, due to this genome closeness it permits scientists to study them and to gain understanding for new drug development, (krinke*etal.,*2000).

Methodology

Adult Wister albino rats were purchased from medical research laboratory in university of Nigeria Teaching Hospital (UNTH) in Enugu State and were allowed to breed in the animal house in Prescocampus. Aspirin (acetylsalicylic acid) tablets containing (300mg/tablet) were purchased from the market, manufactured by Kunimed Pharmachem LTD, Lagos Nigeria.

Experimental Animal

Ten(10) 8 –weeks old albino rat of the genus *Rattus norvergicus* which comprised of both sexes were allowed for 3 days to acclimatize before the administration of the drug. The rats were housed 5 per cage and allowed free access to food water throughout the experiment. Aspirin was suspended in normal saline and administered orally with dose regime of 300mg/kg of aspirin was administered twice daily for 7 days running. The control animals received equivalent amounts of saline only.

Sample Collection

At the end, the animals were bled and blood was obtained by ocular puncture using EDTA as an anticoagulant.

Method Used (Adopted From Cheesbrough 2006)

White blood cell count and platelet count was estimated using improved neubauer chamber; packed cell volume was measured using microhematocritmethod. Blood films were fixed in methanol, stained with leishman stain". Absolute WBC counts were estimated by extrapolating the percent differential counts with that of total leucocyte counts.

Estimation of Packed Cell Volume (PCV)

Principle of Test (using, microhaematocritmethod)

PCV is that proportion of whole blood occupied by red-cells expressed as a ratio (litre). Anticoagulated blood in a glass capillary tube of specificlength bore size and wall thickness is centrifuged in a microheamatocrit centrifuge at RCF 12000g for 5 minutes to obtain constant packing of the red cells.

A small amount of plasma remains trapped between the packed red cells. The PCV value is read from the scale using a microhematocritreader

Total white blood cell count (using improved Neubauer counting chamber)

Principle of the test

Whole blood is diluted 1 in 20 in an acid reagent (Turks solution), which hemolyzes the red cells and platelets leaving the white cells to be counted. White cells are counted microscopically using improved neubauer chamber and the number of white blood cells per litre of blood calculated.

Platelet Count

Principle of the Test

Blood is diluted 1 in 20 in a filtered solution of ammonium oxalate reagent which lyses the red cells, platelets are counted microscopically using improved neubauer counting chamber and the number of platelets per litre of blood calculated.

Haemoglobin Estimation
(Using Cyanmethaemoglobin Method)

Principle of the Test

Whole blood is diluted 1 in 201 in a modified Drabkin'ssolution which contains potassium ferricyanide and potassium cyanide. The red cells are hemolyzed and the hemoglobin is oxidized by the ferricyanide to methemoglobin (Hemiglobin, Hi). This is converted by the cyanide to stable hemiglobin cyanide (HiCN). Absorbance of the HiCN solution is read in a spectrophotometer at wavelength 540nm.

RESULTS

Ten adult wistar rats were used to carry out the investigation on the sub-acute effect of aspirin on hematological parameters. The results of this study are shown in table 1 as well as figure 1-4. In this study the animal treated with aspirin had significantly lower (P>0.05) packed cell volume (PCV), hemoglobin (Hb) and platelet as compared to the controls(Table 1) whereas there was an increase in lymphocyte as compared to that of control [Table 1 . The total white blood cell count (WBC) was significantly increased in rats treated with aspirin as compared to control.

In fig 1, mean packed cell volume of test animal shows a decrease compared to control. However, independent sample t- test indicates a significant difference (p=0.03) in mean packed cell volume between both groups

The mean neutrophil count shows a decrease compared to that of control. However, independent sample t-test indicates no significant difference (P=0.14) in mean neutrophil count between both groups.

The mean percentage lymphocyte count of test animal shows an increase compared to that of control animals. However independent sample t-test indicates a no significant difference (P=0.17) in both groups.

In fig 2, mean a white blood cell count shows an increase in test animal compared to that of control. However, independent sample t-test indicates a significant difference (p=0.01) in both groups.

In fig 3, the mean platelet count of test animal shows a decrease compared to that of control. However, independent sample t-test indicates a significant difference (P=0.01) between both groups.

In fig 4, mean hemoglobin concentration of test animal shows a decrease compared to control. However, independent sample t-test indicates a significant difference (p=0.02) in mean hemoglobin concentration between both groups.

Table 1: Result of sub-acute effect of aspirin in hematological parameters.

Parameters	Test (n=5)	Control (n=5)
PCV (%)	48.0± 2.59*	51.6 ± 0.55
Hb (g/dl)	15.7± 1.45*	17.5 ± 0.36
Platelet (x 109/l)	283.4 ± 1.82*	378.4 ± 45.51
WBC (x 109/l)	11.7 ± 1.68*	8.3 ± 0.39
Neutrophil (%)	24.6 ± 2.89	30.0 ± 6.36
Lymphocyte (%)	74.8 ± 2.89	70.0 ± 6.20

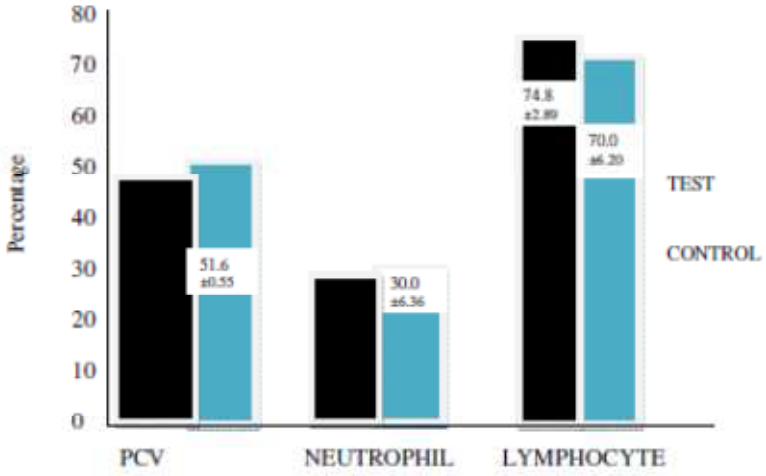

Figure 1:

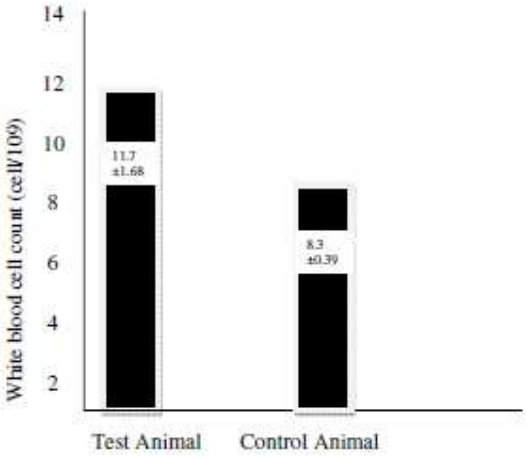

Figure 2: Mean white blood cell count ion test and control animals

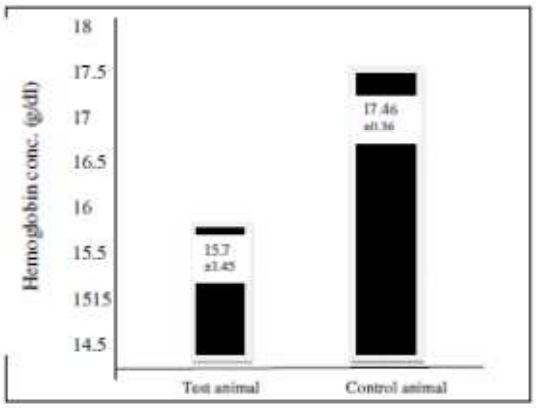

Figure 3: Mean white blood cell count ion test and control animals

DISSCUSSION

The results of the present study indicate that aspirin have adverse effect on some hematological parameters. Prolongation of bleeding time was one of the first clinically recognized hematological side effects of aspirin administration (Cerletti *et al*, 1988) hemolytic anemia, thromobocytopenia, and agranulocytosis are some of the most frequently reported adverse effect of aspirin (Tripathi,2008). In the present study, aspirin treatment significantly reduced the packed cell volume, hemoglobin concentration and platelet of the treated animals whereas there was a slight decrease in neutrophil and slight increase in lymphocyte with a significant increase in total white blood cell count. These results are in agreement with the published reports in rats (Merchant *et al,* 2004)

The rate of erythropoiesis in the treated animals appeared depressed, probably indicating erythropoietin deficiency. The speculation is supported by the fact that since there is direct relationship between the renal prostaglandin level and erythropoietin production, inhibition of prostaglandin production by aspirin (through inhibition of cyclooxygenase-2) may reduce renal erythropoietin production and it is possible that the reduction in erythropoietin production may further aggregate the anemia already induced by aspirin in the treated animals.

CONCLUSION

From the whole study, the effect of aspirin on hematological parameters has been established. It can be concluded that aspirin significantly reduces the packed cell volume (PCV), hemoglobin (Hb) and platelet as compared to that of the control and a significant increase in its total white blood cell count (WBC), there was also a slight decrease in neutrophil and a slight increase in lymphocyte.

RECOMMENDATION

Due to the difficulty in feeding the rats and drug compliance as my limitation, it is recommended that the research should be conducted in humans, to verify if the effect is similar.

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